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Sekcije . Sessions

Sekcija . Session		Voditelj sekcije Session moderators
0	Plenarna izlaganja Plenary Session	
1	Agroekologija, ekološka poljoprivreda i zaštita okoliša Agroecology, Ecological Agriculture and Environment Protection	Doc.dr.sc. Aleksandra Perčin
2	Agroekonomika i ruralni razvoj Agricultural Economics and Rural Development	Doc.dr.sc. Josip Juračak
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4	Povrčarstvo, ukrasno, aromatično i ljekovito bilje Vegetable Growing, Ornamental, Aromatic and Medicinal Plants	Prof.dr.sc. Nina Toth
5	Ratarstvo Field Crop Production	Izv.prof.dr.sc. Darko Uher
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9	Voćarstvo Pomology	Doc.dr.sc. Goran Fruk
10	Poljoprivredna tehnika Agricultural Engineering	Doc.dr.sc. Vanja Jurišić

Contents

Session 0 | Plenary Session

- 3 **Minja Zorc, Peter Dovč**
| Advances in lactation biology

Session 1 | Agroecology, Ecological Agriculture and Environment Protection

- 11 **Ivan Širić, Valentino Držaić, Ivica Kos, Dalibor Bedeković, Bojana Božac, Rina Petric**
| Bioakumulacijski potencijal poljske pečurke *Agaricus campestris* L.
| Bioaccumulation potential of field mushroom *Agaricus campestris* L.
- 16 **Helena Žalac, Vladimir Zebec, Miro Stošić, Domagoj Radić, Andrea Špoljarić, Jurica Jović, Ivan Paponja, Vladimir Ivezić**
| Buckwheat yields in intercropped systems of walnut and buckwheat
| Prinos heljde u konsocijaciji nasada oraha i heljde
- 21 **Violina Angelova**
| Uptake of heavy metals by enhanced tobacco grown in industrially polluted soils in Bulgaria
- 27 **Domina Delač, Ivica Kisić, Paulo Pereiraz**
| Dynamics of runoff pH and electrical conductivity after pile burns
- 33 **Renata Erhatic, Ivka Kvaternjak, Branimir Jambrišak, Mirjana Mužić**
| Sadržaj mikroelemenata u listu i cvijetu prave lavande (*Lavandula angustifolia* Mill.) i lavandina (*Lavandula × intermedia* Emeric ex Loisel.)
| Content of microelements in leaf and flower of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.)
- 38 **Marija Galić, Darija Bilandžija, Milan Mesić, Aleksandra Perčin, Željka Zgorelec**
| Seasonal variability of soil respiration during maize vegetation
| Sezonska varijabilnost disanja tla tijekom vegetacije kukuruza
- 44 **Danijela Jungić, Petar Unger, Stjepan Husnjak**
| Humoznost i kiselost lesiviranih tala na lesu u Panonskoj agoregiji Hrvatske
| Humus content and acidity of Luvisols on loess in Pannonian agoregion of Croatia
- 49 **Hrvoje Kutnjak, Ivona Žiža, Nela Jantol, Josip Leto**
| Seasonal phenology dynamics of alfalfa consociation with Italian ryegrass based on NDVI from Sentinel-2
| Sezonska fenološka dinamika smjese lucerne i talijanskog ljujla bazirana na NDVI-u sa Sentinel-2
- 54 **Ivka Kvaternjak, Renata Erhatic, Miomir Stojnović, Alanais Louis, Antoine Jouve**
| Macroelement content of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.) in organic fertilization
| Sadržaj makroelemenata u lavandi (*Lavandula angustifolia* Mill.) i lavandinu (*Lavandula × intermedia* Emeric ex Loisel.) pri organskoj gnojdbi

- 59 **Ivan Magdić, Ranka Popović, Vedran Rubinić**
Mogućnost pojednostavljenja postupka određivanja gustoće čvrstih čestica tla piknometarskom metodom
Possibilities for simplifying soil particle density determination by pycnometer method
- 64 **Ana Mrgan, Helena Marčetić, Arijana Penava, Kornelija Perković**
Ocjena kvalitete komunalne otpadne vode s područja aglomeracije Lipik – Pakrac na mjestu ispuštanja u kanal Iliđa
Quality assessment of municipal waste water from the area of the agglomeration Lipik-Pakrac measured on the site of discharge to Iliđa canal
- 70 **Ana Nimac, Ivana Štajcer, Zlatko Šatović, Klaudija Carović-Stanko, Boris Lazarević**
Effects of nitrogen deficiency on some physiological parameters and root traits of three Croatian common bean landraces
Utjecaj nedostatka dušika na neke fiziološke parametre i svojstva korijenovog sustava triju hrvatskih tradicijskih kultivara graha
- 76 **Péter Ragályi, Zdenko Lončarić, Andrijana Rebekić, Márk Rékási, Barbara Borsányi, Sándor Molnár, Anita Szabó, Eszter Draskovits, Nikolett Uzinger**
The effect of sewage sludge and sludge compost on soil fertility, organic matter content and yield of perennial ryegrass (*Lolium perenne*)
- 81 **Sanja Grubišić, Marija Kristić, Miroslav Lisjak, Katarina Mišković Špoljarić, Zdenko Lončarić, Andrijana Rebekić**
In vitro bioaccessibility of Ca, K, Mg, Mn, Fe and Zn from wheatgrass juice
In vitro biodostupnost Ca, K, Mg, Mn, Fe i Zn iz soka pšenične trave
- 86 **Aida Šukalić, Maida Đapo Lavić, Alma Rahimić, Vedrana Komlen, Alma Mičijević**
Carcinogenic hazard index of heavy metals from agricultural soil in the southeastern part of Bosnia and Herzegovina
- 91 **Diana Kikić, Ivica Kisić, Željka Zgorelec, Aleksandra Perčin**
Portable X-ray fluorescence as a tool for characterization of nutrient status in soil
Prijenosna rendgenska florescencija kao alat u karakterizaciji opskrbljenosti tla hranivima

Session 2 | **Agricultural Economics and Rural Development**

- 97 **Ivan Spužević, Saša Zovko**
Značaj troškova mineralnih gnojiva za cijenu koštanja i isplativost proizvodnje lubenice
Significance of mineral fertilizer costs for cost price and cost effectiveness of watermelon production
- 104 **Milna Tudor Kalit, Matea Šuš, Marina Tomić Maksan, Samir Kalit**
Stavovi potrošača o sirevima tradicionalne i industrijske proizvodnje
Consumer attitudes about traditional and industrial cheese production
- 109 **Alexander Wirsig, Wolfgang Heisrath**
Welcome to the club? The significance of protected geographical indications and designations of origin in Europe, with special consideration of the Croatian situation

- 114 **Tihana Sudarić, Krunoslav Zmaić, Vinka Čepo**
Razvoj eno-gastronomске turističke destinacije
Development of wine and gastronomic tourist destination
- 119 **Željka Mesić, Matea Šaban**
Obilježja online kupnje ekoloških prehrambenih proizvoda u Hrvatskoj
Characteristics of online purchases of organic food products in Croatia
- 123 **Krisztián Kovács, István Szűcs, Vesna Očić, Josip Juračak**
Efficiency comparison of the main Croatian and Hungarian animal husbandry sectors
- 128 **Marin Čagalj, Marko Ivanković, Ivo Grgić**
Vanjskotrgovinska razmjena Republike Hrvatske i Bosne i Hercegovine poljoprivrednoprehrambenim proizvodima u razdoblju od 2013. do 2018. godine
External trade of agricultural and food products between the Republic of Croatia and Bosnia and Herzegovina in the period 2014-2018
- 133 **Sanja Jelić Milković, Zrinka Tolušić, Ružica Lončarić**
Analiza mišljenja domaćih proizvođača vina o konkurenciji
Analysis of domestic winemakers' opinions on competition
- 139 **Branka Šakić Bobić, Ornella Mikuš, Nataša Šenjug, Zoran Grgić, Vesna Očić**
Iskustva poljoprivrednika Krapinsko-zagorske županije s fondovima Europske Unije
Experiences of farmers from Krapina-Zagorje County with EU funds
- 144 **Ljiljana Božić-Ostojić, Slavica Antunović, Mihaela Blažinkov, Teuta Benković Lačić**
Stavovi proizvođača Brodsko-posavske županije o stanju poljoprivrede i poljoprivredne politike
Attitudes of producers of Brod-Posavina county on the state of agriculture and agricultural policy
- 149 **Ana Čehić, Martina Begić, Marija Cerjak, Milan Oplanić**
Maslinarstvo kao dio turističke ponude
Olive growing as a part of tourism supply
- 153 **Zoran Grgić, Mato Čačić, Zlatko Pavičić, Branka Šakić Bobić**
Osnovni činitelji ekonomike proizvodnje mlijeka na farmama različitog kapaciteta
Basic factors of economics of milk production on farms of different capacity
- 158 **Forest David, Tünde Kovács**
Introducing QSPM Analysis to Agribusiness Firms
- 162 **Damir Kovačić, Marina Čurčić, Marija Cerjak, Željka Mesić**
Mogućnost uvođenja jednokratne ambalaže za ajvar na domaće tržište
The possibility of introducing a disposable ajvar packaging on the domestic market

Session 3 | **Genetics, Plant Breeding and Seed Production**

- 167 **Anita Bošnjak Mihovilović, Ivanka Habuš Jerčić, Marijana Barić, Ivana Tomaz, Tatjana Prebeg, Kristina Bekavac, Snježana Kereša**
Utjecaj osvjetljenja različitih spektralnih karakteristika na formiranje puči kapara (*Capparis orientalis* Veill.) u *in vitro* i *in vivo* uvjetima
Effect of different light spectra on stomata formation of caper (*Capparis orientalis* Veill.) *in vitro* and *in vivo*

- 172 **Aida Dervishi, Doriana (Bode) Xhulaj, Ilda Kaso**
| Genetic diversity of some important wheat (*Triticum aestivum* L.) genotypes in Albania based on morphological traits and RAPD markers
- 177 **Ivanka Habuš Jerčić, Snježana Kereša, Anja Gotić, Anita Mihovilović Bošnjak, Marijana Barić**
| Reakcija genotipova pšenice na različite gustoće sjetve
| Response of wheat genotypes to different sowing densities
- 182 **Ana Lovrić, Nikolina Novaković, Bruno Rajković, Marko Maričević, Ivica Ikić, Hrvoje Šarčević**
| Otpornost na priježetveno proklijavanje u potomstvima dvaju križanja ozime pšenice
| Resistance to pre-harvest sprouting in progenies of two winter wheat crosses
- 187 **Monika Vidak, Ana Nimac, Zlatko Šatović, Jerko Gunjača, Boris Lazarević, Klaudija Carović-Stanko**
| Chlorophyll fluorescence as a method for the prediction of germination success in common bean (*Phaseolus vulgaris* L.)
| Fluorescencija klorofila kao metoda za određivanje klijavosti sjemena graha (*Phaseolus vulgaris* L.)

Session 4 | **Vegetable Growing, Ornamental, Aromatic and Medicinal Plants**

- 193 **Kristina Batelja Lodeta, Davorka Poljak, Lucijana Kovačević, Gvozden Dumičić, Smiljana Goreta Ban**
| Morfološka svojstava kultivara mini lubenice
| Morphological traits of mini watermelon cultivars
- 198 **Božidar Benko, Mislav Đurić, Sanja Radman, Boris Lazarević, Nina Toth, Ivanka Žutić, Nevena Opačić**
| Morfometrijska svojstva ploda i prinos krastavca tretiranog biljnim biostimulatorima
| Fruit morphometric properties and yield of cucumber treated with plant biostimulants
- 203 **Elena Liliana Chelariu, Lucia Draghia, Maria Apostol, Bogdan-Vlad Avarvarei, Mirela Cojocariu, Nicoleta Luminița Paraschiv**
| Vermicompost influence on seedlings quality of *Kniphofia uvaria* species
- 208 **Nina Kacjan Maršić, Katarina Košmelj, Ana Slatnar**
| The impact of planting date and cultivar on yield and morphological traits of garlic (*Allium sativum* L.) bulbs: data from a small-scale experiment
- 213 **Erjon Mamoci, Kostlend Mara, Ilir Lloha, Maria Fe Andrés, Sonia Olmeda, Azucena González- Coloma**
| Chemical composition and antimicrobial activity of essential oils from Albanian conifer plants
- 218 **Nevena Opačić, Tamara Brlek, Sanja Slunjski, Kristina Kljak, Sanja Radman, Sanja Fabek Uher**
| Količina makroelemenata u mladim izdancima cikle i brokule
| Macroelements content of red beet and broccoli microgreens
- 223 **Anisa Peculi, Nertil Xhaferaj, Sara Serbo, Anila Kopali, Alketa Shehaj**
| Determination of caffeine content by UV-VIS spectrophotometer in coffee and tea samples available on Albanian market

Contents

- 227 **Sanja Radman, Antonia Skomrak, Božidar Benko, Sanja Fabek Uher, Nevena Opačić, Ivanka Žutić**
| Utjecaj pre-sjetvenih tretmana na klijavost sjemena mrkve, peršina i kopra
| Effect of pre-sowing treatment on seed germination of carrot, parsley and dill
- 232 **Monika Tkalec Kojić, Tomislav Vinković, Boris Ravnjak, Jasna Kraljičak, Mario Đurić**
| Ispitivanje klijavosti sjemena pustenaste divizme (*Verbascum phlomoides* L.) na različitim podlogama
| Germination test of woolly mullein (*Verbascum phlomoides* L.) on different growth media
- 237 **Nina Toth, Leopold Prlić, Lepomir Čoga, Ivanka Žutić, Sanja Fabek Uher, Božidar Benko**
| Učinak vermikomposta na morfološka svojstva, prinos i mineralni sastav ploda paprike
| Effect of vermicompost on morphological characteristics, yield and mineral content of pepper fruit
- 243 **Nertil Khaferaj, Anisa Pečuli, Sara Serbo, Albjona Brahollari, Erjola Kopali, Anila Kopali**
| Optimization of the extraction of caffeine from coffee and tea samples
- 248 **Ivanka Žutić, Kristina Bajlo, Božidar Benko, Nina Toth, Sanja Radman**
| Klijavost sjemena kapare (*Capparis orientalis* Veill.) pod utjecajem različitih kemijskih tvari
| Germination of capper (*Capparis orientalis* Veill.) seeds affected by different chemical treatments

Session 5 | Field Crop Production

- 255 **Klara Barić, Zlatko Svečnjak, Josip Lakić, Ana Pintar, Tomislav Torić**
| Učinak pokrovne kulture na kontrolu korova na strništu
| Contribution of cover crops to weed suppression
- 260 **Ivan Horvatić, Martina Kovačević, Dario Jareš, Zlatko Svečnjak, Ivana Radojčić Redovniković, Darko Uher**
| Influence of intercropping maize with climbing bean and fertilization with clinoptilolite on forage yield and quality
- 265 **Ivan Horvatić, Martina Kovačević, Dario Jareš, Dubravko Maćešić, Zlatko Svečnjak**
| Influence of intercropping maize with cowpea on forage yield and quality
- 270 **Dario Iljkić, Saša Šormaz, Mirta Rastija, Josip Galić, Ivana Varga, Luka Drenjančević**
| Agronomska i gospodarska svojstva sorata pšenice Bc instituta d.d. Zagreb
| Agronomic and economic properties of wheat varieties of Bc institute d.d. Zagreb
- 275 **Dubravko Maćešić, Zlatko Svečnjak, Darko Uher**
| Effects of seeding rate, seed inoculation and Bioalgeen application on common vetch-wheat forage yield in Western Slavonia
- 279 **Darko Uher, Ivana Radojčić Redovniković, Dario Jareš, Ivan Horvatić**
| Influence of intercropping maize with soybean on forage yield and quality
- 284 **Darko Uher, Ivan Horvatić, Dario Jareš**
| Influence of intercropping sweet sorghum with soybean on yield and crude protein content of fresh fodder
- 289 **Jelena Vančetović, Dragana Ignjatović-Mićić, Marija Kostadinović, Nenad Delić, Goran Stanković, Sofija Božinović, Olivera Đorđević Melnik**
| Agronomic traits of QPM maize hybrids adapted to temperate regions

Session 6 | Fisheries, Wildlife Management and Apiculture

- 295 Brigita Novosel, Neven Iveša, Ivana Balenović, Alexis Conides, Jurica Jug-Dujaković, Ana Gavrilović**
 | Utjecaj vremena nasađivanja mladi na rast orade i lubina u kaveznom uzgojnom sustavu
 | Impact of stocking time on the growth of sea bream and sea bass in a cage rearing system
- 300 Krešimir Krapinec, Krešimir Pauković, Robert Sajfert, Darko Uher**
 | Browsing intensity of some Mediterranean plants by the European mouflon
 | Razina brštenja pojedinih sredozemnih biljnih vrsta od strane europskog muflona
- 306 Mate Malenica, Krunoslav Pintur**
 | Procjena brojnosti populacije jarebice kamenjarke (*Alectoris graeca*) na području značajnog krajobraza vodenog toka i kanjona rijeke Čikole
 | Estimation population size of the rock partridge (*Alectoris graeca*) in the area of protected landscape of the water course and the canyon of the Čikola River
- 311 Ivan Pervan, Matija Konječić, Tomislav Dumić**
 | Kranimetrijska obilježja šljuke bene (*Scolopax rusticola* L.) s područja Dalmatinske zagore
 | Craniometrical features of Eurasian woodcock (*Scolopax rusticola* L) in the area of Dalmatian Hinterland
- 316 Juraj Petravić, Marin Jarnjak, Krešimir Kuri, Martina Andrašić, Ivana Kaleb Vuletić, Margarita Maruškić Kulaš, Helena Jajčević, Goran Jakšić**
 | Fish assemblage of the artificial flood protection channel Kupa-Kupa
 | Sastav riblje zajednice rasteretnog poplavnog kanala Kupa-Kupa
- 322 Tin Batur, Matej Orešković, Antonio Viduka, Tena Radočaj, Ivan Špelić, Danilo Mrdak, Marina Piria**
 | Kompeticija u prehrani između unesene kalifornijske pastrve (*Oncorhynchus mykiss*) i ugrožene Europske jegulje (*Anguilla anguilla*) iz rijeke Žrnovnice
 | Competition for food between introduced rainbow trout (*Oncorhynchus mykiss*) and endangered European eel (*Anguilla anguilla*) from the Žrnovnica River
- 328 Neven Iveša, Ivan Špelić, Martina Gelli, Antonio Castelletichio, Marina Piria, Ana Gavrilović**
 | Analiza ulova ribe mrežom poponicom u Medulinskom zaljevu
 | Fish catch analysis of the “poponica” net in Bay of Medulin
- 334 Marin Kovačić, Nikola Raguž, Ivana Majić, Boris Lukić, Ankica Sarajlić, Zlatko Puškadija**
 | Results of the first generation of Carniolan honey bees (*Apis mellifera carnica*) selection to *Varroa destructor* resistant traits
 | Rezultati selekcije prve generacije sive pčele (*Apis mellifera carnica*) na svojstva otpornosti na grinju *Varroa destructor*
- 339 Marina Piria, Filip Petek, Luana Velagić, Ivana Lisica, Ivan Špelić, Tena Radočaj, Tamara Kanjuh, Ana Marić, Ivana Buj, Marko Čaleta, Davor Zanella, Predrag Simonović**
 | Povijest i pregled istraživanja potočne pastrve crnomorskog sliva u Hrvatskoj
 | History and review of research of brown trout from Black Sea Basin in Croatia
- 345 Ena Spremo, Ana Bratoš Cetinić, Marina Brailo**
 | Učinkovitost različitih metoda ekstrakcije mikroplastike iz mediteranske dagnje *Mytilus galloprovincialis* (Lamarck, 1819)
 | Efficiency of different methods for microplastics extraction from Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819)

- 350 **Jadranka Sulić Šprem, Tatjana Dobrosravić, Sanja Grđan, Kruno Bonačić, Nenad Antolović**
| Indeks kondicije bežmeka *Uranoscopus scaber* Linnaeus, 1758 u južnom Jadranu
| The condition index of the stargazer *Uranoscopus scaber* Linnaeus, 1758. from the southern Adriatic
- 354 **Lea Balen Percela, Saša Prđun, Marica Dražić, Dragan Bubalo**
| Utjecaj snage pčelinje zajednice na skupljanje pčelinjeg otrova metodom elektrostimulacije
| Influence of the honey bee colony strength on collecting bee venom by electro stimulation method
- 358 **Zrinka Tolušić, Vlado Jumić, Tihomir Florijančić**
| Financijski učinci lovnog turizma u istočnoj Hrvatskoj
| Financial effects of hunting tourism in eastern Croatia
- 362 **Željana Đodo, Nenad Antolović, Nikša Glavić, Jadranka Sulić Šprem**
| Length-weight relationship of grass goby *Zosterisessor ophiocephalus* from Novigrad Sea - eastern Adriatic Sea
| Dužinsko - maseni odnos glavoča travaša *Zosterisessor ophiocephalus* u Novigradskom moru – istočno Jadransko more
- 367 **Irena Glavor, Vlasta Bartulović, Tatjana Dobrosravić**
| Analiza prisutnosti čestica mikroplastike u želucima triju komercijalnih vrsta riba na području južnog Jadrana
| Analysis and the presence of microplastic particles in the stomachs of three commercial fish species in the southern Adriatic
- 372 **Sanja Grđan, Ana Bratoš Cetinić, Marija Crnčević, Jadranka Sulić Šprem**
| Morphometric characteristics of the snail *Phorcus turbinatus* (Born, 1778) (Mollusca: Gastropoda) from the marine lake Mrtvo more (Lokrum Island, South Adriatic)
| Morfometrijske značajke puža *Phorcus turbinatus* (Born 1778) (Mollusca: Gastropoda) iz Mrtvog mora (otok Lokrum, južni Jadran)
- 376 **Nikolina Ledenko, Tea Tomljanović, Slavica Čolak, Danijel Mejdandžić, Matko Kolega, Renata Barić, Daniel Matulić**
| Uzroci obojenja mišićnog tkiva svježeg brancina (*Dicentrarchus labrax*) žučnim bojama
| Causes of coloration of muscular tissue at fresh sea bass (*Dicentrarchus labrax*) with bile pigments
- 383 **Mario Prečanica, Kruno Bonačić, Jadranka Sulić Šprem, Josip Mikuš**
| Effects of silicate concentrations on the growth of the diatom *Chaetoceros* cf. *neogracilis* in aquaculture conditions
| Utjecaj različitih koncentracija silikata na abundanciju dijatomeje *Chaetoceros* cf. *neogracilis* (Schutt, 1985) u uzgojnim uvjetima

Session 7 | **Animal Husbandry**

- 389 **Dubravko Škorput, Ivan Torček, Sven Menčik, Željko Mahnet, Vedran Klišanić, Danijel Karolyi, Zoran Luković, Krešimir Salajpal**
| Čimbenici plodnosti krmača banijske šare svinje
| Factors influencing litter size sows in Banija spotted pig
- 394 **Zvonko Antunović, Ozren Erceg, Željka Klir, Josip Novoselec**
| Fenotipske odlike različitih dobnih kategorija hrvatske bijele koze
| Phenotypic traits of Croatian white goats in various ages

- 399 **Dalibor Bedeković, Marko Jelen, Zlatko Janječić, Tatjana Jelen, Marija Meštrovic, Marijana Vrbančić, Gordana Duvnjak, Ivan Širić, Ivica Kos**
| Pokazatelji proizvodnje mesa križevačke kukmaste kokoši
| Meat production indicators of Križevci crested hen
- 405 **Darija Bendelja Ljoljić, Šimun Zamberlin, Iva Dolenčić Špehar, Viktorija Luetic, Dubravka Samaržija**
| The concentration of zinc, iron and manganese in the sheep's milk and yogurt
- 410 **Krešimir Bošnjak, Marina Vranić, Ivana Čačić, Tomislav Mašek, Ivica Andrić, Paula Jurčić**
| Utjecaj boje polietilenske folije na kvalitetu biljne mase silirane u velike bale
| The effect of the colour of polyethylene film on the forage quality ensiled in big bales
- 414 **Krešimir Bošnjak, Marina Vranić, Ivana Čačić, Tomislav Mašek, Ana Beljan**
| Utjecaj primjene krutog stajskog gnoja na sadržaj sirovih proteina i nitrata u krmi s poluprirodnog travnjaka
| The effect of farmyard manure application on the crude protein and nitrate content of forage from semi-natural grassland
- 419 **Iva Dolenčić Špehar, Darija Bendelja Ljoljić, Šimun Zamberlin, Dubravka Samaržija**
| Jogurt u funkciji zdravlja starijih osoba
| Yoghurt as a function of health in the elderly people
- 424 **Valentino Držaić, Jelena Ramljak, Ante Kasap, Ivan Širić, Zvonko Antunović, Boro Mioč**
| Polimorfizmi MTNR1A gena u populaciji istarske ovce
| MTNR1A gene polymorphisms in Istrian sheep population
- 429 **Zlatko Janječić, Zlata Kralik, Dalibor Bedeković**
| Utjecaj alternativnih sustava držanja na oštećenja prsnih kostiju kokoši nesilica
| The influence of alternative rearing systems on breast bone damages of laying hens
- 434 **Ivica Kos, Ivana Mijač, Zlatko Janječić, Blanka Sinčić Pulić, Marko Musulin, Radka Langová, Zoran Luković, Ivan Širić, Dalibor Bedeković**
| Utjecaj dodatka mikroalgi u hranu kokoši nesilica na senzorska svojstva jaja
| Effect of microalgae addition in laying hen diet on egg sensory traits
- 440 **Zlata Kralik¹, Gordana Kralik, Danica Hanžek**
| Mišljenje potrošača u Hrvatskoj o konzumaciji omega-3 obogaćenih jaja
| Consumers' opinion in Croatia on consumption of omega-3 enriched eggs
- 444 **Miloš Marinković, Predrag Perišić, Dušica Ostojčić Andrić, Nenad Mičić, Nenad Stojiljković, Dragan Nikšić, Vlada Pantelić**
| The effect of age on semen quality of Holsteinfriesian bulls
- 449 **Ben Moljk, Janez Jeretina, Jure Brečko**
| Utjecaj automatskog sustava mušnje na proizvodnju mlijeka
| The influence of the automatic milking system on milk production
- 454 **Dominik Petričević, Dubravko Škorput, Danijel Karolyi, Ivica Kos, Ana Kaić, Zoran Luković**
| Klaonički pokazatelji i kakvoća mesa tovljenika banijske šare iz poluotvorenog sustava držanja
| Slaughtering performance and meat quality of Banija spotted fatteners from semi-outdoor system
- 459 **Zvonimir Prpić, Katarina Huzanić, Danijel Mulc, Ivan Vnučec, Branislav Galik, Boro Mioč, Zdravko Barać**
| Porodna masa i odlike rasta muške jaradi izvornih i inozemnih pasmina koza u Hrvatskoj
| Birth weight and growth traits of male goat kids of indigenous and foreign goat breeds in Croatia

- 464 **Nikola Raguž, Boris Lukić**
| Potential gain of genome editing for improved animal breeding
- 469 **Zvonimir Steiner, Drago Bešlo, Stipo Benak, Goran Gavić, Ana Aračić, Ines Rica, Petra Musa, Miljenko Konjačić, Mario Ronta**
| Utjecaj hranidbe teladi sa starterom na bazi proizvoda kvasaca na dnevne priraste i zdravstveno stanje teladi
| Influence of feeding calves with starter based on yeast products on daily weight gain and calf health
- 474 **Nenad Stojiljković, Dragan Radojković, Radomir Savić, Marija Gogić, Vladimir Živković, Miloš Marinković, Krstina Zeljić**
| Influence of genotype, farm, and test year on the variability of traits monitored in the performance test of gilts
- 479 **Josipa Štavalj, Tina Bobić, Ranko Gantner, Pero Mijić**
| Rotacijsko napasivanje u sustavu krava-tele
| Rotational grazing in cow-calf system
- 484 **Marijana Vrbanić, Ivan Vnučec, Tatjana Jelen, Jelena Ramljak, Miljenko Konjačić**
| Povezanost polimorfizma DGAT₁ gena sa mesnim odlikama janjadi
| Polymorphism of DGAT₁ gene on some meat characteristics of lambs

Session 8 | **Viticulture and Enology**

- 491 **Iva Šikuten, Sara Šima Matković, Petra Štambuk, Domagoj Stupić, Željko Andabaka, Zvezdana Marković, Jasminka Karoglan Kontić, Edi Maletić, Darko Preiner,**
| Utjecaj djelomične defolijacije na prinos i sastav grožđa sorte vinove loze 'Belina starohrvatska' (*Vitis vinifera* L.)
| Influence of partial defoliation on yield and grape content of grapevine cultivar 'Belina starohrvatska' (*Vitis vinifera* L.)
- 496 **Sara Rossi, Sanja Radeka, Marijan Bubola, Tomislav Plavša, Ivana Horvat, Igor Lukić, Ana-Marija Jagatić Korenika**
| Influence of different vinification techniques on stilbenes and total polyphenolic content in Teran wines
| Utjecaj različitih vinifikacijskih tehnologija na sadržaj stilbena i ukupnih polifenola u vinima sorte 'Teran'
- 501 **Marina Lavrić, Tihomir Prusina**
| The influence of the vintage year on Blatina wine quality
| Utjecaj godine berbe na kvalitetu vina Blatina
- 506 **Josip Mesić, Valentina Obradović, Helena Marčetić Brankica Svitlica, Ivan Malčić, Tomislav Soldo**
| Impact of cluster thinning on Merlot and Cabernet Sauvignon (*Vitis vinifera* L.) must quality
| Utjecaj prorjeđivanja grozdova kultivara Merlot i Cabernet Sauvignon (*Vitis vinifera* L.) na kakvoću mošta
- 510 **Ivana Rendulić Jelušić, Marina Anić, Ivana Puhelek, Mirela Osrečak, Branka Šakić Bobić, Zoran Grgić, Marko Karoglan**
| Pregled novih tehnologija za praćenje vinogradarske proizvodnje i primjenu preciznog vinogradarstva
| Overview of new technologies for monitoring viticulture production and the application of precision viticulture

Session 9 | **Pomology**

- 517 **Kristina Batelja Lodeta, Maja Zagorc, Vesna Očić, Ines Pohajda, Đani Benčić, Snježana Bolarić, Aleš Vokurka, Snježana Kereša, Jelena Gadže, Josip Gugić, Zlatko Čmelik**
 | Pomološka svojstva sorata šljive na lokalitetu Jazbina
 | Pomological properties of plum varieties at the Jazbina site
- 522 **Željko Perković, Robert Benković, Krunoslav Miroslavljević, Teuta Benković-Lačić**
 | Dinamika porasta mladice kruške sorata 'Williams' i 'Santa Maria'
 | The growth dynamics of branches on pears cultivars 'Williams' and 'Santa Maria'
- 527 **Dora Klisović, Anja Novoselić, Igor Lukić, Karolina Brkić Bubola**
 | Evolution of phenolic compounds and quality parameters after storage of Istarska bjelica and Buža cv. extra virgin olive oil
 | Utjecaj skladištenja na parametre kvalitete i fenolni sastav ekstra djevičanskih maslinovih ulja sorti Istarska bjelica i Buža
- 533 **Anja Novoselić, Dora Klisović, Igor Lukić, Ivana Horvat, Karolina Brkić Bubola**
 | The strategies for antioxidant enrichment of Buža cv. virgin olive oil
 | Strategije antioksidacijskog obogaćivanja djevičanskog maslinovog ulja sorte Buža
- 538 **Ivica Šnajder, Dinko Zima, Mario Jakobović, Valentina Mudrinović**
 | Preferencije potrošača o konzumaciji ekološkog voća u Požeško-slavonskoj županiji
 | Consumer preferences on Organic Fruit Consumption in Požega Slavonia County
- 542 **Sandra Voća, Jana Šic Žlabur, Jasmina Čaušević, Martina Skendrović Babojelić, Nadica Dobričević, Stjepan Plietić, Ante Galić**
 | Biološki potencijal ploda kivija s različitih lokacija
 | Biological potential of kiwi fruit from different locations
- 547 **Mladen Zovko, Ivan Ostojić, Tihomir Miličević, Luka Bošnjak, Jurica Primorac**
 | First record of the honeysuckle whitefly *Aleyrodes loniceræ* (Hemiptera: Aleyrodidae) in Bosnia and Herzegovina and its incidence on cultivated strawberry
 | Prvi nalaz i intenzitet pojave jagodinog štitastog moljca *Aleyrodes loniceræ* (Hemiptera: Aleyrodidae) na kultiviranoj jagodi u Bosni i Hercegovini

Session 10 | **Agricultural Engineering**

- 553 **Alan Antonović, Tajana Krička, Vanja Jurišić, Branimir Šafran, Suzana Antolović, Juraj Stanešić, Josip Ištvančić**
 | Utjecaj različitih polihidričnih alkohola na utekućenje trave *Miscanthus x giganteus*
 | Influence of various polyhydric alcohols on the liquefaction of grass *Miscanthus x giganteus*
- 558 **Mateja Grubor, Vanja Jurišić, Nikola Bilandžija, Zorana Kovačević, Tajana Krička**
 | *Arundo donax* L. kao sirovina u biorafinerijskom procesu
 | *Arundo donax* L. as raw material in the biorefinery process
- 563 **Nives Jovičić, Sanja Kalambura, Alan Antonović, Ana Matin, Tajana Krička**
 | Biokompoziti kao proizvodi u biogospodarstvu
 | Biocomposites as products in the bioeconomy

Contents

- 568 **Vanja Jurišić, Mislav Kontek, Katarina Jakšić, Alan Antonović**
| Usporedba empirijskih i teorijskih ogrjevnih vrijednosti miskantusa
| Comparison of empirical and theoretical heating values of miscanthus
- 573 **Ana Matin, Nikola Bilandžija, Neven Voća, Josip Leto, Sandra Bischof**
| Proizvodnja energije iz sječke *Sida hermaphrodite* kao čvrstog biogoriva
| Energy production from *Sida hermaphrodita* chips as solid biofuel
- 578 **Anamarija Peter, Dubravka Dujmović Purgar, Terezija Međugorac, Mateja Grubor, Neven Voća**
| Upotreba biomase invazivne biljne vrste amorfe (*Amorpha fruticosa* L.) u proizvodnji energije
| Use of invasive plant species Indigobush (*Amorpha fruticosa* L.) biomass in energy production



Session

0 Proceedings

Plenary session

55
Hrvatski

15
Međunarodni
Simpozij
Agronoma

Zbornik radova

Plenarna izlaganja

Advances in lactation biology

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Abstract

Lactation is an important physiological function in mammals aiming to provide nutrition and immune protection to the new-borns in their first period of life. The biology of lactation in farm animals is dealing with mammary gland function and health in livestock species that were substantially changed by genetic selection for production traits. Development of the mammary gland, hormonal stimulation of lactation, optimisation of milk yield and quality, modification of milk composition and improvement of manufacturing properties of the milk are important research challenges in different animal species. The availability of genomic data for the majority of farm animal species opened the possibility for comparative study approach of lactation associated genes and genetic mechanisms, which are involved in development and function of the mammary gland. Rapid development of molecular genomic methodology and bioinformatics tools recently opened a completely new research horizon in lactation biology.

Keywords: cattle genome, mammary gland, primary cell culture, RNA splicing, single cell RNA sequencing

Introduction

Lactation is an important physiological function in mammals, devoted to production of optimized nutrition and immune protection for the new-borns in their first period of life. In some ruminant species, domestication and breeding have increased milk yield in dairy animals significantly over the nutritional needs of the progeny (Ritter et al., 2019). However, in some other species, the increased number of offspring requires an exceptional lactation performance, which in some cases limits the reproductive capacity. The expansion of research in the field of lactation allowed rapid increase of milk produced per dairy cow in the second half of the 20th century (Collier and Bauman, 2017). This development helped significantly to meet the permanently growing nutritional needs of the human population (Fig. 1). Biology of lactation in farm animals is therefore a complex and important research field focused on mammary gland function and health, especially in livestock species that experienced substantial changes caused by intense selection for milk production.

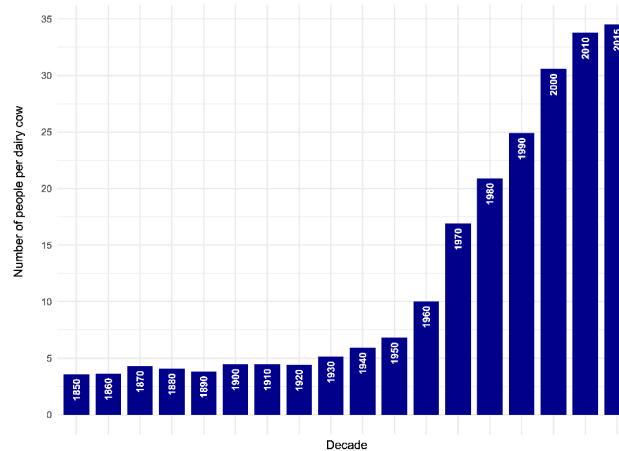


Figure 1: The number of people per dairy cow in the US, adapted from “Triennial lactation symposium/Bolfa: Historical perspectives of lactation biology in the late 20th and early 21st centuries” by (Collier and Bauman, 2017)

Development of the mammary gland, hormonal stimulation of lactation, optimisation of milk yield and quality, modification of milk composition and improvement of technological properties of the milk are research challenges in different farm animal species. The ability of the mammary gland to produce large amounts of high quality bioactive peptides is an important prerequisite for a number of biotechnological applications, especially for the pharmaceutical industry. Therefore, the mammary gland is since almost three decades considered also as a potent bioreactor for efficient production of high quality recombinant proteins using genetically modified animals (Henninghausen, 1994).

Mammary gland development and function

The evolutionary origin of the mammary gland represent the apocrine sweat glands associated with hair follicles (Ofstedal, 2002). The modification of milk composition during lactation in monotremes and some marsupials, progressing from milk with low nutritional value and high proportion of water at the beginning of lactation, to the high-oligosaccharide and high-lipid milk in the subsequent lactation stages, may reflect the evolution of milk composition within the class of Mammalia (Urashima et al., 2001). The evolutionary record of milk proteins suggests that appearance of milk protein genes preceded the development of mammals and mammary glands. The core mammary gland expressed genes are highly conserved in the class Mammalia, especially those associated with milk secretion (Capuco and Akers, 2009). However, a greater mutation rate was characteristic for protein genes associated with immune function of the mammary gland and nutritional value of milk (Lemay et al., 2009a). In the lactating mammary gland, five main cell types are present: i) secretory and ductal epithelial cells, ii) myoepithelial cells, iii) adipocytes, iv) fibroblasts and v) smooth muscle cells. These five cell types represent the building bricks of parenchyma (i) and stroma (ii – v). The organisation of alveolar and ductal structures, contributing to the formation of lobules is very similar in the majority of mammalian species, but the number of mammary glands, their size and capacity to store milk varies significantly among species. Since the metabolic rate of mammary secretory tissue is limited, the increase in milk output can be only achieved by higher growth rate of the mammary tissue (Martin, 1984). In the development and function of the mammary gland play important role also transcription factors *STAT3* and *STAT5*, which are necessary for the initiation of transcription of many mammary gland expressed genes (Akers, 2017)2017. Milk composition varies significantly among mammalian species as well as during the course of lactation, and is adapted to the growth rate of the neonate.

The major hormonal stimuli governing development of the mammary gland are oestrogen, progesterone, matrix metalloproteinase, plasminogen activator (Rabot et al., 2007), somatotropin and IGF-1 (Sejrsen, 1989). The most important lactogenic hormones are prolactin, glucocorticoids, growth hormone and insulin. The stimulation of the central nervous system and pituitary secretion of oxytocin is necessary for contraction of myoepithelial cells and milk secretion. Recently, it has been shown that adaptive immune system participates in remodelling of the mammary gland during pubertal and post pubertal development through the action of antigen presenting cells, CD4+ T cells and interferon g signalling (Plaks et al., 2015).

Impact of genetic variants of lactoproteins on milk manufacturing traits

Six major milk proteins in ruminants are encoded by four casein genes, beta lactoglobulin- (β -LG) and alpha lactalbumin- (α -LA) gene. In all mammalian species the four casein genes (α S1-CN, β -CN, α S2-CN, κ -CN) are clustered on the same chromosome occupying a genomic region of about 250 kb. The gene order and transcriptional orientation are highly conserved among ruminant species (Rijnkels et al., 1997)¶. While casein genes are present in ruminants only as single copy loci, pseudogenes for β -LG exist in cattle, goat and sheep. In addition, a pseudogene for α -LA, flanked by two directly repeated LINE sequences, was found in cattle. The availability of genomic sequences for all lactoprotein genes in different species allowed identification of genetic variants at all lactoprotein loci in different mammalian species. In cattle, some 40 genetic variants at six major lactoprotein loci have been identified (Dovc, 2000). In addition to mutations in the coding regions, several mutations have been found also within the non coding regions. It has been shown that the deletion of 13 aa in bovine *aS1-CN A* is caused by exon 4 skipping due to the point mutation within the splice donor site and not by deletion of genomic DNA (Mohr et al., 1994)¶¶. Similarly, shorter variants of ovine and caprine α S1-CN are associated with exon 16 skipping and differential splicing of exons (Ferranti et al., 1997). The presence of relatively conserved splicing regulatory elements has been shown in the equine β -CN gene, leading to co-transcriptional alternative splicing of β -CN primary transcripts

(Lenasi et al., 2006). The consequence of this mechanism is increased number of milk protein variants in mare's milk, due to alternative splicing. Mutations within the noncoding regions might have important quantitative effects as demonstrated by the occurrence of the LINE element in the exon 19 of the goat α S1-CN E allele. The presence of the LINE element could be responsible for the reduced mRNA stability and consequently for the threefold reduction of α S1-CN E expression (Perez et al., 1994). Allele A and B specific polymorphisms were identified in the proximal promoter region of the β -LG gene, causing differential expression of both alleles due to the different AP-2 binding affinity (Lum et al., 1997). It has been shown that this mutation significantly affects the β -LG level in the milk of Holstein Friesian and Simmental dairy cows (Kuss et al., 2003)2003. Significant research effort was focused on the identification of associations between lactoprotein genetic variants and manufacturing properties of the milk. The significant positive effect on rheological properties of the milk could be shown for κ -CN allele B and β -CN allele B (reviewed in (Buchberger and Dovic, 2000)). The analysis of different lactoprotein gene promoters revealed similar organisation of transcription factor binding sites suggesting similar mechanism for induction of transcription of different lactoprotein loci (Debeljak et al., 2005). The growing body of genomic information and milk traits associated markers led to the identification of important candidates for quantitative trait loci (QTL) for lactation traits. A point mutation K232A in the bovine *DGAT1* gene was the first significant lactation associated QTL affecting milk yield and composition (Grisart et al., 2002). The genome regions, affecting lactation traits and susceptibility for mastitis, are present at all bovine chromosomes and represent a considerable part of the cattle genome (Ogorevc et al., 2009).

Bovine genome revealed

The availability of genomic data for all major dairy species, opened the possibility for comparative study approach focusing on lactation associated genes and genetic mechanisms, which are involved in development and function of the mammary gland (Elsik et al., 2009). The significant reorganisation of milk protein coding genes in cattle resulted in the insertion of the histatherin (*HSTN*) gene in the casein gene cluster on BTA6. The *HSTN* gene is in cattle genome juxtaposed to a regulatory element important for *CSN2* expression, therefore is *HSTN* gene regulated like the casein genes during the course of lactation. In cattle, two members of serum amyloid A gene cluster, *SAA3.1* and *SAA3.2*, located at BTA29, are expressed in the mammary gland. The *SAA3.2* is important inhibitor of microbial growth and contributes to the prevention of microbial infections of the mammary gland. IgG is the predominant immunoglobulin in cow's milk, allowing calves to acquire passive immunity by ingestion of IgG in milk. A core set of 6,469 genes (Lemay et al., 2009b), associated with mammary gland development and function, was established in cattle to allow molecular interpretation of about 240 milk quantitative trait loci, associated with lactation.

The four significant milk protein gene clusters comprise the immunoglobulin genes, casein genes, fibrinogen genes, and genes that encode milk fat globule proteins. Only 6.6% of the milk protein genes were found within the milk protein-specific cluster, however, 27.9% could be found within one of the mammary gene clusters. The milk protein genes in principle do not form clusters with each other but rather with other mammary genes. Milk protein genes are regulated along with other lactation genes without regards to the final destination of the gene product. Different alignments of the bovine genome (UMD3.1, Btau4.6, BtOM1.0), originating from the same animal, were published and can now be used as a comprehensive guide for the comparative genomic studies of different cattle breeds (Zhou et al., 2015).

Mammary cell culture models

Several mammary epithelial cell cultures (mEC) were established in the past, however, the permanent cell cultures often change their phenotype and adapt to cell culture conditions. They also often lose their ability to react to stimulation by lactogenic hormones. In order to enable the study of cellular mechanisms, involved in development of the mammary gland and lactation *in vitro*, primary mECs represent a valuable surrogate for complex and expensive animal experiments (Ogorevc et al., 2011). The mEC model is especially suitable for studying the hormonal responsiveness of mECs and their ability to respond to infections. The responsiveness to infections can be studied successfully at different time points after infection and in the same genetic background. Using the primary mammary cell culture model and RNA sequencing we can establish expression profiles for almost all genes expressed in the mammary epithelium (Ogorevc et al., 2015). Primary mEC cultures have an advantage over established mammary gland cell lines also because of their retained lactogenic hormone responsiveness. They are able to organize spontaneously into alveolar and duct-like structures to a greater extent than permanent cell cultures. Primary cultures usually contain

several cell types (alveolar and ductal epithelial cells, myoepithelial cells and fibroblasts). In combination with animal models, we can study the regeneration / repopulation ability of mECs in recipient's biological niche. A particularly interesting is murine model where the mammary structures were previously surgically removed, so called cleared fat pad model, where the development of mammary gland structures after mEC transplantation can be observed. The characterisation of different cell types in the primary mammary cell culture allowed identification of the mammary progenitor cells (Prpar et al., 2012), which showed impressive regeneration potential in the heterologous mouse system. Using specific markers, different cell types in the primary mammary gland cell culture can be distinguished. Cytokeratin 14, for instance, indicates myoepithelial cells and cytokeratin 18 denotes luminal epithelial cells, whereas EpCAM is an appropriate marker for luminal cells.

Mammary gland gene expression

During the last three decades, different study approaches were applied to dissect physiological, genetic and environmental effects on mammary gland function and health (Fig. 2). These research efforts contributed significantly to better understanding of mammary gland biology and opened numerous possibilities for further improvement of lactation performance. Whole-genome level gene expression profiling of mammary tissue has been extensively used to characterize roles of genes expressed in different physiological states of the mammary gland, such as different stages of lactation, mammary gland development, tumorigenesis and intramammary infections. Studies reported differences in expression level of several hundreds of genes in response to infection of mammary gland by pathogens such as *Escherichia coli* (Mitterhuemer et al., 2010) [Animal/*metabolism/microbiology](#) <keyword>Mastitis, Bovine/*genetics/metabolism </keyword> <keyword>Oligonucleotide Array Sequence Analysis </keyword> </keywords> <isbn>1471-2164 </isbn> <custom2>PMC2846913 </custom2> <titles> <title>Escherichia coli infection induces distinct local and systemic transcriptome responses in the mammary gland </title> <secondary-title>BMC Genomics </secondary-title> </titles> <pages>138 </pages> <contributors> <authors> <author>Mitterhuemer, S. </author> <author>Petzl, W. </author> <author>Krebs, S. </author> <author>Mehne, D. </author> <author>Klanner, A. </author> <author>Wolf, E. </author> <author>Zerbe, H. </author> <author>Blum, H. </author> </authors> </contributors> <edition>2010/02/27 </edition> <language>eng </language> <added-date format="utc">1576416573 </added-date> <ref-type name="Journal Article">17 </ref-type> <auth-address>Laboratory for Functional Genome Analysis (LAFUGA and *Mycoplasma agalactiae* (Ogorevc et al., 2015). Expression profiles for subpopulations of mammary epithelial cells were analysed at a population level using expression microarrays and RNA-sequencing (Kendrick et al., 2008; Sheridan et al., 2015) revealing different gene expression patterns and transcription regulators in mammary epithelium. In mammary gland transcriptomic studies, biopsies of tissue, laser dissection as well as milk cells have been used. Milk transcriptome studies revealed thousands of polymorphisms associated with lactation which can be used as markers for marker assisted selection (Cánovas et al., 2010). Milk fat globules and antibody captured milk mammary epithelial cells allowed precise separation of cell-subtypes (Divari et al., 2018). Recently, it has become possible to investigate transcriptomes at the single cell level (Tang et al., 2009). Since then, mammary single-cell transcriptomes in humans (Bach et al., 2017) and mice (Han et al., 2018) have been examined and revealed much higher heterogeneity of the mammary epithelium cell composition than previously reported (Cristea and Polyak, 2018).

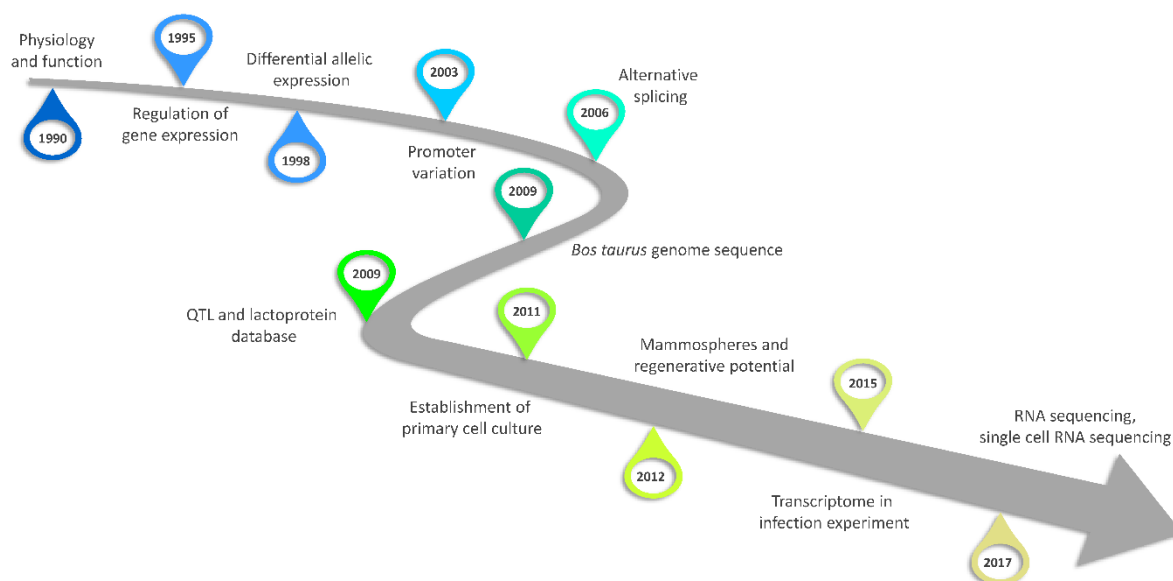


Figure 2: The road map of different research approaches in mammary gland biology in the last three decades.

Future perspectives

Rapid development of molecular genomic methodology and bioinformatics tools opened recently a completely new research horizon in lactation biology. Identification of transcriptomic profiles of single mammary gland cells demonstrated the complexity of gene expression in the mammary gland and shed a new light on the genetic background of lactation (Zorc et al., 2018). Transcriptomic profiling of individual cells, establishment of methylation profiles, detection of local and genomic histone modifications and better understanding of the regulatory role of non-coding RNAs will allow much more complex understanding of genetic and environmental mechanisms, involved in development and function of the mammary gland. The high-density genotyping using SNP chip technology paved the fundament for effective genomic selection, however, the next generations of high throughput DNA sequencing could replace SNP chip genotyping with at least shallow DNA sequencing of virtually entire breeding populations.

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Bioakumulacijski potencijal poljske pečurke *Agaricus campestris* L.

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Sažetak

Cilj istraživanja bio je utvrditi koncentracije teških metala Fe, Zn, Cu, Cr, Ni, Pb, Cd i Hg te bioakumulacijski potencijal jestive gljive poljske pečurke (*Agaricus campestris* L.) na području kontinentalne Hrvatske. Analiza teških metala provedena je na optičkom emisijskom spektrometru s induktivno spregnutom plazmom – ICP-OES, dok je koncentracija žive određivana na živinom analizatoru AMA-254. Koncentracija esencijalnih metala bila je znatno veća u odnosu na toksične metale na svim lokalitetima uzorkovanja. Najveća prosječna koncentracija toksičnih metala u analiziranoj vrsti ustanovljena je za Cd (2,87 mg kg⁻¹), Pb (1,84 mg kg⁻¹) i Hg (1,49 mg kg⁻¹). Bioakumulacijska svojstva vrste *A. campestris* utvrđena su prema metalima Zn, Cu, Cd i Hg (BCF>1).

Ključne riječi: teški metali, jestive gljive, bioakumulacijski potencijal, ekologija

Uvod

Gljive su zasebna mikrobiološka skupina organizama značajne nutritivne, farmaceutske i ekološke vrijednosti. Mnogobrojne samonikle jestive gljive su cijenjene zbog jedinstvenog okusa, arome i prehrambene vrijednosti, gdje se ubraja analizirana vrsta *Agaricus campestris* L. S ekološkog stajališta gljive su važni biološki indikatori onečišćenja okoliša (Garcia i sur., 2005) i biološki čistači tla zagađenog policikličkim aromatskim ugljikovodicima (Eggen i Šašek, 2002). Poznato je da samonikle gljive akumuliraju visoke koncentracije teških metala, metaloida i radionuklida (Kalač, 2010, Širić i sur., 2016, Tucaković i sur., 2018). Akumulacija teških metala u gljivama je kompleksno svojstvo koje ovisi o brojnim vanjskim čimbenicima; mehanizmima unutar gljiva, njihovoj interakciji i genetskim karakteristikama vrste. Micelij gljiva sposoban je akumulirati sve teške metale u znatno većoj koncentraciji nego što ih sadrži supstrat na kojem se razvija i živi (Campos i sur., 2009). Gustoća i dubina micelija koji živi u tlu nekoliko mjeseci ili godinama utječe na sadržaj teških metala u plodnim tijelima gljiva. Pored toga, razni ekološki čimbenici i svojstva tla (pH-vrijednost i organska tvar) mogu utjecati na koncentraciju teških metala u gljivama (Garcia i sur., 2009). Iako se koncentracija teških metala u gljivama (jestivim) povezuje s mineralogijom supstrata (Aloupi i sur., 2012) ili s jako zagađenim područjima poput velikih gradova ili industrijskih središta (Petkovšek i Pokorny, 2013), sadržaj teških metala između vrsta i područja prikupljanja gljiva je nedovoljno proučavan. Spoznaje o akumulacijskom potencijalu jestivih saprotrofnih gljiva u Republici Hrvatskoj su jako skromne. Aktualna znanstvena istraživanja ukazuju na mogućnost korištenja vrijednosti sadržaja teških metala u pojedinim vrstama gljiva (*Agaricus* sp., *Boletus* sp., *Macrolepiota* sp.) kao bioindikatora onečišćenja okoliša. Pored toga, izrazito visoke koncentracije toksičnih teških metala u jestivim gljivama mogu imati negativne učinke na zdravlje ljudi. Stoga je cilj ovog istraživanja utvrditi koncentracije teških metala (Fe, Zn, Cu, Ni, Cr, Pb, Cd i Hg) u jestivoj gljivi *A. campestris* te prikladnost navedene vrste gljiva kao biološkog pokazatelja onečišćenja okoliša.

Materijali i metode

Uzorci gljiva i supstrata tla prikupljeni su na tri lokaliteta kontinentalne Hrvatske (Trakošćan, Medvednica i Petrova gora). Na navedenim lokalitetima prikupljeni su uzorci vrste *Agaricus campestris* slučajnim odobrom. Ukupno je prikupljeno 57 uzoraka, od čega 19 na području Trakošćana, 17 na području Medvednice i 21 na području Petrove gore. Istodobno s prikupljanjem uzoraka gljiva, prikupljeni su uzorci supstrata tla gornjeg horizonta (0-10 cm) nakon uklanjanja površinskog sloja. Nakon prikupljanja, uzorci gljiva su očišćeni i osušeni na 50 °C kroz 48 h do konstantne

težine. Nakon sušenja uzorci su samljeveni na laboratorijskom mlinu (Retsch SM 200) kroz sito promjera otvora 1,00 mm i spremljeni u hermetičke vrećice do očitavanja koncentracije teških metala. Prikupljeni uzorci supstrata rastreseni su u plastične posude i prosušeni na sobnoj temperaturi kroz dva tjedna te samljeveni i prosijani kroz sito promjera otvora 2,0 mm. U pripremljenim uzorcima supstrata izvršeno je očitavanje pH-vrijednosti supstrata u H₂O, sadržaj organske tvari te koncentracija teških metala Fe, Zn, Cu, Ni, Cr, Pb, Cd i Hg.

Laboratorijsko posuđe korišteno u pripremi uzoraka za određivanje teških metala namakano je 24 h u otopini etilendiamintetraoctene kiseline (EDTA; Kemika, Hrvatska) koncentracije 5 % (w/v) i nakon toga 24 h u 10 % (v/v) HNO₃ (Merck, Njemačka). Masa uzorka od 0,5 g razgrađena je s 5 mL HNO₃ (65 %, Suprapur, Merck, Njemačka) u zatvorenim PTFE posudama u mikrovalnoj peći za razaranje (Milestone microwave laboratory system, MLS 1200 mega, SAD). Program razaranja sadržavao je nekoliko koraka: snaga razaranja od 100 W i trajanje 5 min, snaga od 0 W i trajanje 2 min, snaga od 250 W i trajanje 5 min, snaga od 400 W i trajanje 5 min, snaga od 600 W i trajanje 5 min. Nakon razaranja uzorci su hlađeni u vodenoj kupelji te nakon toga odmjereni u tikvice od 25 mL, nakon čega su tikvice dopunjene destiliranom vodom. Iz odmjernih tikvica uzorci su prebačeni u epruvete od umjetne mase za mjerenje na optičkom emisijskom spektrometru s induktivno spregnutom plazmom (eng. Inductively coupled plasma – optical emission spectrometry, ICP-OES, Optima 8000, Perkin Elmer, SAD) opremljenim automatskim uređajem za uzorkovanje, na kojem su analizirani metali. Živa u uzorcima gljiva i tla je mjerena bez razgrađivanja u kiselini koristeći AAS analizator žive (AMA-254, Advanced Mercury Analyser, Leco, Poland) koji koristi neposredno izgaranje uzorka u atmosferi bogatoj kisikom. Vrijednost pH uzoraka supstrata određena je potenciometrijski u suspenziji supstrata tla i destilirane vode (aktualna kiselost) u omjeru 1:5, a napravljena je prema Thomas (1996), pomoću prijenosnog pH-metra IQ 150 (IQ Scientific Instruments, USA). Sadržaj organske tvari u uzorku supstrata određen je gravimetrijski nakon spaljivanja organske tvari (2 g supstrata osušenog na zraku) na 550 °C kroz 16 h u peći (Select – Horn. SELECTA).

Rezultati i rasprava

Osobine tla (pH-vrijednost i sadržaj organske tvari) te prosječne koncentracije analiziranih metala na lokalitetima kontinentalne Hrvatske prikazani su u tablici 1. Iz navedene tablice vidljivo je da se prosječne vrijednosti navedenih svojstava tla razlikuju između lokaliteta uzorkovanja. Tako je prosječna pH-vrijednost na lokalitetu Trakošćan iznosila 6,75, zatim na Medvednici 7,22, a na Petrovoj gori 6,38. Ustanovljene srednje pH-vrijednosti ukazuju na blago kiselu reakciju tla. Postotak organske tvari na lokaciji Trakošćan iznosio je 11,34 %, na Medvednici 4,78 % te 6,94 % na Petrovoj gori. Ustanovljene prosječna vrijednost organske tvari u tlu odgovara kategoriji jako humoznih tala (>5,00 %). Analizom koncentracija teških metala u tlu na istraživanim lokalitetima ustanovljena je znatno veća koncentracija esencijalnih elemenata Fe, Zn i Cu u odnosu na toksične (tablica 1). Najveća prosječna koncentracija olova zabilježena su u uzorcima tla na lokalitetu Petrova gora (22,72 mg kg⁻¹), dok su na području Medvednice ustanovljene najveće koncentracije kadmija (0,61 mg kg⁻¹) i žive (0,086 mg kg⁻¹).

Tablica 1. Koncentracija teških metala u tlu na istraživanim lokalitetima, mg kg⁻¹

	Trakošćan	Medvednica	Petrova gora
pH H ₂ O	6,57 ± 1,13	7,22 ± 0,70	6,38 ± 0,51
O.T., %	11,34 ± 12,52	4,78 ± 3,56	6,94 ± 7,87
Željezo - Fe	8839 ± 310	7673 ± 218	8622 ± 231
Cink - Zn	52,36 ± 34,15	75,17 ± 15,26	87,22 ± 29,18
Bakar - Cu	21,39 ± 8,41	19,38 ± 4,12	28,79 ± 2,01
Nikal - Ni	14,92 ± 6,14	12,53 ± 1,71	11,97 ± 1,34
Krom - Cr	18,73 ± 8,13	17,95 ± 1,38	9,22 ± 0,78
Olovo - Pb	18,37 ± 6,21	14,71 ± 2,26	22,72 ± 1,95
Kadmij - Cd	0,57 ± 0,24	0,61 ± 0,17	0,39 ± 0,06
Živa - Hg	0,055 ± 0,01	0,086 ± 0,004	0,067 ± 0,02

Rezultati su prikazani kao prosjeci suma najmanjih kvadrata ± standardna devijacija.

Prosječne koncentracije analiziranih teških metala u vrsti *A. campestris* na tri lokaliteta uzorkovanja prikazane su u tablici 2. Iz navedene tablice razvidno je kako je analizirana vrsta akumulirala različite koncentracije teških metala između lokaliteta uzorkovanja. Značajno najveća ($P < 0,05$) prosječna koncentracija željeza utvrđena je u uzorcima s područje Medvednice. Vrsta *A. campestris* akumulirala je najveće prosječne koncentracije cinka i bakra na lokalitetu Petrova gora. Uzorci sa područja Trakošćana sadržavali su najveće koncentracije nikla ($3,88 \text{ mg kg}^{-1}$) i kroma ($3,67 \text{ mg kg}^{-1}$). Također, na navedenom lokalitetu utvrđene su značajno najveće koncentracije ($P < 0,05$) toksičnih metala kadmija i žive ($2,87$ i $1,49 \text{ mg kg}^{-1}$). Suprotno tome, najniže koncentracije navedenih metala ustanovljene su u uzorcima na lokalitetu Petrova gora ($2,31$ i $1,03 \text{ mg kg}^{-1}$). Koncentracija toksičnog metala olova bila je različita u uzorcima poljske pečurke između istraživanih lokaliteta, pri čemu je najveća prosječna koncentracija utvrđena u uzorcima sa Petrove gore ($1,84 \text{ mg kg}^{-1}$).

Tablica 2. Koncentracija teških metala u gljivi *Agaricus campestris* L. (mg kg^{-1})

	Trakošćan	Medvednica	Petrova gora
Željezo - Fe	$111,25 \pm 66,23b$	$151,22 \pm 33,53a$	$117,25 \pm 27,54b$
Cink - Zn	$72,84 \pm 21,41b$	$89,56 \pm 14,23a$	$96,33 \pm 16,92a$
Bakar - Cu	$41,33 \pm 13,21b$	$38,56 \pm 12,47b$	$47,87 \pm 16,33a$
Nikal - Ni	$3,88 \pm 0,53a$	$3,63 \pm 0,64ab$	$3,27 \pm 2,48b$
Krom - Cr	$3,67 \pm 0,94a$	$3,04 \pm 0,85b$	$2,69 \pm 0,77b$
Olovo - Pb	$1,56 \pm 0,41ab$	$1,38 \pm 0,34b$	$1,84 \pm 0,47a$
Kadmij - Cd	$2,87 \pm 0,53a$	$2,71 \pm 0,46b$	$2,31 \pm 0,39c$
Živa - Hg	$1,49 \pm 0,21a$	$1,29 \pm 0,24b$	$1,03 \pm 0,12c$

^{a,b,c}: Vrijednosti označene različitim slovom značajno se razlikuju ($P < 0,05$)

U tablici 3. prikazane su vrijednosti biokoncentracijskog faktora (BCF) analiziranih metala. Najviše vrijednosti biokoncentracijskog faktora vrste *A. campestris* utvrđene su za živu ($27,09$) na lokalitetu Trakošćan, zatim $15,00$ na Medvednici i $15,37$ na Petrovoj gori. Također, vrijednosti BCF za kadmij bile su visoke, a iznosile su $5,04$ (Trakošćan), $4,44$ (Medvednica) i $5,92$ (Petrova gora). S druge strane, prema utvrđenim vrijednostima biokoncentracijskog faktora analizirana vrsta gljive ima jako slab bioakumulacijski potencijal za željezo, krom, nikal i olovo (tablica 3).

Tablica 3. Vrijednosti biokoncentracijskog faktora

	BCF		
	Trakošćan	Medvednica	Petrova gora
Željezo - Fe	0,01	0,02	0,01
Cink - Zn	1,39	1,19	1,10
Bakar - Cu	1,93	1,99	1,66
Nikal - Ni	0,26	0,29	0,27
Krom - Cr	0,20	0,17	0,29
Olovo - Pb	0,08	0,09	0,08
Kadmij - Cd	5,04	4,44	5,92
Živa - Hg	27,09	15,00	15,37

BCF – biokoncentracijski faktor

Koncentracije analiziranih metala u vrsti *A. campestris* bile su različite između lokaliteta uzorkovanja. Razvidno najveća zastupljenost metala u analiziranoj vrsti ustanovljena je za metale željezo, zatim cink te bakar (esencijalni elementi), dok su među toksičnim elementima najzastupljeniji olovo i kadmij. Navedeno je u skladu sa rezultatima

istraživanja brojnih autora (Kalač, 2010, Hartikainen i sur., 2012, Širić i sur., 2016). Hertikainen i sur. (2012) u provedenom istraživanju o utjecaju cinka i bakra na rast gljiva zaključuju kako različite grupe gljiva imaju veću toleranciju na cink u odnosu na bakar, što je vidljivo i u većim koncentracijama cinka kojeg usvajaju. Koncentracije toksičnih metala olova, kadmija i žive bile su različite između lokaliteta, ali i između samih metala unutar analizirane vrste. Tako su najviše koncentracije između navedenih metala ustanovljene za kadmij > olovo > živu. Pritom je bitno naglasiti kako su koncentracije olova utvrđene u analiziranoj vrsti znatno niže u odnosu na koncentracije u supstratu tla, dok je za metale kadmij i živu utvrđen suprotan omjer. Slične rezultate za metale olovo, kadmij i živu navode i drugi autori (Garcia i sur., 2009, Melgar i sur., 2009, Kalač i sur., 2010, Širić i sur., 2017). Međutim, bitno je napomenuti da koncentracija metala u gljivama može biti znatno različita između lokaliteta uzorkovanja, a što potvrđuju rezultati istraživanja Petkovšeka i Pokornya (2013). Autori navode znatno veće koncentracije kadmija i olova u brojnim vrstama gljiva, a čiji su uzorci prikupljeni u blizini izvora onečišćenja kao što su topionice i talionice. Nadalje, iako analizirana vrsta akumulira veće koncentracije esencijalnih u odnosu na toksične metala, njen bioakumulacijski potencijal prema esencijalnim elementima je uglavnom slab, a razlog nevedenom su znatno veće koncentracije teških metala utvrđene u tlu. Suprotno tome, dobra bioakumulacijska svojstva ustanovljena su za metale kadmij i živu, pri čemu je živa okarakterizirana kao metal iznimno pristupačan vrsti *A. campestris*. Navedeno potvrđuju rezultati istraživanja (Melgar i sur., 2009, Kalač, 2010, Širić i sur., 2016, Širić i sur., 2017). S druge strane, iznimno slab bioakumulacijski potencijal ustanovljen je za toksični metal olovo, pri čemu su vrijednosti BCF znatno niže od 1. Na temelju navedenoga može se reći da je bioakumulacijski potencijal analizirane vrste uvjetovan samim metalom, ali i lokalitetom uzorkovanja, odnosno stupnjem onečišćenja pojedinih lokaliteta.

Zaključak

Provedenim istraživanjem utvrđene su koncentracije teških metala te bioakumulacijska potencijal vrste *A. campestris*. Ustanovljene su znatno veće koncentracije esencijalnih elementata željeza, cinka i bakra, u odnosu na toksične kadmij, olovo i živu, a što analiziranu vrstu čini odličnom nadopunom prehrani. Koncentracije toksičnih teških metala utvrđene u ovom istraživanju odgovaraju razinama na nezagađenom području te konzumacija analizirane vrste ne predstavlja opasnost za zdravlje ljudi. Utvrđene vrijednosti biokoncentracijskog faktora ukazuju da vrsta *A. campestris* ima velika odstupanja bioakumulacijskog potencijala prema pojedinom metalu, pri čemu su dobra bioakumulacijska svojstva ustanovljena za metale cink, bakar, kadmij i živu (BCF > 1).

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Bioaccumulation potential of field mushroom *Agaricus campestris* L.

Abstract

The aim of this study was to determine the concentration of heavy metals Fe, Zn, Cu, Cr, Ni, Pb, Cd, Hg and bioaccumulation potential of edible mushroom (*Agaricus campestris* L.) in continental Croatia area. The analyses were carried out by Inductively Coupled Plasma– Optical Emission Spectrometry (ICP-OES), while the concentration of mercury was determined on mercury analyzer AMA-254. The concentration of essential metals was considerably higher than toxic metals at all sampling areas. The highest concentrations of toxic metals in analyzed species have been shown for Cd (2.87 mg kg⁻¹), Pb (1.84 mg kg⁻¹) and Hg (1.49 mg kg⁻¹). Bio-accumulation features were established in analyzed species for metals Zn, Cu, Cd and Hg (BCF>1).

Keywords: heavy metals, edible mushroom, bioaccumulation potential, ecology

Buckwheat yields in intercropped systems of walnut and buckwheat

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Abstract

Intercropping involves combining more plant species on the same parcel of land at the same time. The significance of intercropping is in the cultivation of plant species in systems that are less susceptible to different stress conditions. The aim of our research is to investigate buckwheat yields intercropped between alleys of grafted walnuts. The field trial was conducted at two sites in eastern Croatia where on one site walnuts were four years old and on the other eleven years old. Buckwheat yields were significantly lower in intercropped 11-yr old orchard compared to the control plot without walnuts. However, in 4-yr old walnut orchard there was no significant difference between buckwheat yields in the intercropped system and on the control plot. Such results suggest that the shading effect could be the driving force controlling buckwheat yields in such intercropped systems.

Keywords: agroforestry, buckwheat, land equivalent ratio, silvoarable, walnut

Introduction

Intercropping agricultural crops with wood species, such as walnut, can have positive effects on crops. Combining permanent wood species with agricultural crops influences the microclimate conditions, which improves the plant resistance to stress conditions such as climatic extremes (drought, flooding), better use of the production area, positive influence on soil fertility, diversity of production in one vegetation, protection against plant disease, pests and weeds, better use of nutrients and water in soil as well as increased biodiversity. However, the question is how profitable or productive intercropped system can be i.e. how would such combined cultivation affect the yields of agricultural crops?

At the same time, this kind of cultivation can promote allelopathic relationships between plants. In intercropping systems with walnut, it is desirable to choose orchards with grafted plants since the walnut tree secretes juglone that may have a toxic effect on the germination of other plants. In the orchards of walnuts with grafted trees, the secretion of juglone is not so intense and the activity of juglone in such orchards has no significant toxic effect in the first 15 years (Scott and Sullivan, 2007). Furthermore, an introduction of organic matter or organic fertilizers is recommended because it promotes microbiological activity that enhances juglone decomposition (Schmidt, 1987). Another important factor in walnut intercroppedsystems is the selection of plants resistant to low-pH, since the walnuts can acidify the soil. By raising such systems we create stable agroecosystems resistant to negative climatic extremes (Quinkenstein et al., 2009).

Buckwheat (*Fagopyrum esculentum* Moench) is the plant from the *Poligonaceae* family. It originates from Central and Northeast Asia, Manchuria and the Himalayas (Farooq et al., 2016). It was brought to Europe at the end of the fourth century, and it is mainly produced in China, Russia, Ukraine and Kazakhstan.. Buckwheat is grown mainly in post-sowing for a short life cycle, mainly as a pre-sowing crop for vegetables, barley and wheat, it is rarely grown as a main crop. The intensity of buckwheat production at the Mediterranean level is in line with the increased search for healthy food with low environmental impact (Small, 2017). Since buckwheat has short vegetation period and low demand for agrotechnical operations, it was chosen in our filed trial as a crop to be intercropped in walnut orchard. The aim of our research is to investigate possibility of intercropping buckwheat between alleys of grafted walnuts and to determine its yields in two orchards of different age. In addition, we wanted to examine the productivity of such systems by calculating the *land equivalent ratio* (LER).

Material and methods

The field trial was set up in eastern Croatia on two sites: Ivankovo with 4-yr walnut orchard and Đakovo with 11-yr old walnut orchard. Alleys in Đakovo were 8m wide while in Ivankovo 10m. Within the alleys a 6m strip of buckwheat was sown in Đakovo and an 8m strip in Ivankovo. Each of two sites consisted of three plots; a) control plot of buckwheat without walnuts, b) walnut orchard with intercropped buckwheat and c) sole walnut orchard without intercropped wheat. Soil and climatic parameters were measured and monitored during the vegetation period.

Land equivalent ratio

From the crop and walnut fruit yields, land equivalent ratio (LER) was estimated. The land equivalent ratio is defined as the ratio of the area under monoculture production to the area under intercropping needed to give equal yields at the same management level (Ong & Kho, 2015). It is calculated as the ratio of tree yield from intercropped system to the tree monoculture yield plus the ratio of crop yield from the intercropped system to the crop monoculture yield, as shown in Equation 1:

$$\text{eq. 1} \quad LER = \frac{\text{walnut intercropped yield}}{\text{walnut pure orchard yield}} + \frac{\text{buckwheat intercropped yield}}{\text{buckwheat monoculture yield}}$$

When $LER \leq 1$, there is no agronomic advantage of intercropping over sole cropping, but when $LER > 1$, production in the intercropped system is higher than in the separate sole crops.

Results and discussion

The walnut orchard in Đakovo has 10 equally long rows of walnuts. However, so far, walnut yield of first five rows was always around 30% of the total walnut yield, while the last five rows had around 70% of total walnut yield i.e. higher productivity. Walnut orchard in Ivankovo is still too young and it has not produce any walnut yield so far. In Đakovo, we have decided to sow buckwheat in the 4 alleys in between first five rows of trees to increase the productivity of this low productive area. After growing buckwheat in the alleys of first five tree rows the system had walnut yield of 378 kg/ha and buckwheat yield 1.8 t/ha. However since only 75% (6m out of 8m) of area in alleys was covered with buckwheat (the rest 25% was walnut rows) the actual buckwheat yield per ha in intercropped system was actually 1.35 t/ha. Walnut control plot had walnut yield of 746 kg/ha and buckwheat control plot had buckwheat yield of 2.5 t/ha. In relative numbers, the walnut yield in intercropped system was 51% (0.51) of the walnut yield in the walnut control plot and buckwheat yield was 54% (0.54) of the buckwheat yield in the buckwheat control plot. Altogether, it comes out that intercropped plot had land equivalent ratio (LER) of 1.05 (eq. 2) which means that by intercropping buckwheat in between these low productivity rows of walnut we have increased the production of this area by 5% in comparison to high productive area (last five rows of walnut trees).

$$\text{eq.2} \quad LER = \frac{378 \text{ kg / ha}}{746 \text{ kg / ha}} + \frac{1.35 \text{ t / ha}}{2.5 \text{ t / ha}} = 1.05$$

a)



b)



Figure. 1 – Đakovo site: a) intercropped walnut orchard, b) buckwheat control plot

Even though the buckwheat yield in intercropped system in Đakovo was significantly lower than in agricultural field (Table 1.), such lower yield still increased the total productivity of the area (eq. 2). In younger orchard - Ivankovo there was no significant difference between buckwheat yields grown in the walnut alleys and on the agricultural control plot (Table 1).

Analysis of soil parameters indicated that in Đakovo there were significantly higher levels of available potassium as well as significantly higher percentage of soil organic matter (SOM) in intercropped orchard than in agricultural field (Table 1.). However, the buckwheat yields were still lower. Such results indicate the importance of light and shading effect, which are more important than soil properties (Dufour et al., 2013; Talbot et al. 2014). In Ivankovo, where the shading effect was negligible due to young trees with small canopy area, there was no significant difference between buckwheat yields in intercropped system in comparison to agricultural field.

Table 1. Soil parameters and buckwheat yields on two sites

		ĐAKOVO		IVANKOVO	
		n		n	
pH	Orchard	8	5.9 ^{ns}	8	7.3 ^a
	Agri. field	16	5.7 ^{ns}	4	6.0 ^b
AL-P ₂ O ₅ mg/100g	Orchard	8	7.9 ^{ns}	8	16.1 ^{ns}
	Agri. field	16	9.3 ^{ns}	4	13.4 ^{ns}
AL-K ₂ O mg/100g	Orchard	8	17.1 ^a	8	18.2 ^a
	Agri. field	16	11.9 ^b	4	21.9 ^b
SOM %	Orchard	8	2.2 ^a	8	1.5 ^a
	Agri. field	16	1.7 ^b	4	1.7 ^b
Buckwheat Yield t/ha	Orchard	8	1.8 ^a	8	2.5 ^{ns}
	Agri. field	16	2.5 ^b	4	1.7 ^{ns}

n-number of samples, ^{ns} indicates no significant differences, ^a and ^b indicate significant difference between orchard and agricultural field for each site separately, SOM –soil organic matter

Further economic analysis is necessary to investigate the economic aspect of such production, i.e. profitability. For how long can intercropping be profitable in walnut orchards? Previous research that conducted investigating effect of light on crop yield has shown that crop yields in agroforestry system usually drastically drop around year 8-10 from planting (Talbot et al. 2014).

Conclusion

Intercropping of buckwheat in low productive rows of walnut orchard can increase the productivity of such area even if the buckwheat yields are significantly lower compared to yields in agricultural land. In younger walnut orchards (4-yr old orchard) there was no difference in buckwheat yields. However, how long can such intercropping in walnut orchards be profitable, needs to be investigated in more details. At some point the walnut trees will provide too much shade and the production of agricultural crops within walnut rows, such as buckwheat, will not be feasible anymore.

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Prinos heljde u konsocijaciji nasada oraha i heljde

Sažetak

Konsocijacija podrazumijeva kombiniranje više biljnih vrsta na istoj površini u isto vrijeme. Značaj konsocijacije je u uzgoju biljnih vrsta u sustavima koji su manje osjetljivi na abiotski i biotski stres. Kombinacija trajnih nasada s poljoprivrednim kulturama na istoj površini pozitivno utječe na mikroklimatske uvjete, što poboljšava otpornost biljaka na ekstremne klimatske uvjete suše i poplave. Cilj našeg istraživanja je odrediti prinose heljde unutar nasada oraha. Pokus je proveden na dvije lokacije. Na jednoj lokaciji orasi su stari četiri godine (Ivankovo), a na drugoj jedanaest godina (Đakovo). Pokus na obje lokacije se sastojao od tri parcele - kontrolne parcele heljde bez oraha, voćnjaka oraha s usijanom heljdom između redova i voćnjakom oraha bez usijane heljde. Svojstva tla i agroklimatski parametri mjereni su i praćeni tijekom vegetacijskog razdoblja. Prinosi heljde u 11-godišnjem nasadu oraha bili su statistički značajno niži u usporedbi s kontrolnom površinom bez oraha. Međutim, u 4-godišnjem voćnjaku oraha nije bilo statističke značajne razlike između prinosa heljde u voćnjaku i na kontrolnoj parceli bez oraha. Takvi rezultati ukazuju na to da je moguće uzgajati poljoprivredne kulture poput heljde u konsocijaciji s orahom, no takva proizvodnja je isplativa do određene starosti oraha.

Ključne riječi: agrošumarstvo, heljda, iskoristivost zemljišta, silvoarabilno, orah

Uptake of heavy metals by enhanced tobacco grown in industrially polluted soils in Bulgaria

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Abstract

A field study was conducted to evaluate the efficacy of enhanced tobacco for phytoremediation of contaminated soils. The experiment was performed on an agricultural field highly contaminated (2544.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd) by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The concentration of heavy metals in different parts of three varieties of enhanced tobacco (NBCu108F3, NBCu104F3, BAGF3) was determined by ICP. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in the organs of tobacco. Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots, Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn- upper leaves > middle leaves > lower leaves > stalks > roots. The translocation factor for all metals is greater than 1 (TF Pb -13.7-14.8, TF Zn -8.8-10.1, TF Cd -5.5-6.5). None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd.

Keywords: heavy metals, enhanced tobacco, translocation factor, bioaccumulation factor, phytoextraction

Introduction

Remediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to decrease their toxicity (Salt et al., 1998). This technique has many advantages compared with other remediation procedures – low economic costs and the possibility of being applied to soils, causing a minimum environmental impact. As a technology based on the use of plants, the success of phytoremediation will mainly depend on the proper selection of plants. An ideal plant for phytoextraction should possess several characteristics: tolerance to excessive heavy metals concentrations in soil, fast growth and high biomass and high efficiency in heavy metals uptake and translocation to aboveground parts, low water requirement, no invasiveness, resistance to pests and pathogens, phytomanagement with low inputs, and ease of harvest (Herzig et al. 2014). Phytoextraction efficiency can be significantly improved by combining selected and genetically improved cultivars of high biomass plants and relevant agronomical techniques (Nehnevajova et al. 2007, Herzig et al. 2014).

Tobacco is a crop that has an exceptional ability to accumulate more Cd in its leaves than any other crop (Mench and Martin, 1991, Mench et al., 1994) and is an efficient cadmium accumulator (Guadagnini, M., 2000). Guadagnini et al. (2000) developed by means of conventional in vitro-breeding and selection techniques, 106 somaclonal variants of tobacco (*Nicotiana tabacum* spp.) with increased metal tolerance, accumulation and extraction properties. The authors found that 17 of these somaclonal tobacco variants showed an improved heavy metal shoot uptake and phytoextracted 1.2–2.0 times more Cd and Zn than non-modified mother-plants. Herzig et al. (2003) confirmed enhanced shoot metal removals up to a factor 1.8 for Cd, 3.2 for Zn and 2.0 for Pb in soil contaminated by industrial sewage sludge and up to a factor 12.4 for Cd, 13.7 for Zn and 13.5 for Pb in acid sandy soil contaminated by deposits from a zinc smelter for best tobacco variants NBCu108F1 and NBCu 104F1 (PHYTAC, 2005).

The main objective of this paper is to conduct a systematic study, which will allow us to determine the uptake of the heavy metals (Pb, Cd and Zn) by three varieties of enhanced tobacco (NBCu108F3, NBCu104F3, BAG F3), as well as the potential of the enhanced tobacco for phytoremediation of heavy metal contaminated soils.

Materials and methods

The test plant were three varieties of enhanced tobacco (NBCu108F3, NB104F3, BAGF3) grown at a distance of 0.5 km from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. Field trials were set under the block method in four repetitions. Seeds of the plants were germinated in the greenhouse under controlled conditions. Three week old seedlings were transferred to pots, acclimatized outside before planting in the field. A planting distance of 15 cm in the row and 55 cm between the rows was followed. Technically senesced leaves of various stem positions were taken for analysis. As the leaves of tobacco ripen sequentially, harvesting is done in stages /harvests/ by following the sequence of senescence of leaves. Harvesting of leaves is carried out for 3 harvests (1-lower leaves, 2-middle leaves, and 3- upper leaves, The collected technically senesced leaves were strung on a single needle and dried in natural conditions, observing the technology of solar drying of oriental tobacco.

The concentration of heavy metals were determined in the different parts of three varieties of enhanced tobacco - roots, stalks and leaves (lower, middle and upper). The plant samples were treated by the method of microwave mineralization.

The pseudo-total concentration of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metals concentration in soil were extracted in accordance with ISO 14870 by a solution of DTPA.

To determine the heavy metal concentration in the plant and soil samples, inductively coupled emission spectrometer (Jobin Yvon Horiba „ULTIMA 2“, France) was used.

Results and discussion

Chemical characteristics of soils are shown in Tables 1 and 2. The soil used in this experiment was slightly alkaline, with moderate concentration of organic matter and essential nutrients (N, P and K).

Table 1. Characterization of the soil used in the experiment.

	pH	EC	Organic matter, %	N, %	P, mg/kg	K, mg/kg
Soil	7.6	0.3	3.99	0.22	731	4675

Table 2. Total and DTPA extractable Pb, Zn and Cd (mg/kg) in soil sample.

Parameter	Pb	Cd	Zn
Total concentration	2429.3	51.5	2544.8
DTPA extractable	868.0	31.7	279.8
DTPA/total, %	35.7	61.6	11.0
MPC (pH 6.0 -7.4)	100	2.0	320

The pseudo-total concentration of Zn, Pb and Cd is extremely high (2544.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb and 2.0 mg/kg Cd). The extremely high soil total metal concentration was a prerequisite for also high available concentration (for all investigated metals), as confirmed by the results of DTPA-extracted Pb, Cd and Zn (moderate for Pb and Zn, and high for Cd).

Table 3 presents the results obtained for the concentration of heavy metals in the vegetative organs of three varieties of enhanced tobacco NBCu108F3, NBCu104F3 and BAGF3. The root system is a major route for the absorption of heavy metals into plants. Once they have entered the roots, they can be stored or moved to the stems. The obtained results show that a significant portion of Pb, Zn and Cd is accumulated at the roots of the enhanced tobacco NBCu108F3, NBCu104F3 and BAGF3. The lead concentration in tobacco roots varies from 69.8 mg/kg in NBCu108F3 to 132.2 mg/kg in BAGF3, Cd from 17.7 mg/kg in NBCu108F3 to 28.1 mg/kg in BAGF3, Zn – from 97.6 mg/kg in NBCu108F3 to 185.1 mg/kg in BAGF3. These results are contrary to the results obtained by del Piano

(2008), who found that Pb accumulated mainly in the roots of tobacco.

The concentration of heavy metals was higher in tobacco stalks than in the root system, with the exception for the BAGF3 cultivar for Pb and Zn. The lead concentration in tobacco stalks varies from 75.6 mg/kg in NBCu104F3 to 100.2 mg/kg in BAGF3, Cd from 28.2 mg/kg in NBCu104F3 to 33.4 mg/kg in NBCu108F3, Zn stalks 164.4 mg/kg in NBCu104F3 to 184.3 mg/kg in BAGF3 (Table 3).

Table 3. Concentration of Pb, Cd and Zn (mg/kg) in vegetative organs of enhanced tobacco (NBCu108F3, NB104F3, BAGF3).

	Roots	Stalks	Leaves			Average
			Lower	Middle	Upper	
Pb						
NBCu108F3	69.8	96.9	927.8	1062.6	888.4	959.6
NB104F3	74.2	75.6	1062.5	1403.1	838.0	1101.2
BAGF3	132.3	100.2	1074.5	1044.3	930.2	1016.3
Cd						
NBCu108F3	19.8	33.4	155.6	120.9	66.4	114.3
NB104F3	17.7	28.2	155.8	129.0	61.1	115.3
BAGF3	28.1	29.2	132.9	135.2	75.8	114.6
Zn						
NBCu108F3	97.6	176.3	860.1	936.7	1147.9	981.6
NB104F3	124.9	164.4	922.2	1173.0	1134.5	1076.6
BAGF3	185.1	184.4	949.2	1000.7	1065.1	1005.0

The concentration of heavy metals were higher in the tobacco leaves than in the root system and stalks, which is in line with the results of other authors (Wagner and Yergan, 1986; Mench et al., 1989; Keller et al., 2003; Fässler et al., 2010). The average Pb concentration in tobacco leaves varied from 959.6 mg/kg in NBCu108F3 to 1101.2 mg/kg in NB104F3 (Table 3). The concentration of lead was the highest in the middle leaves with the exception for the BAGF3 cultivar. Visible symptoms, caused by high levels of Pb, which occur in tobacco (dark green leaves, leafroll of old leaves, dark brown and short roots), were not observed in our experiments. Obviously, Pb can be incorporated in plant tissues in enhanced tobacco in a way so that they do not pose an issue for plant physiological processes. According to Tso (1991), the concentration of Pb in tobacco leaves varies widely from 0 to 200 mg/kg, and depends largely on the soil characteristics, the type and variety of tobacco, as well as the place of cultivation and total concentration of Pb in soil (Lugon-Moulin, 2004).

The average Cd concentration in tobacco leaves varies from 114.3 mg/kg in NBCu108F3 to 115.3 mg/kg in NB104F3. Visible symptoms caused by the increased concentration of Cd in plants, such as growth arrest, damage to the root system, chlorosis on leaves, reddish to dark brown colour on their edges, were not observed. Obviously, Cd can be incorporated in plant tissues in enhanced tobacco in a way so that they do not pose an issue for plant physiological processes. According to Golia et al. (2007) the concentration of Cd in tobacco ranges from 0.5 to 3.5 mg/kg, while Tso (1991) reports values reaching up to 11.6 mg/kg. According to Mench et al. (1994) and Sappin-Didier et al. (1997), Cd concentration in tobacco leaves varies from 40 to 120 mg/kg depending on the soil characteristics and Cd soil total concentration, plant cultivar and environmental conditions (which affect temperature, moisture, etc.). The results obtained were consistent with the values reported in other literature sources (Adamu et al., 1989, Bell et al., 1992) and higher than the concentrations considered critical for plants to have developmental issues (which were not visually observed) (5-30 mg/kg) (Kabata Pendias and Pendias, 2001). The greater accumulation of Cd in tobacco leaves is probably due to Cd absorption from the soil through the root system of the plant and their movement through the conductive system. It has been found that cadmium can be accumulated in tobacco leaves in an amount 10 times higher than that in the soil (Anonymous, 1995). This is consistent with what was found by Yeargan et al. (1992), who found that tobacco had an extraordinary ability to accumulate Cd as compared to other plants when

grown on Cd contaminated soils.

The concentration of cadmium is the highest in the lower leaves with the exception for the BAGF3 cultivar. Our results confirm the results of Wagner (1994), according to whom the highest values of Cd are found in the leaves from the lower zone of tobacco and significantly lower in the leaves of top zone, assuming gradual accumulation over time.

The average Zn concentration in tobacco leaves varies from 981.6 mg/kg in NBCu108F3 to 1076.6 mg/kg in NB104F3. According to Jones et al. (1991) and Campbell (2000) the optimum amount of Zn in the tobacco is in the range from 20 to 60 (80) mg/kg, and excess were observed in values above 80 to 100 mg/kg. Symptoms of zinc toxicity which manifest themselves as chlorosis and necrosis at the edges of the leaves, inter-veinal chlorosis in young leaves, plant growth arrest as a whole, damage to the roots, were not observed as well. The concentration of zinc is higher in the upper leaves, in comparison with the leaves of the lower and middle leaves with the exception for NB104F3.

The distribution of heavy metals in the organs of enhanced tobacco has selective character specific for individual elements. Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots, Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn- upper leaves > middle leaves > lower leaves > stalks > roots (Table 3).

For comparing phytoextraction efficiencies of enhanced tobacco, translocation and bioconcentration factors were calculated. The translocation Factor ($TF = C_{shoots}/C_{roots}$) provides information on the ability of plants to uptake heavy metals through the roots and to move them to the above-ground mass (leaves). The translocation factor for all metals is greater than 1. The results we obtained show that, with respect to Pb, the translocation factor for plants varies from 7.7 to 14.8, for Cd from 4.1 to 6.2 and for Zn from 5.4 to 10.1 (Table 4).

Table 4. Translocation (TF) and Bioconcentration factors (BCF roots, BCF shoots) in enhanced tobacco (NBCu108F3, NB104F3, BAGF3).

	TF			BCF		
	NBCu108F3	NB104F3	BAGF3	NBCu108F3	NB104F3	BAGF3
Pb	13.7	14.8	7.7	1.1	1.3	1.2
Zn	10.1	8.6	5.4	3.5	3.8	3.6
Cd	5.8	6.5	4.1	3.6	3.6	3.6

Translocation factor (TF=Cshoots/Croots),

Bioconcentration factor (BCFshoots=Cshoots/Cavailable soil concentration)

When compared with the BAGF3, the NBCu108F3 and NB104F3 showed an improved translocation from roots to shoots that was 2 times greater for lead and zinc and 1.5 times for cadmium, the bioconcentration factor ($BCF_{shoot} = C_{shoots}/C_{available\ soil\ concentration}$) values reached to 1.1-1.3 for Pb, 3.5 - 3.8 for Zn and 3.6 for Cd. Higher values for Pb, Cd and Zn are probably a consequence of the greater ability of these elements to accumulate in the above-ground mass than in the roots, which is consistent with the results of Fässler et al. (2010) for Cd. The high concentration of Cd, Zn and Pb in the leaves and the high translocation factor indicate the possibility of enhanced tobacco to be used in phytoextraction of Pb, Cd and Zn from highly contaminated soil. Higher root to shoot translocation of investigated metals indicated that enhanced tobacco have vital characteristics to be used for phytoextraction of these metals. None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd. Further studies are required to determine the utilization of residual products of the enhanced tobacco when growing on industrially polluted soils.

Conclusions

Based on the results obtained regarding the uptake of the heavy metals by enhanced tobacco, as well as the potential of the plant for phytoremediation of heavy metal contaminated soils, the following conclusions can be made:

1. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in the organs of enhanced tobacco. Generally, Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots,

Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn - upper leaves > middle leaves > lower leaves > stalks > roots.

2. The high concentration of Cd, Pb and Zn in the leaves and the high translocation factor (TF Pb - 13.7-14.8, TF Zn - 8.8-10.1, TF Cd -5.5-6.5) indicate the possibility of enhanced tobacco to be used in phytoextraction of these metals.
3. The two varieties of enhanced tobacco NBCu108F3 and NB104F3 showed an improved translocation from roots to shoots than BAGF3, that was 1.8 times greater for lead and zinc and 1.5 times for cadmium.
4. The concentration of Cd in upper, middle, and lower leaves reached to 62.1-75.8 mg/kg, 120.9-135.2 mg/kg, 132.0-155.8 mg/kg; Pb - to 838.0-930.2 mg/kg, 1044.3-1403.1 mg/kg, 927.8-1074.5 mg/kg and Zn - to 1065.1-1147.9 mg/kg, 936.7-1173.0 mg/kg, 860.1-949.2 mg/kg, respectively. None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd. Further studies are required to determine the utilization of residual products of enhanced tobacco when growing on industrially polluted soils.

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Dynamics of runoff pH and electrical conductivity after pile burns

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Abstract

Pile burns are commonly used as a tool to manage agricultural residues. In this management a high amount of biomass was carried out to mimic high severity (HS) and low severity (LS) burn, and the impacts of burn on soil and water properties. A control plot was established to assess the impact of both treatments. Experiment was set up near Vrana (43°58'05.9"N 15°31'26.5"E), Croatia. Mean annual temperature is 15,1 °C, and the annual precipitation is 851 mm. The soil type is classified as *Leptosols*. Slope of filed site is average 18°. Runoff pH and electrical conductivity (EC) were taken as the first indicator of the soil and water changes affected by the pile burns. The sampling time of runoff was 1 month, 3 months and 6 months after burning. Values of both, pH and EC were significant higher 1 month after burn in HS treatment, followed by LS and control ($p < 0.05$). After 3 and 6 months, a continuous decrease in runoff pH and EC was identified with LS and HS treatment. Control treatment had a similar runoff pH and EC at each sampling date without significant difference ($p > 0.05$). Runoff pH was significant different in LS and HS treatments among all sampling date ($p < 0.05$). Runoff EC was significant different in HS treatment among all sampling date ($p < 0.05$). In LS treatment, runoff EC was significant difference among 1 and 3 months, and 1 and 6 months after burn. Significant difference was no observed among LS and HS treatments 6 months after burn for runoff pH and EC values ($p > 0.05$). Pile burn is being consider as alternative to manage agriculture residues without permanent impact on soil and water properties.

Keywords: pile burn, low severity, high severity, runoff pH, runoff EC.

Introduction

Burning woody residues, in a pile, is a common practice in agriculture areas (Rhoades and Fornwalt, 2014). Farmers often used pile burn, as an inexpensive method to remove agricultural residues on the field. When a pile is burned, the soil is heated for a long time, longer than a wildfire and often has impacted at deeper soil layers (Miller et al., 2015). Different amounts of burned fuel lead to a different impact on soil, and that can be expressed as burn severity. Burn severity is a qualitative measure of burn effects on-site resources (Harford and Frandsen, 1992; Ryan and Noste, 1983). Part of the nutrients accumulated in aboveground biomass, after burning, are deposited as ash, which contains different amounts of available nutrients depending on the severity (Marion et al., 1991; Gimeno-Garcia et al., 2004). The ash-bed effects strongly influence soil and water properties in the period immediately after the burning (Beyers et al., 2005; Pereira et al., 2019). After burning, variable amounts of ash are left remaining on the soil surface until the ash is either redistributed by wind and water erosion or is leached into the soil (Beyers et al., 2005; Johansen et al., 2001; Pannkuk and Robichaud, 2003; Larsen et al., 2007). Ash is a mixture of different organic and inorganic materials and has a different impact on soil and water (Brook and Wittenberg, 2016). As a reflection of hydrochemical characteristics, pH is one of the important indicators to evaluate soil (Smith and Doran, 1996) and water quality (Feng et al., 2017). pH is usually changed due to OH⁻ losses, the complete oxidation of soil organic matter and the release of cations (Arocena and Opio, 2003; Certini, 2005). Another indicator to get insight into soil and water quality is electrical conductivity (EC). EC is related to the total amount of cations and anions in soil solution. EC tends to increase in the immediate period after burning, owing to the release of soluble ions during the combustion of soil organic matter and the incorporation of ash into the soil (Certini, 2005). In many research

higher EC values was noted in the burning area compared to unburned (Heydari et al. 2016; Badia and Marti, 2008; Alcaniz et al., 2016). Increase in EC values is explained as the release of base cations (Ca^{2+} , Mg^{2+} and K^+) due to the progressive loss of structural OH-groups in the exchange complex during burning (Badía and Martí 2003; Sertsu and Sánchez 1978). The measurement of both parameters, pH and EC, provides a more complete indication of the chemical properties of soil and water. When measured together, burn effects will be detected more easily (Smith and Doran, 1996). Few types of research were carried out about runoff pH and EC in areas affected by pile burns. The objective of this work is to study the effects of low severity (LS) and high severity (HS) pile burn on the quality of runoff pH and EC.

Material and methods

The site for the experimental pile burn is located near Vrana (43°58'05.9"N 15°31'26.5"E), Croatia. Mean annual temperature is 15.1 °C, and the annual precipitation of 851 mm (data from 1971-2000) (Zaninović, et al. 2008). The soil type is classified as Leptosols (IUSS Working Group WRB, 2015). The location is selected due to common frequent burning agricultural residues in this region. The experiment was located on agricultural land abandoned 40 years ago. The field sites for experimental pile burn were divided into three experimental treatments; low severity (LS), high severity (HS) and a control plot. Burned plots and control were covered with undisturbed vegetation (*Foeniculum vulgare* Mill., *Elymus repens* (L.) Gould, *Digitaria sanguinalis* (L.) Scop.). Before the burn, five metal rings with an area of 0,2 m² were set up on each experimental plot (fifteen rings in total). Two experimental burn treatments were applied to reach different severities. The plot that represents LS treatment is filed with 100 kg/10m² straw and HS treatment is filed with 100kg/10m² straw and 150 kg vine stem (vineyard pruning residues). The piles were burned on 18th March 2019 at 10:13 h by the local time and it was burning for 40 min. The slope of the experimental filed site is average 18° (min 9°, max 25°). After burning, plastic tanks were set up under rings to collect runoff. Sampling of runoff water was carried in April (1 month after burn), June (3 months after burn), and August (6 months after burn). In the laboratory, runoff was filtrated thoughts active carbon and prepared for pH and EC analyse. The runoff pH was measured with the Beckman pH-meter Φ72 according to HRN ISO 11464:2004 norm. The runoff EC was measured with The Lab 960 conductivity meter according to HRN ISO 11265:2004 norm. The mean runoff pH and EC of 5 repetitions per treatment were taken and represented the value of the treatment. Statistical analysis was used to identify differences in the sampling period and between treatments for pH and EC parameters. The two-way ANOVA and Tukey post-hoc tests were applied to identify the statistically significant difference. All statistical tests were performed at the standard 5% significance level. Statistical analyses were carried out with SigmaPlot 11. software (Systat Software Inc.).

Results and discussion

Runoff pH was not significantly different among sampling dates for control ($p < 0.05$), but was for LS and HS treatment ($p > 0.05$). HS treatment had a significant highest mean runoff pH values (9.40, 8.60, 8.08), followed by LS (8.90, 8.16, 7.85), and control (7.88, 7.60, 7.50) (Fig.1.). In post-burn period a continuous decrease in runoff pH was observed. Runoff pH was a significantly higher in LS and HS treatments among all sampling date ($p < 0.05$). Runoff pH was significant different among treatments HS and control, and LS and control among all sampling dates ($p < 0.05$). Significant difference was no observed among LS and HS treatments 6 months after burn for runoff pH values ($p > 0.05$).

Dynamics of runoff pH and electrical conductivity after pile burns

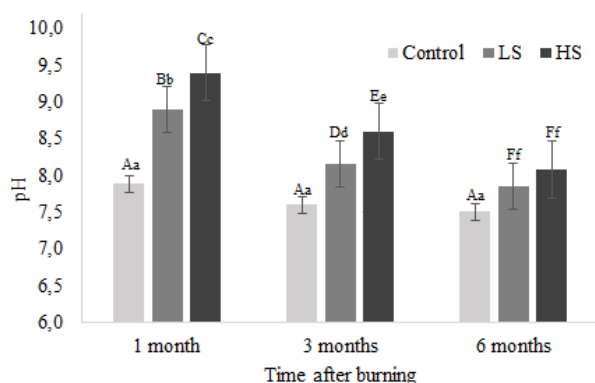


Fig. 2. EC of control, LS and HS. Different letters indicate significant differences at $p < 0.05$, between treatments (capital letters) and time (low-case letters). Bars show standard error. EC (electrical conductivity), LS (low severity), HS (high severity).

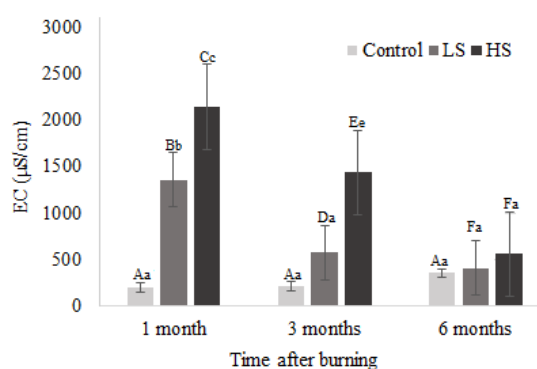


Fig. 1. pH of control, LS and HS. Different letters indicate significant differences at $p < 0.05$, between treatments (capital letters) and time (low-case letters). Bars show standard error. LS (low severity), HS (high severity).

The increased runoff pH after burning is usually temporary depending upon the original soil pH, amount and type of ash produced and post-burning weather conditions (Wells et al., 1978). Previous research was observed an increase in soil pH after the burning (Arocena and Opio, 2003; Ubeda et al., 2005). In our experiment, an explanation for the higher runoff pH of LS and HS can be the production of the ash. In HS treatments a larger amount of biomass was burned and that lead to thickest layer of ash. After burning, ash is remaining on the soil surface (Ulery et al., 1993; Wells et al., 1978; Beyers et al., 2005). The incorporation of ash into the soil's top layer has been referred to as the main cause of changes in soil chemistry (Badia et al., 2014; Thomaz et al., 2014). After the first rainfall, it is assumed that ash particles were transported into the tanks together with runoff. According to Pereira et al. (2019), it is not possible to differentiate the contributions from ash and soil to runoff chemistry in burned areas. Ash pH is directly related to the impact of burning severity on the chemical properties of the topsoil layer (Henig-Sever et al., 2001). Soil pH increases the result of organic acid denaturation during biomass burning (Certini, 2005). Arocena and Opio (2003) observed that during a burning H^+ and Al^{3+} cations are increasing the soil pH by displacing the H^+ and Al^{3+} ions adsorbed on the negative charge of the soil colloids. In our experiment, the thickest layer of ash was noticed in HS treatment which is attributed to a higher amount of biomass burned. According to Arocena and Opio (2003), a larger amount of burned fuel results in more severe burning and that is generally related to higher concentrations of exchangeable cations. Ash produced at LS is mainly composed of incomplete combustion of organic matter (Santin et al., 2012), while at HS calcium carbonate is formed (Ulery et al., 1993). In our experiment runoff pH of HS had the highest values. Usually, ash mixed with water is higher than the water extract pH of the unburned areas (Blank and Zamudio, 1998). This explains the higher runoff pH of LS and HS, comparing to the unburned plot, control. Pereira et al. (2012) observed that ash pH increases with burning severity. In our experiment, 6 months after setting up the experiment, runoff pH of LS and HS were lower than in 1 months. This is attributed to the ash leaching and erosion (Ulery et al., 1993). EC of both burn treatments, LS and HS, was highest in 1 month after burning comparing to control. Pardini et al. (2004) described an increment of both parameters, pH and EC, as production of ash after burning which is collated to the organic matter denaturation. In our experiment, HS had the highest mean runoff EC value in each sampling date (2140.2 $\mu S/cm$, 1431.5 $\mu S/cm$, 555.4 $\mu S/cm$), followed by LS (1357.6 $\mu S/cm$, 574 $\mu S/cm$, 402.6 $\mu S/cm$). Control had the lowest runoff EC (193.3 $\mu S/cm$, 208 $\mu S/cm$, 349.5 $\mu S/cm$) (Fig.2.). Runoff EC was significantly different among sampling date in LS and HS treatments ($p < 0.05$). In control, no significant difference was observed among sampling date ($p > 0.05$). Runoff EC was significantly higher in HS treatment than in LS treatment among sampling date 1 and 3 months, and 1 and 6 months after burn ($p < 0.05$). In HS treatment a significant difference was observed among all sampling date ($p < 0.05$). In LS treatment a significant difference was observed among sampling date 1 and 3, and 1 and 6 months after burn ($p < 0.05$). Significant difference was no observed among LS and HS treatments 6 months after burn for runoff EC values ($p > 0.05$). Decreasing value of runoff EC can be explained as erosion removal of ash particles after burn. Higher runoff EC values in HS treatment, compared to LS and control is accordance with a higher biomass burned. In research by Alcaniz et al. (2016),

immediately after burning, EC was significantly increased by comparing burn and control treatment. Certini (2005) explained increased EC after burning, as the release of soluble inorganic ions during combustion of soil organic matter, as well as the formation of black carbon and the incorporation of ash into the soil. According to Ubeda et al. (2005) decreased EC after burning is related to leaching of soil ions. In our experiment, a decrease of runoff EC was noted in post-burn period. Reduction in EC, after burning, is usually the result of overland flow (Badia and Marti, 2008). In our experiment 3 and 6 months after burning, runoff EC had lower values compared to the values 1 month after burning but without significant difference ($p > 0.05$). In research by Brye (2006), a decrease in EC after burning was explained due to nutrient export. Badia and Marti (2003) state that the increase in EC or total dissolved solutes in burned soil is not related to the ash from burned biomass, so it should be related to the mineralization on the soil organic matter by heating. In research by Heydari et al. (2016), EC was higher in HS compared to LS and control. EC was higher in HS treatments due to the release of mineral ions generated by the higher burning temperatures of organic matter which is accordance with higher amount of biomass burned (Certini 2005; Heydari et al. 2016).

Conclusion

The most notable difference was with HS treatment between 1 and 6 months after burn and for both measured parameters, runoff pH and EC. After 3 months, values of LS and HS were lower than 1 month after burn but still higher than 6 months after burn. Control on each sampling date had similar runoff pH and EC values. During 6 months of sampling, both measured parameters of LS and HS were decreasing and approaching to the values of control. Considering decreasing values, the impact of pile burns on soil and water is not permanent. Pile burn is being considered as alternative to manage agriculture residues without permanent impact on soil and water properties. Future measured parameters are needed to get a wider view of pile burns effects on soil and water quality.

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Sadržaj mikroelemenata u listu i cvijetu prave lavande (*Lavandula angustifolia* Mill.) i lavandina (*Lavandula × intermedia* Emeric ex Loisel.)

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Sažetak

Obzirom na veliku zastupljenost uzgoja prave lavande (*Lavandula angustifolia* Mill.) i lavandina (*Lavandula × intermedia* Emeric ex Loisel.) u Republici Hrvatskoj kao i činjenicu da se upravo te vrste pretežno koriste za proizvodnju eteričnog ulja, cilj ovog istraživanja bio je utvrditi sadržaj mikroelemenata u cvijetu i listu prave lavande i lavandina. Postavljen je dvofaktorijski pokus (prava lavanda i lavandin) po slučajnom bloknom rasporedu u četiri ponavljanja. Kemijskim analizama utvrđena su agrokemijska svojstva tla i koncentracije mikroelemenata u biljkama. Kemijskom analizom istraživanih mikroelemenata u listu i cvijetu utvrđeno je da se njihove vrijednosti smanjuju slijedom $Fe > Zn > Mn > Cu$, pri čemu je bakar bio prisutan u najmanjoj količini (2,38 do 4,88 mg/kg), a željezo kao esencijalni element bilo je najzastupljenije (186,8 do 353,2 mg/kg). Jaka negativna korelacijska povezanost utvrđena je između sadržaja bakra u cvijetu prave lavande i listu lavandina sa bakrom u tlu ($r = -0,969$; $r = -0,834$).

Ključne riječi: lavanda, željezo, cink, mangan, bakar

Uvod

Lavande i lavandini su višegodišnje, kserofitne, polugrmolike biljke iz porodice *Lamiaceae* (Kolak i Šatović, 2003). Prvi zapisi o lavandi (*Lavandula angustifolia* Mill.) govore da potječe iz XIII. stoljeća i to vrijeme se smatra početkom širenja te vrste u srednjoj Europi, dok se uzgoj hibridne lavande (*Lavandula × intermedia* Emeric ex Loisel.) spominje u XVI. stoljeću (Šilješ i sur, 1992). Pripadaju skupini ljekovitih i aromatičnih biljaka porijeklom iz zapadnog dijela Sredozemlja, a uzgajaju se zbog cvjetova koji sadrže eterično ulje. Naziv "lavandin" obuhvaća križance prave ili uskolisne lavande (*Lavandula angustifolia* Mill.) s drugim vrstama istog roda, širokolisnom lavandom (*Lavandula latifolia* Medik.). Različite vrste i sorte lavande i lavandina ne razlikuju se međusobno samo po vanjskom izgledu i ekološkim zahtjevima, nego i po količini i sastavu eteričnog ulja. Eterična ulja prave lavande i lavandina imaju sličan kemijski sastav, no udio je pojedinih komponenti različit i uvelike ovisi o podneblju uzgoja (Žutić, 2007; Kara i Baydar, 2013). Eterična ulja se danas intenzivno koriste u kozmetičkoj, prehrambenoj i parfemskoj industriji (Lis-Balchin i Hart, 1999). Uporaba organskih gnojiva, poboljšava svojstva tla, posebice strukturu što ima za posljedicu bolji vodozračni odnos te veću raspoloživost svih hraniva (Bohloul i sur, 2016). Iako su biljne potrebe za mikroelementima znatno niže u usporedbi s onima za makroelemente, mikroelementi su bitna hraniva potrebna za pravilan rast i razvoj biljaka. Manjak mikroelemenata u biljkama najprije rezultira smanjenjem otpornosti biljaka na štetne čimbenike okoliša, nakon čega slijedi smanjenje prinosa i njihove kvalitete (Alloway, 2008). Dugotrajna mineralna i organska gnojidba može značajno izmijeniti svojstva tla kao što su pH, sadržaj organskih tvari ili drugo bogatstvo tla dostupnim oblicima makroelemenata, koji određuju dostupnost mikroelemenata biljkama (Li i sur, 2007).

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Nekoliko studija pokazalo je da gnojidba fosforom ograničava dostupnost cinka za biljke (Li i sur, 2007; Fan i sur, 2011). Rutkowska i sur. (2014) navode da su se koncentracije cinka, bora i željeza u otopini tla značajno povećale uz primjenu stajskog gnoja. Također navode da sustavna i dugotrajna primjena stajskog gnoja rezultira povećanjem udjela organske tvari u tlu. Određene količine mikroelemenata poput kobalta, bakra, mangana, molibdena i siena neophodne su za održavanje bioloških funkcija u biljnom i životinjskom organizmu, veće količine mogu djelovati toksično. Elementi poput željeza, kroma, bakra i mangana mogu utjecati na stabilnost ulja tijekom skladištenja, uslijed njihovog katalitičkog djelovanja (Abu-Arab i Abou-Donia, 2000; Massadeh i Snook, 2002; Johnsson, 2003).

S obzirom na veliku zastupljenost uzgoja prave lavande i lavandina u Republici Hrvatskoj, kao i činjenicu da se te vrste pretežno koriste za proizvodnju eteričnog ulja, cilj ovog istraživanja bio je utvrditi utjecaj organske gnojidbe na sadržaj mikroelemenata u cvijetu i listu prave lavande i lavandina.

Materijal i metode

Istraživanje je provedeno 2019. godine na pokušalištu Visokog gospodarskog učilišta u Križevcima. U jesen 2018. godine izvršena je osnovna obrada tla za sadnju lavande i lavandina, te gnojidba. Prije osnovne obrade, uzet je prosječni uzorak tla koji je analiziran u laboratoriju Visokog gospodarskog učilišta u Križevcima. Kemijskom analizom je utvrđena neutralna reakcija tla (pH 6,94 u 1 M KCl-u), dobra opskrbljenost humusom (4 %), bogata opskrbljenosti biljkama pristupačnim fosforom (25,55 mg P₂O₅ 100g⁻¹ tla) i kalijem (27,04 mg K₂O 100 g⁻¹ tla). U jesen s osnovnom obradom primijenjeno je peletirano organsko mineralno gnojivo Proeco 5:10:10. Uneseno je 20 kg dušika, 40 kg fosfora, i 40 kg kalija. U proljeće 2019. izvršena je priprema tla za sadnju i gnojidba organskim dušičnim gnojivom Fertil Supernova 12,5. Navedenim gnojivom uneseno je 25 kg/ha organskog dušika. Na površini 306 m² postavljen je dvofaktorijski pokus (prava lavanda, lavandina) po metodi slučajnog blokno rasporeda u četiri ponavljanja. Veličina pokusne parcele iznosi 34 m² (10 x 3,4 m). Sadnja prave lavande i lavandina obavljena je u travnju 2019. Svaka pokusna parcela zasađena je sa 20 biljaka, od kojih su slučajnim odabirom uzeti uzorci lista i cvijeta za kemijsku analizu. Na svakoj pokusnoj parceli posađeno je po 10 biljaka u dva reda. Uzorkovanje tla, lista i cvijeta sa svake pokusne parcele obavljeno je u srpnju 2019. godine. Uzorkovano je po deset biljaka sa svake parcele. Priprema uzoraka tla za kemijske analize obavljena je sukladno normi HRN ISO 11464. Analiza željeza, mangana, cinka i bakra u tlu provedena je ISO 11466 (1995) metodom. Uzorci su osušeni i usitnjeni za daljnje analize. Nakon digestije sa smjesom kiselina (1/3 HNO₃+2/3 HCl), željezo, mangan, cink i bakar određeni su na atomskom apsorpcijskom spektrometru (AAS). Podaci su obrađeni statistički analizom varijance u Statistica 13.4.0.14. programu. Srednje vrijednosti istraživanih parametara između tretmana testirane su t-testom. Utvrđeni su i korelacijski odnosi istraživanih parametara izračunom Pearsonovog korelacijskog koeficijenta.

Rezultati i rasprava

Rezultati opskrbljenosti tla mikroelementima prikazani su na tablici 1. Veći sadržaj željeza, mangana i cinka utvrđen je na varijantama gdje je uzgajana lavandin, dok je veći sadržaj bakra utvrđen na varijantama gdje je uzgajana prava lavanda. Međutim, utvrđene razlike sadržaja istraživanih mikroelemenata u tlu između varijanata na kojima se uzgajala prava lavanda i varijanata lavandina nisu bile statistički opravdane (p>0,05).

Tablica 1. Sadržaj mikroelemenata u tlu neposredno prije cvatnje lavande i lavandina

Varijante	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Prava lavanda	31888	613,5	119,5	30,4
Lavandin	32439	636,8	123,4	29,3

Provedena je i kemijska analiza na sadržaj mikroelemenata u cvijetu i listu prave lavande i lavandina. Razlike u koncentracijama mikroelemenata utvrđenih u listu i cvijetu prave lavande i lavandina nisu statistički opravdane (tablica 2). Jurišić i sur. (2015.) navode da sadržaj mikroelemenata utječe na stabilnost ulja tijekom skladištenja te na katalitička svojstva ulja. Sadržaj istraživanih mikroelemenata u listu i cvijetu prave lavande i lavandina smanjuje se ovim slijedom: Fe>Zn>Mn>Cu. Utvrđene vrijednosti željeza, mangana i cinka u cvijetu prave lavande i lavandina u skladu su s istraživanjima Jurišić i sur. (2015) i Tokalioğlu (2012), dok Grzeszczuk i sur. (2018) navode znatno veće

Sadržaj mikroelemenata u listu i cvijetu prave lavande (*Lavandula angustifolia* Mill.) i lavandina (*Lavandula × intermedia* Emeric ex Loisel.)

vrijednosti bakra. Iz tablice 2. razmjerno je da se sadržaji mikroelemenata u listu prave lavande i lavandina smanjuje istim slijedom kao i u cvijetu Fe>Zn>Mn>Cu. Relativno veće vrijednosti željeza i mangana zabilježene su u listu prave lavande, dok je sadržaj cinka i bakra relativno veći u listu hibridne lavande. Imelouane i sur. (2011.) navode u cvijetu i listu vrste *Lavandula dentata* (8447 mg kg⁻¹ željeza, 367,4 mg kg⁻¹ mangana i 480,4 mg kg⁻¹ cinka).

Tablica 2. Sadržaj mikroelemenata u listu i cvijetu lavande i lavandina

Varijante		Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Lavanda	List	353,2	38,9	45,7	2,38
	Cvijet	230,9	9,85	68,0	3,96
Lavandin	List	350,5	37,7	48,5	3,19
	Cvijet	186,8	10,8	49,5	4,88

Na temelju izračunatih korelacijskih koeficijenata utvrđena je jačina povezanosti između sadržaja određenih mikroelemenata u tlu sa sadržajem mikroelemenata u listu i cvijetu prave lavande i lavandina. U tablici 3. je vidljivo da je sadržaj cinka u cvijetu prave lavande u jakoj pozitivnoj korelaciji sa sadržajem cinka u tlu ($r = 0,887$), željeza u srednje jakoj pozitivnoj korelaciji sa sadržajem željeza u tlu ($r = 0,590$), sadržaj mangana u slaboj pozitivnoj korelaciji sa sadržajem mangana u tlu ($r = 0,491$), dok je sadržaj bakra u negativnoj i jakoj korelaciji ($r = -0,969$) sa sadržajem bakra u tlu. Singh i sur. (2010), te Fan i sur. (2011) navode da se s povećanjem organske tvari primjećuje i veća pristupačnost cinka, dok je pristupačnost bakrom i manganom smanjena.

Tablica 3. Vrijednosti Pearsonovih korelacijskih koeficijenata istraživanih mikroelemenata kod prave lavande

Tlo	Fe		Mn		Zn		Cu	
	List	Cvijet	List	Cvijet	List	Cvijet	List	Cvijet
Fe	-0,018	0,590						
Mn			-0,010	0,491				
Zn					0,191	0,887		
Cu							0,203	-0,969*

* $p > 0,05$

U tablici 4. prikazana je korelacijska povezanost između istraživanih mikroelemenata u listu i cvijetu lavandina sa sadržajem istih mikroelemenata u tlu. Utvrđena je jaka pozitivna korelacijska povezanost mangana u cvijetu lavandina sa sadržajem mangana u tlu ($r = 0,778$), dok je sadržaj željeza i bakra u slaboj korelaciji sa sadržajem željeza i bakra u tlu ($r = 0,307$; $r = 0,437$). Također, utvrđena je slaba pozitivna korelacija sadržaja mangana u listu lavandina sa sadržajem mangana u tlu ($r = 0,120$). Jaka negativna korelacijska povezanost prisutna je kod sadržaja bakra u listu lavandina sa sadržajem bakra u tlu ($r = -0,834$) što se može povezati slabom pokretljivošću bakra iz korijena u nadzemne dijelove, a djelomično i slabijim usvajanjem bakra iz tla što raste paralelno sa porastom pH vrijednosti (Dučić i Polle, 2005). Sadržaj cinka u cvijetu lavandina je u srednje jakoj negativnoj korelaciji sa sadržajem cinka u tlu ($r = -0,675$). Nedostatak cinka djelomično se može povezati s bogato opskrbljenim tlom fosforom i slabom pokretljivošću cinka u biljci. Pozitivna korelacijska povezanost vidljiva je samo između sadržaja mangana u listu i tlu ($r = 0,120$). Jaka negativna korelacijska povezanost izražena je između bakra u tlu i listu ($r = -0,834$), dok je srednje jaka negativna korelacija prisutna kod sadržaja željeza u tlu i sadržaja željeza u listu ($r = -0,619$).

Tablica 4. Vrijednosti Pearsonovih korelacijskih koeficijenata istraživanih mikroelemenata kod lavandina

Tlo	Fe		Mn		Zn		Cu	
	List	Cvijet	List	Cvijet	List	Cvijet	List	Cvijet
Fe	-0,619	0,307						
Mn			0,120	0,778				
Zn					-0,302	-0,675		
Cu							-0,834	0,437

* $p > 0,05$

Zaključak

Istraživanjem je utvrđeno da razlike između sadržaja istraživanih mikroelemenata u listu i cvijetu prave lavande i lavandina uzgajane s organskom gnojibom nisu statistički opravdane. Rezultati pokazuju da je sadržaj pojedinog mikroelementa u tlu utjecao na mineralni sastav biljke, te je utvrđena jaka negativna korelacijska povezanost između sadržaja bakra u cvijetu prave lavande i listu lavandina sa bakrom u tlu. Vrlo jaka pozitivna korelacijska povezanost zabilježena je između sadržaja cinka u tlu i cvijetu kod prave lavande te sadržaja mangana u tlu i cvijetu kod lavandina.

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Content of microelements in leaf and flower of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.)

Abstract

In view of the high prevalence of cultivation of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.) in the Republic of Croatia, as well as the fact that they are mainly used for the production of essential oil, the aim of this study was to determine the presence of microelements in flower and leaf of true lavender and lavender. Two-factor experiment (true lavender and lavender) was set in randomized block design in four repetitions. Agrochemical properties of soil and the concentrations of microelements in plants were determined by standard chemical analyses. Results revealed that concentrations of the investigated microelements in leaf and flower decrease in order Fe > Zn > Mn > Cu, with copper present in the smallest amount (2.38 to 4.88 mg/kg) while the iron, as essential element, was the most abundant (186.8 to 353.2 mg/kg). A strong negative correlation was determined between the copper content of the true lavender flower and leaf of the lavender and the copper in the soil ($r = -0.969$; $r = -0.834$).

Keywords: lavender, iron, zinc, manganese, copper

Seasonal variability of soil respiration during maize vegetation

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Abstract

As a source of greenhouse gas emissions, the agricultural sector directly affects the global problem of climate change. A research study was conducted in Western Pannonian sub-region of Croatia to measure soil respiration and its seasonal variability influenced by vegetation presence (maize - *Zea mays* L.). Soil C-CO₂ flux was measured 14 times during the investigated period in a field experiment with 4 different fertilization treatments. Average daily C-CO₂ flux was the highest in June 2017 (38.2 kg ha⁻¹ day⁻¹) and the lowest in November 2017 (0.76 kg ha⁻¹ day⁻¹). The results imply that season and vegetation presence has a significant effect on soil respiration rates.

Keywords: C-CO₂ emission, vegetation, fertilization, season

Introduction

Over the past few decades, climate trends have been fairly rapid in many agricultural regions around the world including Croatia too. Of the six major climate forcing radiative sources (carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), tropospheric ozone (O₃), halocarbons and black carbon) agriculture has direct influence on CO₂, CH₄, N₂O, O₃ and black carbon (IPCC, 2001). These sources are together responsible for approximately 80% of contemporary climate forcing, and agricultural activities affect some GHGs more than others (Robertson, 2014). According to Bilandžija et al. (2016), carbon dioxide represents an important compound that affects the processes of global warming and is considered as an initiator of global climate change. Depending on cropping history and contemporary management, soil carbon in agricultural lands can represent a net sink or source of CO₂ (Robertson, 2014). The primary process responsible for emissions releasing from the soil into the atmosphere is diffusion which is affected by plant roots, organisms of soil respiration and soil organic matter decomposition (Buchmann, 2000; Raich and Tufekcioglu, 2000; Epron et al., 2006; Xu and Qi, 2001). Numerous studies have shown that factors including excessive tillage, irrigation practices, fertilization, soil temperature, soil moisture, climatic factors, crop presence and crop density influence the GHG production and emission rates from the soil surface (Lal, 2003; Bilandžija et al., 2014; Ball, 2013; Skiba and Ball, 2002; Ludwig et al., 2001). According to the fact that vegetation affects climate and weather patterns, the objective of this research was to determine the seasonal fluctuations of soil respiration under crop presence and crop absence, and determine the resulting changes in soil C-CO₂ emission.

Materials and methods

Study area and cover crop

A field experiment with 4 different fertilization treatments was located in the Western Pannonian sub-region of Croatia, in Popovaca (N 45° 33' 21.42", E 16° 31' 44.62"). According to Husnjak (2014) soil is classified as deep distric pseudogley (Stagnosol). The fertilization treatments were: I) control treatment-no fertilization; II) 250 kg N ha⁻¹+P+K+ 40 t ha⁻¹ of mixed solid farmyard manure; III) 300 kg N ha⁻¹+P+K and IV) black fallow-no vegetation. The size of the plot of each treatment was 30 × 130 m² including blank space.

The cover crop at the experimental plot was maize (*Zea mays* L. – P0412 Pioneer). Maize was seeded on 12 April 2017 and harvest was conducted on 15 September 2017.

Meteorological conditions

The meteorological conditions for the reference period (1961–1990) and the studied period (08.2016 – 04.2018) were presented according to the official meteorological data from the main meteorological station of the Meteorological and Hydrological Service of Croatia located in Sisak (DHMZ, 2019). The meteorological conditions are described by Lang's rain factor and Walter climate diagram. According to Gracanin's climate classification, interpretation of Lang's rain factor is conducted (Gracanin, 1950).

Measurements of soil CO₂ concentration and agro-ecological factors

To measure the seasonal variability of soil respiration, data from winter wheat harvest (09 July 2016) to soybean sowing (27 April 2018) were considered. During the mentioned period, soil CO₂ concentrations were measured 14 times (8 times in 2017) in three repetitions on each treatment. Concentrations were measured by *in situ* closed static chamber method (Figure 1). At the beginning of measurement, circular frames were inserted in the soil where initial CO₂ concentration close to the soil surface was measured. Afterwards, the chambers were closed and the incubation time was 30 minutes. After 30 minutes, the soil CO₂ concentration was also measured with a portable infrared CO₂ detector (GasAlerMicro5 IR, 2011). The soil carbon dioxide flux was calculated according to Bilandzija et al. (2014). At the beginning and the end of each measurement date, air temperature and relative air humidity were measured with a Testo 610 humidity and temperature meter and air pressure with a Testo 511 absolut pressure meter. Soil temperature and soil moisture were measured with an IMKO HD2 – probe Trime, Pico64, 2011 at 10 cm depth in the vicinity of the chambers in three replications (Figure 1c).

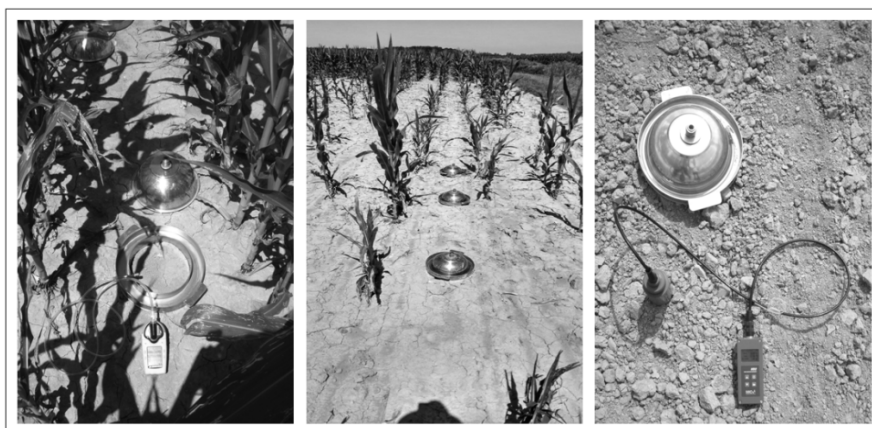


Figure 1. (a) Measurement of soil initial CO₂ concentration; (b) Chambers during the incubation time; (c) Field measurement of soil parameters (soil temperature and soil moisture)

Statistical analysis

All measured data were analyzed using statistical Software SAS (SAS Institute Inc., USA). Variability between months for each treatment was evaluated with analysis of variance (ANOVA) and tested with Fisher's least significant difference procedure. In all statistical tests the significance level was 5 %.

Results and discussion

Meteorological conditions of study area

During the 30-year-long reference period (1961–1990), mean annual amount of precipitation in Sisak was 865 mm. The mean annual temperature was 10.6 °C indicating temperate continental climate. According to Lang's rain factor (Lf), the reference period was characterized by a semi-humid climate (Lf = 82) (Butorac, 1988). In the studied period the mean precipitation amount was 1631 mm with mean temperature of 11.0 °C. According to Lang's rain factor (Lf), studied period was characterized by a humid climate (Lf = 148.6). According to Walter's climate diagram, the average climate conditions are without dry periods during the referent period (Figure 2). In the studied period, water deficit was recorded in June, July and August in 2017 (Figure 3).

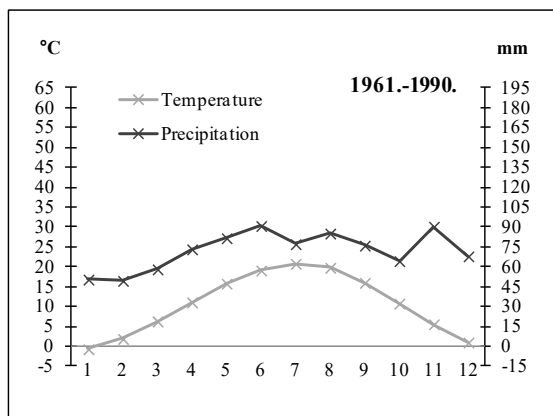


Figure 2. Weather conditions according to Walter's climate diagram for the referent period

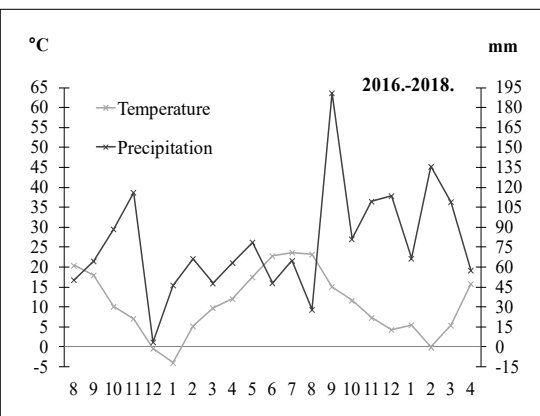


Figure 3. Weather conditions according to Walter's climate diagram for investigated period 2017

Influence of vegetation on C-CO₂ emission

According to Schlesinger and Andrews (2000), CO₂ flux from soils is closely tied to plant growth, which supplies organic residues for decomposers. Therefore, vegetation presence and type have a great influence on soil CO₂ emissions. Considering that microbial activity and root respiration are the major sources of CO₂ production, developed plants with powerful roots system surrounded by numerous microorganisms are responsible for high soil respiration (Brito et al., 2009; Galic et al., 2019). Accordingly, during the investigated period, a vegetation peak is noticeable in Figure 4 due to the presence of vegetation (02.06.17.) in a phase where nodal roots are established in the lowest, below-ground nodes of the plant. Thus, it was significantly higher in comparison to other months where vegetation transits to reproductive phase, not developed or absent. In this research, emissions amounted to 8.7 kg ha⁻¹ day⁻¹ (Figure 4) which is lower than the results obtained by Ussiri and Lal (2009). During this research, in period without vegetation emissions amounted to 6.4 kg ha⁻¹ day⁻¹ while in period with vegetation to 12.8 kg ha⁻¹ day⁻¹ (Figure 4). According to Bilandžija et al. (2016), the higher planting crop density in wheat resulted in higher and denser root biomass contributed to a 40.5 % higher emission in wheat vegetation compared to maize vegetation.

Seasonal fluctuations of soil C-CO₂ emissions

With the change of the seasons, soil C-CO₂ emissions are also changing and show dependence on meteorological conditions (Galic et al., 2019). According to the relevant literature, the seasonal fluctuation of carbon-dioxide emission changes according to the variations in the abiotic factors meaning that precipitation and temperature variations determine it (Brito et al., 2009; Liebig et al., 2013). Soil respiration is usually the highest in summer, decreases in the colder months and is the lowest in winter (Bilandžija et al., 2016). Accordingly, in this research the highest emission was recorded in June 2017 (38.2 kg ha⁻¹ day⁻¹) and the lowest in November 2017 (0.76 kg ha⁻¹ day⁻¹) and they were significantly different (Figure 4). In harmony with the results of Gaumont-Guay et al. (2006) and Martin et al. (2007) the increased emission in October 2017 can be attributed due to the extremely high rainfall during September and first half of October, which occur in higher soil moisture (Figure 3 and 4).

In this research, during the investigated period, a significant difference between the treatments was recorded in the followed months: September 2016, November 2016, April 2017, May 2017, June 2017, July 2017, August 2017, September 2017, January 2018, March 2018 and April 2018.

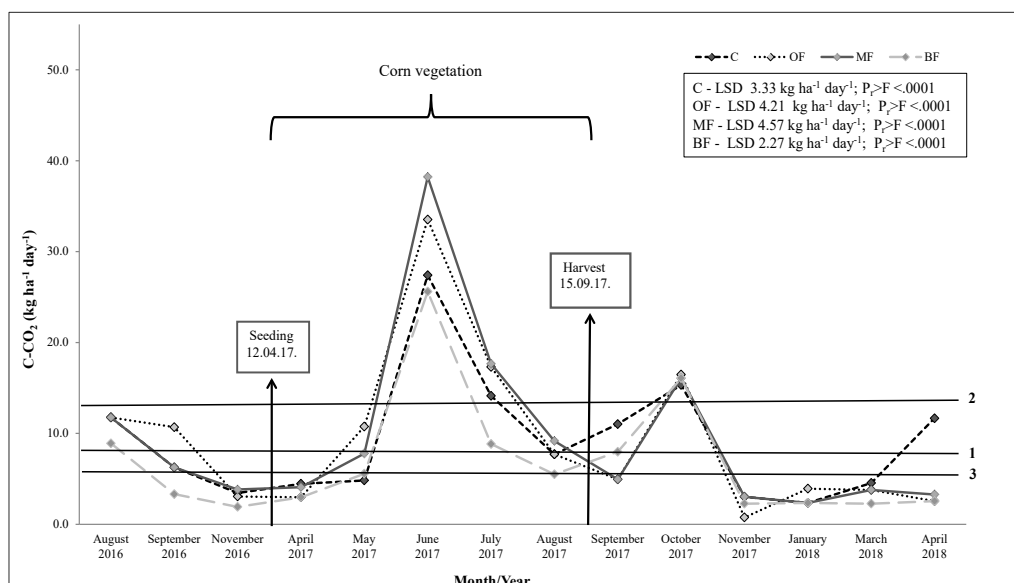


Figure 4. Average daily soil C-CO₂ emissions (kg ha⁻¹ day⁻¹) during the investigated period. Three horizontal lines represent mean C-CO₂ emission during investigated period (1), mean C-CO₂ emission during the vegetation presence (2) and mean C-CO₂ emissions during vegetation absence (3)

Conclusion

In this research conducted in Western Pannonian sub-region of Croatia, it was found that the time of the measurement and vegetation presence have a significant influence on soil C-CO₂ emissions. The C-CO₂ flux was higher during the second half of spring and in the first half of summer where the highest emission was recorded (June 2017 – 38.2 kg ha⁻¹ day⁻¹) and was significantly different in comparison to the lowest emission in November (0.76 kg ha⁻¹ day⁻¹). Considering that carbon dioxide from the soil does not depend only on factors observed in this research, it is very important to explore many other factors in agro-ecosystem by which climate change could be mitigated.

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Sezonska varijabilnost disanja tla tijekom vegetacije kukuruza

Sažetak

Kao izvor emisija stakleničkih plinova, poljoprivredni sektor izravno utječe na globalni problem klimatskih promjena. Istraživanje je postavljeno u zapadno panonskom dijelu Hrvatske u svrhu mjerenja disanja tla i njegove sezone varijabilnosti pod utjecajem prisutnosti vegetacije (kukuruz - *Zea mays* L.). CO₂ fluks izmjeren je 14 puta tijekom istraživanih razdoblja na pokusnom polju s 4 različita gnojidbena tretmana. Prosječne dnevne vrijednosti CO₂ fluksa bile je najviše u lipnju 2017. godine (38,2 kg ha⁻¹ dan⁻¹), a najniže u studenom 2017. godine (0,76 kg ha⁻¹ dan⁻¹). Dobiveni rezultati pokazuju da sezona i prisustvo vegetacije značajno utječe na brzinu disanja tla.

Ključne riječi: emisija C-CO₂, vegetacija, gnojidba, sezona

Humoznost i kiselost lesiviranih tala na lesu u Panonskoj agroregiji Hrvatske

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Sažetak

Cilj ovog rada bio je utvrditi stanje humoznosti i kiselosti Lesiviranog tla na lesu u Panonskoj agroregiji Hrvatske, te procijeniti utjecaj promjene klimatskih prilika na utvrđeno stanje. Osnovni pedološki podaci o sadržaju humusa i reakciji tla (pH) dobiveni su iz listova Osnovne pedološke karte RH, mjerila 1:50.000. Generalno, utvrđen je trend povećanja kiselosti površinskog sloja tla od istoka prema zapadu (od 4,99 do 3,99), te povećanja prosječnog sadržaja humusa (1,83 % do 3,90 %), što je u korelaciji s povećanjem količina oborina odnosno humidnijom klimom. Radi sprječavanja degradacije tih tala, nužno ih je koristiti na održivi način, uvažavajući pri tome klimatske promjene.

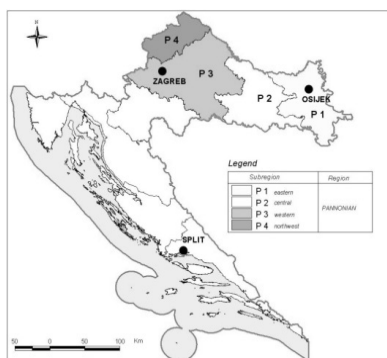
Ključne riječi: sadržaj humusa, reakcija tla, Panonska agroregija, lesivirano tlo

Uvod

Tlo je vrlo kompleksan medij, na koji, posebice u današnje vrijeme, utječu različiti degradacijski procesi, uzrokujući pritom njegovo djelomično ili trajno oštećenje. Oštećenje tala kod nas i u svijetu predstavlja vrlo ozbiljan problem današnjice, budući da je tlo prirodno i dinamičko tijelo koje ima višestruku ulogu u okolišu. Prema Husnjaku i sur., (2011) u posljednje vrijeme svjedoci smo različitim vrstama oštećenja tla koja sve češće nastaju kao posljedica klimatskih promjena (acidifikacija, dehumifikacija, erozija, desertifikacija). Degradacijski procesi s najizraženijim posljedicama u Panonskoj agroregiji Hrvatske su dehumizacija i zakiseljavanje tla (Bašić i Bašić, 2007). Posljedice dehumizacije su pogoršanje strukture tla, vodozračnih odnosa i biološke aktivnosti u tlu, dok zakiseljavanje tla uz navedeno uzrokuje još i smanjenje biopristupačnosti hraniva i povećanje mobilnosti toksičnih elemenata aluminija, željeza i mangana. Osim navedenog, opadanjem sadržaja humusa, smanjuju se i zalihe organskog ugljika u tlu (Constantini i sur., 2013). Navedeni procesi su posebno izraženi na izvorno kiselim tlima, poput lesiviranog tla na lesu, koje je sa 11,75 % najzastupljeniji tip tla u Republici Hrvatskoj. Također, ova tla su znatno više zastupljena u Panonskoj agroregiji, u odnosu na ostale agroregije u Hrvatskoj (Husnjak, 2014). Osim kisele reakcije, lesivirana tla na lesu karakterizira i slaba humoznost, pa se tako ovisno o načinu korištenja sadržaj humusa kreće od 3 % na poljoprivrednim oraničnim površinama do 4-6 % pod prirodnom vegetacijom (Husnjak, 2014). Na promjenu pH i sadržaja humusa u ovim tlima, u najvećoj mjeri utječu različite klimatske prilike u kombinaciji sa specifičnim reljefnim obilježjima, kao i značajkama matičnog supstrata. Brzina mineralizacije organske tvari i količina humusa u tlu usko je povezana sa povećanjem temperature zraka (Kirschbaum, 1995). S druge strane se, s povećanjem količine oborina ubrzava zakiseljavanje, a u aridnim uvjetima, uz povećanu evapotranspiraciju, prisutno je nakupljanje Ca^{+2} iz CaCO_3 (les) te porast pH i do vrijednosti 8,0 (Slessarev i sur., 2016). S tim u svezi ciljevi ovog rada bili su: utvrditi stanje humoznosti i reakcije tla u Panonskoj agroregiji, procijeniti utjecaj klimatskih prilika na utvrđeno stanje, te procijeniti promjene navedenih kemijskih značajki uvažavajući promjene klimatskih značajki.

Materijal i metode

S obzirom na različitost klimatskih značajki u Panonskoj agroregiji, pH tla i humoznost lesiviranih tala na lesu prikazane su za svaku podregiju, slika 1.



Slika 1. Panonska agroregija Hrvatske, s podregijama (Bašić, Bogunović, Husnjak, 2001.)

Panonska agroregija je podijeljena na četiri podregije: P-1 (Istočna), P-2 (Središnja), P-3 (Zapadna) i P-4 (Sjeverozapadna). Osnovni izvor pedoloških podataka (pH i sadržaj humusa) predstavljali su listovi Osnovne pedološke karte RH, mjerila 1:50:000 i tumači tih karata. Iz postojećih karata i tumača izdvojeno je 52 reprezentativna profila lesiviranih tala na lesu, s tim da su podaci o reakciji tla i sadržaju humusa uzeti iz A (humusno-akumulativnog horizonta) i E (eluvijalnog horizonta) tla. Prosječne vrijednosti pH (KCl) i sadržaja humusa (%) u svakoj podregiji statistički su obrađeni, pomoću GLM /LSMEANS procedure statističkog programa SAS STAT 9.4. (SAS Institute Inc.,2002.-2012.), a razlike između navedenih kemijskih parametara analizirane su pri razini signifikantnosti $p < 0,05$. Podaci o srednjim mjesečnim i godišnjim količinama oborina, te srednjim mjesečnim i godišnjim vrijednostima temperature zraka za višegodišnja razdoblja (1959.-1988. i 1989.-2018.), za svaku podregiju dobiveni su od DHMZ-a. Podaci su prikupljeni s meteoroloških postaja Osijek (P-1), Požeга (P-2), Bjelovar (P-3) i Varaždin (P-4), tablice 1. i 2. Navedena razdoblja uzeta su u razmatranje radi usporedbe stanja klimatskih parametara (oborina i temperature zraka) tijekom uzorkovanja tla (1978.-1985.), sa stanjem navedenih parametara u posljednjih 30 godina, s ciljem procjene utjecaja njihove promjene na utvrđeno stanje humoznosti i kiselosti lesiviranih tala na lesu.

Tablica 1. Srednje mjesečne i godišnje količine oborina za razdoblja (1959.-1988. i 1989.-2018.) u Panonskoj agroregiji Hrvatske

Razdoblje	Meteo postaje	Oborine, mm												Suma
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
1959.-1988.	Osijek	51,5	42,2	46,0	54,9	58,5	88,8	65,3	58,3	44,9	41,6	58,8	54,1	664,8
1989.-2018.		43,2	41,8	43,5	50,6	71,1	81,4	63,9	66,1	66,3	59,8	55,8	52,9	694,7
1959.-1988.	Požeга	51,9	45,6	51,8	62,7	68,9	93,2	83,7	76,8	68,8	55,9	72,7	65,7	792,4
1989.-2018.		47,2	45,6	46,7	56,9	72,6	82,1	80,3	64,3	88,6	75,0	63,6	54,4	771,1
1959.-1988.	Bjelovar	53,9	47,0	52,5	63,5	77,6	97,6	81,0	79,1	64,3	55,8	86,6	64,7	823,6
1989.-2018.		44,2	46,6	51,1	54,7	75,8	78,6	67,7	80,9	99,9	72,4	71,9	58,7	802,6
1959.-1988.	Varaždin	46,2	44,9	54,5	70,1	83,3	99,2	95,0	97,7	78,2	68,9	84,4	62,8	885,2
1989.-2018.		38,7	47,9	49,1	60,4	75,9	86,3	86,4	86,3	108,5	81,2	74,9	52,0	847,7

Tablica 2. Srednja mjesečna i godišnja temperatura zraka za razdoblja (1959.-1988. i 1989.-2018.) u Panonskoj agroregiji Hrvatske

Razdoblje	Meteo postaje	Temperatura zraka °C												Sred.
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
1959.-1988.		-1,3	1,2	5,9	11,3	16,4	19,5	21,0	20,3	16,6	11,1	5,5	1,1	10,7
1989.-2018.	Osijek	0,6	2,3	7,0	12,3	17,2	20,6	22,4	21,9	16,7	11,8	6,4	1,4	11,7
1959.-1988.		-1,0	1,8	6,3	10,8	15,6	19,0	20,4	19,5	16,0	10,8	5,7	1,2	10,5
1989.-2018.	Požega	0,8	2,5	7,1	11,8	16,6	20,3	21,9	21,5	16,2	11,5	6,2	1,5	11,6
1959.-1988.		-1,1	1,3	5,8	10,7	15,5	18,8	20,3	19,4	15,7	10,4	5,2	0,8	10,2
1989.-2018.	Bjelovar	0,7	2,7	7,4	12,2	17,0	20,6	22,3	21,7	16,3	11,3	6,3	1,4	11,6
1959.-1988.		-1,3	1,0	5,3	10,3	15,0	18,3	19,8	18,8	15,3	10,1	5,0	0,6	9,9
1989.-2018.	Varaždin	0,6	2,3	6,6	11,5	16,2	19,7	21,3	20,6	15,7	10,9	6,1	1,3	11,1

Rezultati i rasprava

Humoznost tla

S obzirom na prosječni sadržaj humusa, lesivirana tla u Panonskoj agroregiji uglavnom su slabo humozna. Utvrđene su značajne razlike u sadržaju humusa između pojedinih podregija. Najmanja prosječna vrijednost humusa u A horizontu ovih tala utvrđena je u P-2 podregiji (1,83 %), a značajno najveća humoznost u P-3 podregiji (3,90 %). Statistički značajne razlike između P-1, P-2 i P-4 podregije nisu utvrđene, tablica 3. Minimalne vrijednosti sadržaja humusa uglavnom se javljaju u P-2 (0,5 %) i P-1 (1,5 %) podregijama. Nizak sadržaj humusa, u površinskom sloju lesiviranih tala sličnog podneblja (0,7-1,5 %) utvrdila je i Filcheva (2015). Idući prema zapadu, s obzirom na povećanje humidnosti, načina korištenja zemljišta, kao i različit biljni pokrov, sadržaj humusa u tlu se povećava do maksimalnih 5,0 % u P-3 podregiji. Interesantno je navesti da je u odnosu na P-1 podregiju, P-3 podregija u razdoblju 1959.-1988. bila za čak 158,8 mm vlažnija, i za 0,5 °C hladnija. Kao rezultat navedenih čimbenika, mineralizacija organske tvari značajno je manja, što u konačnici rezultira i većim sadržajem humusa u tlu. Navedeno su potvrdili i Koinov i sur., 1998, u lesiviranim tlima na lesu u Bugarskoj, koji su utvrdili također povećanje sadržaja humusa od 1,5 % u aridnijim područjima do 2,0 % u huminijim područjima. Sličan trend povećanja sadržaja humusa u humidnim uvjetima u odnosu na aridne uvjete, u različitim tipovima tala dobili su i Hristov i Filcheva (2017). Stanje humoznosti u Panonskoj agroregiji može se objasniti činjenicom da su u aridnijim uvjetima koje obilježavaju više temperature zraka i manje količine oborina povoljniji uvjeti za mineralizaciju organske tvari. Na isti način, u humidnijim uvjetima koje obilježavaju niže temperature zraka i veće količine oborina povoljniji su uvjeti za humifikaciju organske tvari a nepovoljniji za njezinu mineralizaciju.

Tablica 3. Sadržaj humusa (%) u lesiviranim tlima na lesu Panonske agroregije Hrvatske

Tip tla	Horizonti tla	Podregije Panonske agroregije											
		P-1			P-2			P-3			P-4		
		Humus (%)											
		min	max	X	min.	max.	X	min.	max.	X	min.	max.	X
Lesivirano na lesu	A	1,50	3,40	1,95 b*	0,50	2,33	1,83 b	1,96	5,00	3,90 a	1,60	4,50	2,36 b
	E	0,52	1,73	0,96 b	0,73	1,08	1,33 ab	0,24	2,29	1,28 ab	0,80	2,10	1,74 a

* različita slova uz prosječne vrijednosti u istom retku upućuju na postojanje signifikantnih razlika, temeljem LSD testa uz $p < 0,05$

** min= minimalne vrijednosti; max= maksimalne vrijednosti; X = prosječne vrijednosti

Kiselost tla

S obzirom na prosječnu vrijednost pH, lesivirana tla Panonske agroregije uglavnom su kisela, osim u P-3 podregiji, gdje su jako kisela. Prosječna pH vrijednost (u KCl) A horizonta lesiviranih tala P-2 podregije (4,99), značajno je bila viša u odnosu na ostale podregije dok je najmanja prosječna vrijednost pH utvrđena je u P-3 podregiji (3,99), tablica 4. Vidljivo je da je na prijelazu iz semiaridnih (P-2) u humidnije klimatske uvjete (P-3) ta razlika u vrijednosti pH tla sve izraženija. To je posljedica znatnijeg ispiranja baznih kationa sa adsorpcijskog kompleksa (zakiseljavanja tla) u uvjetima većih količina oborina (885,2 mm godišnje u P-4) u odnosu na krajnji istok Hrvatske (664,8 mm P-1 podregiji). Stanje kiselosti tla u Panonskoj agroregiji također se može s općeg aspekta objasniti činjenicom da su u aridnijim uvjetima koje obilježavaju manje količine oborina nepovoljniji uvjeti za ispiranje tvari, u odnosu na humidnije uvjete.

Tablica 4. Reakcija tla (pH) u lesiviranim tlima na lesu Panonske agroregije Hrvatske

Tip tla	Horizonti tla	Podregije Panonske agroregije											
		P-1			P-2			P-3			P-4		
		pH (KCl)											
		min.	max.	X	min.	max.	X	min.	max.	X	min.	max.	X
Lesivirano na lesu	A	3,80	5,84	4,80 a*	4,32	5,80	4,99 a	3,40	4,70	3,99 b	3,10	5,50	4,55 a
	E	4,16	6,90	5,07 a	3,70	4,66	4,38 b	3,50	4,40	3,85 b	3,70	5,00	4,21 b

* različita slova uz prosječne vrijednosti u istom retku upućuju na postojanje signifikantnih razlika, temeljem LSD testa uz $p < 0,05$

Procjena utjecaja promjena značajki klime na stanje humoznosti i kiselosti tla

Usporedbom podataka o značajkama klime iz razdoblja 1959.-1988. sa razdobljem 1989.-2018., utvrđen je opći trend smanjenja količina oborina i porasta temperature zraka. Najveće smanjenje prosječnih godišnjih količina oborina od 4,2 % zabilježeno je u P-4 podregiji, dok je porast srednje godišnje temperature zraka bio najizraženiji u P-3 podregiji i iznosio je 1,4 °C. Procjenjujemo da su navedene promjene mogle utjecati na povećani intenzitet mineralizacije organske tvari i na smanjenje intenziteta zakiseljavanja u odnosu na ranije razdoblje. Također procjenjujemo da će se takav trend nastaviti i u budućnosti. Navedeno je nužno provjeriti temeljem podataka o današnjih stanju humoznosti i kiselosti tla.

Zaključak

Lesivirana tla na lesu u Panonskoj agroregiji slabo su humozna i uglavnom kisela. Prosječni sadržaj humusa u A horizontu najmanji je u P-2 podregiji (1,83%), a najveći u P-3 podregiji (3,90%), dok je pH najniži u P-3 podregiji (3,99) a najviši u P-2 podregiji (4,99). Idući od istoka prema zapadu, utvrđen je trend porasta sadržaja humusa, te povećanja kiselosti tla. Tijekom razdoblja 1989-2018 utvrđen je trend smanjenja količina oborina i porasta temperature zraka (od istoka prema zapadu), pa se procjenjuje da će takve promjene u budućnosti dovesti do povećanog intenziteta humifikacije organske tvari i smanjenja zakiseljavanja tla. Pri tome je novim istraživanjima nužno utvrditi današnje stanje humoznosti i kiselosti tla, u cilju provjere stvarnog utjecaja navedenih promjena klimatskih prilika na ove značajke tla. Radi sprječavanja daljnje degradacije ovih tala, nužno ih je koristiti na održivi način uvažavajući pri tome klimatske promjene.

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Humus content and acidity of Luvisols on loess in Pannonian agroregion of Croatia

Abstract

The aim of this study was to determine the humus content and acidity of Luvisol on loess of the Pannonian agroregion of Croatia and to assess the effect of climate changes on these soil properties. Basic pedological data on humus content and soil reaction (pH) were obtained from sheets of the Basic Pedological Map of the Republic of Croatia, scale 1: 50,000. In general, there is a trend of increasing acidity of the surface layer of soil from east to west (from 4.99 to 3.99), and an increase in average humus content (1.83% to 3.90%), which is correlated with an increase in rainfall. In order to prevent the degradation of these soils, it is necessary to use them in a sustainable way, while respecting climate change.

Keywords: humus content, soil reaction, Pannonian agroregion, luvisol

Seasonal phenology dynamics of alfalfa consociation with Italian ryegrass based on NDVI from Sentinel-2

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Abstract

Understanding of the specificities of phenological dynamics is essential for the reliable estimation and classification of crops by satellite imagery. This study analysed the seasonal dynamics of the normalized differential vegetation index (NDVI) of alfalfa and Italian ryegrass from the Maksimir test field at University of Zagreb Faculty of Agriculture, and explained its dynamics through utilisation and weather conditions during 2018 and 2019. Temporally and spatially, the NDVI values ranged from 0.28 to 0.82. The results showed the specificities of vegetation index dynamics caused by mowing and regeneration, with the highest values recorded in April and very strong positive correlation of NDVI to dry matter of aboveground biomass $y = 13.2 * x - 9.2$ ($r = 0.951$, $p < 0.05$) suggesting the possibility of estimating the yield of alfalfa consociation with the Italian ryegrass using Sentinel 2 imagery. By connecting meteorological data and phenological dynamics of NDVI, the extent to which thermal stress can influence NDVI values and dynamics of primary production respectively is shown.

Keywords: NDVI, Sentinel-2, monitoring, phenological dynamics, satellite imagery

Introduction

Insight into the specificities of the phenological dynamics of agricultural crops is important for their reliable estimation and classification by remote sensing techniques using satellites and drones. Dynamics is driven by environmental factors and human influence, biological traits of crops and crop rotation which again influence biophysical processes (Fengshan et al., 2017). Understanding of phenological dynamics is crucial for the reliable assessment, classification, mapping and modelling of crops using remote sensing methods, and the greater availability of satellite image series such as MODIS has intensified research and comparative analyses of phenological curves of major crops such as maize, wheat, soybeans, cereals, alfalfa, etc. (Reeed et al., 1994; Xin et al., 2002; Kathuroju et al., 2007; Masialeti et al., 2010; Atzberger, 2013; Wei et al., 2019). In order to advance the Common Agricultural Policy (CAP), the European Commission adopted new rules in 2018 that will, for the first time, explicitly allow the use of a range of modern arable crop checking technologies for the calculation of direct payments in agriculture, including the possibility to completely replace physical checks on the farm with an automatic verification system based on the analysis of data obtained from satellite systems (EC, 2018). One of the main prerequisites for this purpose was achieved through Copernicus program of the European Commission and the European Space Agency, which established the constellations of the Sentinel satellite fleet to observe Earth from space. Recordings and data are free and publicly available. The Sentinel-2 satellite system, with its specifications, such as high spatial (wavelength dependent, 10 - 60 m/pixel), high temporal resolution (5-day revisit) and 13 spectral channels in the visible, near-infrared and short-wave infrared region of the electromagnetic spectrum, is suitable and meets the requirements of agricultural monitoring. So far, little has been done in Croatia regarding remote sensing in agriculture (Ljubičić et al., 2008; Kutnjak et al., 2015; Kutnjak and Karanović, 2019). Although knowledge and techniques of interpreting satellite imagery exist, there is room for progress in crop monitoring through understanding the local specificities of climate-vegetation zones (Becker-Reshef et al., 2010). Several studies have used satellite remote sensing images and ground-based sensors as non destructive tools for yield prediction of alfalfa and grass (Schut et al., 2002, Kayad et

al., 2016, Noland et al., 2018).

The aim of this paper is to analyse the seasonal dynamics of the normalized difference vegetation index (NDVI) of alfalfa and Italian ryegrass from the test field of the Maksimir Agronomy Faculty in Zagreb and to explain through agricultural utilization and weather during 2018 and 2019.

The hypothesis of this work is the existence of a trans-seasonally strong correlation of NDVI values and above-ground biomass of alfalfa consociation with Italian ryegrass.

Materials and methods

In this study the seasonal dynamics of normalized difference vegetation index (NDVI) of alfalfa and Italian ryegrass from the Maksimir test field of the Faculty of Agriculture, University of Zagreb, with nett productive area of 8.8 ha (N 45° 49'55", E 16° 01'52") were analyzed. At the end of September 2017 the Italian ryegrass (*Lolium multiflorum* Lam.) was directly sown in alfalfa (*Medicago sativa* L.) crop and in 2018 the crop mixture was mowed three times and in 2019 four times (16 May 2018, 9 July 2018, 19 September 2018, 22 May 2019, 2 July 2019, 12 August 2019, 17 September 2019). The mowing period was presumably determined according to the optimal phenological stage for mowing, that is, before the full flowering of alfalfa. The mowed plant material was dried baled as haylage except second cutting in 2018 which was harvested and stored as hay. The dry matter content of haylage bales was determined on a one-off basis in May 2018 when subsamples of vegetable mass were collected from an area of 1 m² at different locations on the field, dried in a drying room, and recalculated to the total surface of the field and divided by the number of bales in that swath (98) to calculate the dry matter content (DM) of 144 kg per bale. A total of 350 haylage bales were collected over the two years and one harvest of hay. Meteorological data on precipitation and temperatures have been collected from the website of the Croatian Meteorological and Hydrological Service (DHMZ, 2019).

Sentinel-2 satellite imagery, consisting of a constellation of two satellites in a polar orbit, that provides observation of the Earth's surface with an equatorial temporal resolution of 5 days, a spatial resolution of 10 m, and a spectral resolution of 4 frequency bands within 448 nm and 912 nm of which red and closely infrared were used for NDVI.

The recordings were accessed through the Google Earth Engine (GEE) online platform (Gorelick et al. 2017), which included the Normalized Difference Vegetation Index (NDVI) for which the red and infrared frequency bands were used (Kriegler et al., 1969, Rouse et al., 1973). The time frame within which the recordings were collected and processed covered 2018 and 2019. Descriptive statistics were calculated for the values obtained, and the results are presented in tables and graphs.

Results and discussion

Between the beginning of 2018 and November 2019, 106 suitable images that were not covered by clouds were included in the analysis. The highest maximums of NDVI values in the calendar years were recorded shortly before the first harvest in May and decreased towards the end of the season together with the yield. The date when the last winter minimum of NDVI was recorded was taken as the start of the growing season. Estimation of the dynamics based on the approximate dry matter (DM) yield per day shows that it is the highest in the beginning and mid-year when it begins to fall and before the end of the season is only 19% (2018) and 11% (2019) of the initial daily increase. The same trend within the growing season was obtained in the correlation of the NDVI and the ordinal day of the year when the field was mowed. A very strong positive correlation was observed between average NDVI (x) and total dry matter mass $f(x)$ in tonnes [t] on the test field through a linear model $y = 13.2 \cdot x - 9.2$ ($r = 0.951$, $p < 0.05$).

Table 1. Dynamics of mowing, yield and maximum NDVI values. * - number of days of growth calculated from the last winter NDVI minimum, ** - not applicable because hay was harvested instead of haylage, Post. min. NDVI shows the post-harvest minimum of NDVI values. dNDVI represents the difference between the maximum and minimum values.

Date	No. bales	Aprox. DM [kg ha ⁻¹]	Days of growth	Aprox DM growth [kg ha ⁻¹ dan ⁻¹]	Max. NDVI	Post. Min. NDVI	dNDVI
16.05.2018.	98	1604	68*	23.6	0.802	0.589	0.213
09.07.2018.	**	**	54	**	0.714	0.331	0.383
19.09.2018.	20	327	72	4.5	0.702	0.289	0.413
22.05.2019.	130	2127	76*	28.0	0.820	0.281	0.539
02.07.2019.	67	1096	41	26.7	0.769	0.306	0.463
12.08.2019.	28	458	41	11.2	0.754	0.381	0.373
17.09.2019.	7	115	36	3.2	0.698	0.446	0.252

Observing the whole phenological curve, it is evident that it reaches its maximum in April and the drop in values accompanies each harvest by an average of 0.4 (Graph 1). The decline in NDVI was in all cases below the absolute value of 0.4 except after the first harvest of 2018 which is likely to be due to the low ground layer of the Italian ryegrass sown in the second half of 2017 and after the last drop of 2019 when again due to the weeds in the ground layer this value was not lower than 0.45 (Table 1).

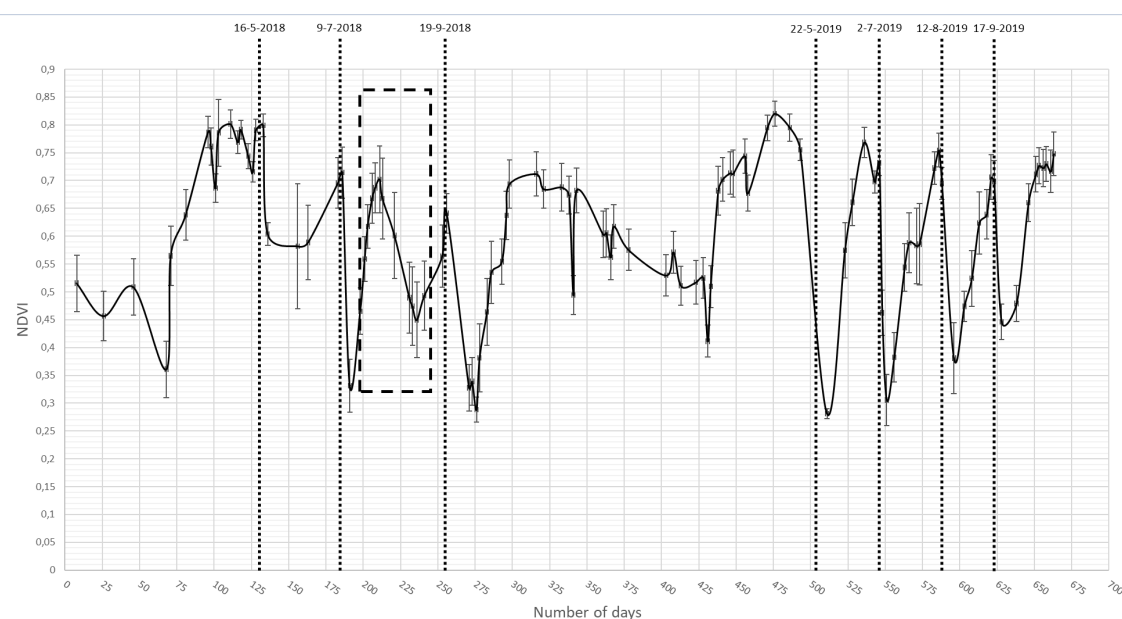


Figure 1. Phenological curve with dynamics of NDVI values during 2018 and 2019.

Vertical range lines represent the standard deviation of NDVI values. Vertical short dashed lines indicate mowing day, and a rectangle with dashed line indicates a period of extremely high average daily temperatures in the period 30 July- 25 August 2018.

According to data from DHMZ, the whole of 2018 was extremely warm with normal rainfall, while in 2019 it was extremely warm in summer and was dry considering rainfall. The manifestation of the weather is illustrated by a phenological curve that shows an interesting detail in the summer of 2018, which is a 25-day decline in the NDVI value from 0.70 to 0.45. During this period an unusually long heat stroke occurred, characterized by high average daily values above 25 °C. Rain amount of 30 mm measured on 26 August 2018 and a drop in average daily temperature below 20 °C resulted in vegetation recovery and its regrowth. With this stress, the cycle between mowing is extended by almost a month (Figure 1).

Conclusions

Monitoring the phenological dynamics of NDVI alfalfa and Italian ryegrass through the analysis of time series of Sentinel 2 satellite images showed the specificities of vegetation dynamics caused by mowing and regrowth, with the highest values recorded in April. The very strong positive correlation of NDVI with respect to the dry matter of aboveground biomass $y = 13.2 * x - 9.2$ ($r = 0.951$, $p < 0.05$) through the season suggests the possibility of estimating the yield of alfalfa consociation with Italian ryegrass by remote monitoring methods and confirms the hypothesis. By connecting meteorological data and phenological dynamics of NDVI, the extent to which thermal stress can influence NDVI values and dynamics of primary production respectively is shown.

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Sezonska fenološka dinamika smjese lucerne i talijanskog ljulja bazirana na NDVI-u sa Sentinela-2

Sažetak

Poznavanje specifičnosti fenološke dinamike je bitno za pouzdanu procjenu i klasifikaciju poljoprivrednih kultura pomoću satelitskih snimaka. U ovom istraživanju je analizirana sezonska dinamika normaliziranog diferencijalnog vegetacijskog indeksa (NDVI) lucerne i talijanskog ljulja sa proizvodne table pokušališta Maksimir Agronomskog fakulteta u Zagrebu te objašnjena kroz način korištenja i vremenske prilike tijekom 2018. i 2019. godine. NDVI se kretao od 0,28 do 0,82. Rezultati su pokazali specifičnosti dinamike vegetacijskog indeksa uvjetovane košnjom i ponovnom regeneracijom pri čemu su najviše vrijednosti zabilježene u travnju i vrlo jaku pozitivnu korelaciju NDVI-a sa suhom tvari nadzemne biomase $y=13,2 \cdot x-9,2$ ($r=0,951$, $p<0,05$) što upućuje na mogućnost procjene prinosa konsocijacije lucerne s talijanskim ljuljem korištenjem snimaka Sentinela 2. Povezivanjem meteoroloških podataka i fenološke dinamike NDVI-a pokazano je u kojoj mjeri toplinski stres može utjecati na vrijednosti NDVI-a.

Ključne riječi: NDVI, Sentinel-2, praćenje, fenološka dinamika, satelitske snimke

Macroelement content of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.) in organic fertilization

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Abstract

The aim of the study was to determine the effect of organic fertilization on the content of macroelements in the leaf and flower of true lavender and lavender. The research was conducted in 2019. The experiment included 2 factors in 4 repetitions. Sampling of soil, leaf and flower samples of true lavender and lavender was performed at the beginning of flowering. A relative higher concentration of K (2,96 %), Ca (0,76 %) and Mg (0,26 %) was recorded in the true lavender leaf compared to the concentration of the same elements in the lavender leaf. Relative higher concentrations of K (2,43 %), P (2,38 %), Ca (1,45 %) and Mg (0,36 %) were detected in lavender flower. A significant positive correlation ($r = 0.969$) $p < 0.05$ was found between plant available K_2O in soil and K in true lavender leaf and a strong negative correlation ($r = -0.503$) was found between Ca in soil and Ca in leaf. A significant positive correlation for lavender was found between plant available P_2O_5 in soil and P concentration in flower ($r = 0.937$) and a significant positive correlation between Ca in soil and Ca concentration in leaf ($r = 0.931$).

Keywords: lavender, nitrogen, calcium, magnesium

Introduction

The genus *Lavandula* is represented by more than 20 species that differ in morphological and chemical characteristics. Commonly grown species are true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.). Lavender has a greater potential for yield of lavender essential oil, however, due to its higher content of camphor, it is more often used to preserve products and less in the cosmetic industry and for therapeutic purposes (Kara and Baydar, 2013; Andrys and Kulpa, 2016). Carovic-Stanko et al. (2016) highlight the wide range of useful functions of plants from family of the *Lamiaceae* and the possibility of using them as functional foods in the healthy diet of consumers.

Generally, application of macronutrient increases yield, growth, and quality of crops (Tripathi et al. 2014). Kucukyumuk et al. (2015) found that amount of nitrogen applied in fertilization affects plant height, length of flowers and P and Ca concentrations in lavender leaf. Biesiada et al. (2008) found that the level of nitrogen supply affects the concentration of phenolic compounds and carotenoids in true lavender flower. Chrysargyris et al. (2017) found that the concentration of K in nutrient solution of hydroponic production of true lavender affects the growth of plants, root development and the quantity and quality of oil. As cited by Adaszynska et al. (2011), cit. Prusinowska and Smigielski (2014) the content of biogenic macroelements depends on the lavender variety. Higher yield and quality of aromatic and medicinal plants were achieved by fertilization with organic and biological fertilizers (Sartip et al., 2015). Due to the slow release of nutrients, organic fertilizers have a positive effect on the quality and yield of essential oil compared to mineral fertilizers (Silva et al., 2017). Research to date indicates widespread use of true lavender and lavender. Emphasis is placed on the yield and quality of essential oils. However, the mineral composition of the leaf and

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flower, which depends on the lavender variety, but also on the soil's supply of biogenic elements, is less explored. Therefore, the aim of the study was to determine the effect of organic fertilization on the content of macroelements in the leaf and flower of true lavender and lavender.

Material and methods

The research was conducted in 2019 at the experimental field of the Križevci College of Agriculture. A soil analysis was performed before the experiment was set up. Neutral soil reaction was found, pH in 1M KCl was 6.94, good humus supply (4 %), rich plant available phosphorus (25.55 mg P₂O₅/100g soil) and potassium (27.04 mg K₂O/100 g soil). Proeco 5-10-10 pelleted organic mineral fertilizer was applied with primary tillage in the fall. The amount of 20 kg ha⁻¹ of nitrogen, 40 kg ha⁻¹ of phosphorus and 40 kg ha⁻¹ of potassium was applied. In the spring of 2019, the soil was prepared for planting and fertilized with Fertil Supernova 12.5 N organic nitrogen fertilizer. This fertilizer supplied soil with 50 kg ha⁻¹ of organic nitrogen. A two-factor experiment was set up on the plot of 306 m² (true lavender and lavender) in 4 repetitions. The size of the test plot is 34 m² (10 x 3.4 m). Planting of true lavender and lavender was done in April 2019. On each experimental plot 10 plants were planted in two rows. Soil, leaf and flower sampling from each experimental plot was conducted in July 2019. Soil samples were taken from 30 cm depth. Preparation of soil samples was carried out in accordance with the standard HRN ISO 11464. Total nitrogen analysis was performed by the modified Kjeldahl method, HRN ISO 11261: 2004, the content of plants available phosphorus and potassium (AL method, Egner et al., 1960) and calcium and magnesium according to ISO 11466, 1995. Plant samples were dried and ground for further analysis. After digestion with a mixture of acids (1/3 HNO₃ + 2/3 HCl) in a microwave device, phosphorus was determined spectrophotometrically, potassium on a flame photometer and calcium and magnesium on an atomic absorption spectrometer (AAS). Data were analyzed by analysis of variance in Statistica 13.4.0.14 program. Mean values of parameters between treatment (true lavender/lavender) were tested using t-test. Partial correlations were computed between the content of macronutrients in the soil and the leaf and flower of true lavender and lavender.

Results and discussion

The results of soil supply to biogenic macronutrients are shown in Table 1, and the effect of organic fertilization on the content of macronutrients in the leaf and flower of lavender and lavender is shown in Table 2.

Table 1. Content of macroelements in soil up to 30 cm of depth at the beginning of flowering of true lavender and lavender

Variants	Total N %	Available form P ₂ O ₅ mg/100 g of soil	Available form K ₂ O mg/100 g of soil	Ca %	Mg %
True lavender	0.23	27.29	29.45	1.25	0.68
Lavender	0.20	25.95	27.21	1.36	0.71

At the beginning of flowering, a rich soil supply with total N, plant available P₂O₅ and plant available K₂O was determined and average amounts of Ca and Mg were recorded in soil on true lavender and lavender variants. As expected, the observed differences in the content of the macroelements in soil between the variants were not statistically justified ($p > 0.05$).

A higher concentration of K, Ca and Mg was found in the true lavender leaf compared to the concentration of the same elements in the lavender leaf. (Table 2). However, these differences were not statistically justified. Regarding to all five investigated nutrients true lavender and lavender leaves contained the most of potassium (2.96 % and 2.59 %, respectively). In the leaves of aromatic and medicinal plants, Imelouane et al. (2011) found a higher concentration of potassium than other biogenic macronutrients. Relative higher concentration of K in the true lavender leaf compared to the concentration in the lavender leaf was probably influenced by the higher available K₂O content in the soil of the true lavender variant. Chrysargyris et al. (2017) found out a significant effect of potassium concentration in the nutrient solution on the K concentration in the true lavender leaf ($p = 0.0014$) by regression analysis and a significant positive correlation between the K concentration in the nutrient solution and the concentration in the true lavender leaf.

Table 2. Influence of organic fertilization on the content of biogenic macronutrients in the leaf and flower of true lavender and lavender

Variants		N (%)	P (%)	K (%)	Ca (%)	Mg (%)
True lavender	Leaf	2.48	0.24	2.96	0.76	0.26
	Flower	2.07	2.12	2.04	0.82	0.32
Lavender	Leaf	2.56	0.25	2.59	0.71	0.18
	Flower	1.75	2.38	2.43	1.45	0.36

The highest concentration of K was also detected in lavender flower compared to other biogenic elements. Grzeszczuk et al. (2018.) reported higher K concentration in flowers of selected ornamental plant species compared to other macroelements. Also, relative higher concentration of P was found in lavender flower than in true lavender flower. Seidler-Lozykowska et al. (2014) reported a significant influence of year and location on P concentration in flower. Ca content in lavender flower was relative higher than in true lavender flower. Also, Felicia et al. (2018) found out higher calcium concentrations in lavender flower. The concentrations of P, Ca and K in lavender leaf presented in table 2 are approximately similar to those reported by Erbas et al. (2017) for fertilization with 50 kg ha⁻¹ of phosphorus. The authors found that lavender fertilization with higher amounts of phosphorus increased concentrations of P, N, K, Ca, and Fe, and significantly reduced the concentrations of Mg, Mn, and Zn in the lavender leaf. Tables 3 and 4 show the correlations between the studied macroelements in the soil and the leaf and flower of true lavender and lavender.

Table 3. Pearson correlation coefficients between macroelements in soil and leaf and flower of true lavender

Soil	N		P		K		Ca		Mg	
	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower
N, %	0.891	0.209								
P ₂ O ₅ , mg/100g			0.209	0.491						
K ₂ O, mg/100g					0.969*	0.698				
Ca, %							-0.503	0.234		
Mg, %									0.410	0.883

* indicates significant correlation $p < 0,05$

A positive correlation was found between the investigated macroelements in soil and the concentration in true lavender leaf and flower except between Ca in soil and Ca in leaf. A significant positive correlation ($r = 0.969$) $p < 0.05$ was found between available K₂O in soil and K in lavender leaf and strong negative correlation ($r = -0.503$) was found between Ca in soil and Ca in leaf. In lavender, mainly positive correlations were recorded between the macroelements in the soil and leaf and flower (Table 4). A negative correlation was found between Ca in soil and Ca in flower and Mg in soil and Mg in flower. This may be due to the limited movement of calcium by phloem and distribution within the plant and the accumulation in the vacuole of older cells in the form of oxalate or other difficultly soluble compounds (Kirkby and Pilbea, 2006) or maybe antagonistic effect of K on Ca. Logically, magnesium is more concentrated in leaves. Similar results were reported by Tuma et al. (2004). A significant positive correlation was found between plant available P₂O₅ in soil and P concentration in flower ($r = 0.937$) and Ca in soil and Ca concentration in leaf ($r = 0.931$). Similar correlations between plant available P₂O₅ in soil and P concentration in flower of medicinal plants have been reported by Konieczynski and Wesolowski (2007).

Macroelement content of true lavender (*Lavandula angustifolia* Mill.) and lavender (*Lavandula × intermedia* Emeric ex Loisel.) in organic fertilization

Table 4. Pearson correlation coefficients between macroelements in soil and leaf and flower of lavender

Soil	N		P		K		Ca		Mg	
	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower
N, %	0.587	0.562								
P ₂ O ₅ ,mg/100g			0.830	0.937*						
K ₂ O,mg/100g					0.119	0.537				
Ca, %							0.931*	-0.441		
Mg, %									0.446	-0.206

* indicates significant correlation $p < 0,05$

Conclusion

The study revealed that one year of organic fertilization did not statistically influenced on macroelements status in the leaf and flower of true lavender and lavender. Significant correlations between soil macroelements and their concentrations in true lavender and lavender leaf and flower were partly result of plant species and fertilization, but also some other factors. As one year of research is not sufficient for the relevant conclusion, it is recommended to continue the research.

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Sadržaj makrolemenata u lavandi (*Lavandula angustifolia* Mill.) i lavandinu (*Lavandula × intermedia* Emeric ex Loisel.) pri organskoj gnojidbi

Sažetak

Cilj istraživanja bio je utvrditi utjecaj organske gnojidbe na sadržaj makroelemenata u listu i cvijetu lavande i lavandina. Istraživanje je provedeno 2019. godine. Pokus je uključivao dva faktora (prava lavanda – lavandin) u četiri ponavljanja. Uzorkovanje tla, lista i cvijeta lavande i lavandina izvršeno je na početku cvatnje. U listu lavande utvrđena je relativno veća koncentracija K (2,96 %), Ca (0,76 %) i Mg (0,26 %) u usporedbi s koncentracijom istih elemenata u listu lavandina. U cvijetu lavandina utvrđena je relativno veća koncentracija K (2,43%), P (2,38%), Ca (1,45 %) i Mg (0,36 %). Značajna pozitivna korelacija ($r = 0,969$) $p < 0,05$ utvrđena je između biljkama pristupačnog K_2O u tlu i K u listu prave lavande, a negativna korelacija ($r = -0,503$) između Ca u tlu i Ca u listu. Značajna pozitivna korelacija kod lavandina utvrđena je između biljkama pristupačnog P_2O_5 u tlu i koncentracije P u cvijetu ($r=0,937$) te značajna pozitivna korelacija između Ca u tlu i koncentracije Ca u listu ($r=0,931$).

Ključne riječi: lavanda, dušik, kalcij, magnezij

Mogućnost pojednostavljenja postupka određivanja gustoće čvrstih čestica tla piknometarskom metodom

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Sažetak

Gustoća čvrstih čestica tla (ρ_c) predstavlja omjer mase i neporoznog volumena apsolutno suhog tla. Ona je jedan od parametara potrebnih za određivanje ukupne poroznosti tla. Standardna piknometarska metoda određivanja ρ_c dugotrajna je i zahtjevna, pa je cilj rada bio, u 20 praškasto ilovastih i slabo humoznih uzoraka tla, ispitati da li pojednostavljenje laboratorijskog postupka utječe na rezultat analize. Razlika između prosječnih vrijednosti ρ_c utvrđenih primjenom standardne ($2,67 \text{ g cm}^{-3}$) i pojednostavljene ($2,64 \text{ g cm}^{-3}$) metode statistički je signifikantna ($p = 0,000003$), ali praktički mala. Stoga se modificirana metoda može koristiti ako je vrijednost ρ_c potrebna prvenstveno za izračun ukupne poroznosti tla.

Ključne riječi: gustoća čvrstih čestica tla, piknometarska metoda, temperiranje, praškasto ilovasta tla, slabo humozna tla

Uvod

Za određivanje ukupne poroznosti tla (P) jedan od ključnih parametara je gustoća čvrstih čestica (ρ_c) koja predstavlja omjer mase apsolutno suhog tla (osušenog na $105 \text{ }^\circ\text{C}$) i neporoznog volumena koje to tlo ima. Vrijednosti ρ_c ovise o sastavu minerala i sadržaju organske tvari u tlu. Gustoća minerala koji se obično nalaze u tlima (silikati i alumosilikati) kreće se od $2,60$ do $2,75 \text{ g cm}^{-3}$. Međutim, ako su u tlu prisutne veće količine oksida željeza i minerala kao što su magnetit i cirkon, gustoća čestica tla može biti veća od $2,75 \text{ g cm}^{-3}$ (Ball i sur. 2006.). Gustoća čestica organske tvari daleko je manja od gustoće mineralnih čestica, a kreće se od $1,3$ do $1,5 \text{ g cm}^{-3}$. S obzirom na navedeno, relativno male količine organske tvari mogu imati značajan utjecaj na ρ_c . Prema Schjonningu i sur. (2017.) vrijednosti ρ_c smanjuju se povećanjem sadržaja organske tvari u tlu, dok se povećanjem sadržaja gline gustoća povećava.

U nedostatku izmjerenih vrijednosti ρ_c pri izračunu P i volumetrijskih odnosa vode i zraka u tlu, nerijetko se može koristiti konstantna vrijednost ρ_c od $2,65 \text{ g cm}^{-3}$ (McBride i sur. 2015.) Ipak, Redding i sur. (2006.) navode kako se vrijednost od $2,65 \text{ g cm}^{-3}$ može primijeniti samo za tla s niskom koncentracijom organskog ugljika i visokom koncentracijom frakcija kvarcnog pijeska. To potvrđuju i istraživanja koja su proveli Bielders i sur. (1990.) te Blanco-Canqui i sur. (2006.). Pojedini znanstvenici navode da se vrijednost ρ_c znatno razlikuje kroz tipove tala i ukazuju na potrebu njenog laboratorijskog određivanja (Bielders i sur. 1990., Ball i sur. 2000., Rühlmann i sur. 2006.).

Postoje različite laboratorijske metode za određivanje ρ_c tla. Tako, osim standardne piknometarske metode (SM) i metode volumetrijske tikvice, postoje i metode plinske piknometrije (Bielders i sur. 1990.), te zamjene volumena vlažnog i suhog uzorka (Yuying i sur. 2014.). Zajedničko svim navedenim metodama je da podrazumijevaju dugotrajne analize koje iziskuju dosta rada. Cilj ovog istraživanja bio je, stoga, ispitati postoji li mogućnost pojednostavljenja određivanja ρ_c i to modificiranjem SM na način da se iz postupka isključi kuhanje/temperiranje uzoraka tla.

Materijali i metode

Za potrebe analiza korišteno je 20 uzoraka tla iz arhive Zavoda za pedologiju Agronomskog fakulteta u Zagrebu. Uzorci tla bili su praškasto ilovaste (PrI) teksture, a tekstura tla određena je prema FAO teksturnom trokutu (FAO, 2006). Svi analizirani uzorci bili su slabo humozni sa 1-3 % humusa. Prije analize uzorci su osušeni u sušioniku na

temperaturi od 105 °C, usitnjeni i prosijani kroz sito promjera 1 mm. Analiza gustoće svakog uzorka odrađena je korištenjem SM (modificirana ISO 11508:1998 i JDPZ, 1971.) i pojednostavljene metode (PM) koja je isključivala predtretman kuhanja/temperiranja uzorka. SM podrazumijeva vaganje 10 g tla te prelijevanje sa 30 ml destilirane vode. Tako pripremljen uzorak kuha se uz povremeno miješanje. Kuhanje se obavlja s ciljem eliminacije zraka iz uzorka. Istovremeno su piknometri od 100 mL napunjeni destiliranom vodom i temperirani na 20 °C. Piknometri su začepljeni zadržavajući vodu, obrisani te izvagani pri temperaturi od 20 °C (). U ispražnjene piknometre kvantitativno je prenesena ohlađena suspenzija tla svakog uzorka te je do vrha dodana destilirana voda. Piknometri sa suspenzijom također su temperirani na 20 °C, obrisani izvana te je izvagana masa svakog piknometra s tlom (). računa se prema formuli:

$$\rho_{\check{c}} = \frac{\rho_v T_t}{T_t + T_{pv} - T_{pt}},$$

gdje su: masa tla (10 g),

gustoća vode pri 20 °C (1 g cm⁻³),

masa piknometra s vodom (g),

masa piknometra s tlom (g).

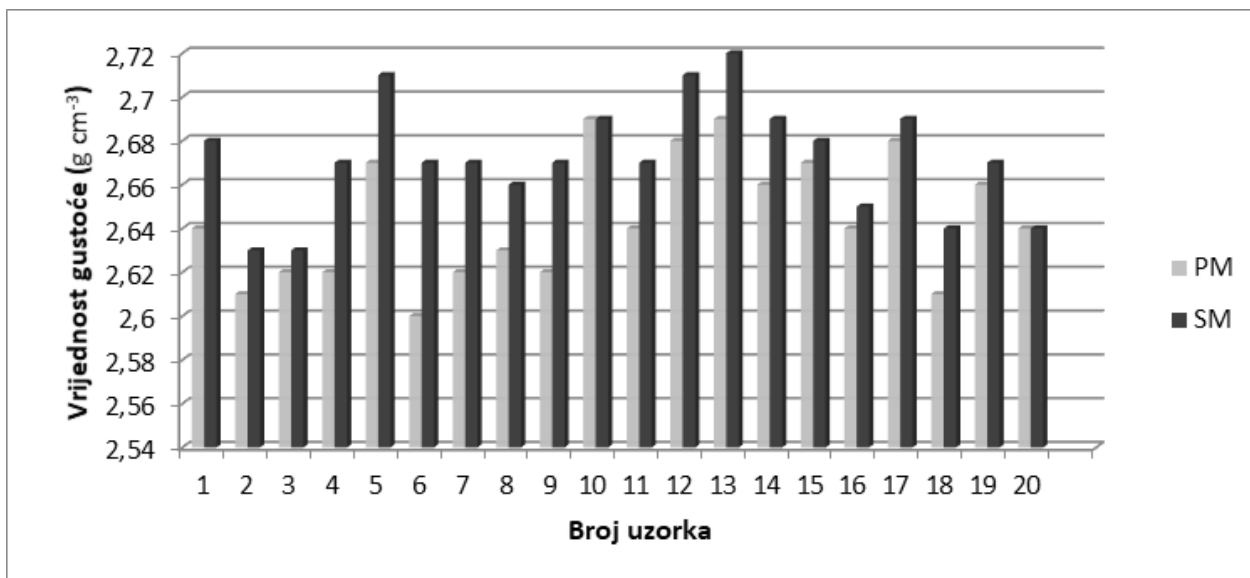
Statistička obrada podataka odrađena je t-testom za zavisne uzorke u statističkom programu SAS System for Win ver. 9.1.3 (Copyright 2002-2003 by SAS Institute Inc., Cary, NC, USA.).

Rezultati i rasprava

Podaci dobiveni deskriptivnom statističkom analizom prikazani su u Tablici 1. Vidljivo je da je prosječna $\rho_{\check{c}}$ nakon primjene PM iznosila 2,64 g cm⁻³, dok je nakon primjene SM ona iznosila 2,67 g cm⁻³. Razlika između prosječnih vrijednosti $\rho_{\check{c}}$ bila je statistički signifikantna (p= 0,000003), ali realno vrlo mala. Iz Grafikona 1, vidljivo je da je najveća razlika između $\rho_{\check{c}}$, obzirom na korišteni analitički postupak, zabilježena kod uzorka 6. S druge strane, kod uzoraka 10 i 20, iznosi $\rho_{\check{c}}$ dobiveni primjenom SM i PM bili su jednaki.

Tablica 1. Deskriptivna statistička analiza za pojednostavljeni (PM) i standardni postupak (SM) određivanja gustoće čvrstih čestica tla

Statistički parametar	Pojednostavljeni postupak (PM)	Standardni postupak (SM)
Broj uzoraka (N)	20	20
Aritmetička sredina (g cm ⁻³)	2,64	2,67
Medijan(g cm ⁻³)	2,64	2,67
Mod (g cm ⁻³)	2,64	2,67
Standardna devijacija (g cm ⁻³)	0,028	0,026
Najmanja vrijednost (MIN) (g cm ⁻³)	2,60	2,63
Najveća vrijednost (MAX) (g cm ⁻³)	2,69	2,72



Grafikon 1. Vrijednosti gustoće čvrstih čestica tla dobivene primjenom pojednostavljenog (PM) i standardnog (SM) postupka određivanja

Za oba seta podataka (PM i SM), dobivene prosječne vrijednosti ρ_c ne izlaze iz raspona ρ_c definiranog prema Hillelu (1998.), koji se kreće između $2,60 \text{ g cm}^{-3}$ i $2,70 \text{ g cm}^{-3}$, odnosno ne odstupaju u velikoj mjeri od konstantne vrijednosti $2,65 \text{ g cm}^{-3}$ pretpostavljene za većinu mineralnih tala (McBride i sur., 2015.). Navedeno je u skladu s činjenicom da su analizirani uzorci bili slabo humozni, praškasto-ilovaste teksture. Brogowski i sur. (2014.) su, nakon izdvajanja granulometrijskih frakcija različitih tala, odredili da prosječna vrijednost ρ_c za prah iznosi $2,58 \text{ g cm}^{-3}$. Ovdje analizirana tla, pored praha kao dominantne frakcije, uglavnom sadrže < 25 % gline te < 5 % pijeska.

Iako su utvrđene razlike između vrijednosti ρ_c određene primjenom SM i PM bile signifikantne, one nisu dovoljno velike da bi bitno utjecale na izračun P. Stoga je primjena PM određivanja ρ_c opravdana u slučajevima u kojima se vrijednost ρ_c koristi prvenstveno za određivanje P, i to temeljem slijedećeg izračuna:

$$P = \left(1 - \frac{\rho_v}{\rho_c}\right) \times 100 [\%],$$

gdje su: volumna gustoća tla (g cm^{-3}),
gustoća čvrstih čestica (g cm^{-3}).

Na temelju vrijednosti ρ_c određenih korištenjem PM i SM i konstantne vrijednosti volumne gustoće tla (ρ_v) ($1,33 \text{ g cm}^{-3}$), koja se podrazumijeva u površinskim slojevima tla ako ona nije laboratorijski određena (Jury i Horton 2004.), izračunat je P tla. Raspon P kod SM kretao se od 49,4 % do 51,1 % sa prosječnom vrijednosti od 49,7 %, dok se kod PM kretao u rasponu od 49,0 % do 50,6 % sa prosječnom vrijednosti od 50,2 %. Iz navedenog je vidljivo da su prosječne razlike između izračunatih hipotetskih vrijednosti P zanemarive, pogotovo ako se uzme u obzir da su interpretacijske vrijednosti za P široke i da se u oba slučaja analizirano tlo klasificira kao porozno (Tablica 2).

Tablica 2. Granične vrijednosti ukupne poroznosti tla (P) sa interpretacijskim vrijednostima prema (Škorić, 1986)

Postotak pora u tlu (% P)	Interpretacijske vrijednosti
P \geq 60 %	Vrlo porozno
P 45 – 60 %	Porozno
P 30 – 45 %	Slabo porozno
P \leq 30 %	Vrlo slabo porozno

Zaključak

Iako je razlika između prosječnih vrijednosti ρ_c dobivenih primjenom SM i PM bila statistički signifikantna, ona je u relativnom smislu vrlo mala. PM određivanja ρ_c stoga se može primijeniti u slučajevima u kojima je vrijednost ρ_c potrebna prvenstveno za izračun P, kao i drugih s njome povezanih značajki (npr. kapaciteta tla za zrak). Primjena PM tako može pridonijeti smanjenju utroška vremena za analize, a samim time i ukupnih troškova istraživanja. Međutim, potrebno je provesti dodatna ispitivanja mogućnosti pojednostavljenja određivanja ρ_c , i to na većem broju uzoraka tala raznolike teksture i širokog raspona sadržaja organske tvari.

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Possibilities for simplifying soil particle density determination by pycnometer method

Abstract

Soil particle density (ρ_p) is the ratio of mass and non-porous volume of oven dry soil. It is one of the parameters required to determine the total soil porosity. Standard laboratory analysis is demanding and time-consuming, so the aim of the study was to examine, in 20 silt loam soils with low organic matter content, whether the simplification of the laboratory procedure affected the result of the ρ_p analysis. The difference between the average values of ρ_p determined by the standard (2.67 g cm^{-3}) and simplified (2.64 g cm^{-3}) method was significant ($p= 0,000003$), but practically small. Therefore, the modified method can be used if the value of ρ_p is required primarily for the calculation of the total soil porosity.

Keywords: soil particle density, pycnometer method, sample tempering, silt loam soils, soils with low organic matter content

Ocjena kvalitete komunalne otpadne vode s područja aglomeracije Lipik – Pakrac na mjestu ispuštanja u kanal Iliđa

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Sažetak

Cilj rada je bio pratiti parametre kvalitete komunalne otpadne vode u periodu od 2016. do 2018. god. s aglomeracije Lipik – Pakrac koja putem odvodnog kanala Iliđa dopijeva u rijeku Pakru. Komunalna otpadna voda prije ispuštanja u kanal prolazi fizikalne postupke obrade prvog stupnja. Iz dobivenih rezultata je vidljivo da analizirani parametri (suspendirana tvar, kemijska potrošnja kisika (KPK), biološka potrošnja kisika (BPK₅), ukupni fosfor i ukupni dušik) na ispusnom mjestu ne odstupaju od zadanih vrijednosti propisanih Pravilnikom (NN 80/13, 43/14, 27/15, 3/16). Ovakva kvaliteta komunalne otpadne vode ne može se postići nakon samo prvog stupnja pročišćavanja, što upućuje na probleme vezane za propuštanje kanalizacijskog sustava.

Ključne riječi: otpadna voda, pročišćavanje, kvaliteta

Uvod

Očuvanje vodnog bogatstva među najvažnijim je zadacima u očuvanju prirodnih bogatstava neke zemlje, a tu se osobito ističe očuvanje kvalitete površinskih voda. Zato jednom upotrijebljenu vodu koja postaje otpadna, prije ispuštanja u prirodne prijemnike potrebno je prethodno pročistiti. Nedovoljno pročišćene otpadne vode koje se ispuštaju u prirodne vodne sustave mogu bitno utjecati na promjenu eko-sustava, kao i mogućnost korištenja vode za različite namjene. Komunalne otpadne vode su visoko opterećene organskim ugljikom, spojevima dušika i fosfora. Ispuštanje otpadne vode opterećene hranjivim tvarima može dovesti do pojave eutrofikacije. Jedan od načina uklanjanja ovih tvari iz vode je biološka obrada otpadne vode (Tušar, 2009).

U Hrvatskoj je nedovoljna izgrađenost odvodne kanalizacijske mreže, tek je 43,6 % stanovništva priključeno na sustav javne odvodnje, a samo 27 % otpadne vode prije ispuštanja se pročišćava (Plišić, 2014.). Osim nedovoljne izgrađenosti kanalizacijske mreže, često je postojeća mreža dotrajala i neadekvatna te zahtjeva rekonstrukciju.

U ovome radu su prikazani parametri kvalitete komunalne otpadne vode propisani Vodopravnom dozvolom (NN 80/13, 43/14, 27/15, 3/16) za ispuštanje otpadnih voda. Ispitivanja se rade kvartalno na ulazu u uređaj za pročišćavanje otpadnih voda (UPOV) i izlazu, odnosno mjestu ispuštanja u prijemni kanal. S obzirom da je u tijeku priprema projekta izgradnje pročistača I, II i III stupnja pročišćavanja s biološkom obradom otpadne vode, prijemom i obradom otpadne vode iz septičkih jama, te postupci obrade aktivnog mulja. Cilj rada je bio prikazati parametre kvalitete (BPK₅, KPK, suspendirane tvari, ukupni dušik i ukupni fosfor) komunalne otpadne vode s područja aglomeracije Lipik – Pakrac koja se ispušta u kanal Iliđa za razdoblje od 2016. do 2018. godine.

Materijal i metode

U radu su prikazani rezultati ispitivanja otpadne komunalne vode prije i poslije prvog stupnja fizikalnog pročišćavanja s aglomeracije Lipik – Pakrac. U ovaj sustav odvodnje skupljaju se otpadne vode iz domaćinstava s područja gradova Lipika i Pakraca, sela Dobrovac, Prekopakra i Filipovac, otpadne voda industrijskih pogona i javnih ustanova, kao i oborinske vode koje se slijevaju s javnih površina ovih naselja. Komunalna otpadna voda prije ispuštanja u kanal

Iliđu, dolazi na UPOV u naselju Dobrovac, gdje prolazi postupke prvog stupnja fizikalnog pročišćavanja (gruba i fina rešetka, fina sita, aerirani pjeskolov i mastolov).

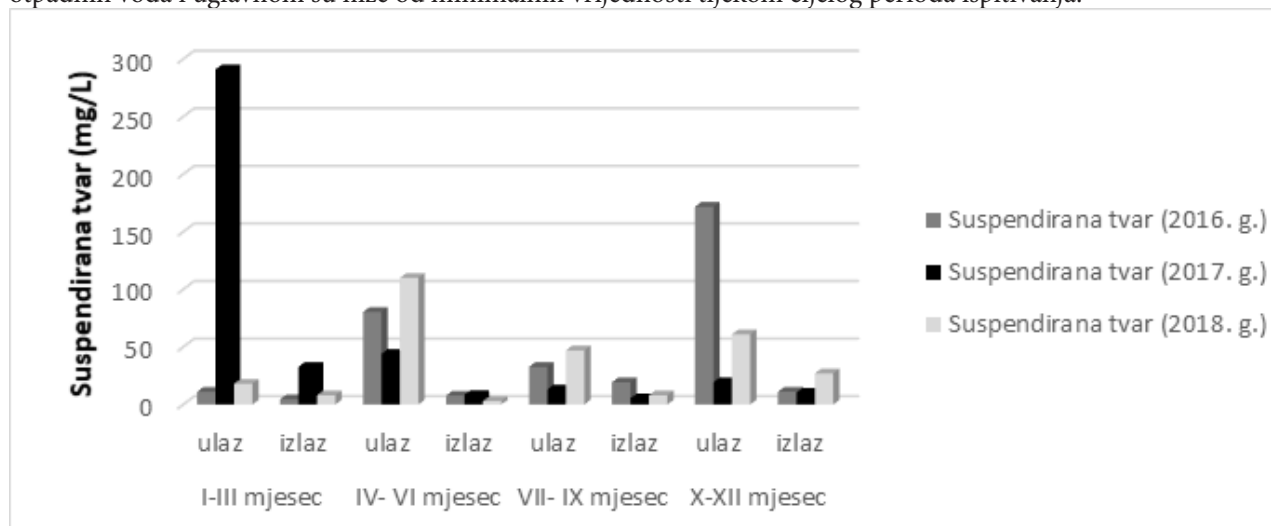
Ispitivani su kompozitni uzorci za vrijeme rada pročišćavača na kontrolnom oknu prije i poslije pročišćavanja, uzorkovani automatskim uzorkivačem tijekom 24 sata uzimani svakih sat vremena. Prema Vodopravnoj dozvoli (NN 80/13, 43/14, 27/15, 3/16) uzorkovanja i ispitivanja se provode četiri puta godišnje u ovlaštenom laboratoriju prema standardnim metodama: 1) ukupne suspendirane tvari sukladno HRN EN 872:2008; 2) BPK₅ sukladno HRN EN 1899: 2004; 3) KPK sukladno HRN ISO 6060:2003; 4) ukupni fosfor sukladno HRN EN ISO 6878:2008 5) ukupni dušik sukladno HRN EN ISO 11905-1:2001. Granične vrijednosti ispitivanih parametara u pročišćenoj otpadnoj vodi propisane su prema Pravilniku o graničnim vrijednostima emisija otpadnih voda NN 80/13, 43/14, 27/15 i 3/16 (Tablica 1).

Tablica 1. Granične vrijednosti emisija otpadnih voda prilikom ispuštanja u površinske vode (NN 80/13, 43/14, 27/15, 3/16)

Pokazatelji	Jedinica	Površinske vode
BPK ₅	mg O ₂ /L	25
KPK	mg O ₂ /L	125
Ukupni fosfor	mg P/L	2
Ukupni dušik	mg N/L	15
Suspendirana tvar	mg /L	35

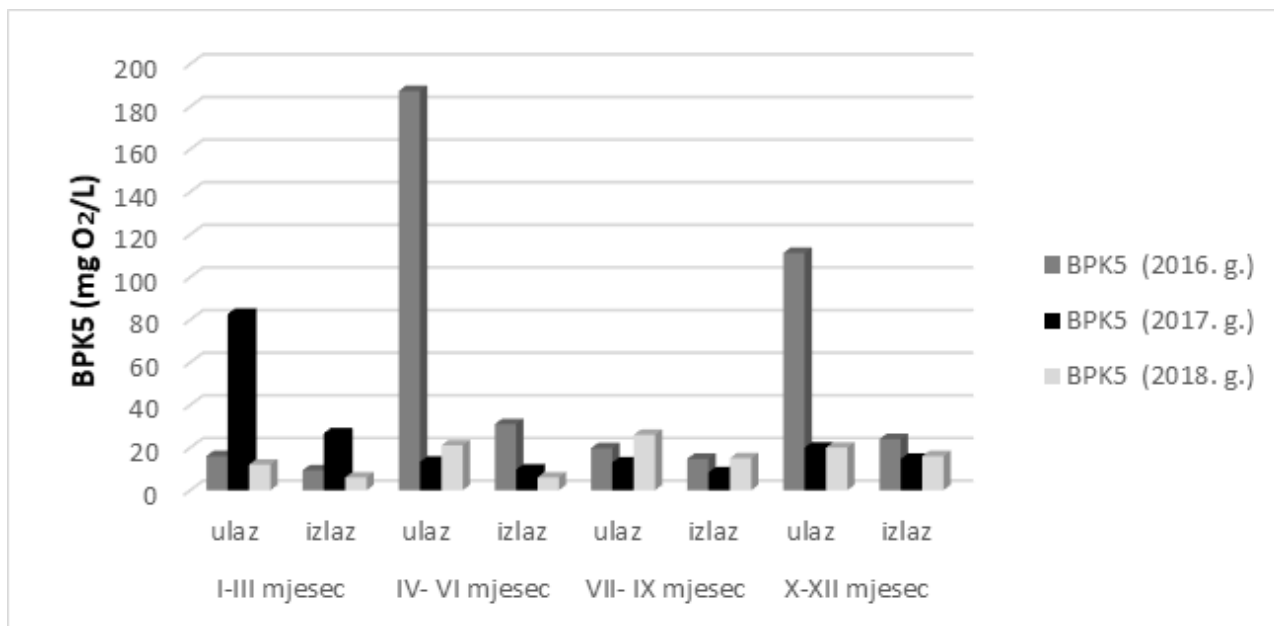
Rezultati i rasprava

Na slici 1 prikazani su rezultati ispitivanja suspendirane tvari u periodu od 2016. do 2018. godine. Vidljivo je da su ulazne koncentracije suspendirane tvari u granicama koncentracija svojstvenih za pokazatelje sastava komunalnih otpadnih voda i uglavnom su niže od minimalnih vrijednosti tijekom cijelog perioda ispitivanja.

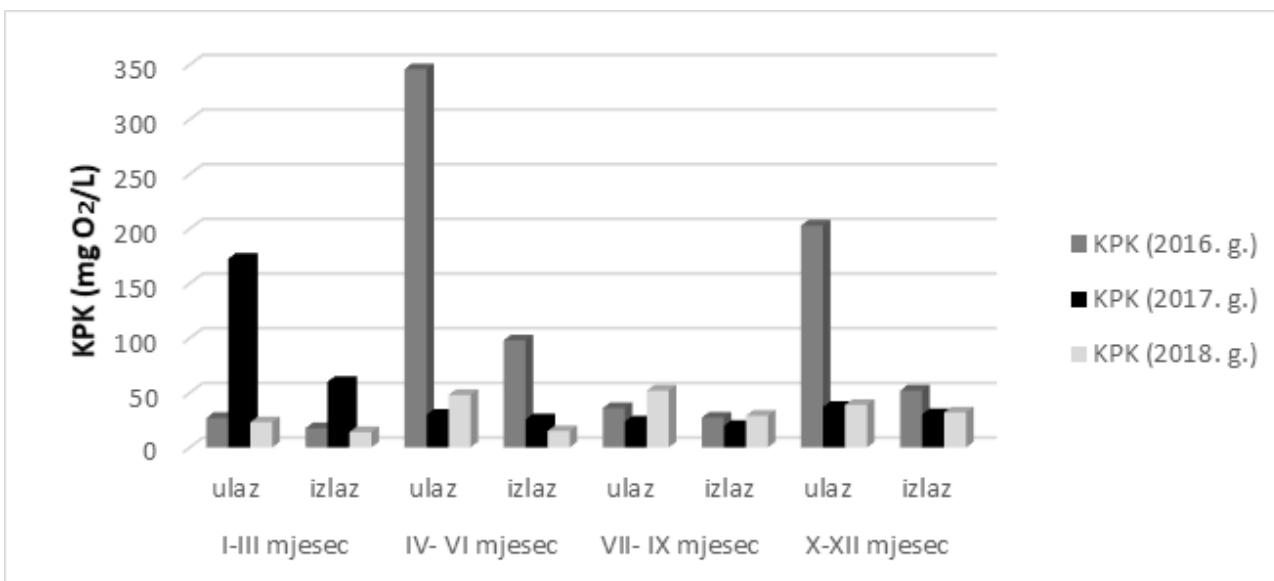


Slika 1. Rezultati ispitivanja suspendirane tvari u otpadnoj vodi na ulazu i izlazu iz pročišćavača 1. stupnja u razdoblju od 2016. do 2018.

Iz prikazanih rezultata na slikama 2 i 3 je vidljivo da su veće koncentracije od minimalnih vrijednosti zabilježene u drugom i četvrtom kvartalu za BPK₅ i KPK, u 2016. godini te kod vrijednosti izlaznih koncentracija uočene su povećane koncentracije u odnosu na MDK vrijednosti za BPK₅ u prvom kvartalu 2017. godine za 6,8 %. Neovisno o povećanoj koncentraciji BPK₅ za 6,8 % u odnosu na MDK vrijednost, ispunjeni su zahtjevi iz Vodopravne dozvole za minimalnim smanjenjem izlazne koncentracije za 20 % u odnosu na ulaznu vrijednost.

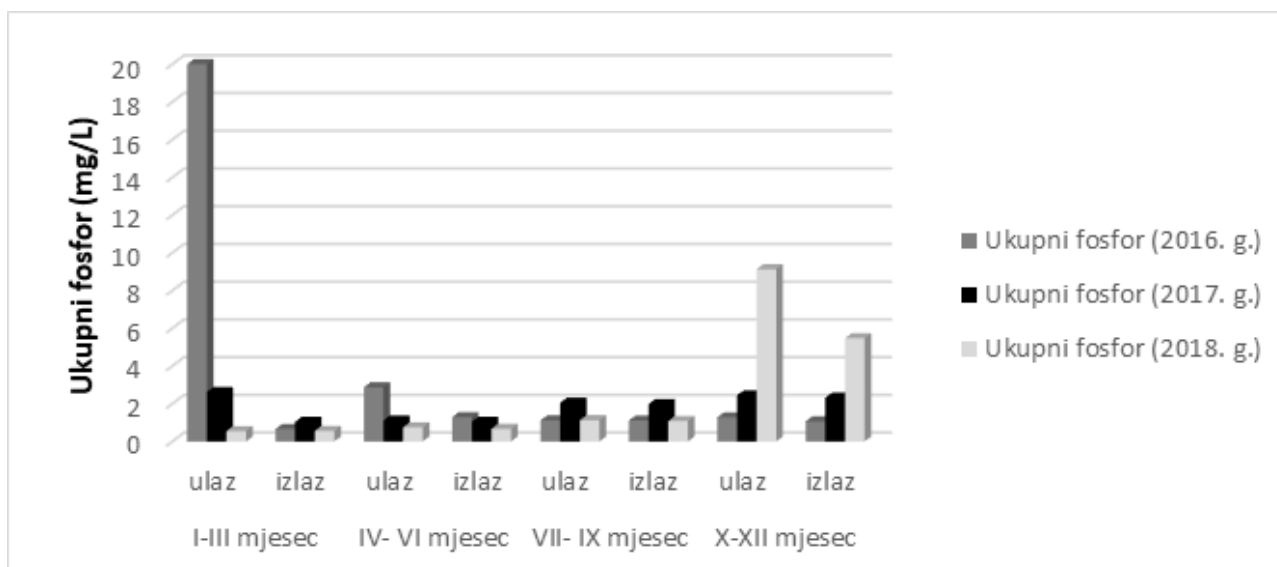


Slika 2. Rezultati ispitivanja BPK₅ vrijednosti u otpadnoj vodi na ulazu i izlazu iz pročištača 1. stupnja u razdoblju od 2016. do 2018.



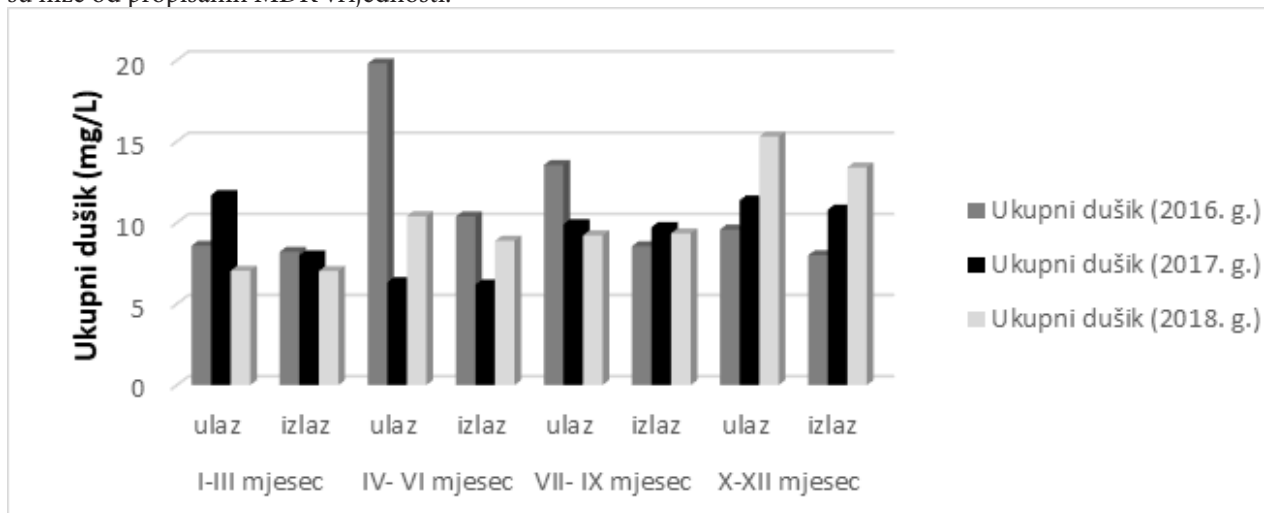
Slika 3. Rezultati ispitivanja KPK vrijednosti u otpadnoj vodi na ulazu i izlazu iz pročištača 1. stupnja u razdoblju od 2016. do 2018.

Slika 4 prikazuje rezultate ispitivanja ukupnog fosfora, gdje su sve ulazne koncentracije niže od koncentracija određenih parametara svojstvenih za komunalnu otpadnu vodu. Kod vrijednosti izlaznih koncentracija uočene su povećane koncentracije u odnosu na MDK vrijednosti u četvrtom kvartalu 2017. godine za 16 %, dok u 2018. godini je zabilježeno povećanje izlazne koncentracije ukupnog fosfora za 173,5 %.



Slika 4. Rezultati ispitivanja ukupnog fosfora u otpadnoj vodi na ulazu i izlazu iz pročistača 1. stupnja u razdoblju od 2016. do 2018.

Na slici 5 je vidljivo da koncentracije ukupnog dušika na ulazu u UPOV ne prelaze minimalne vrijednosti koncentracija svojstvenih za komunalnu otpadnu vodu osim u drugom kvartalu 2016. godine. Izlazne koncentracije su niže od propisanih MDK vrijednosti.



Slika 5. Rezultati ispitivanja ukupnog dušika u otpadnoj vodi na ulazu i izlazu iz pročistača 1. stupnja u razdoblju od 2016. do 2018.

Iz prikazanih rezultata je vidljivo da su ulazne koncentracije analiziranih tvari niže od uobičajenih vrijednosti koncentracija pokazatelja kakvoće komunalnih otpadnih voda prema Tušar (2009), ali su u granicama vrijednosti za: 1) krutine od 300 do 1200 mg/L; 2) BPK₅ od 100 do 400 mg O₂/L; 3) KPK od 200 do 1000 mg O₂/L; 4) ukupni fosfor od 5 do 20 mg/L; 5) ukupni dušik od 15 do 90 mg/L.

Svi privredni subjekti koji ispuštaju vodu u gradsku kanalizaciju ili direktno u prirodni prijemnik moraju posjedovati Vodopravnu dozvolu za ispuštanje otpadnih voda, gdje im je propisano učestalost analiza kao i vrsta i koncentracija određenih parametara. Vodopravnom dozvolom za ispuštanje otpadne vode s aglomeracije Lipik – Pakrac, propisane su analize četiri puta godišnje uzimanjem kompozitnog uzorka i postotci smanjenja koncentracija za BPK₅ i ukupnu suspendiranu tvar.

Zaključak

Rezultati ispitivanja komunalne otpadne voda u razdoblju od 2016. do 2018. godine na aglomeraciji Lipik-Pakrac, pokazuju oscilacije u vrijednostima emisija otpadnih voda na ulazu u pročištač, ali u granicama koncentracija karakterističnih za komunalne otpadne vode. Ovakve oscilacije mogu biti uzrokovane količinom oborina u vrijeme uzorkovanja odnosno količinom oborinskih voda koje dopijevaju u odvodni sustav, oscilacijama u dinamici industrijske proizvodnje, kao i nekim izvanrednim situacijama. Koncentracije ispitivanih parametara u pravilu su niže od minimalnih pokazatelja koncentracija za komunalne otpadne vode, izuzev u 2016. godini kada su zabilježena povećanja za BPK₅, KPK, i ukupni fosfor u drugom kvartalu, te BPK₅ i KPK u četvrtom kvartalu.

Iz ovog istraživanja je vidljivo da koncentracije analiziranih parametara otpadne komunalne vode ne potvrđuju očekivane vrijednosti karakteristične za ovakvu vrstu otpadne vode, budući da je primijenjen samo prvi stupanj fizikalnog pročišćavanja. Uzroci ovakvih rezultata mogu biti posljedica velikih razrjeđenja otpadne vode, uzrokovane oštećenjem kanalizacijske mreže i infiltracije riječne vode u sustav.

Prema studijama analiza položaja korita rijeke Pakre i položaja kanalizacijske mreže vjerojatna je znatna infiltracija riječne vode u kanalizacijski sustav.

Izgradnja novog pročištača za otpadnu vodu neće biti dostatno rješenje za postojeći problem, nego je potrebno obnoviti kanalizacijsku mrežu radi sprečavanja mogućeg zagađenja podzemnih voda.

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Quality assessment of municipal waste water from the area of the agglomeration Lipik-Pakrac measured on the site of discharge to Iliđa canal

Abstract

The aim of the paper was to monitor municipal waste water quality parameters from 2016. to 2018. from the Lipik - Pakrac agglomeration which reaches the Pakra river via the Iliđa drainage channel. Municipal waste water undergoes first-degree physical processing procedures before being discharged into the sewer. From the obtained results show that the analyzed parameters (suspended substance, chemical oxygen demand (COD), biological oxygen demand (BOD5), total phosphorus and total nitrogen) at the discharge point do not deviate from the set values prescribed by the Regulation (NN 80/13, 43 / 14, 27/15, 3/16).

Such quality of waste water cannot be characteristic of municipal water after only the first stage of treatment, which indicates problems related to the sewage system.

Keywords: waste water, purification, quality

Effects of nitrogen deficiency on some physiological parameters and root traits of three Croatian common bean landraces

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Abstract

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes in the world and the lack of nitrogen in its cultivation can cause major negative changes leading to decrease in leaf protein content, such as RuBisCO, which causes a decrease in photosynthesis capacity and ultimately results in leaf damage and senescence. The aim of this study was to analyze the effect of nitrogen deficiency with 'N-' treatment, Hoagland solution with no added nitrogen, on the chlorophyll content index, 'greenness', digital biomass, quantum yield of photosystem II (Y (II)), maximum quantum yield of photosystem II (F_v/F_m) and the root system traits of Croatian common bean landraces 'Trešnjevac', 'Zelenčec' and 'Biser'. Nitrogen deficiency treatment caused a decrease in the chlorophyll content index and 'greenness', digital biomass and changes in root architecture for all three landraces. The landrace 'Zelenčec' developed the largest root volume, and the landrace 'Trešnjevac' the largest average root diameter, digital biomass and highest chlorophyll content index in nitrogen treatment (N-).

Keywords: Common bean, nitrogen deficiency, root architecture, photochemical reactions

Introduction

For normal physiological functions and development, plants need macro and micro nutrients. The lack of nutrients such as nitrogen, potassium and phosphorus is a big problem in agricultural production (Aleksandrov, 2019). Nitrogen is a major component of amino acids and is important in biochemistry of photosynthetic pigments and co-enzymes (Aleksandrov, 2019; Maathuis, 2009) magnesium, nitrogen, phosphorous, potassium and sulfur in relatively large amounts (>0.1% of dry mass. Common bean (*Phaseolus vulgaris* L.) forms a relationship with nitrogen-fixing rhizobia and through a process termed symbiotic nitrogen fixation provides the plant a source of nitrogen (George and Singleton, 1992; Wilker et al., 2019), but efficiency of process depends on soil fertility (Muñoz-Azcarate et al., 2017) and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. Therefore, optimization of nitrogen fixation of bean-rhizobia symbioses and selection of efficient rhizobial strains should be a priority, which begins with the study of the natural diversity of the symbioses and the rhizobial populations associated. Natural rhizobia biodiversity that nodulates common bean may be a source of adaptive alleles acting through phenotypic plasticity. Crosses between accessions differing for nitrogen fixation may combine alleles that never meet in nature. Another way to discover adaptive genes is to use association genetics to identify loci that common bean plants use for enhanced biological nitrogen fixation and, in consequence, for marker assisted selection for genetic improvement of symbiotic nitrogen fixation. In this review, rhizobial biodiversity resources will be discussed, together with what is known about the loci that underlie such genetic variation, and the

potential candidate genes that may influence the symbiosis' fitness benefits, thus achieving an optimal nitrogen fixation capacity in order to help reduce reliance on nitrogen fertilizers in common bean.,"author":[{"dropping-particle":"","family":"Muñoz-Azcarate","given":"Olaya","non-dropping-particle":"","parse-names":false,"suffix":""}],{"dropping-particle":"","family":"M González","given":"Ana","non-dropping-particle":"","parse-names":false,"suffix":""}],{"dropping-particle":"","family":"Santalla","given":"Marta","non-dropping-particle":"","parse-names":false,"suffix":""}],container-title":"AIMS Microbiology","id":"ITEM-1","issue":"3","issued":{"date-parts":["2017"]},"page":"435-466","publisher":"American Institute of Mathematical Sciences (AIMS. Because of the variability of nitrogen that can be ensured through symbiotic fixation it is necessary to add nitrogen as mineral fertilization for the initial growth of the beans (Muñoz-Azcarate et al., 2017)and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. 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Assuming that N persists in the root zone, N deficiency can act as external signal that affects root growth and development (Muñoz-Azcarate et al., 2017)and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. 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Certain morphological traits such as length, volume, ramification and root diameter, can increase the efficiency of the root in nutrient acquisition from the soil (Lazarević, 2018; Lynch, 2013; Sinclair and Ruffy, 2012)root systems with rapid exploitation of deep soil would

optimize water and N capture in most maize production environments. The ideotype Specific phenes that may contribute to rooting depth in maize include (a. The need for nitrogen is also increased at the end of the growing season due to the synthesis of proteins in the seed (Sinclair and Rufty, 2012) improved plant genetics is viewed as the path to increased crop yields. However, in this manuscript, we argue that yield increases most often result from a combination of improved genetics and increased availability of nitrogen and water resources. At this time, it is likely that resource availability is the main impediment to yield increase in many cropping systems. In developing regions, it appears that nitrogen availability limits crop yield. In developed regions, rainfall and water availability commonly impose a substantial constraint on further crop yield increase. Strategies are examined to enhance resource accumulation and use in cropping systems of the future. © 2012 Elsevier B.V.;author":[{"dropping-particle":"","family":"Sinclair","given":"Thomas R.","non-dropping-particle":"","parse-names":false,"suffix":""},{dropping-particle":"","family":"Rufty","given":"Thomas W.","non-dropping-particle":"","parse-names":false,"suffix":""}],container-title:"Global Food Security",id:"ITEM-1",issue:"2",issued":{"date-parts":["2012","12"]},page:"94-98",title:"Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics",type:"article",volume:"1"},uris:["http://www.mendeley.com/documents/?uuid=58beb228-fa18-3d4d-9353-5a1b6dea9871"],mendeley":{"formattedCitation":"(Sinclair & Rufty, 2012. Because of nitrogen deficiency in the soil, common bean plants become weaker and more susceptible to diseases, insects and adverse weather conditions (Wilker et al., 2019). Under nitrogen deficiency conditions breakdown of RuBisCO occurs, which leads to decrease in photosynthetic rate and reduction of plant growth (Aleksandrov, 2019).

Materials and methods

Plant material and experimental set up

The seeds of traditional Croatian common bean landraces; 'Trešnjevac', 'Zelenčec' and 'Biser' were obtained from the Department of Seed Science and Technology Collection at University of Zagreb Faculty of Agriculture. Experiment was conducted in a greenhouse. Seeds were surface-sterilized using 1.5% sodium hypochlorite for five minutes and 70% ethanol for 30 seconds and then washed with distilled water three times. The experiment was set in randomized design with three replications. Seeds were sown into 2L plastic pots filled with vermiculite. Three seeds were planted in each pot and 15 days after emergence two plants were removed. Half of the plants was irrigated with ½ Hoagland solution (N+) and the other half with ½ Hoagland solution without nitrogen (N-) (Hoagland and Arnon, 1950).

Measurements

Leaf chlorophyll content index (CCI), greenness (GR), digital biomass (DB) and chlorophyll fluorescence parameters maximum quantum yield of PSII (F_v/F_m) and quantum yield of PSII (Y(II)) were measured non-destructively once a week during four weeks of experiment (measurement time) on the same plants. Chlorophyll content index was measured on the first fully expanded leaf from the top of the plant (CCM-200, Opti-Sciences Inc, USA) and chlorophyll fluorescence measurements were done in dark and light adapted leaves using modulated fluorometers (Plant Stress Kit, Opti-Sciences Inc, USA). GR and DB were measured using PlantEye multispectral 3D scanner (Phenospex B.V., The Netherlands). At the end of the experiment roots were washed from the substrate and scanned with an Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan). Root images were used to analyze root morphological traits (length, volume, average diameter and number of tips) using WinRHIZO Pro software (Regent Instruments Inc., Quebec, QC, Canada).

Statistical analysis

The analysis of variance (ANOVA) was conducted in order to determine effect of nitrogen treatment, genotype, measurement time on measured physiological traits and effect of nitrogen treatment and genotype on root system traits. The general linear model in R software (R core team, 2017) was used and mean differences between the values of the variables were determined by the Tukey test ($P < 0.05$).

Results and discussion

N- caused significant decrease ($P < 0.01$) of CCI through measurement time in all three examined common bean landraces (data not shown). Hence, the lowest CCI was observed in last week of measurement time for all three landraces (Figure 1). The decrease of GR through measurement time was also observed where N- significantly ($P < 0.01$) decreased GR (data not shown). DB has been calculated as the product of height and 3D leaf area assuming that the plant is a regular body of which the volume can be computed by taking into account height and length. Decrease in DB in last week of measurement occurred as a result of epinasty of leaves which can be caused by stress conditions (Lazarević and Poljak, 2019), in this case nitrogen deficiency stress and changes in environmental conditions while measuring (transferring plants from greenhouse to phenotyping laboratory). Lowered DB was observed in all weeks of measurement time for plants grown in N- compared to N+ (Figure 2). The differences among landraces in DB have also been observed where landrace 'Trešnjevac' both in N+ and N- had the largest DB, and the landrace 'Biser' had the smallest. N- reduced ($P < 0.05$) F_v/F_m through the measurement time on average for 14% (data not shown). No significant differences have been observed for the parameter Y(II).

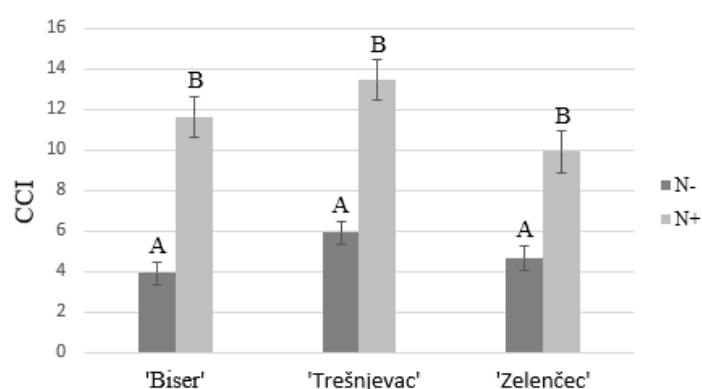


Figure 1. Chlorophyll content index (CCI) of three Croatian common bean landraces grown with (N+) or without nitrogen (N-). Vertical bars denote mean \pm S.E. of means.

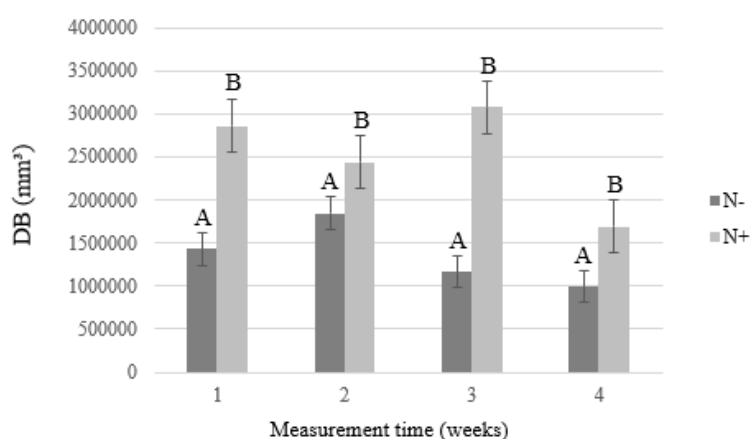


Figure 2. Differences in total digital biomass (DB) of all three genotypes between nitrogen treatment (N-) and control (N+) through measurement time. Vertical bars denote mean \pm S.E. of means.

The differences among genotypes for root traits volume (V) and average root diameter (D) and between N+ and N- for traits length (L), D, number of tips (NT) have been observed (Table 1) (Figure 3). The interaction genotype \times treatment showed significant negative effect on root trait V for all three landraces (Table 1). The landrace 'Zelenčec' has developed largest V (39.8 cm^3) followed by landrace 'Trešnjevac' (38.75 cm^3), while landrace 'Biser' developed the root with smallest V (18.68 cm^3) in N-. The landrace 'Trešnjevac' developed root with larger D (0.48 mm) compared to landrace 'Zelenčec' (0.46 mm) and the landrace 'Biser' (0.39 mm) in N-.

	DF	L (cm)	V (cm ³)	D (mm)	NT
Genotype	2	ns	*	***	ns
Treatment	1	*	ns	*	***
Genotype x Treatment	2	ns	*	ns	ns

Table 1. Analysis of variance (ANOVA) results for measured root traits

L (cm) – length, V (cm³) – root volume, D (mm) – average root diameter, NT – number of tips, DF – degrees of freedom; ns- not significant; *- significant at $P < 0.05$, **- significant at $P < 0.01$, ***-significant at $P < 0.001$.

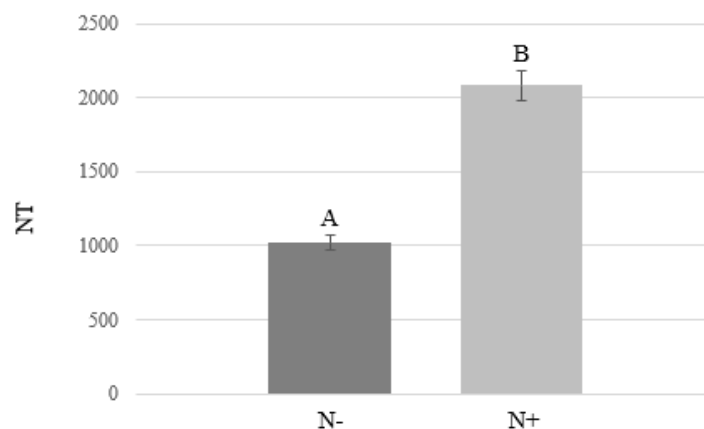


Figure 3. Total number of tips at the end of the experiment for control (N+) and nitrogen treatment (N-). Vertical bars denote mean \pm S.E. of means.

Taken together, results of this study indicate that nitrogen deficiency reduced DB of all three genotypes. The decrease of CCI was followed by lowered GR in N-. Lima et al. (1999) isolated or in combination, on leaf gas exchange and fast chlorophyll (Chl reported similar reduction of CCI in 28 days old Negrito bean variety irrigated with low-dose nitrogen, compared to plants irrigated with control ('N+' Hoagland's solution). NT was significantly lowered in nitrogen deficiency treatment. The similar results occurred in nitrogen deficiency experiment with soybean conducted by Castell (2018) where N-deficient soybean had smaller root system; diameter of roots, evidence of root hairs, depth of taproot. While some studies, in a term of photosystem II (PSII) photochemistry, have demonstrated that N-deficiency has no effect on the maximum quantum yield of PSII (Khamis et al., 1990), others have shown that the lack of nitrogen reduces the maximum quantum yield of PSII photochemistry (F_v/F_m), indicating that N-deficiency causes damage to PSII (Huang et al., 2004; Verhoeven et al., 1997) which also occurred in this research.

Conclusions

Nitrogen deficiency decreased the CCI, GR and DB of common bean plants of all three landraces. Through the experiment the small changes of F_v/F_m occurred which can indicate that nitrogen deficiency caused stress that affected photosystem II in a dark adapted state. The changes in root architecture were observed through the reduction in L, V, D and NT when exposed to nitrogen deficiency stress. The landrace 'Zelenčec' developed the largest V, and the landrace 'Trešnjevac' the largest D, DB and highest CCI in N-.

Acknowledgement

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Utjecaj nedostatka dušika na neke fiziološke parametre i svojstva korijenovog sustava triju hrvatskih tradicijskih kultivara graha

Sažetak

Grah (*Phaseolus vulgaris* L.) predstavlja važnu mahunarku u svijetu, a nedostatak dušika u njegovom uzgoj može uzrokovati velike negativne promjene vodeći do smanjenja sadržaja proteina u listovima, poput RuBisCO-a, što uzrokuje smanjenje kapaciteta fotosinteze i na kraju rezultira oštećenjem listova i starenjem. Cilj ovog istraživanja bio je analizirati utjecaj nedostatka dušika, tretmanom Hoaglandovom otopinom bez dodanog dušika, na indeks sadržaja klorofila, 'greenness', digitalnu biomasu, kvantni prinos fotosustava II (Y (II)), maksimalni kvantni prinos fotosustava II (F_v/F_m) i svojstva korijenovog sustava triju hrvatskih tradicijskih kultivara graha 'Trešnjevac', 'Zelenčec' i 'Biser'. Tretman nedostatkom dušika uzrokovao je pad indeksa sadržaja klorofila. Manjak dušika također je uzrokovao pad u 'greenness'-u, digitalnoj biomasu te promijene u arhitekturi korijena za sva tri kultivara. Kultivar 'Zelenčec' razvio je najveći volumen korijena, a kultivar "Trešnjevac" najveći prosječni promjer korijena, digitalnu biomasu i najviši indeks sadržaja klorofila u N- tretmanu.

Ključne riječi: Grah, nedostatak dušika, arhitektura korijena, fotokemijske reakcije

The effect of sewage sludge and sludge compost on soil fertility, organic matter content and yield of perennial ryegrass (*Lolium perenne*)

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Abstract

The effect of anaerobically stabilized sewage sludge and sludge compost on the chemical properties, organic matter (OM) content and quality, and macro-nutrient amounts of acidic sand and calcareous chernozem soils was studied. The impact on the yield and nutrient uptake of perennial ryegrass test plant was also evaluated. The amendments were added to the soils in a proportion of 1 % and 3 % rates. The composting process and the compost additive (green waste) increased the pH and decreased the organic carbon and nitrogen contents of the sewage sludge. The pH of the acidic soil was increased especially by compost treatments, while the sewage sludge application lowered the pH of the calcareous chernozem from the alkaline range to near neutral. The OM and dissolved organic carbon (DOC) showed moderate, total N slight, while NH₄-N, NO₃-N and P substantial increases as a result of the applied treatments. Biomass yields followed a similar trend on both soils: sewage sludge amendment showed a strong effect even at the 1 % treatment rate while increasing the compost addition to soil led to a more gradual growth of ryegrass yields. As a result of the studied treatments, the plant N contents also increased in some cases.

Keywords: sewage sludge, compost, organic matter quality, perennial ryegrass

Introduction

The municipal sewage sludge is a by-product of wastewater treatment process. Studies demonstrated its positive effect on crop production as a fertiliser, primarily as an N source (Zebarth et al., 2002). However, the instability of organic matter, high mineral nitrogen concentration as well as pollutants may hinder its application. Composting of sewage sludge can solve these problems, resulting a material that provides plant nutrients for a more extended period, increase soil organic matter content and improves its quality (Smith, 2009).

In our experiment anaerobically stabilized municipal sewage sludge and compost derived from the same sludge and green waste were tested as soil amendments by considering their effect on soil OM and efficiency of fertilisation on sandy and loamy soils. The hypothesis was that on a shorter period (one growing season) sewage sludge may provide more beneficial effects as a fertiliser, while the compost can improve OM quality more efficiently.

Materials and Methods

The pot experiment was performed under greenhouse conditions according to standard designs in plant biometry. Anaerobically stabilized municipal sewage sludge from domestic source was collected from wastewater treatment plant of the city of Érd (Hungary, 47° 23' 31" N; 18° 54' 16.3" E) and used as a soil amendment. Furthermore, the sludge compost produced from sewage sludge and municipal green waste (in 5:6 dry matter ratio) was also used as organic fertilizer. For substrate preparation, sewage sludge and compost were added to soil in a proportion of 1 % w/w and 3 %

The effect of sewage sludge and sludge compost on soil fertility, organic matter content and yield of perennial ryegrass (*Lolium perenne*)

w/w amendment rates in three replicates. Before setting up the experiment, the soils and the organic amendments were dried, sieved, and soils were ground for the homogenization. The basic properties of calcareous chernozem and acidic sandy soils are shown in the tables reporting the results of the treatments in the “Control” rows.

After 2 weeks of incubation of the studied soils and organic fertilisers, the seeds of perennial ryegrass (*Lolium perenne*) test plant were sown into pots filled with the amended soils. Four weeks after the emergence, plants were harvested and the biomass was weighted and total element contents were measured.

The OM quality of soils and fertilisers was investigated by two methods. The E_4/E_6 ratio is a well-known and widely used method (Kononova 1966; Chen et al. 1977). Generally, its value is favourable below 5. The Hargitai's two-solvent humus stability number (Q) was determined as follows: crude OM and humus fractions with less favourable properties are primarily soluble in NaOH solution whereas high quality, high molecular weight humus material bound to calcium ions are dissolved in NaF. The relative light absorption value of the solutions is the humus stability number (Q). This characterises the ratio of “real” humic substances to crude OM with a lower degree of humification. For the humic substance analysis, 20 ml of 1 % NaF or 20 ml of 0.5 % NaOH was added to 2 g of soil. The absorbance of the prepared solutions was read on a spectrophotometer between 400 nm and 750 nm at every 50 nm. At each wavelength, the absorbance of a given sample in NaF solution was divided by that of in NaOH. This gives a stability number (Q value) for each wavelength. The more the Q stability number exceeds 1, the better quality humic substances are present in the sample (Buzás, 1988). Unfortunately, the E_4/E_6 and Q value methods can give contradictory results.

The total element contents of organic fertilisers and soils were extracted using the *aqua regia* method (a mixture of nitric acid, hydrochloric acid and peroxide solution), the easily soluble element content was extracted with ammonium-lactate and acetic acid (Egner et al. 1960), and the water-soluble copper was determined in extracts of distilled water. The element contents of the extracts were measured with an ICP-OES instrument (JY Ultima 2). For DOC measurement, soil extracts were prepared and filtered through a membrane and then analysed with “Elementar vario TOC cube” device. The nitrogen (N) content of the plants was measured after digestion with sulfuric acid + hydrogen peroxide, while the contents of phosphorous (P) and potassium (K) were measured after digestion with nitric acid and hydrogen peroxide. The data obtained in the experiment were evaluated using analysis of variance with Statistica 13.0 programme.

Results and Discussion

The characteristics of the organic fertiliser additives are summarised in Tables 1 and 2. The sewage sludge has an acidic pH, and the highest organic carbon and total N content. Its total P and Cu contents are also outstanding. The pH of the compost was higher, and its organic carbon and N content were lower. The total K, Ca and Mg contents increased in the compost compared to the sewage sludge and the total Cu content significantly decreased (Table 1).

Table 1. Chemical parameters and total element content of the additives in dry weight basis

	pH _{H2O}	Salt %	C _{org} m/m%	N _{total} m/m%	P	K	Ca	Mg	Cu
					mg·kg ⁻¹				
Sewage sludge	6.31	0.30	29.8	4.93	27087	3132	39364	7129	255
Compost	7.35	0.41	22.9	1.80	7640	8167	54199	8701	75

The sewage sludge had high ammonium and low nitrate content, whereas nitrification processes in the compost increased nitrate content that can be most efficiently utilized by the plants, and decreased the amount of ammonium. While practically there is no difference in the amount of easily soluble phosphorus content, the amount of easily soluble potassium is much higher in the compost than in the sewage sludge. In sewage sludge, the amount of total copper is high, but the ratio of available fraction is much less. The total and easily available copper content is already much lower in compost (Table 2).

Table 2. The easily soluble element contents of the additives

	NH ₄ -N	NO ₃ -N	AL-P ₂ O ₅	AL-K ₂ O	AL-Ca	KCL-Mg	DW-Cu
	mg·kg ⁻¹						µg/l
Sewage sludge	349	56.4	7783	2558	18084	1808	48.1
Compost	291	392	7639	7615	32314	1302	35.6

AL-ammonium-lactate soluble; DW- distilled water soluble

The Q value below 1 indicates larger proportion of inferior, acidic, less complex humic substances, while above 1 refers to higher quality composite humic substances. The Q value of the sewage sludge was 0.74, while that of the compost was slightly higher: 0.82, thus its OM quality was better. The E₄/E₆ ratio shows just the opposite, as it is favourable below 5, the value of 6.1 for compost indicates a deterioration in quality compared to the value of 3.7 for sewage sludge, which has more favourable humic substances according to this method.

The change in the C/N ratio reflects the transformation and decomposition of organic waste during the composting process. The ratio of 6 in the sewage sludge shows that the easily biodegradable constituents predominate, nitrogen content is high. Due to composting process the total nitrogen content decreased significantly, the organic carbon content decreased to a lesser extent; therefore the C/N ratio increased to 13 in compost.

The low pH of the acidic sandy soil was increased only up to 5.31 by the 3 % rate of the sewage sludge application, while both the 1 % and 3 % doses of compost treatment raised the pH value above 6. The effect of compost was significantly different compared to sewage sludge at both treatment levels. The very low OM content was increased by sewage sludge treatment only at the highest dose by 20 %, while the compost application increased the soil OM content by 75 %, so at the 3 % level, compost was more efficient. However, the approximately doubling of DOC content did not show such a difference between the two fertilisers. The increase of total and easily soluble P contents of the acidic sandy soil was similar for the two substances, while the NH₄-N and NO₃-N contents increased strongly due to sewage sludge treatment. Concerning the K content, only the easily soluble K fraction could be increased significantly by the highest level of compost addition (Table 3).

The calcareous chernozem soil generally had more favourable characteristics. The high pH of 8.14 was significantly reduced only by the highest dose of sewage sludge application. The already high soil OM content increased by approximately 15 % in the case of the addition of both organic amendments at the highest rate. The DOC showed only a 20 % increase due to the treatments. The total N showed a 15-20 % increase for both fertilisers, but the NH₄-N and especially the NO₃-N contents increased strongly only under the influence of sewage sludge treatment. The increase of P content is more pronounced for sewage sludge fertilization. Although the total P content is much higher in sewage sludge than in compost (27087 vs 7640 mg·kg⁻¹), there is practically no difference between the easily soluble P contents. The nearly 6-fold increase in AL-P₂O₅ content in calcareous soil can probably also be explained by a decrease in soil pH. In the case of K, only the easily soluble fraction increased in this soil due to the higher dose of compost treatment (Table 3).

On the acidic sandy soil, the 3 % rate of compost addition significantly doubled the poor Q value of 0.36. However, it also caused a negative increase in E₄/E₆ ratio. On calcareous chernozem soil, the sewage sludge amendment reduced the Q value of 21.1 by 20 % and the compost addition by more than 50 %. The E₄/E₆ ratio increased only due to compost treatment, i.e. it deteriorated, but only to a minimal extent (Table 4).

The effect of sewage sludge and sludge compost on soil fertility, organic matter content and yield of perennial ryegrass (*Lolium perenne*)

Table 3. The effect of different treatments on properties and macronutrient content of the soils

	pH _{H₂O}	OM	DOC	N _{tot}	NH ₄ -N	NO ₃ -N	P _{tot}	AL-P ₂ O ₅	K _{tot}	AL-K ₂ O
		m/m%	mg·L ⁻¹	m/m%	mg·kg ⁻¹					
Acidic sand										
Control	5.18	0.55	5.8	0.04	1.92	0.59	248	105	1365	49.5
Sewage sludge										
1%	5.17	0.62	6.4	0.05	2.80	0.88	281	152	1180	30.8
3%	5.31	0.66	10.6	0.06	8.65	102.8	394	336	1189	37.1
LSD _{5%}	0.13*	n.s.	2.85*	n.s.	0.77***	27.5**	75.0*	63.0**	n.s.	n.s.
Compost										
1%	6.32	0.64	8.3	0.04	1.92	1.03	280	170	1166	48.8
3%	7.16	0.96	12.8	0.07	1.92	1.33	374	299	1285	106.9
LSD _{5%}	0.3***	0.14**	1.21**	0.03*	n.s.	n.s.	65.0*	45.9**	n.s.	31.0*
Calcareous chernozem										
Control	8.14	2.83	13.2	0.20	3.00	2.21	807	114	5905	165
Sewage sludge										
1%	8.05	2.84	16.2	0.21	3.11	2.14	932	324	5575	151
3%	7.66	3.23	15.0	0.24	6.96	126	1139	653	5391	141
LSD _{5%}	0.12**	0.41*	n.s.	0.02*	3.44*	45.2**	203*	337*	n.s.	15*
Compost										
1%	8.14	3.03	14.6	0.21	3.78	3.11	857	225	5393	192
3%	8.08	3.35	16.2	0.23	2.84	1.74	958	392	5810	244
LSD _{5%}	n.s.	0.18**	1.47*	0.01***	n.s.	n.s.	68*	35***	212**	28**

AL-ammonium-lactate soluble; *- $p < 0,1$; **- $p < 0,05$; ***- $p < 0,01$; n.s. – not significant

This shows that sewage sludge compost may improve OM quality on a poor acidic sandy soil but may degrade that of on a chernozem soil, because its organic compounds are less complex than the original humus molecules of the chernozem soil. Thus, in this case both sewage sludge and compost treatments have negative effect on OM quality.

Table 4. The effect of treatments on OM quality and C/N ratio of soils

	Acidic sand			Calcareous chernozem		
	Q	E ₄ /E ₆	C/N	Q	E ₄ /E ₆	C/N
Control	0.36	4.8	7.5	21.1	3.7	8.4
Sewage sludge						
1%	0.41	4.7	7.9	19.3	3.7	8.0
3%	0.41	4.8	6.8	16.7	3.9	7.8
LSD _{5%}	n.s.	n.s.	n.s.	2.67*	n.s.	n.s.
Compost						
1%	0.49	4.8	8.6	14.5	3.92	8.4
3%	0.62	5.1	8.3	10.2	4.08	8.5
LSD _{5%}	0.058**	0.25*	n.s.	1.52***	0.172*	n.s.

*- $p < 0,1$; **- $p < 0,05$; n.s. – not significant

On acidic sandy soil, the effect of the 1 % dose was more pronounced for the sewage sludge fertilization, which increased the dry air biomass production of the perennial ryegrass more than two and a half times, but the 3 % dose resulted only a slight further increase. In the case of compost application, the lower treatment dose resulted in a relatively moderate increase of 50%, which was further increased to more than twice the control over the 4-week growing period. The rapid, immediate stimulating effect of raw sewage sludge addition is due to its higher N content

and bioavailable element amounts compared to sludge compost. The N content of the plants increased significantly with the sewage sludge treatment, while the K and P contents did not show a consistent change (Table 5).

Table 5. The effect of treatments on biomass production (g D.M. per pot) and nutrient content of perennial ryegrass test plant

	Acidic sand				Calcareous chernozem			
	Biomass (g) D.M.	N m/m%	K mg·kg ⁻¹	P mg·kg ⁻¹	Biomass (g) D.M.	N m/m%	K mg·kg ⁻¹	P mg·kg ⁻¹
Control	0.72	2.81	42346	9257	1.48	2.80	55013	3292
Sewage sludge								
1%	1.94	5.02	26502	7797	2.23	2.23	46966	3972
3%	2.03	6.44	41108	9076	2.69	2.69	54673	5087
LSD _{5%}	0.36**	0.73**	5800**	n.s.	0.45**	0.45**	6491*	1098*
Compost								
1%	1.08	2.03	40050	7840	1.85	3.00	56319	4163
3%	1.63	2.10	47236	8106	2.29	3.56	62299	4638
LSD _{5%}	0.22**	0.48*	5194*	1331*	0.173**	0.37*	n.s.	937*

*- $p < 0,1$; **- $p < 0,05$; n.s. – not significant

On calcareous chernozem soil, trends in biomass yield were similar to those on the sand, i.e. sewage sludge application showed a strong effect already at 1 % rate while increasing compost doses caused a more gradual increase. The general yield increase was lower because on the more favourable chernozem soil the untreated control already produced higher yields. In terms of total element contents of perennial ryegrass, only the compost addition resulted in a significant increase in N levels. The P content showed an increase especially for soil with sewage sludge amendment.

Conclusions

With its higher pH, the compost raised the pH of the acidic sandy soil to a near-neutral range and significantly increased the soil OM content. At the same time, the sewage sludge application was much more favourable in terms of the mineral N content, and it provided a fast nitrogen source for the studied plants, which was also reflected in the plant biomass yield. Thus, for short-term crop production purposes, sewage sludge amendment might be preferable, whereas for long-term soil-based purposes, the compost addition may be ideal for acidic sandy soils. The calcareous chernozem had better original properties, so the stronger effect of sewage sludge fertilization proved to be more favourable for the increase of N and P contents of soil and plant biomass production. In order to better understand the effects of municipal sewage sludge and composted sludge, further studies are needed on composts prepared with different sewage sludge and green waste ratios added to soil types other than examined in this present study.

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In vitro bioaccessibility of Ca, K, Mg, Mn, Fe and Zn from wheatgrass juice

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Abstract

Due to its nutritional value, wheatgrass is increasingly used as dietary food supplement in the form of fresh wheatgrass juice (WGJ), powder or tablets. The aim of this research was to examine the difference in total and *in vitro* bioaccessible concentrations of K, Ca, Mg, Mn, Fe and Zn in fresh WGJ of 37 wheat genotypes. The results of this research indicates that a selection of genotype for cultivation of wheatgrass is important since genotype has significant effect on total and *in vitro* bioaccessible concentrations of examined elements. Also, the effect of antinutrients and enhancers of bioaccessibility should be taken into consideration when selecting genotypes for the cultivation of wheatgrass.

Keywords: bioavailability, antinutrients, functional food, biofortification

Introduction

It is well known that diet has a significant effect on human health. Nevertheless, many people's diet is irregular and incomplete which can cause a lack of certain nutrients in the body. Because of this, many choose to supplement their daily diet with synthetic or natural dietary supplements. Young wheat shoots, known as wheatgrass, are used as a natural dietary supplement in a form of fresh juice, powder or tablets.

Wheatgrass is rich in minerals (calcium, phosphorus, magnesium, potassium, zinc, boron and molybdenum), vitamins (A, C, E and B complex), and has a high content of amino acids (Padalia et al. 2010) and chlorophylls (Rana et al. 2011). Due to its nutritional quality, wheatgrass helps in the treatment and prevention of various diseases, such as high blood pressure, obesity, diabetes, and pancreatic and liver problems (Wigmore, 1985). Besides, WGJ facilitates treatment therapy for patients in the terminal phase of cancer (Dey et al., 2006) and it has an antiproliferative effect on different human carcinoma cell lines (Gore et al., 2017, Singh et al., 2012). Beside total nutrient concentrations, bioaccessibility and bioavailability of nutrients are also important. The definitions of bioaccessibility and bioavailability are different regarding the area of research. From the nutritional point of view, bioaccessibility is defined as the quantity of a compound that is released from its matrix in the gastrointestinal tract, becoming available for absorption. Bioaccessibility is often assessed by simulation of static *in vitro* digestion (drawbacks and strengths of *in vitro* simulation methods are given in Fernández-García et al., 2009) that includes simulation of oral, gastric and intestinal digestion.

The aim of this research was to evaluate the differences in total and *in vitro* bioaccessible concentrations of K, Ca, Mg, Mn, Fe and Zn in fresh WGJ of 37 wheat genotypes grown in controlled conditions.

Materials and methods

In this research 37 winter wheat genotypes, which are the part of gene collection of the Faculty of Agrobiotechnical Sciences Osijek, were tested for their total and *in vitro* bioaccessible concentrations of K, Ca, Mg, Mn, Fe and Zn in fresh WGJ. The seeds of the tested genotypes were stored in the seed storage chamber. Before sowing, the seeds were washed three times with autoclaved water for five minutes in order to disinfect the seed surface to prevent mildew occurrence. After the surface disinfection, the seeds were placed in jars, covered with a net, and placed

upside down so that any remaining water can drain. The jars with seeds were kept in a dark room for two days, with the occasional moistening of the seeds. After 48 hours seeds were sown in shallow plates filled with Brill substrate Bio start. Wheatgrass was grown for 12 days in a plant growth chamber with a day/night cycle of 12 hours at the temperature of 20 °C. On the twelfth day, leaves of wheatgrass were cut about 2 cm above the substrate surface and were used for making juice on a manual wheatgrass juicer BL-30. Freshly squeezed juice was used for simulation of *in vitro* digestion. Simulation of *in vitro* digestion has been carried out according to the Kiers et al. (2000). After the end of the simulation of digestion, samples were centrifuged at 4,000 rpm at 4 °C for 15 minutes, and supernatant has been transferred to 15 ml tubes and stored at -80 °C. Determination of the total concentrations of the elements in the WGJ has been done by the standard procedure for plant samples. WGJ samples (1 ml) were pipetted into the cuvettes and wet digested with 6 ml 65% (v/v) HNO₃ and 2 ml 30% H₂O₂ in microwave vessels (Kingstone and Lassie, 1986). The total concentrations and *in vitro* bioaccessible concentrations of K, Ca, Mg, Mn, Fe, and Zn has been determined by inductively coupled plasma – optical emission spectrometry (ICP-OES) technique. The percentage of bioaccessibility was calculated as follows: B (%) = (*in vitro* bioaccessible concentration * 100) / total concentration of element. The obtained results were analysed in the SAS 9.4 for Windows and Enterprise Guide 7.1. The experiment was set up according to completely randomized design with four replicates. The normality of distribution of tested variables was checked with Shapiro-Wilks test (p < 0.05). The effect of genotype on the total, *in vitro* bioaccessible and on the percentage of bioaccessibility of tested elements was tested with one-way ANOVA. Correlation between total and *in vitro* bioaccessible concentrations was obtained by Pearson's correlation coefficient.

Results and discussion

Total concentrations of examined minerals in fresh WGJ were decreasing in following order K > Ca > Mg > Mn > Fe > Zn. (Table 1). Similar ordering of elemental concentrations in wheatgrass has been found by Ghumman et al. (2017), Ozkose et al. (2016) and Kulkarni et al. (2006). In present research wheatgrass has been grown for 12 days in controlled conditions, on organic substrate without fertilizer. During that time, the root system of young plants has not been developed completely so the concentration of elements in wheatgrass depended mostly on the amount of elements that have been stored in grains. The accumulation of minerals in wheat grain is specific regarding the genotype (Clark, 1983, Sarić, 1981), so selection of genotypes with higher amount of minerals in grain will produce wheatgrass richer in minerals. This is supported by the results of analysis of variance that showed that examined genotypes differed significantly in total concentration of K, Ca, Mg, Fe and Zn in fresh WGJ (Table 1), indicating that the content of the minerals in the WGJ significantly depends on the genotype. Besides, duration of cultivation, precisely, the time of harvest had significant effect on the amount of specific element in WGJ (Kulkarni et al., 2006, Ozkose et al., 2016).

Table 1. Total concentrations of K, Ca, Mg, Mn, Fe and Zn (mg kg⁻¹) in fresh wheatgrass juice

Element	Min.	Max.	Mean	St. Dev.	C.V.	F value	p
K	1930	5408	3824	804	21	1.91	< 0.01
Ca	220.3	530.4	359.8	56.7	15.8	13.01	< 0.001
Mg	139.5	303.5	230.2	32.1	13.9	4.37	< 0.001
Mn	2.78	6.20	3.59	0.55	15.2	1.15	0.28
Fe	2.26	5.16	3.81	0.50	13.2	7.09	< 0.001
Zn	1.53	2.98	2.22	0.27	12.1	6.19	< 0.001

The total concentration of the elements in fresh WGJ is an important trait in the production of wheatgrass, but from the nutritional point of view, bioaccessibility of those elements is even more important. Similarly as with total concentrations, concentrations of elements after *in vitro* digestion of fresh WGJ were arranged in the same order as total concentrations: K > Ca > Mg > Mn > Fe > Zn (Table 2). Again, wheat genotypes differed significantly in Ca, Mg, Fe and Zn bioaccessible concentrations (Table 2), pointing the importance of choosing the right genotype for cultivation of wheatgrass. In comparison to the total concentrations, *in vitro* bioaccessible concentrations were more variable in the examined set of genotypes (Table 2).

Table 2. *In vitro* bioaccessible concentrations of K, Ca, Mg, Mn, Fe and Zn (mg kg⁻¹) from fresh wheatgrass juice

Element	Min.	Max.	Mean	St. Dev.	C.V.	F value	p
K	1088	6762	3589	1030	15.2	1.44	0.076
Ca	165.4	427.2	290.3	55.5	19.1	3.74	< 0.001
Mg	122	291.7	200.5	31	15.4	5.02	< 0.001
Mn	1.96	5.14	3.04	0.52	17.1	1.76	0.013
Fe	0.72	2.43	1.43	0.38	26.2	4.35	< 0.001
Zn	0.05	1.35	0.64	0.28	43.6	4.07	< 0.001

For example, variability of the Fe and Zn *in vitro* bioaccessible concentrations were 2 and 3.6 fold (respectively) higher compared to the variability of total Fe and Zn concentrations in the examined set of genotypes. Also, Fe and Zn had low correlation coefficient between total and *in vitro* bioaccessible concentrations (Table 3), where only 37 % of variability in bioaccessible concentrations of Fe and Zn, could be explained with variability of Fe and Zn total concentrations.

Table 3. Pearson's correlation coefficient between total and bioaccessible concentration of K, Ca, Mg, Mn, Fe and Zn in wheatgrass juice (n = 37)

	K	Ca	Mg	Mn	Fe	Zn
r	0.82	0.89	0.93	0.60	0.61	0.61
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

This results indicates that bioaccessibility of Fe and Zn is under the strong effect of other factors, most probably antinutrients, which were not under the scope of this research. Unlike K (88.4 %), Ca (78.7 %), Mg (85.7 %) and Mn (83.4 %) which had high percentage of *in vitro* bioaccessibility, the *in vitro* bioaccessibility of Fe (37.6 %) and Zn (28.5 %) was much lower (Figure 1). Kulkarni et al.(2007) found that the *in vitro* bioaccessibility of Zn, Fe and Mn are under the effect of growing conditions and are in range between 42 -48 % for Zn, 45 – 65 % for Fe and 52 – 69 % for Mn in fresh wheatgrass. Same authors confirmed that bioavailability of those elements is higher in fresh wheatgrass in comparison to commercial wheatgrass tablets. Furthermore, percentage of *in vitro* bioaccessibility of Fe (F = 3.07; p < 0.01) and Zn (F = 2.82; p < 0.01) was under the effect of genotype, while genotype had no significant effect on the percentage of bioaccessibility of other examined elements (K - F = 1.26; p = 0.178; Ca - F = 0.89; p = 0.65; Mg - F = 0.88; p = 0.65; Mn - F = 0.96; p = 0.54).

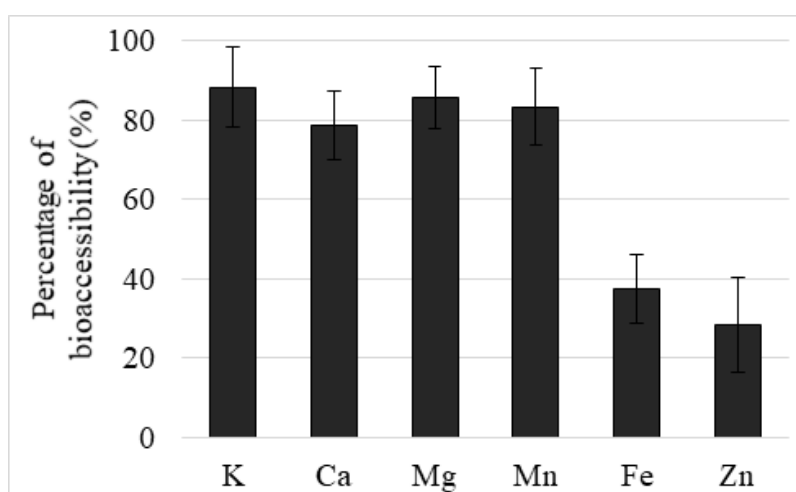


Figure 1. Percentage of *in vitro* bioaccessibility of K, Ca, Mg, Mn, Fe and Zn from wheatgrass juice

Conclusions

Wheatgrass could be a valuable source of minerals. The elemental composition of wheatgrass is highly dependent on growing conditions and, as it is shown in this research on genotype used for the cultivation of wheatgrass. Wheat genotypes differ significantly in the total and *in vitro* bioaccessible concentrations of K, Ca, Mg, Fe, and Zn in fresh wheatgrass juice. On the other hand, only Fe and Zn percentage of *in vitro* bioaccessibility were under the effect of genotype. Percentage of *in vitro* bioaccessibility of K, Ca, Mg and Mn is high and indicates a potential of wheatgrass juice as a dietary source of those elements. However, the percentage of *in vitro* bioaccessibility of Fe and Zn is much lower, indicating that further research is needed to establish which factors limiting the bioaccessibility of Fe and Zn and what measures could be used to alleviate it.

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In vitro biodostupnost Ca, K, Mg, Mn, Fe i Zn iz soka pšenične trave

Sažetak

Zbog svoje hranjive vrijednosti, pšenična trava se sve češće koristi kao dodatka prehrani u obliku svježeg soka, praha ili tableta. Ovo istraživanje, provedeno je s ciljem ispitivanja razlika u ukupnim i *in vitro* bioraspoloživim koncentracijama K, Ca, Mg, Mn, Fe i Zn u soku pšenične trave 37 sorata pšenice. S obzirom da genotip ima značajan utjecaj na ukupne i *in vitro* biodostupne koncentracije elemenata, izbor odgovarajućeg genotipa pšenice za uzgoj pšenične trave je izuzetno značajan. Uz to, antinutrijenti i promotori bioraspoloživosti značajno utječu na bioraspoloživosti pojedinih elemenata, te bi prilikom odabira genotipova za uzgoj pšenične trave i njihov sadržaj trebalo uzeti u obzir.

Ključne riječi: bioraspoloživosti, antinutrijenti, funkcionalna hrana, biofortifikacija

Carcinogenic hazard index of heavy metals from agricultural soil in the southeastern part of Bosnia and Herzegovina

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Abstract

The aim of the paper was to determine the carcinogenic risk for the health of adults and children by oral, inhalation and dermal intake of heavy metals via soil into the body. The survey included three locations in the south part of Herzegovina, namely Čapljina, Stolac, and Mostar. The carcinogenic risk was calculated for carcinogenic and potentially carcinogenic elements (Pb, Co, Cd, and Ni). The US Environmental Protection Agency (USEPA) considers carcinogenic risk in the range of 1×10^{-6} to 1×10^{-4} to be acceptable. By calculating the carcinogenic hazard index, the values determined for oral and inhalation intake, and dermal contact of the metals through the soil for adults and children were within the limit values that USEPA considers acceptable.

Keywords: Heavy metals, carcinogenic risk, soil, oral intake, inhalation, dermal contact

Introduction

Contamination of agricultural soil is caused by the entry of harmful substances by water, air or their accumulation on or in the soil at concentrations above the permitted levels. Hazardous substances include: heavy metals (cadmium, mercury, nickel, cobalt, lead, chromium, zinc, copper, arsenic), polycyclic aromatic hydrocarbons, as well as other harmful substances introduced into the soil and which, due to improper use or application in large quantities at an inappropriate time can lead to a variety of adverse effects on the environment. The use of mineral and organic fertilizers (Cu, As) and the use of processed sewage sludges (Cu, Cd, Fe, Pb) contribute to the pollution of agricultural soil by heavy metals (Gimeno-Garcia et al., 1996). In the second half of the last century, lead - arsenate was widely used as an insecticide, while today copper and inorganic-organic fungicides based on tin, mercury, manganese, and zinc are used (Đurišić - Mladenović, 2012). Exposure to metals can result in adverse health effects, which is why European and international food safety agencies define a set of health-based limit values and a risk assessment methodology to protect human health. The risk assessment methodology for chemical contaminants is defined by WHO (World Health Organization), US EPA (Environmental Protection Agency) and EFSA (European Food Safety Authority) (Dorne et al., 2011) Absorption of heavy metals into the body cannot be completely avoided, but when body deposits can cause very serious health problems (Singh, et al. 2003).

Material and method

This research included an analysis of the total form of six heavy metals (Pb, Co, Cu, Cd, Zn, and Ni) in the soil according to the Guidelines on Determination of Allowed Hazardous Substances in Soil and Methods of Testing (FBiH Official Gazette, No. 72/09). Soil sampling was conducted on two depths (0-30 cm and 30-60 cm) at three locations (Mostar, Stolac, Čapljina) in 2015 and 2016. A total of 32 samples were collected by random sampling (12 samples from Mostar, 12 samples from Čapljina and 10 samples from Stolac) on which two cultivars of nectarine were cultivated. After that, the samples were submitted to the Agropedological Institute in Sarajevo for analysis of the total form of heavy metals.

Determination of heavy metals content in soil

Samples for instrumental analysis of heavy metal content in soil were prepared to utilize an aqua regia, and then content was determined in the extract by atomic absorption spectrometry (AAS). The extraction of heavy metals in the aqua regia solution was performed according to the international standard ISO11464. Table 1 represents maximum permitted values of six investigated heavy metals in soil according to Official Gazette (No. 72/09).

Table 1. Limit values for individual heavy metals in soil according to Official Gazette of FBiH, No. 72/09

Element	Limit values (mg/kg)		
	Sandy soil	Silty loam soil	Clay soil
Cadmium (Cd)	0.5	1	1.5
Copper (Cu)	50	65	80
Lead (Pb)	50	80	100
Zink (Zn)	100	150	200
Cobalt (Co)	30	45	60
Nickel (Ni)	40	40	50

Assessment of carcinogenic risk to human health

Human health risk assessment is a procedure used to evaluate the health effects that may result from exposure to carcinogenic and non-carcinogenic chemicals. The risk assessment process consists of four basic steps: hazard identification, exposure assessment, toxicity (dose-response) and risk characterization (USEPA, 2011). Hazard identification aims to investigate the chemicals present at any site, their concentrations, and spatial distribution. Pb, Cd, Co, Ni, Cu, and Zn have been identified in the test field as potential hazards to the community (Šukalić et al. 2018). In the case of exposure assessment, a specific approach characteristic for human exposure to agricultural soil was applied, taking particularly care of the different exposure rates for children and adults (usually expressed as exposure factors). In addition, the magnitude of exposure the intake or dose (consumed or inhaled amount) of contaminated soil is almost always different for different individuals. Therefore, the risk was calculated for the lifetime exposure, the total exposure to a substance that a human would receive in a lifetime – usually assumed to be 70 years. All other parameter that may be site characteristic were taken to be constant through the whole calculating procedure for all elements and all sites, since their importance becomes less significant in case of the lifetime exposure approximation. The purpose of exposure assessment is to estimate the intensity, frequency, and duration of human exposure to environmental pollution. In the study, exposure estimation is performed by calculating the acceptable daily intake (ADI) of heavy metals due to swallowing, inhalation and dermal contact between adults and children in the study area. Adults and children were separated due to their behavior and physiological differences. Given the various adverse effects of heavy metals on human health, the corresponding carcinogenic risks were calculated for children and adults by the USEPA (1989) risk assessment model:

$$ADI_{ing} = \frac{C \times IR \times EF \times ED \times CF}{BW \times AT} \quad ADI_{inh} = \frac{C \times IR_{air} \times EF \times ED}{PEF \times BW \times AT} \quad ADI_{der} = \frac{C \times SA \times FE \times ABS \times EF \times ED \times CF}{BW \times AT}$$

where ADI_{ing}, ADI_{inh}, ADI_{dermal} are chronic daily intakes or doses administered orally (mg/kg/d), inhalation (mg/m³ for non-cancerous and g/m³ for carcinogenic elements) and dermal route (mg/kg/d). C concentration of heavy metals in mg/kg in soil, IR in mg/d ingestion factor, IR_{air} in m³/d inhalation factor, EF in days/years exposure frequency, ED duration of exposure per year, BW body weight in kg, AT period in to which the average dose is expressed in days, SA skin area exposed in cm², FE ratio of dermal exposure, AF soil adhesion factor for skin in mg/cm², ABS is dermal absorption factor, CF chronic conversion factor in kg/mg.

In table 2 and 3 are presented exposure parameters for different routes of heavy metal uptake from soil for children and adults but also cancer slope factors for calculation risk assessment models.

Tabel 2. Exposure parameters for different routes of heavy metal uptake from soil for children and adults (according to Kamunda et.al, 2016)

Parameters	Units	Definition	Values	
			Children	Adults
ABS	--	Dermal absorption factor	0.1	0.1
AF	mg/cm ²	Soil adhesion factor for skin	0.2	0.07
BW	Kg	Average weight	15	70
ED	Year	Exposure time	6	30
EF	d/year	Exposure frequency	350	350
FE	--	dermal exposure ratio	0.61	0.61
IngR	mg/d	Soil ingested factor	200	100
IR air	m ³ /d	Inhalation factor	10	20
SA	cm ² /event	Exposed skin surface	2800	5700
ATca	D	Average time for cancerous hazards	70 × 365	
CF	kg/mg	Calculation factor	10 ⁻⁶	
PEF	m ³ /kg	Soil particulate emission factor - air	1.36 × 10 ⁹	

For carcinogens, the risk is estimated as the incremental likelihood that an individual will develop cancer during life, as a result of exposure to a potential carcinogen. The equation for calculating lifetime cancer risk is:

In which Risk is the likelihood that an individual will develop cancer throughout a lifetime due to an individual metal introduced into the body. The ADI (mg/kg/day) represents the average daily intake, the CSF (mg/kg/day) factor for the carcinogenic calculation, k represents the heavy metal and n the number of heavy metals.

Tabel 3. CSF for different heavy metals

Element	Cancer slope factor (mg/kg)			
	Oral CSF	Inhalation CSF	Dermal CSF	Cancerogenic classification by USEPA ^c
Cadmium(Cd)	3,8x10 ^{-1a}	6,3 ^a		1
Copper (Cu)				D
Lead (Pb)	8,5x10 ^{-3a}	4,20x10 ^{-2a}	8,5x10 ^{-6a}	B2
Zink (Zn)				D
Cobalt (Co)		9,8 ^b		B2
Nickel (Ni)		9,1x10 ^{-1a}		B2

a) USEPA, 2012

b) Kamunda et al., 2016.

c) 1 – proven cancerogenic to humans, B2 – possible cancerogenic to humans, D –no cancerogenic effects

Results and Discussion

The concentration of heavy metals at locations

The average concentrations of heavy metals at the Čapljina site in increasing range were in order: Cd (0.75 mg/kg) < Co (31.75 mg/kg) < Ni (36.45 mg/kg) < Cu (36.45 mg/kg) < Pb (58.35 mg/kg) < Zn (116.1 mg/kg); on site Stolac: Cd (0.8 mg/kg) < Ni (32.55 mg/kg) < Co (35.4 mg/kg) < Pb (38.85 mg/kg) < Cu (49.5 mg/kg) < Zn (99.25 mg/kg); and at the Mostar: Cd (0.7 mg/kg) < Co (28.35 mg/kg) < (28.65 mg/kg) < Cu (31.85 mg/kg) < Pb (39.85 mg/kg) <

Carcinogenic hazard index of heavy metals from agricultural soil in the southeastern part of Bosnia and Herzegovina

Zn (79.9 mg/kg). From the above it can be seen that the concentration of Cd exceeds the limit values at the locations. Xiao-San Luo et al. (2012) state average values of heavy metals content in soil Cd 0.04–1.25 (0.3), Co 1.3–10 (4.1), Cr 3.7–27 (14), Cu 4.5–97 (26), Mn 184–746 (347), Ni 0.8–24 (8.4), Pb 10–270 (36), and Zn 40–233 (100) in mg/kg.

Carcinogenic risk to the health of adults and children

Tables 4 and 5 list the carcinogenic risk values for adults and children in all locations. USEPA considers carcinogenic risk in the range of 1×10^{-6} to 1×10^{-4} to be acceptable for regulatory purposes. Calculating the carcinogenic hazard index the values determined for oral intake of Cd, and oral intake and dermal contact of Pb by soil for adults and children were close to the threshold recommended by USEPA. The values of possible carcinogenic risk by inhalation for Ni and Co were also calculated and found to be of no concern for carcinogenic effects.

In a study by Kamunda et al. (2016), the carcinogenic risk was 1.7×10^{-4} , suggesting that one person may be affected of 5882 adults. Besides, from 2725 children one child may suffer (3.67×10^{-4}). These carcinogenic risk values were more than acceptable. Huabin et al. (2019) reported a total carcinogenic index for Cr, Cu, Zn, As, Cd, Pb, and Hg for adults ($2.80 \pm 0.79 \times 10^{-5}$) and children ($1.36 \pm 0.39 \times 10^{-5}$).

Table 4. Calculated carcinogenic risk values for adults

Location		Pb	Co	Cd	Cu	Ni	Zn
Čapljina	ingestion	2.91E-06		2E-06			
	inhalation	4.28E-14	2.69E-08	4E-10		2.86E-09	
	dermal	3.50E-06					
	total	6.41E-06		2E-06			
Stolac	ingestion	1.94E-06		2E-06			
	inhalation	2.85E-14	3.00E-08	4E-10		2.56E-09	
	dermal	2.33E-06					
	total	4.27E-06		2E-06			
Mostar	ingestion	1.99E-06		2E-06			
	inhalation	2.92E-14	2.40E-08	4E-10		2.25E-09	
	dermal	2.39E-06					
	total	4.38E-06		2E-06			

Table 5. Calculated risk values for children

Location		Pb	Co	Cd	Cu	Ni	Zn
Čapljina	ingestion	5.44E-06		3.12E-06			
	inhalation	2.00E-14	1.25E-08	1.90E-10		1.34E-09	
	dermal	4.59E-06					
	total	1.002E-05		3.12E-06			
Stolac	ingestion	3.62E-06		3.33E-06			
	inhalation	1.33E-14	1.40E-08	2.03E-10		1.19E-09	
	dermal	3.05E-06					
	total	6.67E-06		3.33E-06			
Mostar	ingestion	4.85E-06		3.33E-06			
	inhalation	1.78E-14	1.15E-08	2.03E-10		1.23E-09	
	dermal	3.13E-06					
	total	7.99E-06		3.33E-06			

Conclusion

The study results show that cadmium concentrations exceed the maximum limit values at all locations. The assessment of possible carcinogenic risk has revealed that the risk of cancer is within the limits recommended by USEPA (10⁻⁶ to 10⁻⁴), and by other regulatory agencies. Although all values of carcinogenic effects do not exceed regulatory values, monitoring of agricultural soil is necessary to minimize the risks. Generally, the present-day exposure risk assessment can be used as a screening method to identify important routes of exposure and to determine the urgency of soil remediation action.

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Portable X-ray fluorescence as a tool for characterization of nutrient status in soil

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Abstract

The goal of this research was to determine the total concentrations of Ca, Fe, Cu and Mo in 43 referent soil samples by means of pXRF method and to determine the accuracy and precision of pXRF analyser. Results indicated that a Vanta handheld (portable) XRF analyser is capable of measuring Ca, Fe and Cu in restricted ranges of concentrations (2441 -219000 mg Ca/kg; 3840 -59610 mg Fe/kg; 25 -158 mg Cu/kg) with excellent (RPD 0-10 %) to good (RPD 10-25 %) accuracy and acceptable precision (RSD < 20 %) especially in clay soil samples. Molybdenum was not detected although several soil samples had concentrations of Mo higher than LOD (> 2 mg/kg). Along with extension of time analysis up to 120 seconds and same additional calibrations which will take in consideration matrix characteristics, pXRF will become an accurate and indispensable tool for nutrient and pollutant determination in soils because of numerous advantages including speed and low cost analysis.

Keywords: pXRF, calcium, iron, copper, molybdenum

Introduction

Soils are a major source of nutrients that are needed for plant growth. A higher plant require inorganic forms of essential nutrients and among of three criteria which must be met in order to consider an element as plant nutrient Mengel and Kirkby (1987) adduce that element has to be directly involved in plant nutrition, for example as a constituent of an essential metabolite. The principal criterion is that the element must be required for a plant to complete its life cycle (Barker and Pilbeam, 2007). Seventeen elements are considered to have met those criteria, three of them are derived from air or water (C, H, O), while other 14 are obtained from soil or nutrient solutions (N, P, K, Ca, Mg, S, Fe, Mn, Cu, B, Zn, Mo, Cl and Ni). Among these fourteen several of them (Cu, Ni, Zn and Mo) in higher amounts and certain conditions in soils are considered as pollutants (Official gazette, 71/19).

In those days, rapid chemical analysis of soils and their accuracy are essential criteria for method selection. Although most of analytical methods for characterization of nutrient status in soil involve extraction of elements and results of those measurements represent amount of available nutrients, techniques which combines speed, real-time, simultaneous multi-(total) element analysis become widely used in soil science for characterization of fertility and pollution status in soil (Stockmann et al., 2016; Wan et al., 2019). One of these techniques is portable X-ray fluorescence (pXRF) spectrometry which offer many advantages over traditional techniques including: no destructiveness in evaluating solid samples, portability, wide dynamic range of elemental quantification, little/no need for sample pre-treatment, simplicity, relatively low detection limits and relatively low cost (Weindorf et al. 2014). Due to all of these advantages pXRF spectrometry is applied in many research areas, such as: soil science, geology, environmental science, climate change, industry, mining, cultural heritage, forensic and clinical investigations (West et al., 2013). Among of all advantages, several disadvantages were also reported, especially attenuation of the pXRF signal with increasing content of organic matter and soil moisture (Ravansari and Lemke, 2018; Ge et al., 2005).

The goal of this research was to determine the concentrations of calcium (Ca), iron (Fe), copper (Cu) and molybdenum (Mo) in referent soil samples by means of pXRF method and to determine the accuracy and precision of pXRF analyser using the referent soil samples.

Materials and method

The research included the analysis of 43 referent soil samples which texture varied from sandy to clayey mostly sampled in the Netherlands, but also in Switzerland, Spain, the Philippines and Saudi Arabia. Soil samples were collected from 2009 to 2019 as part of participation of Analytical laboratory of Department of General Agronomy in worldwide Wageningen Evaluating Programme for Analytical Laboratories (WEPAL) conducted by Wageningen University (Netherlands) within International Soil-analytical Exchange (ISE) program.

Before the pXRF measurement, dried, ground and homogenized soil samples were transferred in measuring cups and measurement was conducted according to loose powder method (Takahashi, 2015) which involves a simple “point and shoot” technique.

A Vanta™ handheld (portable) XRF analyser C Series (Olympus, Waltham, MA, USA, 2019) equipped with a silicon drift detector and an Rh anode X-ray tube (50 keV) as excitation source was used during this research. Measurement principle is based on excitation of soil sample by X-ray photons generated from Rh anode X-ray tube which result in excitation of secondary X-ray photons characteristic for each atom present in the soil sample. After the processing of resulted X-ray spectrum by Si-drift detector concentrations of analysed elements were displayed in ppm (mg/kg). The detection limits of the Vanta pXRF analyser were: 30 mg/kg for Ca, 15 mg/kg for Fe, 4 mg/kg for Cu and 2 mg/kg for Mo. When concentration was lower than above detection limits, the displaying window showed LOD (limit of detection).

Each soil sample was measured 3 times for 30 seconds. Statistical data analysis included the calculation of: median, relative standard deviation (RSD), Pearson correlation coefficient (r), coefficient of determination (R^2) and root mean square error (RMSE). Linear regression was used to correlate theoretical and measured concentrations of three nutrients (Ca, Fe and Cu). The accuracy was estimated by the relative percent difference (RPD) between the theoretical concentration in the referent material (RM value) and the concentrations measured by pXRF (pXRF value) as follow: $(C_{\text{pXRF}} - C_{\text{RM}}) / C_{\text{RM}} \times 100$.

The theoretical values of the nutrient content represent the median of the results achieved in each year of inter-laboratory comparison for each element with respect to the number of participants and they were detected by the means of XRF and ICP-MS techniques.

Results and discussion

Figure 1 represents regression models of relationship between measured (pXRF values) and theoretical concentrations (RM values) for Ca, Fe and Cu, but not for Mo. As it is already stated detection limit for Mo was 2 mg/kg. In 37 of 43 analysed referent soil samples theoretical concentrations of Mo were lower than 2 mg/kg and they were in range from 0.242 mg/kg to 1.84 mg/kg and due to that fact not detectable. While in six remaining soil samples theoretical values varied from 2.21 mg/kg to 4.63 mg/kg but still not detectable in this measuring conditions. For example, each measurement lasted 30 seconds and considering these results it is recommendable to repeat measurements with increase time of analysis in those six samples with Mo content higher than 2 mg/kg. According to US EPA (2007) shorter source measurement times (30 seconds) are generally used for initial screening and hot spot delineation while longer measurement times (up to 300 seconds) are typically used to meet higher precision and accuracy requirements. The detection limit is usually dependent on measurement time or counting-time (time of sample exposure to excitation source) (Glanzman and Closs, 2007) and it decreases by half as the counting time is increased by a factor of four. All of that leads to conclusion that measurement time for low Mo concentrations should be set up for minimum 120 seconds. However, Kilbride et al. (2006) reported that precision and accuracy was not gained by extension of analysis time beyond 120 s.

Theoretical concentrations of other selected nutrients in referent soil materials (RM values) were in range from 300 to 219000 mg/kg for calcium, from 2105 to 59610 mg/kg for iron and from 5.4 to 158 mg/kg for copper (Figure 1), while values measured by pXRF analyser (pXRF values) varied from 546.5 to 226133 mg/kg for calcium, from 3227 to 52174 mg/kg for iron and from 11 do 182.5 mg/kg for copper. Results of regression analysis reveal that within presented concentration range, slope greater than 1 determined for Cu (1.1407) indicates that values obtained by pXRF measurements were generally higher than theoretical RM values, while a slope less than 1 determined for Fe (0.8663) demonstrates that values obtained by pXRF measurements were generally lower than theoretical RM values. Slope value of 1.0208 reveals that concentrations of Ca determined by pXRF were generally consistent with

theoretical values in referent material (RM value). The coefficients of determination (R^2) for Ca, Fe and Cu were 0.9869, 0.9947 and 0.9862, respectively, which means that more than 98 % of observed variation for all three nutrients can be explained by linear models presented on figure 1. High positive correlations were also observed between measured and theoretical values according to presented coefficients of correlation (r) which varied from 0.9931 (Cu) to 0.9974 (Fe).

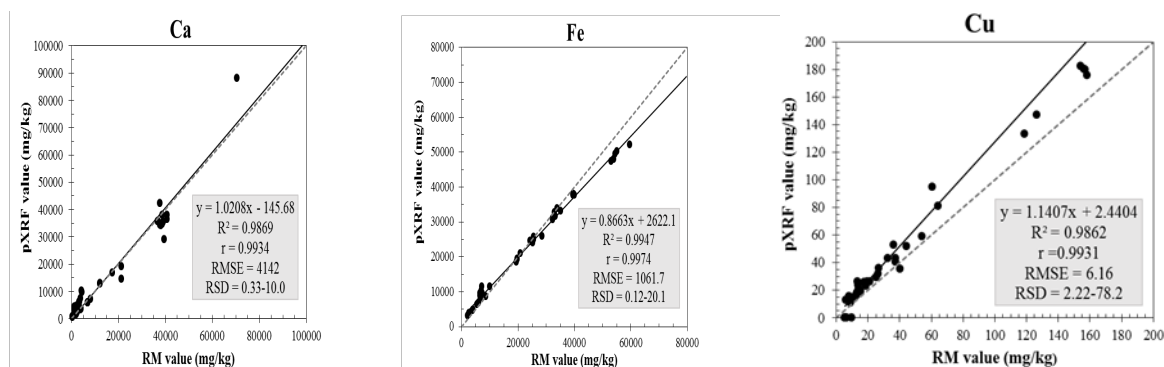


Figure 1. Regression of pXRF measurements of nutrient concentrations against RM values along with Pearson correlation coefficients (r), root mean square errors (RMSE) and RSD values

The relative standard deviation (RSD) was used to assess precision of analyser performance. For pXRF data to be considered adequately precise, the RSD should not be greater than 20 % (US EPA, 2007). In terms of Ca and Fe from measured data it is evident that precision was acceptable (0.33-10.0 and 0.12-20.1 respectively), while for Cu RSD values were ranged from 2.22 % to 78.2 %. It has to be point out that in 31 of 43 analysed samples, mainly clay samples with theoretical range of Cu from 12.4 to 158 mg/kg, RSD values were lower than 20 %, while in 12 analysed referent material mostly sand samples with content of Cu < 9 mg/kg, RDS was greater than 20 %. Durance et al. (2014) explained that poorer results in precision can be related to the fact that the concentrations of measured elements are close to the LOD of pXRF analyser. Except for precision, RSD values along with R^2 values can be used to allocate a data quality level to each presented relationship. According to US EPA (1998) the highest quality level, definitive data, are data with $R^2 = 0.85$ to 1.0 and $RSD \leq 10$ %, second level are quantitative data with $R^2 = 0.7$ to 1.0 and $RSD \leq 20$ % while third level are qualitative data with $R^2 < 0.7$ and $RSD > 20$ %. All together indicate that definitive level data was achieved across the entire range of samples tested for Ca and quantitative level for entire range of Fe. For Cu quantitative data level was achieved in restricted range from 12.4 to 158 mg Cu/kg ($R^2 = 0.9862$, $RSD \leq 20$) and qualitative level for Cu content < 9 mg/kg ($RSD > 20$ %). For example, quantitative data provide confirmed analyte identification with relatively imprecise quantification (US EPA, 1998). These results are comparable with results of other authors (Kilbride et al., 2006, Potts et al., 1995, Rouillon and Taylor, 2016). Data quality level is partly related to the accuracy of measurement. In terms of Ca measurement (Figure 2a), 25 (58%) referent materials mostly clay soil samples had an excellent to good accuracy in theoretical range of Ca from 2441 to 219000 mg/kg while 34 (79%) measured referent soil samples in the range from 3840 to 59610 mg Fe/kg had an excellent to good accuracy (Figure 2b). Fair and poor accuracy was determined in the remaining samples especially due to fact that analyser overestimated Ca and Fe at low and high concentrations in sandy soils. In the range from 25 to 158 mg Cu/kg results indicated an excellent to good accuracy in 17 (40%) clay referent soil samples (Figure 2c). Fair and poor accuracy was determined in restricted range of Cu (< 20 mg/kg) in 60% of investigated samples where overestimation of concentrations was observed for sandy but also in clay soils. Although Kilbride et al. (2006) reported that particle size (<2 μm , 2-63 μm , >63 μm) did not have a significant influence on the pXRF performance, these results indicate that accuracy decrease with increment of particle size and it was lower in sandy soils. Glanzman and Closs (2007) explained that as the grain size increases, the potential variability increases geometrically because of both grain size and lack of true homogeneity of the sample exposed to the x-ray beam. Also, determined overestimations were result of fact that X-rays that were returned to the detector are derived from all matrix material include in measured soil sample, including the larger-grained quartz, feldspar, lithic minerals, metal complexes, and organics (US EPA, 1998).

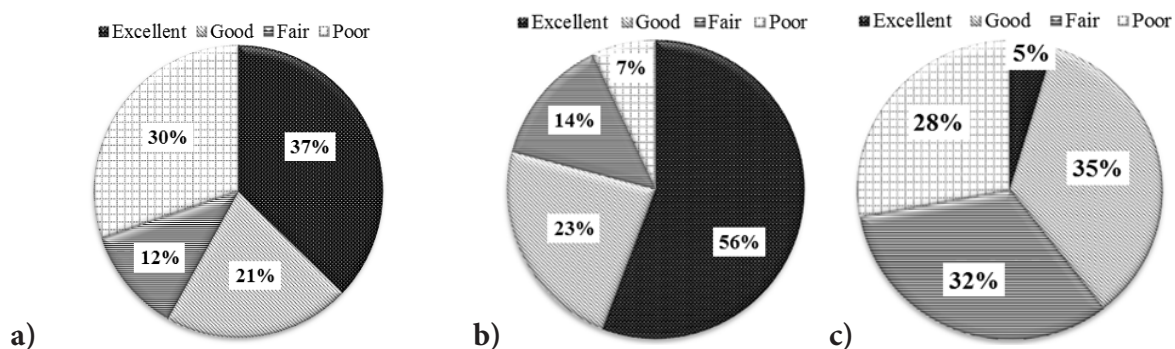


Figure 2. Accuracy of pXRF measurements according to relative percent difference (RPD) for calcium (a), iron (b) and copper (c) due to following categories: excellent (RPD 0-10 %), good (RPD 10-25 %), fair (RPD 25-50 %) and poor (RPD > 50 %)

Conclusions

Results indicated that a Vanta handheld (portable) XRF analyser is capable of measuring calcium, iron and copper in restricted ranges, but for soil suitable, concentrations with excellent to good accuracy and acceptable precision especially in clay soil samples. Molybdenum was not detected although several soil samples had concentrations of Mo higher than LOD (> 2 mg/kg). Along with extension of time analysis up to 120 seconds and same additional calibrations which will take in consideration matrix characteristics (particle size), pXRF will become an indispensable tool for nutrient and pollutant determination in soils because of numerous advantages including speed and low cost analysis.

Acknowledgments

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Prijenosna reagenska fluorescencija kao alat u karakterizaciji opskrbljenosti tla hranivima

Sažetak

Cilj ovog istraživanja bio je utvrditi ukupne koncentracije Ca, Fe, Cu i Mo u 43 referentna uzorka tla pomoću pXRF metode i odrediti točnost i preciznost pXRF analizatora. Rezultati su pokazali da je Vanta ručni (prijenosni) XRF analizator sposoban izmjeriti Ca, Fe i Cu u ograničenom rasponu koncentracija (2441 mg Ca/kg do 219000 mg Ca/kg; 3840 mg Fe/kg do 59610 mg Fe/kg; 25 mg Cu/kg do 158 mg Cu/kg) s izvrsnom (RPD 0-10 %) do dobrom (RPD 10-25 %) točnošću i prihvatljivom preciznošću (RSD < 20 %) osobito u glinenim tlima. Molibden nije detektiran iako je u nekoliko uzoraka tla koncentracija Mo bila veća od granice detekcije (> 2 mg / kg). Uz produljenje vremena analize na 120 sekundi i dodatnu kalibraciju koje će uzeti u obzir i karakteristike analiziranih uzoraka, pXRF analizator bi zbog brojnih prednosti, uključujući brzinu i niske troškove analize, mogao poslužiti kao točan i neophodan alat za određivanje hraniva ali i onečišćujućih tvari u tlu.

Ključne riječi: pXRF, kalcij, željezo, bakar, molibden

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Značaj troškova mineralnih gnojiva za cijenu koštanja i isplativost proizvodnje lubenice

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Sažetak

U radu je analizirana financijska isplativost u proizvodnji lubenica na području dalmatinske zagore. Prikazani su ukupni troškovi pripreme zemljišta, sadnje lubenica, te kalkulacija pokrića varijabilnih troškova pri redovitoj proizvodnji lubenica. Posebno su analizirani troškovi mineralnih gnojiva i njihov utjecaj na prinose. Svi podaci za ovaj rad su dobiveni na primjeru Obiteljskog poljoprivrednog gospodarstva, koje se već duži niz godina bavi uzgojem lubenica i koje ima iznad prosječne prinose. Cilj rada je istražiti utjecaj troškova mineralnih gnojiva na cijenu koštanja lubenice kao i na isplativost njene proizvodnje. Pri obradi podataka korištena je metoda kalkulacije pokrića varijabilnih troškova. Dobiveni rezultati ukazuju da je proizvodnja lubenica ekonomski opravdana s ekonomičnošću od 1,81, stopom rentabilnosti poslovanja od 44,9 i cijenom koštanja po jednom kilogramu od 0,08 €.

Ključne riječi: proizvodnja lubenica, investicije, ekonomska opravdanost.

Uvod

Lubenica (*Citrulus vulgaris*, sin. *Citrulus lanatus*) je pripadnik familije tikava, tj. Cucurbitaceae, porijeklom iz Centralne i Južne Afrike. U perifernim oblastima pustinje Kalahari i danas se mogu naći divlje forme ove vrste, koje se brzo razvijaju nakon obilnih kiša. Kao kulturna biljka prvo se počela gajiti u Egiptu, zatim u Indiji, a u X vijeku prije nove ere počeli su je uzgajati Kinezi. Stari Grci i Rimljani bili su upoznati sa lubenicama početkom nove ere (Lešić, 2002.) Značajnije širenje lubenice na europskom kontinentu počinje u XVI vijeku.

U komercijalnoj proizvodnji lubenica koriste se gotovo isključivo hibridi. Čjenjeniji su hibridi tanje kore, slađeg okusa i intenzivno crvene boje unutrašnjosti ploda (Matotan, 2004.)

Prema Statističkom ljetopisu Republike Hrvatske iz 2017. godine, domaća proizvodnja dinja i lubenica na površinama od 830 ha je iznosila 23,4 tisuće tona, sa prosječnim prinosom od 28,2 t/ha, što je blizu svjetskog prosječnog prinosa od 30,8 t/ha. Za zadovoljenje domaćeg tržišta, te godine, uvezeno je još oko 15 tisuća tona.

Što se tiče prinosa i kvaliteta najpogodniji lokaliteti za proizvodnju lubenica u Hrvatskoj su u donjoj Neretvi, međutim zbog vrlo sličnih agroekoloških uvjeta lokaliteti Vrgoračkog polja su također izuzetno povoljni za tu vrstu proizvodnje. Prema dostupnim podatcima za Vrgorac (DHMZ za razdoblje 1981-2005. god.), srednja mjesečna temperatura najhladnijeg mjeseca (siječanj) je 5,1 °C, a najtoplijeg (srpanj) 24,6 °C, dok je srednja godišnja temperatura 14,0 °C. Na postupno smanjenje maritimnog utjecaja ukazuje godišnja amplituda (18,9 °C) te razmjerno velika apsolutna maksimalna (40,5 °C), odnosno apsolutna minimalna temperatura zraka (-12,0 °C). Kao najtopliji dijelovi ističu se jugoistočni dijelovi vrgoračkog područja, odnosno zavale krških polja. U toplijim područjima, a naročito na Mediteranu, uzgojem lubenica može se postići dobar ekonomski rezultat (Lešić, 2002.). Povoljnost agroekoloških uvjeta uz primjenu suvremenih tehnoloških postupaka proizvodnje omogućuje razvoj intenzivne proizvodnje lubenica na području Hercegovine (Spužević i Sefo, 2018.).

Suvremena proizvodnja lubenica iziskuje visoke troškove koje je moguće pokriti jedino visokim prinosima, te se za tržište gotovo isključivo koriste hibridi (Matotan, 2006.). Zbog ekonomičnosti skupog hibridnog sjemena hibridni kultivari se uzgajaju iz presadnica. (Lešić i sur., 2004.) Za presađivanje su najbolje biljke sa otvorenim kotiledonima i prvim pravim listom (Lešić, 2002.). U intenzivnom uzgoju na manjim površinama korisno je ograničiti broj plodova po biljci na 3 do 4, a svi ostali se odstranjuju da bi se odabrani plodovi bolje razvili (Lešić, 2002.). Lubenica vrlo dobro

podnosi sušu, ali previše vode smanjuje kvalitetu prinosa - sitni plod, debela kora, smanjen sadržaj šećera (Matotan, 2004.). Radi boljeg toplinskog djelovanja tlo se pokriva polietilenskom folijom te se na taj način ubrzava sazrijevanje plodova. Japickino i Gagliano (1986.) su proučavali utjecaj malčiranja crnog i prozirnog PE filma u proizvodnji lubenica, te su utvrdili značajno povećanje ranije zrelosti i ukupnog prinosa i kod crnog i kod prozirnog PE filma. Dušična gnojiva temelj su za dobivanje visokih prinosa. Osim toga, primjena dušičnih gnojiva utječe i na količinu vitamina, minerala, proteina i esencijalnih aminokiselina u biljci (Lešić, 2002.).

Cilj ovoga rada je prikazati ekonomsku isplativost proizvodnje lubenica i istražiti utjecaj troškova mineralnih gnojiva na cijenu koštanja lubenice kao i na isplativost njene proizvodnje na lokalitetu Orah-Banja u vrgoračkom području.

Materijal i metode

Svi podaci, koji su korišteni u ovome radu, su prikupljeni na obiteljskom poljoprivrednom gospodarstvu i predstavljaju stvarne troškove nastale pri pripremi tla, sadnji i proizvodnji lubenica. U radu je prikazan proračun troškova pripreme tla, troškova sadnje te troškova proizvodnje lubenica s posebnim osvrtom na troškove gnojiva sorte Fantasy F1 na otvorenom. Od metoda je korištena metoda kalkulacije pokrića varijabilnih troškova, odnosno obračunska kalkulacija. Troškovi vlastite mehanizacije, koji inače pripadaju fiksnim troškovima sastoje se iz tehničkog održavanja i amortizacije, a mogu im se dodati i troškovi registracije i osiguranja traktora. Oni su izračunati metodom proračuna troškova po satu rada traktora s priključnim strojem, te se u kalkulaciji oduzimaju pri izračunu pokrića varijabilnih troškova (PVT-a).

Fantasy F1 je srednje rani hibrid. Biljku odlikuje izuzetno snažan korijen koji duboko prodire u zemlju te u najtoplijim ljetnim danima osigurava dovoljnu količinu vode i hranjiva. Snažna biljka, brzog porasta omogućava lisnoj masi dobro zatvaranje i na taj način brani plodove od sunčevih ožegotina. Plodovi su ovalnog oblika, jarko crvene unutrašnjosti i izuzetnog mirisa i okusa. Sadrži vrlo visoki udjel **šećera, i u** standardnim agrotehničkim uvjetima plodovi teže 9-12 kg. U intenzivnoj proizvodnji plodovi su težine 13-17 kg. Dobro se skladišti i kvalitetan je proizvod za izvoz na strana tržišta. Iako ima tanku koru nije sklona pucanju, vrlo dobro podnosi duge transporte i prirodna oštećenja kore.

Rezultati i rasprava

Proizvodnja lubenica se obavlja na dvije parcele ukupne površine od jednog hektara. Na toj površini ukupno je posađeno 4.000 biljaka na 4 tisuće metara folije. Sadnja je obavljena u dva termina - 21.04. i 28.04., po 2.000 biljaka. Lokacija parcela se nalazi na samoj granici Hrvatske i Hercegovine između sela Banja i Orah, koja su u sastavu grada Vrgorca. Nadmorska visina je 81m. Klima je kombinacija blage mediteranske i kontinentalne klime.

Osnovna gnojidba je obavljena sa 600 kg kombiniranog multikompleksa i hidrokompleska, odnosno po 300 kg jedne i druge kombinacije. U osnovnoj gnojidbi su korištene mikrogranule - Physiostart za bolje ukorjenjivanje u dozi od 25 kg.

Tijekom vegetacije ukupno je obavljeno 15 prihrana, od kojih je samo jedna bila folijarna prihrana, sve ostale (14) su bile fertirigacijom. Prije svake prihrane obavljao se vizualni nadzor proizvodnje i fotografiranje. Nakon obilaska proizvodnje, davane su preporuke za prihranu - formulacija i doza (g/sadnici).

Opisanom tehnologijom gnojenja su postignuti visoki prinosi u proizvodnji lubenica, znatno viši od hrvatskog prosjeka koji po Statističkom ljetopisu Republike Hrvatske iz 2017. godine iznose 28,2 t/ha. Također je ustanovljeno da su ovako visoki prinosi povoljno utjecali na cijenu koštanja lubenice kao i na ekonomičnost proizvodnje i rentabilnost poslovanja.

Značaj troškova mineralnih gnojiva za cijenu koštanja i isplativost proizvodnje lubenice

Tablica 1. Troškovi sata rada traktora		u/€
A:	Nabavna vrijednost	20.000,00
B:	Likvidacijska vrijednost	2.500,00
C:	Amortizacijska vrijednost (A – B)	17.500,00
D:	Amortizacijski razdoblje	10 godina
E:	Godišnji iznos amortizacije C/D	1.750,00
F:	Planirano radnih sati godišnje	800
G:	Amortizacijski iznos po satu rada E/F	2,1875
H:	Godišnji iznos kamata na uložena sredstva (5% od 20.000,00)	1.000,00
I:	Kamata po satu rada H/F	1,25
J:	Troškovi goriva po satu rada (10l x 0,75 €)	7,50
K:	Troškovi tehničkog održavanja (motorno ulje, filtri, redovito servisiranje, potrošni materijal, akumulator, gume, remenje) - 50% od troškova goriva	3,75
Cijena sata rada traktora = G+I+J+K = 14,7 + PDV = 18,4 €		

Tablica 2. Troškovi sata rada priključnog stroja		dovde	u/€
A:	Nabavna vrijednost		2.500,00
B:	Likvidacijska vrijednost		400,00
C:	Amortizacijska vrijednost (A – B)		2.100,00
D:	Amortizacijska vrijednost		10
E:	Godišnji iznos amortizacije (C/D)		210,00
F:	Planirano radnih sati godišnje		100
G:	Amortizacija po satu rada (E/F)		2,10
H:	Godišnji iznos kamata na uložena sredstva (5% od 2.500,00)		125,00
I:	Kamata po satu rada (H/F)		1,25
J:	Godišnji troškovi održavanja		50,00
K:	Troškovi tehničkog održavanja po satu rada (J/F)		0,50
Cijena sata rada priključnog stroja = G +I+K = 3,85 + PDV = 4,8 €			

Tablica 3. Potrebno radno vrijeme za agrotehniku proizvodnje lubenica, ha

	Brzina (km/h)	Radni zah- vat (m)	Teoretski učinak (ha/sat)	Iskoristivost r. vremena %	Učinak ha/sat	Broj iz- vođenja	Ukupan broj sati
Duboko jesensko oranje	6	0,70	0,42	65	0,27	1	3,70
Proletno oranje	7	0,70	0,49	65	0,32	2	6,20
Drljanje	5	2,20	1,10	50	0,55	1	1,80
Tanjuranje	5	2,20	1,10	50	0,55	1	1,80
Rasipanje gnojiva	5	7,00	3,50	30	1,05	2	1,90
Zaštita	5	8,00	4,00	30	1,20	12	10,00
Ukupno sati							25,40

Izvor: izrada autora prema Turšić (2001.)

Tablica 3. Izračun materijalnih troškova pri proizvodnji lubenica

Troškovi materijala	Jedinica mjere	Količina	cijena	€/ha
Presadnice	kom	4.000	0,55	2.200,00
Mineralna gnojiva:				
a) YaraMila Complex	vreća (25 kg)	300 (12 vreća)	18,75	225,00
b) Multi – Comp Base	vreća (25 kg)	300 (12 vreća)	19,75	237,00
Mikrogranule:				
a) Physiostart	vreća (25 kg)	25 (1 vreća)	65,50	65,50
Vodotopiva gnojiva za prihranu				
a) KSC PHYT-actyl I	vreća (25 kg)	25 (1 vreća)	81,50	81,50
b) KSC PHYT-actyl II	vreća (25 kg)	25 (1 vreća)	53,00	53,00
c) KSC PHYT-actyl III	vreća (25 kg)	25 (1 vreća)	65,50	65,50
d) KSC PHYT-actyl XX	vreća (25 kg)	25 (1 vreća)	70,00	70,00
e) KSC PHYT-actyl VII PERLA	vreća (25 kg)	25 (1 vreća)	59,50	59,50
f) KSC PHYT-actyl V	vreća (25 kg)	75 (3 vreće)	73,65	221,00
g) Co-Actyl H	vreća (10 kg)	10 (1 vreća)	62,50	62,50
h) Polyfeed 20-20-20	vreća (25 kg)	25 (1 vreća)	40,50	40,50
i) Magnezij sulfat	vreća (25 kg)	25 (1 vreća)	15,00	15,00
j) Amonijev nitrat	vreća (25 kg)	25 (1 vreća)	19,50	19,50
Tekuća folijarna gnojiva za prihra.				
Fertiactyl Starter	kom (1l)	2 (2 litre)	17,00	34,00
Fertileader Gold	kom (1l)	2 (2 litre)	15,00	30,00
Mineralna gnojiva ukupno				1.279,50
Sredstva za zaštitu bilja od bolesti				
a) Ridomil Gold MZ Pepite	kg	2	18,00	36,00
b) Topas 100 EC	100ml	1 kom.	9,00	9,00
c) Amistar Opti	l	3	24,50	73,50
d) Ortiva Top	500ml	6 kom.	36,00	216,00
e) Alietta flash	kg	4	26,88	107,50
f) Nativo 75 WG	200g	3 kom.	32,00	96,00

Značaj troškova mineralnih gnojiva za cijenu koštanja i isplativost proizvodnje lubenice

g) Quadris	100ml	2 kom.	9,50	19,00
Sredstva za zaštitu bilja od štetnika				
a) Actara 25 WG	250g	2 kom.	42,50	85,00
b) Karate Zeon	50ml	1 kom.	3,00	3,00
c) Mospilan 20 SP	50g	9 kom.	5,50	49,50
d) Zoom 11SC	50ml	8 kom.	15,13	121,00
e) Chromogor 40	l	2	13,00	26,00
f) Force 1,5G	1500 g	10 kom.	13,50	135,00
Sredstva za zaštitu bilja ukupno				976,50
Troškovi ostalog materijal				
Crna folija	kg	100	3,95	395,00
Agril folija	m ²	5000	0,80	400,00
Cijevi za navodnjavanje (φ16)	m	5000	0,15	750,00
Cijevi za navodnjavanje (φ32)	m	114	0,58	66,00
Ambalaža (box palete 1000 kg)	kom	50	25,00	1.250,00
Troškovi ostalog materijal ukup.				2.861,00
Troškovi materijala ukupno				7.317,00

Tablica 4. Izračun troškova ljudskog rada

Troškovi ljudskog rada	Jedinica mjere	Količina	Cijena	€/ha
a) postavljanje crne folije	sat	10	3,00	30,00
b) Sadnja	sat	70	3,00	210,00
c) Postavljanje agril folije	sat	10	3,00	30,00
d) Kontrola i uklanjanje korova	sat	10	3,00	30,00
e) Berba plodova	sat	30	3,00	90,00
Ukupno				390,00

Tablica 5. Kalkulacija pokrića varijabilnih troškova proizvodnje lubenica

Kalkulacija pokrića varijabilnih troškova (PVT)	€/ha	Udjel
Prinos kg/ha (Q)	103.609	
Cijena, 1 kg	0,15	
UKUPNI PRIHOD	15.541,50	
Presadnice (4.000 kom x 0,55 €)	2.200,00	25,7
Mineralna gnojiva	1.279,50	14,9
Sredstva za zaštitu bilja	976,50	
Ostali materijalni troškovi	2.861,00	
Troškovi ljudskog rada	390,00	
Ostali troškovi	150,00	
UKUPNI VARIJABILNI TROŠKOVI	7.857,00	
PVT	7.684,50	
Troškovi vlastite mehanizacije (25,4 sata x 23,2 €) i troškovi registracije i osiguranja (120,00 €)	709,00	
PVT 1	6.975,5	

Troškovi vlastite mehanizacije uključuju: duboko jesensko oranje, proljetno oranje, drljanje, tanjuranje, rasipanje gnojiva i zaštitu od bolesti i štetnika.

Ekonomski pokazatelj	u €
Ekonomski pokazatelj	Vrijednost
Ukupni prihod	15.541,50
Ukupni varijabilni troškovi	7.857,00
PVT	7.684,50
Troškovi vlastite mehanizacije s troškovima registracije i osiguranja traktora	709,00
Ukupni troškovi	
PVT 1	6.975,50
Ekonomičnost proizvodnje	1,81
Stopa rentabilnosti poslovanja	44,9
Cijena koštanja po 1 kg Q)	0,08

Zaključak

Na osnovu tehnološko-ekonomske analize s ciljem utvrđivanja ekonomske opravdanosti investicije u podizanju nasada i proizvodnji lubenica na području Vrgoračkog kraja zaključuje se kako je potvrđena ekonomska opravdanost proizvodnje lubenica.

Povoljnost agroekoloških uvjeta uz primjenu suvremenih tehnoloških postupaka proizvodnje, uključujući vodotopiva mineralna gnojiva omogućuje intenzivnu proizvodnju lubenica s visokim prinosima od 103,609 t/ha. U proizvodnji su korištena mineralna gnojiva sa biljnim stimulatorima, a njihov udjel u ukupnim troškovima je iznosio 14,9 %. Visoki prinosi su povoljno utjecali na cijenu koštanja lubenice od 0,08 €/kg. Ostali ekonomski pokazatelji su također polučili dobre rezultate, ekonomičnost s koeficijentom od 1,81 i rentabilnost poslovanja sa stopom od 44,9 i na taj način valorizirali komparativne prednosti mediteranske klime i primijenjene agrotehnologije.

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Significance of mineral fertilizer costs for cost price and cost effectiveness of watermelon production

Abstract

This paper analyzes the financial viability of the production of watermelons in the Dalmatian hinterland. The total costs of land preparation, watermelon planting and the calculation of variable cost coverage for regular watermelon production are shown. The costs of mineral fertilizers and their impact on yields were analyzed separately. All data for this paper were obtained from the case study on the family farm which has been producing watermelons for many years, and which has above average yields. The aim of this study is to investigate the impact of mineral fertilizer costs on the cost of watermelon cost and the cost-effectiveness of its production. When processing the data, the variable cost coverage calculation method was used. The obtained results indicate that the production of watermelons is economically justified with a revenue/cost ratio of 1.81, a return on sale of 44.9% and a cost of production of one kilogram of € 0.08.

Keywords: watermelon production, investment, cost-effectiveness

Stavovi potrošača o sirevima tradicionalne i industrijske proizvodnje

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Sažetak

Cilj istraživanja bio je utvrditi ponašanje potrošača u kupnji sira, znanje o pojmovima tradicionalnog i industrijskog načina proizvodnje sireva te stavove o sirevima tradicionalnog načina proizvodnje u odnosu na sireve industrijskog načina proizvodnje. On-line anketno ispitivanje provedeno je na uzorku od 200 ispitanika u Hrvatskoj. Ispitanici najčešće kupuju sireve u supermarketima i na tržnicama, te češće kupuju sireve industrijske proizvodnje nego sireve tradicionalne proizvodnje. Pri kupnji sira ispitanicima su najvažnija obilježja okus i kvaliteta. Ispitanici imaju pozitivnije stavove o tradicionalno proizvedenom siru u odnosu na industrijski proizveden sir.

Ključne riječi: sir, tradicionalna proizvodnja, industrijska proizvodnja, potrošači, stavovi

Uvod

Tradicionalna hrana proizvodi se specifičnim načinom proizvodnje koji je dio gastronomske baštine određenog lokalnog područja, regije ili države, što rezultira karakterističnim senzorskim svojstvima proizvoda (Guerrero i sur. 2009., Balogh i sur. 2016.). Interes potrošača za tradicionalnom hranom u stalnom je porastu, posebice u zemljama Europe. Pozitivan stav potrošača prema tradicionalnoj hrani povećava se kada proizvodi nose oznaku koja ukazuje da je taj proizvod proizveden upravo u tom području gdje je i kupljen, kao što su oznaka izvornosti ili zemljopisnog porijekla (Boza i Muñoz, 2016.). Sirevi proizvedeni tradicionalnim načinom proizvodnje specifični su za određenu regiju. Takva proizvodnja najčešće se povezuje uz proizvodnju na obiteljskim poljoprivrednim gospodarstvima (OPG) te je povezana s tradicijskim običajima i prehrambenim navikama lokalnog stanovništva, klimom, reljefom, zemljopisnim položajem, botaničkim sastavom prirodnih livada i pašnjaka, pasminom, vrstom uzgoja i prijenosom znanja i iskustava o njihovoj proizvodnji (Barukčić i Tudor Kalit, 2019.). Brojni sirevi sa svjetskim ugledom upravo su potekli od lokalnih proizvodnji te se danas proizvode u industriji prema prilagođenoj tehnologiji proizvodnje (Barukčić, 2015.). Industrijski sirevi proizvode se suvremenim načinom proizvodnje koji uključuje primjenu postupka pasterizacije kako bi se uništili svi nepoželjni i patogeni mikroorganizmi te postupak standardizacije mlijeka kojim se optimizira količina mliječne masti i proteina uz korištenje moderne opreme. Tradicionalni sirevi proizvode se bez postupaka pasterizacije i standardizacije mlijeka, s mnogo ručnog rada (bez suvremene opreme) te su po svom okusu, mirisu, aromi i teksturi drugačiji od industrijski proizvedenih sireva. Sirarski gurmani uživaju u tradicionalno proizvedenim sirevima od sirovog mlijeka te smatraju industrijsko sirarstvo "ubojicom" okusa sira (Kalit, 2015). Sirevi dobiveni industrijskim načinom proizvodnje ujednačenog su izgleda i kvalitete, a aroma i okus su im slabije izraženi. Tradicionalno proizvedeni sirevi intenzivnijeg su, raznolikijeg, i uzbudljivijeg okusa i arome. Cilj ovoga rada je utvrditi ponašanje potrošača (kupaca sira) u kupnji sira, njihovo znanje i stavove o sirevima tradicionalne i industrijske proizvodnje.

Materijal i metode

On-line anketno ispitivanje provedeno je na prigodnom uzorku od 200 kupaca sira u Hrvatskoj u razdoblju od 8. srpnja do 21. kolovoza 2019. Anketni upitnik distribuiran je putem društvene mreže Facebook i e-mailom te je obuhvaćao pitanja vezana uz znanje i stavove ispitanika o sirevima tradicionalne i industrijske proizvodnje, ponašanje u kupnji sira te sociodemografska pitanja. Pitanja vezana uz znanje ispitanika o pojmovima tradicionalnog i industrijskog načina proizvodnje sireva bila su otvorenog tipa („Što bi po Vašem mišljenju bio sir industrijske

proizvodnje/proizveden na tradicionalan način?“), a ostala pitanja bila su zatvorenog tipa. Važnost obilježja sira prilikom kupnje mjerena je ljestvicom od 5 stupnjeva (1= potpuno nevažno obilježje, 5= vrlo važno obilježje). Za mjerenje stavova ispitanika o sirevima tradicionalne i industrijske proizvodnje korištena je kognitivna komponenta putem 7 izjava, od kojih je njih 5 preuzeto iz rada Colonna i sur. (2011.), a dvije izjave kreirane su za potrebe ovog istraživanja. Ispitanici su za svaku izjavu iskazali stupanj slaganja, koji je mjeran Likertovom ljestvicom od 5 stupnjeva (1-uopće se ne slažem, 5-potpuno se slažem). Analiza podataka provedena je u programu Excel, pri čemu je korištena jednovarijatna (frekvencije i distribucija) analiza.

Rezultati i rasprava

Opis uzorka

U istraživanju je sudjelovalo 67% žena i 33% muškaraca. Većina ispitanika pripada dobnoj skupini od 18 do 25 godina (44%), slijede ispitanici starosti od 26-35 godina (22,5%) i 36-45 godina (15,5%). Manji udio ispitanika ima 46-55 godina (12%), 56 do 65 godina (4,5%) i više od 65 godina (1,5%). S obzirom da dominiraju mlađi ispitanici dobna struktura ispitanika ograničenje je uzorka što utječe na rezultat i zaključak istraživanja. Za buduća istraživanja potrebna je homogenija raspodjela uzorka s obzirom na dobnu strukturu. Preko polovice ispitanika (59,5%) je zaposleno, slijede studenti/učenici (35,5%), dok je najmanji postotak nezaposlenih i umirovljenika (2,5%). Prema stupnju obrazovanja najviše ispitanika navodi VŠS/VSS (52%), zatim SSS (32,5%), te magisterij/doktorat (15,5%). Trećina ispitanika (32%) ima mjesečna primanja manja od 3.500 kn, slijede ispitanici koji imaju mjesečna primanja od 3.500-5.500 kn (24%) i od 5.501-7.500 kn (23%). Najmanje ispitanika ima primanja veća od 9.500 kn (11%) i od 7.501 – 9.500 kn (10%). Čak 75% ispitanika stanuje u gradskim sredinama, dok 25% stanuje u ruralnim sredinama.

Ponašanje u kupnji sireva

Na pitanje gdje najčešće kupuju sireve (mogućnost više odgovora), najviše ispitanika navelo je supermarkete (75,5%) i tržnice (42%), dok 33% ispitanika najčešće kupuje sireve direktno od proizvođača. Kupnja sireva na sajmovima je zastupljena kod 17% ispitanika, kupnja u specijaliziranim trgovinama kod 10% ispitanika, a 1% ispitanika navelo je da sami proizvode sireve. Colonna i sur. (2011) također navode da većina ispitanika sireve kupuje u supermarketima. S obzirom na način proizvodnje ispitanici češće kupuju sireve industrijske proizvodnje (56%) nego sireve tradicionalne proizvodnje (44%). Kao najvažnija obilježja sira prilikom kupnje, ispitanici su istaknuli okus (srednja vrijednost 4,77) i kvalitetu (srednja vrijednost 4,60), kao što je dobiveno u istraživanju Auty (1992). Slijede aroma (srednja vrijednost 4,43) i miris (srednja vrijednost 4,32), dok im je najmanje bitna tržna marka (srednja vrijednost 2,85) - Tablica 1.

Tablica 1. Važnost obilježja sira prilikom kupnje

Obilježje sira	Srednja vrijednost	Standardna devijacija	Obilježje sira	Srednja vrijednost	Standardna devijacija
Okus	4,77	0,50	Porijeklo sira	3,91	0,95
Kvaliteta	4,60	0,60	Konzistencija	3,73	0,90
Aroma	4,43	0,70	Način proizvodnje	3,51	0,91
Miris	4,32	0,78	Boja	3,43	0,94
Odnos cijene i kvalitete	4,28	0,73	Nutritivna vrijednost	3,31	0,98
Rok trajanja	4,25	0,97	Nutritivna deklaracija	3,12	0,95
Ujednačenost kvalitete pri svakoj kupnji	4,15	0,80	Pakiranje	2,89	0,94
Cijena	4,09	0,79	Tržna marka	2,85	0,97

Znanje ispitanika o pojmovima tradicionalne i industrijske proizvodnje sira

Na pitanje što bi bio sir proizveden na tradicionalan način, ispitanici su većinom odgovarali kako je to sir proizveden na malim OPG-ima te sir proizveden bez primjene konzervansa, različitih dodataka i aditiva (Tablica 2). Ispitanici su odgovorili kako je sir industrijske proizvodnje sir proizveden u industriji prema industrijskoj recepturi, u velikim količinama, uz primjenu različitih dodataka i tvari za produljenje roka trajanja (Tablica 3). Za zaključiti je da je znanje ispitanika o pojmovima tradicionalnog i industrijskog načina proizvodnje sira veliko jer je većina odgovora ispravna. Sličan rezultat navode Weichselbaum i sur. (2009.): potrošači tradicionalnu hranu definiraju kao hranu njihovih baka i djedova, proizvedenu prema tradicionalnom receptu od domaćih sirovina, kao prirodnu hranu bez značajnijih procesa prerade te specifičnih senzorskih svojstva. Slično, ispitanici u istraživanju Colonna i sur. (2011.) navode kako se sir iz pastereziranog mlijeka proizvodi isključivo u velikim industrijskim postrojenjima, a sir iz nepasteriziranog mlijeka je male, tradicionalne proizvodnje.

Tablica 2. Odgovori ispitanika na pitanje „Što bi po Vašem mišljenju bio sir proizveden na tradicionalan način?“

Što bi po Vašem mišljenju bio sir proizveden na tradicionalan način?	N	%
Sir proizveden po receptu koji se prenosi s koljena na koljeno, prema dugogodišnjoj tradicionalnoj recepturi i tradicionalnom načinu proizvodnje na malim OPG-ovima.	48	24,0
Sir proizveden bez primjene konzervansa, različitih dodataka te aditiva.	32	16,0
Sir proizveden od mlijeka proizvedenog na tradicionalan način uz primjenu lokalnih, domaćih sastojaka.	24	12,0
Sir specifičan za određenu regiju, geografsko područje.	22	11,0
Sir manjeg kapaciteta proizvodnje.	22	11,0
Sir ručne izrade.	15	7,5
Kvalitetniji i zdraviji sir.	14	7,0
Sir karakterističnog okusa i arome.	9	4,5
Sir proizveden bez uporabe suvremene opreme i strojeva.	7	3,5
Sir proizveden bez primjene postupaka pasterezacije i standardizacije mlijeka.	7	3,5

Tablica 3. Odgovori ispitanika na pitanje „Što bi po Vašem mišljenju bio sir industrijske proizvodnje?“

Što bi po Vašem mišljenju bio sir industrijske proizvodnje?	N	%
Sir proizveden u industriji prema industrijskoj recepturi.	43	21,5
Sir proizveden u velikim količinama.	42	21,0
Sir proizveden uz primjenu različitih dodataka poput aditiva, konzervansa, pojačivača okusa, emulgatora, tvari za produljenje roka trajanja.	41	20,5
Sir proizveden uz primjenu suvremene opreme.	23	11,5
Sir proizveden uz primjenu postupaka pasterezacije i standardizacije mlijeka.	15	7,5
Sir proizveden od mlijeka konvencionalne proizvodnje.	11	5,5
Sir slabije izraženog okusa i arome.	10	5,0
Sir slabije kvalitete.	9	4,5
Sir ujednačenog sastava.	6	3,0

Stavovi ispitanika o sirevima tradicionalne proizvodnje u odnosu na sireve industrijske proizvodnje

Ispitanici općenito imaju pozitivne stavove o sirevima tradicionalne proizvodnje. Najviše se slažu s izjavom da je tradicionalno proizveden sir ukusniji od industrijski proizvedenog sira što je u skladu s rezultatima istraživanja Guerrero i sur. (2009.). Nadalje, smatraju da je tradicionalno proizveden sir kvalitetniji od industrijski proizvedenog sira, te da ima veću nutritivnu vrijednost. Ipak, smatraju da je tradicionalno proizveden sir skuplji od industrijski proizvedenog sira, dok su neutralnog stava oko sigurnosti tradicionalno proizvedenog sira u odnosu na industrijski proizveden sir (Tablica 4), što možemo povezati s činjenicom da su u ovoj fazi ispitanici bili upoznati s činjenicom da se tradicionalni sir proizvodi od nepasteriziranog mlijeka što je u skladu s istraživanjem Le i sur. (2014.). Ispitanici

u istraživanju Colonna i sur. (2011) smatraju da je industrijski sir sigurniji, ima slabije izražen okus i cjenovno prihvatljiviji.

Tablica 4. Stavovi ispitanika o sirevima tradicionalne proizvodnje u odnosu na sireve industrijske proizvodnje

Stavovi ispitanika o sirevima tradicionalne proizvodnje u odnosu na sireve industrijske proizvodnje	Srednja vrijednost	Standardna devijacija
Tradicionalno proizveden sir ukusniji je od industrijski proizvedenog sira.	4,30	0,77
Tradicionalno proizveden sir kvalitetniji je od industrijski proizvedenog sira.	4,09	0,95
Tradicionalno proizveden sir ima veću nutritivnu vrijednost od industrijski proizvedenog sira.	4,09	0,91
Tradicionalno proizveden sir skuplji je od industrijski proizvedenog sira.	4,02	0,96
Radije se odlučujem za kupnju tradicionalno proizvedenih sireva nego industrijskih.	3,61	1,10
Imam više povjerenja u tradicionalno proizvedene sireve nego u industrijski proizvedene sireve.	3,48	0,96
Tradicionalno proizveden sir sigurniji je (zdravstveno ispravniji) od industrijski proizvedenog sira.	3,04	0,96

Zaključak

Ispitanici češće kupuju sireve industrijske proizvodnje, no smatraju da su tradicionalno proizvedeni sirevi ukusniji i kvalitetniji te imaju veću nutritivnu vrijednost. Znanje ispitanika o pojmovima tradicionalnog i industrijskog načina proizvodnje sira je veliko odnosno ispitanici su upoznati sa specifičnostima tradicionalne i industrijske proizvodnje sira. Rezultati ovog istraživanja daju vrijedne informacije proizvođačima sira u svrhu planiranja proizvodnje i marketinških aktivnosti.

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Consumer attitudes about traditional and industrial cheese production

Abstract

The aim of the study was to determine consumers' behaviour in cheese purchase, knowledge about the concepts of traditional and industrial cheeses and their attitudes about cheeses of traditional production compared to cheeses of industrial production. An online survey was conducted on a sample of 200 respondents from Croatia. Respondents most often buy cheeses in supermarkets and at the open marketplaces, with more frequent purchases of industrial cheeses than traditional cheeses. When buying cheese, the most important characteristics for respondents are taste and quality. Respondents have positive attitudes about traditionally produced cheese over industrially produced cheese.

Keywords: cheese, traditional production, industrial production, consumers, attitudes

Welcome to the club? The significance of protected geographical indications and designations of origin in Europe, with special consideration of the Croatian situation

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Abstract

Geographical indications (GIs) and traditional specialties guaranteed (TSGs) are a major pillar of the EU quality scheme. The objective of this study was to determine their significance in Europe, with special consideration of the Croatian situation. A decade after their accession EU11 Central, Eastern and Baltic European member states appear to use EU quality schemes only on a limited extent and rather for legal protection against fraud and misuse than to obtain EU co-funded promotional programs. We assume that member states without a tradition in PGIs and TSGs lack public institutions which support the promotion and marketing of these products.

Keywords: protected designation of origin (PDO), protected geographical indication (PGI), common agricultural policy (CAP), traditional speciality guaranteed (TSG), EU quality scheme

Introduction

For over 20 years, geographical indications (GIs) are a major pillar of the Common European Agricultural Policy (CAP). Protected geographical indications (PGIs) and protected designations of origin (PDOs) aim at protecting the names of specific products to promote their unique characteristics, linked to their geographical origin as well as traditional know-how used during the production process (Wirsig et al. 2014). Traditional specialties guaranteed (TSGs) in turn highlight the traditional aspects such as the way the product is made or its composition, without being linked to a specific geographical area. The registration of a product as PDO, PGI, or TSG protects the use of the name against falsification and misuse. According to estimates (AND International 2012), a total annual sales volume of approximately €54.3 billion is generated with GIs and TSGs registered in the EU, with France and Italy being the main profiteers. Whereas GIs have a long tradition in southern European member countries, for example in France, Italy and Spain, including the legal protection and financial support by the state (Profeta et al. 2010), no such tradition exists in major Central, Eastern and Baltic European member states which joined the EU succeeding later. The objective of this study was to determine the significance of GIs and TSGs overall in Europe, in Central, Eastern and Baltic European member states with special consideration of the Croatian situation.

Materials and methods

Data on three aspects were analysed in order to estimate the significance of GIs and TSGs in the respective member states: 1. awareness of PDO, PGI and TSG logos; 2. amount of GI and TSG products registered, 3. participation in EU promotional programs focusing on quality schemes. In the study publicly available data from the European Commission were used: the DOOR database for food and agricultural products (EC 2019a), the eAmbrosia database for wine & spirit drinks (EC 2019b), and the register for aromatised wines (EC 2019c). Furthermore, the Campaigns map and statistics database of the EU's consumers, health, agriculture and food executive agency (Chafea 2019) and

results of Eurobarometer (DG Agri 2018) were employed. However, due to the absence of official economic data on GI and TSG products - particularly for Croatia and other Eastern and Baltic European member states - the study does not deal with the market value of these products (Török & Moir, 2018). In order to identify differences in the significance of GIs and TSGs in the member states they were grouped by their date of EU accession. EU28 means all the present member states as of October 2019. The group of EU15 comprises all member states from 1 January 1995 to 1 May 2004. The EU15 includes Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, and United Kingdom. EU11 refers to the Central, Eastern and Baltic European member states that joined in 2004, 2007 and 2013: in 2004 the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, the Slovak Republic, and Slovenia; in 2007 Bulgaria, Romania; and since 2013 Croatia.

Results and discussion

Overall awareness of EU quality schemes in respect to GIs and TSGs in EU11 countries is comparable high for PGIs, whereas values for TSGs are slightly above and values for PDOS are slightly below to that in EU15 and EU28 countries. Within EU11 countries, awareness of PDO, PGI and TSG logos are highest in Czech and Slovakia followed by Croatia. Values for awareness of GIs and TSGs in these countries are above those of EU28 (Table 1).

Table 1. Awareness of awareness of PDO, PGI and TSG logos in the EU

	PDO	PGI	TSG	Interviews*
	%	%	%	Number (n)
Estonia	12%	15%	13%	1005
Latvia	6%	9%	12%	1000
Lithuania	13%	19%	29%	1013
Poland	12%	15%	14%	997
Slovak Republic	26%	22%	27%	1089
Slovenia	19%	26%	17%	1042
Czech Republic	25%	30%	30%	1032
Hungary	13%	13%	10%	1038
Bulgaria	14%	12%	12%	1040
Romania	5%	9%	16%	1005
Croatia	20%	24%	22%	1.031
EU11	14%	17%	18%	1.2302
EU 15	21%	17%	14%	15676
EU 28	18%	18%	15%	28.031

Source: own composition based on DG Agri (2018). * Face-to-face.

Apparently, the number of GI and TSG registrations are significantly lower in EU11 countries in comparison to that in EU15 and EU 28 (Table 2). Whereas, EU15 show an average number of 83 GI registrations per member state, EU11 have only a poor average number of 17 GI registrations per member state. In contrast the average number of TSG registrations are comparable to that in EU11. Within EU11 Croatia has the highest number of PDO registrations, Poland and Czech the highest number of PGI registrations and Poland followed by Slovakia the highest number of TSG registrations. In total Poland and Czech Republic contribute the highest number of registrations with Croatia being in the middle (Table 2).

Table 2. GI and TSG products registered on agricultural products and foodstuffs

	PDO	PGI	TSG	Total	Ø/ MS*
Estonia	0	0	0	0	
Latvia	1	2	3	6	
Lithuania	1	5	2	8	
Poland	8	23	10	41	
Slovak Republic	2	10	7	19	
Slovenia	9	13	3	25	
Czech Republic	6	23	5	34	
Hungary	6	8	2	16	
Bulgaria	1	2	5	8	
Romania	1	4	0	5	
Croatia	12	11	0	23	
EU11	47	101	37	185	16,8
EU 15	585	629	29	1243	82,9
EU 28	633	734	66	1433	51,2

Source: own composition based on Door database (2019). *Member state.

Croatia in particular made its first submission to register a food product under the EU quality scheme shortly before its EU accession in 2013 (Figure 1). In 2015 first Croatian agricultural and food products were registered by the EU. Nevertheless, Croatia already had by the time of its EU membership 16 registrations for wine and further six registrations of spirits drinks and one for aromatized wine products.

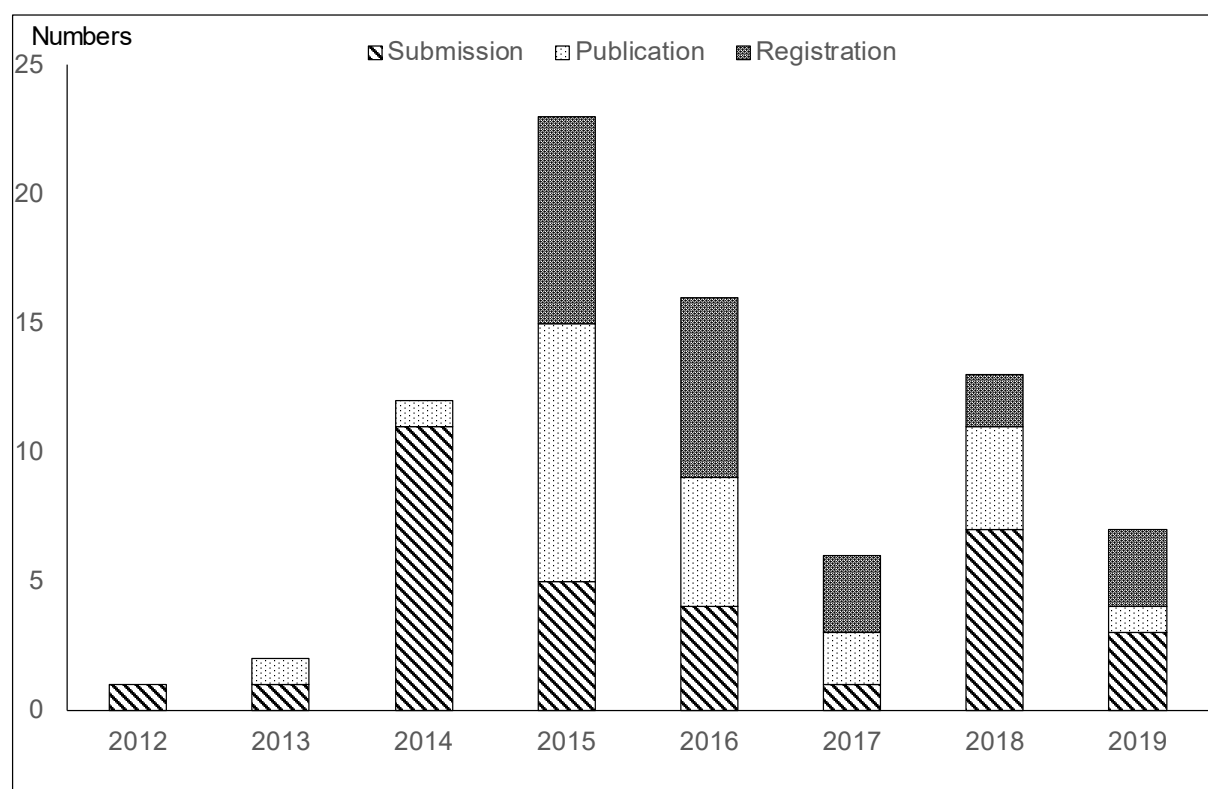


Figure 1. Annual number of Croatian applications on agricultural products and foodstuffs in the EU registration process (Source: EC 2019a).

In 2014 a peak of eleven submissions occurred which were prepared in the framework of multi- and bilateral cooperation in support to the Croatian accession process (GTZ 2004, GIZ 2011, Wirsig et al. 2011). A lower peak of submissions happened in 2018, partly as an outcome of the previous work done (MPS 2015). In total, Croatia made 33 submissions to register agricultural products and foodstuffs under the EU quality schemes of which ten recent submissions are still pending in the EU registration process (Figure 1).

The EU policy on information provision and promotion measures for agricultural products aims at helping producers to communicate the quality of their products. The European Commission and Chafea granted EU11 Central, Eastern and Baltic European member states hitherto 16 campaigns on EU Quality schemes with an overall funding of €12.6 million from the EU agricultural budget (Table 3) – with Slovenia owing the highest number of accepted proposals. In 2019 the European Commission and Chafea approved 81 campaigns with an overall funding of €200 million from the EU agricultural budget. Out of the 81 campaigns approved, 35 are focusing on EU Quality schemes, two of them proposed from EU11 countries. Hitherto, Croatia does not participate in any promotional program on EU Quality schemes, although it is a target market for EU co-funded campaigns from other EU member countries (Chafea 2019).

Table 3. Participation in EU promotional programs focusing on quality schemes (2005-2019)

	Number of proposals	PDO/ PGI/ TSG Product(s)	EU contribution	Target market
			in Euro	Countries
Estonia	1	Spirit drinks	278.043 €	EE, DE, LV, LT, PL
Latvia	0	*	*	*
Lithuania	1	Spirit drinks	1.775.710 €	EE, DE, LV, LT, PL
Poland	2	Apples	3.520.314 €	Third Countries
Slovak Republic	0	*	*	*
Slovenia	7	Oil, vegetables, bread, honey, meat products	2.326.694 €	SI, AU, HR, Third Countries
Czech Republic	1	Basket of products	1.881.740 €	CZ, SK
Hungary	2	Sheep and goat meat	900.000 €	Third Countries
Bulgaria	1	Wine	733.251 €	Third Countries
Romania	1	Meat preparations	1.145.175 €	IT, RO, ES
Croatia	0	*	*	*
EU11	16		12.560.927 €	*

Source: Chafea, (2019). * Not applicable.

Conclusions

A decade after its accession to the European Union the EU11 Central, Eastern and Baltic European member states appear to use EU quality schemes only on a limited extent - in contrast to member states with a long tradition with

GIs and TSGs - and rather for legal protection against fraud and misuse than to obtain EU co-funded promotional programs. Although awareness of EU quality schemes in EU11 countries is comparable or even better to that in traditional EU15 and EU28 member countries, the number of EU registered GIs and TSGs is low. Furthermore participation of EU11 member states on EU promotional programs is low in comparison to that of EU15 and EU28 countries, Slovenia being the only exception. The authors believe that member states without a tradition in PGIs and TSGs lack public institutions which support the promotion and marketing of GI and TSG products. The validation of this assumption and the identification of the underlying determining factors will be a question of further research.

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Razvoj eno-gastronomске turističke destinacije

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Sažetak

Cilj rada je prepoznati i analizirati ponudu Slavonije i Baranje kroz enofilski i gastronomski turizam. Anketni uzorak obuhvatio je 24 ispitanika, ruralna turistička subjekta koji u svojoj ponudi imaju hranu. Ispitanici smatraju da je Slavonija i Baranja prepoznata po svojoj eno-gastronomskoj ponudi, ali da je nedovoljno valorizirana i promovirana iako je već dugi niz godina sastavni dio Strategije razvoja turizma (MINT 2013). Od ukupnog broja ispitanika, 83,3% smatra kako je eno-gastronomija osnovni motiv dolaska u ruralnu sredinu, te 46% ispitanika najvećim dijelom sami proizvode sastojke za pripremu hrane. Komplementarnost usluga u ruralnom turizmu je neophodna kako bi posjetitelji doživjeli određenu destinaciju kroz razvoj tradicionalnog graditeljskog izričaja, regionalnih običaja, lokalne kulture kao i eno-gastronomskog nasljeđa.

Ključne riječi: eno-gastronomija, destinacija, Slavonija i Baranja, ruralni turizam

Uvod

Prema Nacionalnoj strategiji razvoja turizma Republike Hrvatske do 2020.godine (MINT, 2013.) eno-gastronomski turizam je prepoznat kao jedan od specifičnih turističkih proizvoda s visokim razvojnim potencijalom. Željena pozicija u ostvarivanju *gourmet* destinacija je poticanje lokalne proizvodnje kroz gastronomiju i enologiju. Eno-gastronomiju Slavonije i Baranje odlikuju kvalitetni suhomesnati proizvodi, specijaliteti pripremljeni od riječnih riba, raznovrsne slastice kao i vrhunska vina. Najveće površine pod vinovom lozom u Republici Hrvatskoj su upravo u Slavoniji (Čepo, 2019.). Hrana, odnosno gastronomija u ruralnom turizmu je značajna i visoko pozicionirana, što potvrđuje istraživanje Lončarić i sur. (2013) koji pokazuju kako 65% ispitanika preferira tradicionalne poljoprivredne proizvode u odnosu na konvencionalne. Autori Leko Šimić i Pap (2016.) analiziraju mogućnosti povećanja dodane vrijednosti turizma kroz razvoj turizma hrane kao posebnog turističkog proizvoda kroz valorizaciju i zaštitu raspoloživih resursa.

Materijal i metode

Cilj rada bio je prepoznati i analizirati ponudu eno-gastronomije u Slavoniji i Baranji. Istraživanje o mogućnostima i smjernicama eno-gastronomije provedeno je svibnju 2019. godine CATI-metodom (*Computer Assisted Telephone Interviewing*). Istraživanje je obuhvatilo 24 turistička seoska obiteljska gospodarstava (TSOG) iz 5 županija (Osječko-baranjska županija, Brodsko-posavska županija, Požeško-slavonska županija, Virovitičko podravska županija i Vukovarsko srijemska županija). Uvjet za anketno ispitivanje je bio da TSOG imaju uslugu smještaja i uslugu hrane, odnosno doručak, ručak i večeru. Istraživanje je temeljeno na analizi primarnih i sekundarnih izvora podataka, a metode sinteze i deskripcije primijenjene su u interpretaciji dobivenih rezultata i formiranja zaključaka.

Rezultati i rasprava

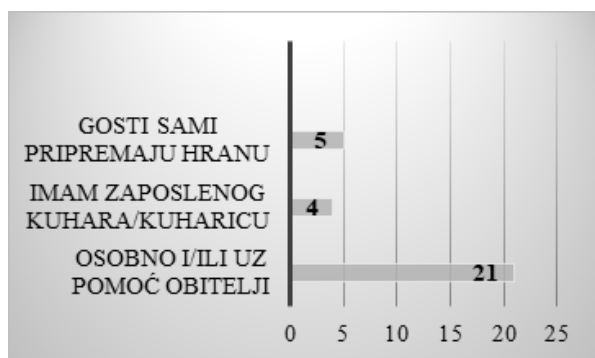
Nalaženje i odabir TSOG za ovo istraživanje provedeno je na temelju podataka Nacionalnog kataloga „Ruralni turizam Hrvatske“ (Mandić i sur., 2015). Ukupan broj potencijalnih ispitanika TSOG, onih koji imaju uslugu noćenja uz puni pansion, je 34 kako je prikazano u tablici 1. Anketni uzorak obuhvatio je 24 ispitanika na TSOG-ima od čega je 62,5% bilo starijih od 50 godina, 20,8% između 36 i 50 godina te 16,7% između 21 i 35 godina. Niti jedan ispitanik nije imao manje od 20 godina iz čega se može zaključiti da su velikim dijelom članovi TSOG osobe starije

dobne skupine. Što se tiče stupnja obrazovanja, 50% ispitanika ima srednju stručnu spremu, 37,5% ima završen preddiplomski ili diplomski studij, te 12,5% ima završenu samo osnovnu školu. Podjednak je omjer bio ženskih (54%) i muških ispitanika (46%).

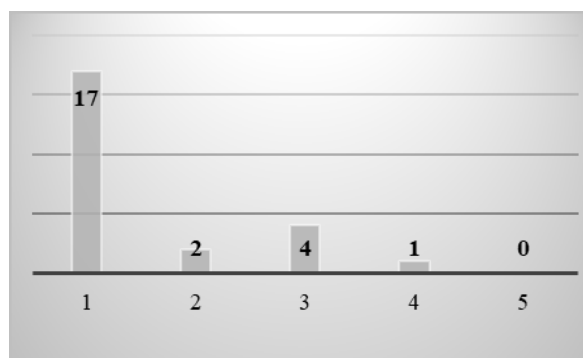
Tablica 1. Ukupan broj TSOG sa uslugom noćenja i punim pansionom

Županija	Ukupan broj TSOG s uslugom punog pansiona	Statistički uzorak	
		n	%
Osječko-baranjska	19	14	58,3
Požeško-slavonska	3	3	12,5
Virovitičko-podravska	3	1	4,2
Vukovarsko-srijemska	5	2	8,3
Brodsko-posavska	4	4	16,7
Ukupno	34	24	100%
Populacija i uzorak, %	100		70,6

Pri ispitivanju eno-gastronomske ponude primijenjena je Likertova skala koja procjenjuje stupanj slaganja odnosno neslaganja sa pojedinom tvrdnjom postavljenom u anketnom upitniku. Najviše ispitanika (83,3%) odgovorilo je kako im je gastronomska ponuda *izrazito važna* na gospodarstvu za privlačenje gostiju jer ostvaruje osjećaj ugone i ponudu doživljava tradicije.

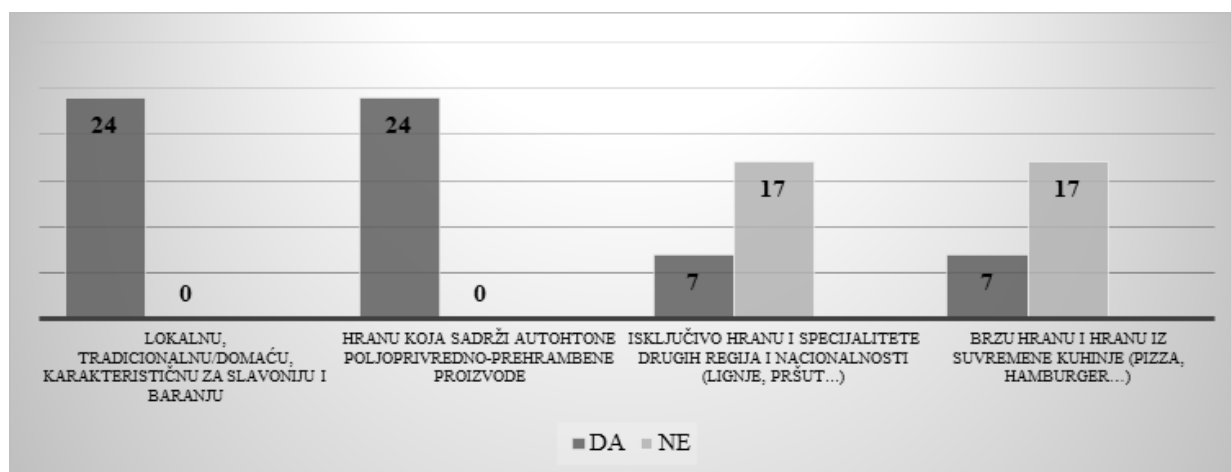


Grafikon 1. Način pripreme hrane na ispitanim TSOG



Grafikon 2. Recepti koje ispitanika TSOG koriste (1 - isključivo tradicionalne, 5 - isključivo suvremene)

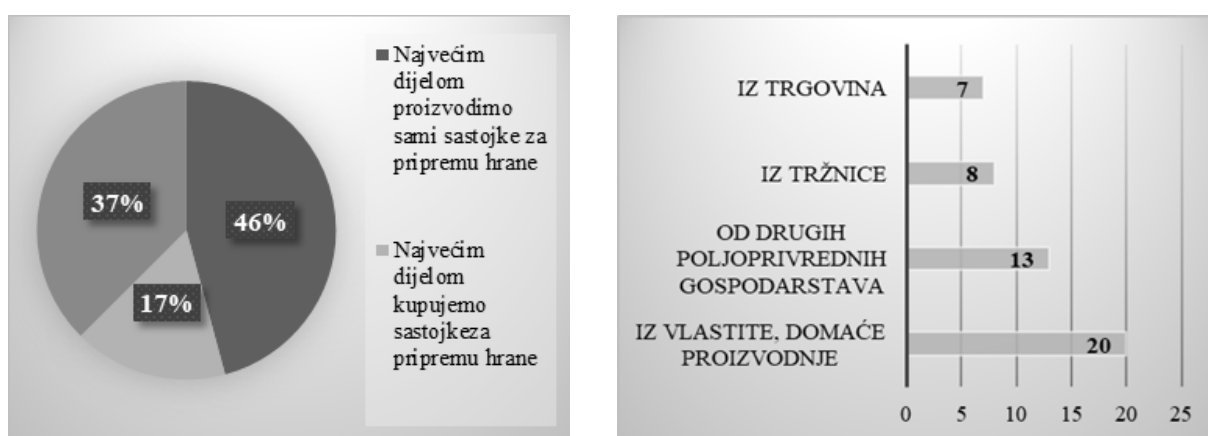
Prema dosadašnjim istraživanjima (Sudarić, 2017.) motiv hrane je dovoljno dobar turistički impuls, ali za kreiranje ekonomije doživljava na osnovu gastronomije potrebno je puno više. Područje mogućeg razvitka treba tražiti u elementima zabave i edukacije, gdje bi turisti mogli biti i aktivni sudionici u pripremi tradicionalnih jela i edukaciji o tradicionalnim recepturama. Distribucija odgovora kod važnosti ponude vina bila je raznovrsnija te ih je samo 58,3% odgovorilo kako im je ponuda vina *izrazito važna* iz razloga što neka gospodarstva nemaju ponudu vina. Isti udio ispitanika (58,3%) smatra kako je Slavonija i Baranja prepoznata po ponudi vina. Druga istraživanja (Tomić Maksan i sur., 2019) naglašavaju kako upravo potrošački etnocentrizam ima snažan i pozitivan utjecaj na stavove o kupnji domaćeg vina. Najveći broj ispitanika tvrdi da hranu pripremaju osobno i uz pomoć obitelji (87,5%) što je prikazano u grafikonu 1. Manji broj ispitanika izjasnio se da su registrirani kao trgovačka društva, te da stoga moraju imati zaposlenog kuhara, dok su se neki izjasnili da kuhara zapošljavaju po potrebi. Samo 20,8% ispitanika tvrdi da gosti sami pripremaju hranu ako žele. Najveći broj ispitanika tvrdi da koristi tradicionalne recepte pri pripremi hrane, dok poneki koriste suvremene recepte, a 4 ispitanika podjednako koriste i jedne i druge recepte (Grafikon 2).



Grafikon 3. Hrana koju ispitanja TSOG pripremaju

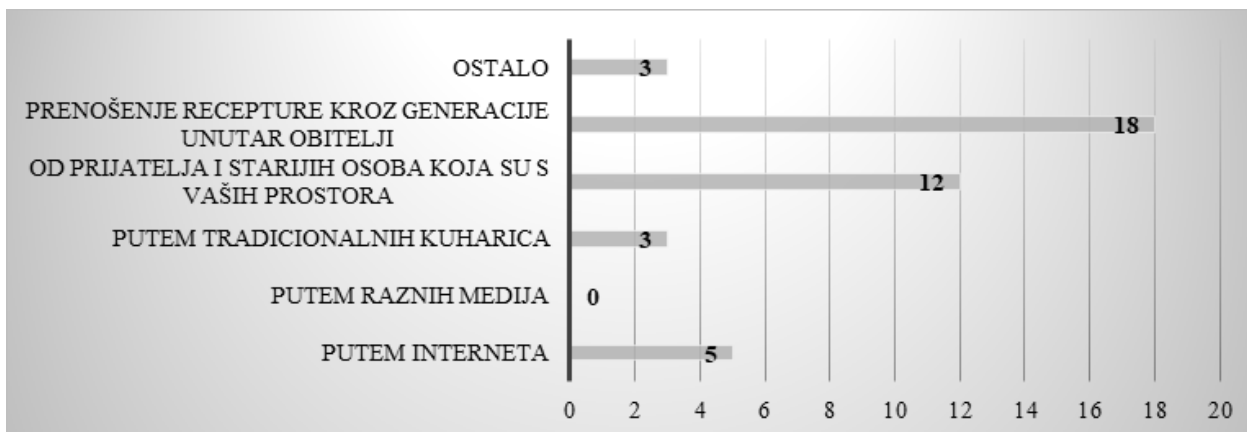
Oni koji u svojoj ponudi imaju i hranu spremljenu po suvremenim receptima, tvrde da takvu hranu pripremaju stranim gostima i to onima koji dolaze organizirano (npr. putničkim brodovima) na nekoliko sati i to na unaprijed dogovoren ručak. Što se domaćih gostiju tiče, ispitanici tvrde da oni dolaze prvenstveno radi kušanja domaće i tradicionalne kuhinje, a ponudu suvremene hrane imaju isključivo zbog gostiju mlađe dobne skupine iz tog segmenta. Svi ispitanici u svojoj ponudi imaju tradicionalnu, domaću hranu i onu koja sadrži autohtone poljoprivredno-prehrambene proizvode Slavonije i Baranje, dok 29% ispitanika u svojoj ponudi ima i hranu i specijalitete drugih regija, brzu hranu i hranu iz suvremene kuhinje (Grafikon 3).

Najveći broj ispitanih pojedine sastojke nabavljaju iz vlastite proizvodnje (83%). Veliki dio ih tvrdi da imaju vlastitu proizvodnju, ali da kupuju ono što ne mogu sami proizvesti od drugih poljoprivrednih gospodarstava u blizini. Nešto manji broj namirnice kupuje u trgovinama i na tržnici. Ispitanici su naveli nekoliko gastronomskih jela specifičnih za Slavoniju i Baranju koja pripremaju: fiš paprikaš, čobanac, sarma, punjena ("filovana") paprika, grah, kulen i drugi slavonski suhomesnati proizvodi, juha od povrća, taške, knedle sa šljivama, šaran na rašljama, gulaš, kotlovina, makovnjače, salenjaci, žganci bijeli, kruh iz krušne peći, divljač, šufnudle, tijesto s krumpirom, patka na starinski način, štruca s krumpirom, granatir, lungić u umaku od šljivovice, flekice s kupusom, pogačice s čvarcima, tarana, požeški vinogradarski ćevap i slično.



Grafikon 4. Sastojci za pripremu hrane koju koriste ispitanici TSOG

Vina nabavljaju od lokalnih proizvođača (91,6%) te nitko od ispitanika nema vlastitu proizvodnju vina. Ukoliko gosti žele vidjeti proizvodnju vina, u mogućnosti su posjetiti obližnje poznate vinarije na koje ih domaćini upućuju. Ispitanici tvrde da gosti izuzetno cijene kušanje tradicionalnih jela i poljoprivredno-prehrambenih proizvoda Slavonije i Baranje, većina ih dodaje da je to jedan od najvažnijih motiva dolaska na TSOG. Najveći broj ispitanika koristi recepte koji se prenose kroz generacije unutar obitelji i starijih osoba s vlastitog lokaliteta. Nitko od ispitanika recepte ne traži preko različitih medija, a manji dio ih recepte traži u tradicionalnim kuharicama i na Internetu (Grafikon 5).



Grafikon 5. Način informiranja o receptima tradicionalnih jela TSOG

U 45,8% slučajeva vole provjerene jelovnike jer tvrde da je tradicionalna hrana koju pripremaju oduvijek iste recepture (kroz generacije) i da se to ne treba mijenjati te da je to ujedno dodatak vrijednost koju takva hrana ima. Preostalih 54,2% ispitanika tvrde da su skloni inovacijama prilikom pripreme hrane, odnosno ukoliko sami otkriju novi recept koji im se sviđa ili novi sastojak u starom receptu, primjene to prilikom pripreme hrane.

Zaključak

Slavonija i Baranja imaju veliku eno-gastronomsku baštinu koja je analizirana u radu te su ispitani stavovi nositelja ruralno-turističkih subjekata o hrani, recepturama i vinu. Ispitanici smatraju da je Slavonija i Baranja prepoznata po svojoj eno-gastronomskoj ponudi, međutim da gastronomska destinacija treba biti promovirana kroz veći utjecaj lokalne i nacionalne institucijske potpore. Razvoj eno-gastronomske destinacije treba se njegovati i razvijati po kvaliteti i prepoznatljivosti proizvoda, cijeni ali i dostupnosti, odnosno, raspoloživosti pojedinih namirnica u dovoljnim količinama. Uz eno-gastronomiju značajan utjecaj na ponudu ogleđa se u prirodnim ljepotama kraja ili mjesta, kulturnoj baštini i tradiciji, atraktivnosti gospodarstva i ljubaznosti domaćina. Komplementarnost usluga u ruralnom turizmu je neophodna kako bi posjetitelji doživjeli određenu destinaciju. Pojedini ispitanici ističu problem nedostatka sadržaja, odnosno različitih aktivnosti (adrenalinski sportovi, ruralni wellness, lokalne manifestacije i slično) koje bi produžile boravak posjetitelja, a istovremeno utjecale na izgradnju imidža i prepoznatljivosti ruralne destinacije.

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Development of wine and gastronomic tourist destination

Abstract

The aim of the paper is to identify and analyse the offer of Slavonia and Baranja through wine and gastronomic tourism. The survey included 24 respondents from rural tourism entities who have food in their offer. Respondents believe that Slavonia and Baranja are recognized for their gastronomic offer, but that it is under-valued and promoted, although it has been an integral part of the national Tourism Development Strategy for many years (MINT 2013). Among all respondents, 83.3% believe that one gastronomy main motive in rural areas, and 46% of respondents mostly themselves produce ingredients for food preparation. The complementarity of services in rural tourism is necessary for visitors to experience a particular destination by respecting traditional architectural expression, preserving regional customs and local culture as well as the wine and gastronomic heritage.

Keywords: gastronomy, wine, destination, Slavonia and Baranja, rural tourism

Obilježja online kupnje ekoloških prehrambenih proizvoda u Hrvatskoj

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Sažetak

Cilj istraživanja je utvrditi obilježja online kupnje ekoloških prehrambenih proizvoda u Hrvatskoj. Online anketno ispitivanje provedeno je na prigodnom uzorku od 222 online kupaca ekoloških prehrambenih proizvoda. Najposjećeniji Internet servisi za onlinu kupnju ekoloških prehrambenih proizvoda su Burza hrane i Fino.hr. Najzastupljenije kategorije prehrambenih proizvoda u online kupnji su ekološki med, voće i povrće. Kao najvažnije prednosti online kupnje ispitanici navode mogućnost kupnje od 0-24h i uštedu vremena. Problemi s kojima se najčešće susreću kod Internet kupnje su kašnjenje isporuke i razlika između očekivanog i dobivenog proizvoda. Visok udio ispitanika smatra da Hrvatska nema dobre zakonske standarde za sigurnost hrane koja su prodaje putem Internet trgovine te da prodaja hrane preko Interneta nije dobro zakonski regulirana.

Ključne riječi: ekološki prehrambeni proizvodi, online prodaja, ponašanje potrošača

Uvod

U većini zemalja Europske Unije ekološki prehrambeni proizvodi prodaju se putem izravne prodaje, u specijaliziranim prodavaonicama i supermarketima. Novi kanali distribucije uključuju Ho.Re.Ca (hoteli-restorani-kafići) i javne ustanove (kantine, škole, bolnice i vojska), kao i distribuciju ekološke hrane putem ekološkog agroturizma (Gajdić i sur., 2018). U posljednjih desetak godina jedan od sve popularnijih kanala prodaje prehrambenih proizvoda je Internet trgovina. Trend rasta internet trgovine hrane bilježi se i u Hrvatskoj, premda je tek u povojima u odnosu na najrazvijenija tržišta EU. Panian (2000) definira Internet prodaju kao *“proces kupnje, prodaje ili razmjene proizvoda, usluga ili informacija putem javno dostupne računalne mreže, interneta, a nudi veliko smanjenje troškova i vremena transakcija”*. Internet trgovina omogućuje ljudima da pretražuju, uspoređuju cijene i lako pristupaju proizvodima i uslugama (Kitsikoglou i sur. 2014). Uspješna internet trgovina obuhvaća jednostavnost korištenja, proizvode razdijeljene u kategorije, veličinu asortimana, informacije o proizvodu, sigurnost i proces kupnje. Prema Kim Dang i sur. (2018) čimbenici koji utječu na ponašanje online kupaca prehrambenih proizvoda su praktičnost, cijena, samoprocjena sigurnosti hrane i preporuke prijatelja. Cilj istraživanja je utvrditi obilježja online kupnje ekoloških prehrambenih proizvoda u Hrvatskoj.

Materijal i metode

Primarni izvori podataka prikupljeni su online anketnim ispitivanjem kupaca ekoloških proizvoda na prigodnom uzorku od 222 ispitanika. Anketni upitnik je uz suglasnost vlasnika portala postavljen na sljedeće web stranice koje se bave online prodajom ekoloških proizvoda: Uberi ovo, Tvornica zdrave hrane, Broskva.hr, Mr. Eko, Špajza, OPG Veselić, te Fino.hr. Anketno istraživanje provedeno je u razdoblju od 25. svibnja do 15. lipnja 2019. godine. Anketnim upitnikom obuhvaćena su pitanja o zastupljenosti pojedinih online servisa u kupnji eko proizvoda, kategorije proizvoda koje se kupuju, mjesečnoj potrošnji, načinu plaćanja, razlozima kupnje putem Interneta, problemima s kojim se kupci susreću u online kupnji, te pitanja o povjerenju u sigurnost hrane koja se prodaje putem Internet trgovine. Posljednja skupina pitanja u anketi odnosila se na sociodemografska obilježja: spol, dob, školsku spremu, osobni mjesečni prihodi, broj djece, mjesto stanovanja. Rezultati su analizirani pomoću jednovarijantne i dvovarijantne analize (hi kvadrat test) u statističkom programskom paketu SPSS21.

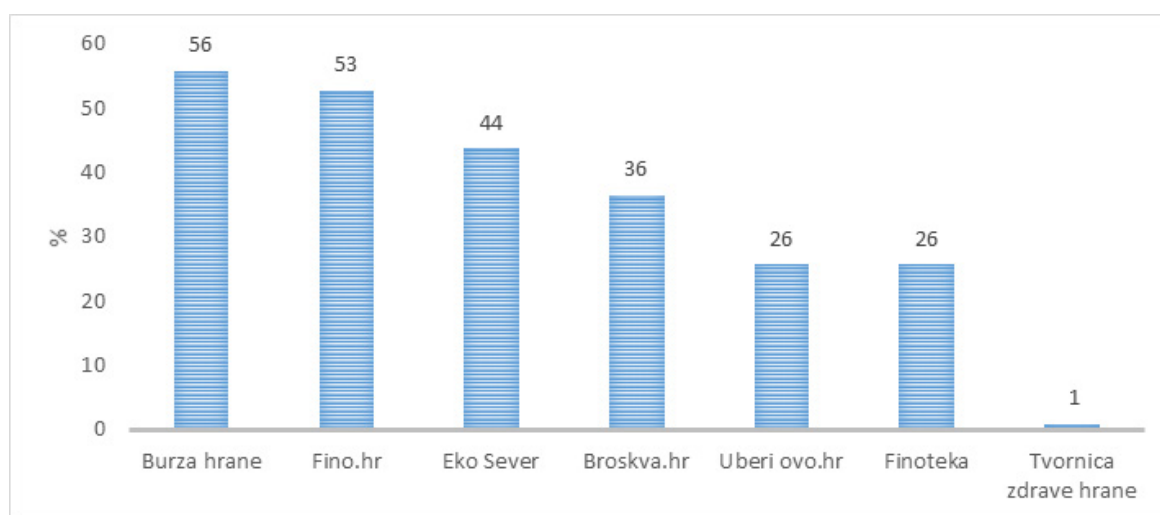
Rezultati i rasprava

Opis uzorka

U istraživanju je sudjelovalo 50,5% ženskih i 49,5% muških ispitanika. Najviše je potrošača u dobi od 30 do 45 godina (45,9%), dok je manji udio ispitanika u dobnim kategorijama između 18 i 29 godina (28,7%) te između 46 – 60 godina (22,5%). Samo 3,2% ispitanika ima više od 60 godina. Najviše ispitanika ima završen magisterij ili doktorat (42,2%), zatim slijede ispitanici s višom i visokom stručnom spremom (39,5%), te završenom srednjom školom (17%). Najmanje je ispitanika sa završenom osnovnom školom (1,3%). Najviše je kućanstva u životnom ciklusu bez djece (43%), slijede ih oni s djecom vrtićke i predškolske dobi (18,8%) i djecom u osnovnoj školi (17,5%). S obzirom na mjesto prebivališta većina ispitanika živi u gradu (74%). Ukupna mjesečna primanja kod najvećeg broja ispitanika su između 6001 do 12000 kn (36,8%), 33,2% ima ukupna mjesečna primanja od 4001-6000kn, a 19,3% do 4 000 kn. Najmanje ispitanika ima primanja više od 12.000 kn (10,8%).

Obilježja potražnje kupaca ekoloških prehrambenih proizvoda putem Interneta

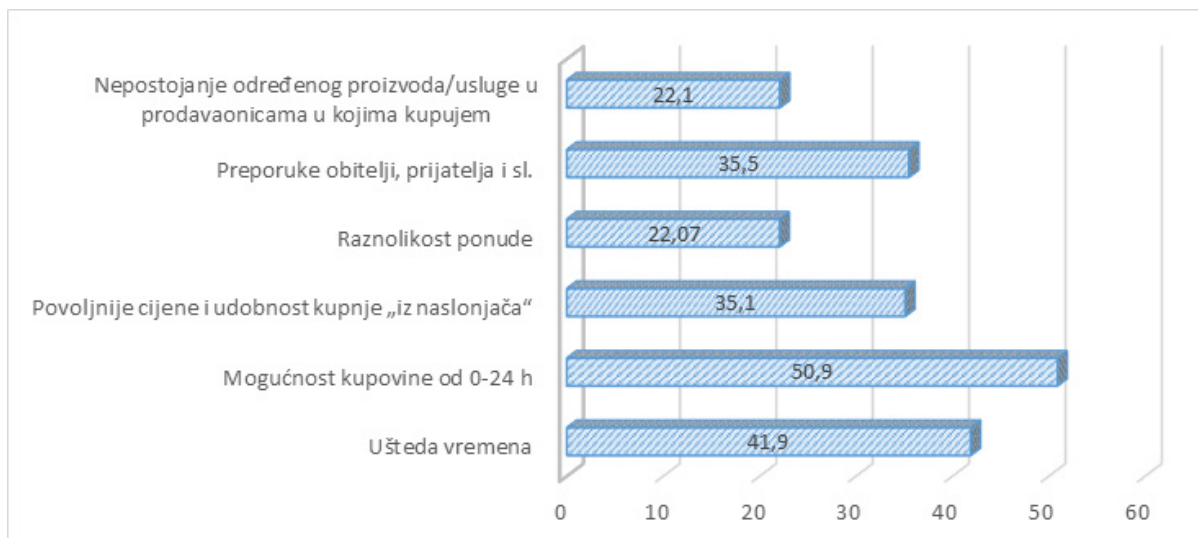
Najveći broj ispitanika ekološke prehrambene proizvode kupuje preko servisa Burza hrane (56% ispitanika), Fino.hr (53% ispitanika), Eko Sever (44%), Broskva. Hr (36%), Uberi ovo.hr (26%), Finoteka (26%), Tvorница zdrave hrane (1%).



Graf 1. Najzastupljeniji internet servisi putem kojih se kupuju ekološki proizvodi

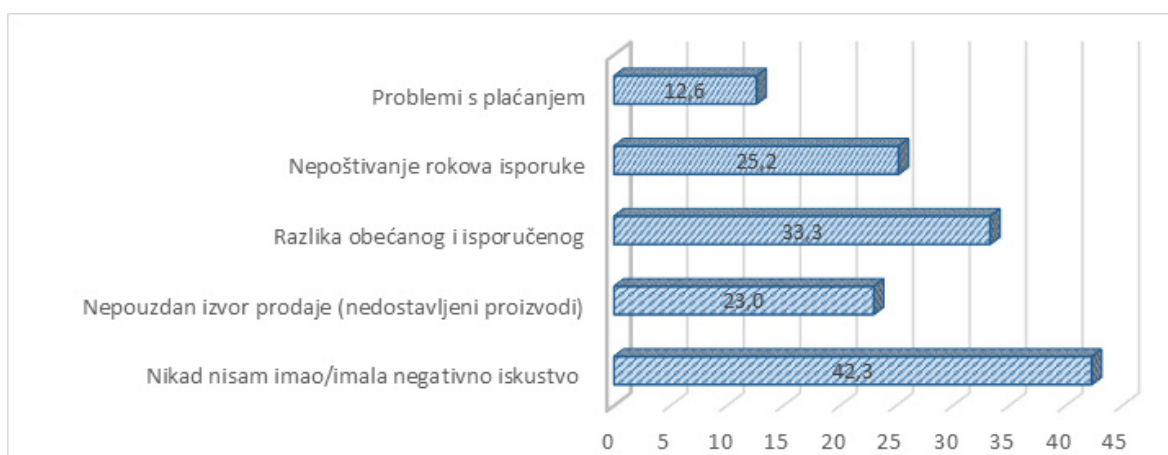
U online kupnji su najzastupljenije sljedeće kategorije proizvoda: med (n=108), voće i povrće (n=77), meso i mesne prerađevine (n=68), mlijeko i mliječne proizvode (n=67), te jaja (n=66). Najmanje je zastupljena riba i morski proizvodi (n=57), te kruh, brašno i pekarski proizvodi (n=51).

Na pitanje o mjesečnim izdvajanjima za onlinu kupnju ekoloških proizvoda najviše ispitanika izdvaja između 501 – 800 kuna (31,1%), zatim približno podjednak udio ispitanika izdvaja između 301 – 500 kuna (22,1%) te između 101 – 300 kuna (20,7%). Manji udio ispitanika (18,9%) troši više od 800 kuna mjesečno za kupnju ekoloških proizvoda. Najmanje ispitanika (7,2%) troši manje od 100 kuna mjesečno. Podjednak udio ispitanika plaćanje kupljenih proizvoda vrši direktno dostavljaču/plaćanje poduzećem (52,5%) te putem kreditne kartice (47,5%). Kao najvažnije razloge online kupnje ekoloških prehrambenih proizvoda ispitanici navode mogućnost kupnje od 0-24h (50,9%) i uštedu vremena (41,9%), preporuku obitelji i prijatelja (35,1%), povoljnije cijene i udobnost kupnje „iz kauča“.



Graf 2. Razlozi kupnje ekoloških proizvoda putem Interneta

Ispitanici su pri online kupnji ekoloških prehrambenih proizvoda najzadovoljniji dostavom (srednja ocjena 3,92), uslugom (3,91), te kvalitetom ponude (srednja ocjena 3,90). Manje su zadovoljni asortimanom, ponudom (3,82) i prodajnom cijenom (3,78).



Graf 3. Problemi s kojim se kupci susreću pri online kupnji

Najveći udio ispitanika nikad nije imao negativno iskustvo u online kupnji (42,3%). Kao probleme s kojima su se susreli u online kupnji ispitanici navode razliku između očekivanog i dobivenog proizvoda (33,3%) te nepoštivanje rokova isporuke (25,2%). Najveći broj ispitanika (46%) smatra da Hrvatska nema dobre standarde za sigurnost hrane koja su prodaje putem Internet trgovine, 22% ispitanika smatra da ima, dok 31% ispitanika ne zna odgovor na postavljeno pitanje. Na pitanje je li online prodaja hrane putem Internet servisa u Hrvatskoj zakonski dobro regulirana, visok udio ispitanika (46%) smatra da nije. Gotovo 38% ispitanika ne zna odgovor na to pitanje, a svega 15,8% njih smatra online prodaju hrane u Hrvatskoj zakonski dobro reguliranu. Rezultati hi kvadrat testa pokazali su da ne postoji statistički značajna povezanost između sociodemografskih obilježja ispitanika (spol, dob, obrazovanje, dohodak, životni ciklus obitelji) u odnosu mjesečna izdvajanja, razloge kupnje putem interneta te zadovoljstvo pojedinim obilježjima online prodaje ($p < 0.05$).

Zaključak

Rezultati istraživanja su pokazali da su najposjećeniji Internet servisi za kupovinu ekoloških prehrambenih proizvoda Burza hrane, Fino.hr i Broskva.hr preko kojih ispitanici najčešće kupuje med, voće i povrće, te meso i mesne prerađevine. Najviše ispitanika mjesečno troši između 501 – 800 kuna za onlinu kupnju ekoloških proizvoda. Kao najvažnije razloge kupnje putem Interneta ispitanici navode mogućnost kupnje od 0-24h i uštedu vremena. Ispitanici su pri online kupnji najviše zadovoljni dostavom i uslugom, a najmanje prodajnom cijenom ekoloških proizvoda. Kao najvažnije probleme izdvajaju vrijeme čekanja isporuke i razliku između očekivanog i dobivenog proizvoda. Približno polovica ispitanika smatra da Hrvatska nema dobre standarde za sigurnost hrane koja su prodaje putem Internet trgovine te da prodaja hrane preko Interneta nije dobro zakonski regulirana. Ovi nalazi daju praktične savjete potrošačima koji kupuju hranu preko interneta, online trgovcima na malo te kreatorima politika u svrhu boljeg informiranja potrošača o standardima sigurnosti hrane koja se prodaje putem Interneta.

Napomena

Podaci iznijeti u radu predstavljaju rezultate istraživanja diplomskog rada studentice Mateje Šaban na Agronomskom fakultetu Sveučilišta u Zagrebu pod nazivom: Profil online kupaca ekoloških prehrambenih proizvoda u Hrvatskoj.

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Characteristics of online purchases of organic food products in Croatia

Abstract

The aim of the research is to determine the characteristics of online purchases of organic food products in Croatia. An online survey was conducted on a convenience sample of 222 online shoppers. The most visited online service for buying organic food is the Burza hrane and Fino.hr. The most common food product in online shopping are eco honey, fruits and vegetables. The most important reasons for online buying are the possibility of buying from 0-24h and saving time. The problems most commonly encountered with online shopping are the waiting time for delivery and the difference between the expected and received product. A high proportion of respondents believe that Croatia does not have good standards for the safety of food sold through an online food services and that the online sale of food in Croatia is not legally well regulated.

Keywords: organic food products, online purchases, consumer behavior

Efficiency comparison of the main Croatian and Hungarian animal husbandry sectors

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Abstract

The animal husbandry sectors play an important role both in Hungarian and Croatian agriculture. To our knowledge, no research has been done so far to determine and compare efficiency levels of different animal husbandry farms in these two countries. The objective of this research was to examine the technical efficiency indicators of dairy, beef, broiler, sheep and pig sectors in Hungary and Croatia using DEA VRS method. We used data from national FADN databases for the period 2014-2017 for the calculations of indicators. The results of the research show us that from five main animal husbandry sectors, Hungarian farms score better in dairy and beef sector. Croatian farms achieve higher technical efficiency level in the sheep sector. In pig and broiler sectors farms from both countries achieve very similar technical efficiency indicators.

Keywords: animal husbandry sectors, technical efficiency, DEA, Croatia, Hungary

Introduction

In terms of the global nutrition supply, it is essential to increase animal and animal products production efficiency in the future to meet the enormous meat and dairy product demand due to the expected population growth (UN 2019). From economic and social point of view, increasing the efficiency level of the food production is a highly important area of the European Union (EU), Croatian and of the Hungarian agriculture as well. In general, the efficiency is a very broad concept, so it is necessary to define accurately what we mean by it, i.e. which factors influence it, what the assessment indicators are and what methods can be used to measure the efficiency of the production. The question of efficiency should be a priority area for both the Croatian and Hungarian animal husbandry farms, since it is frequently applied for determination of strength and weaknesses of the production units (Kočišová 2015).

The general objectives of this research were to explore the main indicators of the animal husbandry sector in Croatia and Hungary, and then to define and systematize their efficiency and relevant concerning factors for the most important animal husbandry sectors.

Material and methods

Efficiency is a widely used concept in economic evaluations, e.g. in measuring farm level performance. Economic (or overall) efficiency can be expressed as a combination of technical and allocative (or price) efficiencies. Technical efficiency is the ability of a farmer to obtain maximal output from a given set of inputs, while allocative efficiency measures the ability of the farmer to use inputs in optimal proportions, given their input prices and technology (Coelli et al. 2005, Begum et al. 2009, Toma et al. 2015). Several scientific methods are suitable for measuring efficiency. One of the generally used methods is data envelopment analysis (DEA) (Kočišová 2015). Since the method does not use a functional relation between inputs and outputs the possibilities of its employment are manifold (Kočišová 2015, Toma et al. 2015). Consequently, DEA has been widely used for the efficiency assessment in agriculture as well (Atici and Podinovski 2015, Kočišová 2015). By using of DEA we are able to explore hidden reserves within the given sectors and countries in different time lines.

In this research, we used input orientation to measure the DEA VRS (variable returns to scale) technical efficiency

in different animal husbandry sectors from two countries. The DEA VRS formula envelopes data points more tightly and provides higher or equal efficiency scores than the CRS (constant returns to scale) model. The difference between VRS and CRS technical efficiency scores represents the scale inefficiency.

In order to measure technical efficiency, we used the radial measures of technical efficiency. The advantage of the radial approach is that its technical efficiency measures are easily interpreted and communicated as the maximum percentage reduction of inputs required to produce a given output bundle, or the maximum percentage expansion of outputs allowed for at given inputs.

DEA VRS (input oriented) model and Directional Input Distance mathematical model is written in the following way (Serra et al. 2011):

$$\begin{aligned} & \underset{\beta, \lambda}{\text{Max}} \beta \\ \text{s.t.} \quad & -y_i + Y\lambda \geq 0 \\ & x_i - \beta g_x - X\lambda \geq 0 \\ & \lambda' \mathbf{1} = 1 \\ & \lambda \geq 0 \end{aligned}$$

where:

β = Technical Inefficiency score

g_x = directional distance vector

λ = vector of parameters (firm weights)

X and Y are matrixes with all outputs and inputs used in the model.

A main result of DEA modelling is the technical efficiency indicator which ranges between 0 and 1. The value 1 means fully efficient firm and there is no place for improvement. The distance from the estimated production frontier (1, or 100% efficient) represents the technical efficiency reserves or improvement potential for a certain unit of analysis.

In our research we used a database from the European Farm Accountancy Data Network (FADN) provided by the Research Institute of Agricultural Economics in Hungary (AKI) and the Croatian FADN Office at the Ministry of Agriculture. From the database, we selected data for specialised dairy, beef, pig, poultry, and sheep farms for the period 2014-2017.

Table 1. The number of farms in Hungarian and Croatian FADN sample per year and sector

Year	Dairy farms		Beef farms		Pig farms		Sheep farms		Broiler farms	
	HUN	CRO	HUN	CRO	HUN	CRO	HUN	CRO	HUN	CRO
2014	100	210	33	76	57	17	30	74	123	4
2015	112	209	33	81	60	23	33	71	121	16
2016	108	200	38	79	98	22	34	61	121	11
2017	109	209	37	76	87	21	28	70	115	19

Source: FADN 2019 database, AKI and Republic of Croatia Ministry of Agriculture.

Depending on the sector, we used one or two outputs in the input orientated DEA models. The output variables in the dairy farm type were: (1) cow's milk and milk products value (in EUR, FADN code: SE216); and (2) beef and veal value (in EUR, FADN code: SE220). The one output variable for the beef farm type was beef and veal value (in EUR, FADN code: SE220). The output of the pig farm type was pig meat value (in EUR, FADN code: SE225). The output variable for the broiler sector was poultry meat value (in EUR, FADN code: SE230) (FADN 2017).

The following five input variables were used for the model (Kovacs 2009 and 2016):

(1) Total fixed assets: value of buildings and land associated with agricultural activity (in EUR). FADN variable code: SE441.

(2) Total current assets: The current assets comprise values of stocks and other short term assets expressed in EUR. FADN variable code: SE465.

(3) Labour input: This is the total number of working hours at annual basis, and the FADN variable code is SE011

(4) Major cost items: This input factor is a sum of the three biggest costs categories expressed in EUR: livestock feed, energy cost and other direct costs. The total value of major cost items is calculated as a sum of the three FADN corresponding variables: SE310, SE330 and SE345.

The fifth input variable depends on the particular farm type or sector:

(5) Dairy cows: The number expressed in the European livestock units (LSU). (FADN code: SE085).

(6) Sheep and goats: The number on the farm expressed in LSU. (FADN code: SE095).

(7) Pigs: The number on the farm expressed LSU. (FADN code: SE100).

(8) Poultry: The number on the farm expressed in LSU. (FADN code: SE105).

Efficiency indicators for all farms were calculated for each year in the period 2014-2017. Beside the global efficiency level, the efficiency was also analysed by 3 farm size groups:

- small farms with the standard output value between 4-25 thousand EUR
- medium farms with the standard output value between 25-500 thousand EUR and
- large farms with the standard output value higher than 500 thousand EUR.

The results were also analysed by countries. Altogether, 160 frontier estimation models were created under different time, spatial and size dimensions.

Results and discussion

Dairy sector

We can observe from Figure 1. that the return to scale technical efficiency for input orientated variables on Hungarian dairy farms is 8-13% higher than in Croatia. The sample size was 200-210 in the Croatian dairy sector and 100-112 in Hungarian. The results show that the worst year was 2016 for both countries regarding the individual farms' technical efficiency. That was the first year after the European Quota System abolishment, when the output prices decreased in this sector.

Table 2. Technical efficiency indicators of Hungarian and Croatian farms according to animal husbandry sectors between 2014–2017.

Year	Dary farms		Beef farms		Pig farms		Sheep farms		Broiler farms	
	HUN	CRO	HUN	CRO	HUN	CRO	HUN	CRO	HUN	CRO
2014	0,816	0,738	0,739	0,650	0,867	0,883	0,739	0,809	0,867	0,952
2015	0,757	0,648	0,698	0,515	0,803	0,761	0,727	0,823	0,804	0,752
2016	0,748	0,614	0,701	0,643	0,846	0,873	0,705	0,805	0,819	0,867
2017	0,782	0,658	0,800	0,601	0,852	0,856	0,759	0,897	0,816	0,717

The results by farm size groups shows that the Hungarian small size dairy farms efficiency is 91.5% on average, while the Croatian farms average is 79.6%. The Croatian farms efficiency was also heavily fluctuating during the examined periods (69.1% to 85.9%).

The medium size dairy farms technical efficiency values are quite close to each other for Croatia and Hungary. The difference in the period 2015–2017 is only 1-2%, and the average values are 80.2% in Hungary and 77.5% in Croatia. It is interesting that in Croatia small scale farms efficiency is higher (84.1%) than for medium size farms (74.8%), which means that medium farms have to improve their performance. There was only one large Croatian dairy farm in our sample, so we could not compare country level results for this size group. The large size Hungarian dairy farms average efficiency indicator is 94.5% for the examined period, which means that there is 5.5% efficiency improvement potential for that farms. It can be done by decreasing the input for the same output or by increasing the output with the same level of inputs.

Beef sector

The Hungarian beef sector is 13.3% more efficient on average than the Croatian. In 2017, the difference between the two countries was the highest (20%). This is probably due to the large number and share of medium size farms in Croatia, while there are no large size beef farms.

Small beef farms average efficiency is higher in Hungary (86.2%) compared to the Croatian farms (74.4%). According to the results, year 2016 was quite unfavourable for the small size beef farms in both countries. The biggest difference between Croatian and Hungarian beef farms was determined for the group of medium size: 63.3% for Croatia and 84.2% for Hungary.

Same as with the dairy sector, technical efficiency of Croatian small size farms is higher (74.4%) than of medium size farms (63.3%). In the beef sector we can observe a similar trend with regard to the Hungarian farms, except for 2016. Both countries do not have enough large beef farms in the FADN sample, thus the technical efficiency indicator for this size category is not available.

Pig sector

The pig sector average technical efficiency indicator is 84.2% for the Hungarian, and 84.3% for the Croatian pig farms. These values indicate that farms had similar average efficiency level in both countries in the examined period.

The difference in the average efficiency between the two countries numbers on small size pig farms is actually small (only 1-2%). Simultaneously, the small farms technical efficiency was over the medium farms efficiency in both countries. The medium size farms efficiency indicator is 2% higher in Hungary on average. However, in 2016 it was higher in Croatia for 6.7%, while in 2017 the difference was again higher for the Hungarian farms for 11.9%. Same as for the dairy farms, there were no large size Croatian pig farms in the sample, while the efficiency of large Hungarian pig farms was increasing from 93.1% to 95.4% in the examined period.

Sheep sector

The sheep sector is the only sector in which the Croatian farms' technical efficiency values dominate. The average technical efficiency during four years is 83.4% in Croatia, in Hungary the average is 73.3%. In 2017 the Croatian farms technical efficiency reached the maximum of the period: 89.7%.

According to the farm size groups, the efficiency of small size sheep farms in Hungary is 1-9% higher than in Croatia. In 2016, this value was the highest in Hungary (97.6%). In this sector, the biggest difference between countries is with medium size farms. The Hungarian farms average technical efficiency is 80.2%, while the Croatian farms average is 93%. It is in line with findings that in Hungary small sheep farms achieve higher efficiency than medium size farms, while in Croatia the situation is the opposite. There were no large size sheep farms in the sample, thus we were not able to analyse them.

Broiler sector

The broiler sector average efficiency is almost the same for the two countries (82.2% and 82.7%). Here we have to mention that the sample size of this type of farms was almost ten times larger in Hungary than in Croatia.

As a consequence of the small sample, there are no small or large broiler farms in the Croatian FADN sample. The technical efficiency of Hungarian small size broiler farms in four years is 96.9%. The worst year for this group was 2015, when technical efficiency decreased to 92.5%.

The efficiency of medium size broiler farms is rather fluctuating. The average technical efficiency value of Hungarian farms is 86.9%, while the value of Croatian farms is 84.1%. The group of large broiler farms in Hungary has average technical efficiency indicator 98.1% in the period 2014-2017 (ranges from 97.2% to 99.2%). The results for large farms suggests that there is actually no space for improvement in the technical efficiency.

Conclusions

In this research, we calculated and compared the technical efficiency indicators of Croatian and Hungarian animal husbandry sectors for the period 2014-2017. Having in mind significant differences among particular sectors as well as farms of different sizes, we determined technical efficiency indicators across different farm types and sizes. According to the results obtained, the Hungarian farms are better at technical efficiency in dairy and beef sectors, while the Croatian farms prevail in sheep sector. The performance of farms in pig and broiler sector is almost the same in both countries. With respect to the farm size groups, there are some sectors where the technical efficiency indicators of small size farms are higher than of medium farms. In Hungary, this is the case with broiler and sheep sector, in Croatia with beef and dairy sector, and in both countries with pig sector.

Future research are needed to examine the reasons for the differences found between countries, both in terms of type and size of farms. There are many factors that could significantly influence the results obtained, ranging from farm characteristics to environmental and socio-economic factors.

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Vanjskotrgovinska razmjena Republike Hrvatske i Bosne i Hercegovine poljoprivredno-prehrambenim proizvodima u razdoblju od 2013. do 2018. godine

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Sažetak

U radu je analizirana vanjskotrgovinska razmjena poljoprivredno-prehrambenih proizvoda između Republike Hrvatske i Bosne i Hercegovine u razdoblju od 2014. do 2018. godine. Bosna i Hercegovina je i dalje najvažniji trgovinski partner, iako je primjetno da se pristupanjem Hrvatske Europskoj Uniji, značaj vanjskotrgovinske razmjene između dvije države smanjio. U analiziranom razdoblju RH najviše uvozi svinjskog mesa (70.000 t) i to najviše uvozimo na razini EU. Primjetno je da RH ima probleme u proizvodnji mlijeka i mliječnih proizvoda, jaja i meda, gdje također imamo velik uvoz i negativnu razliku u odnosu prema izvozu od 144 milijuna €. Proizvodnja mlijeka svedena je na 470 milijuna litara, a potrebe su gotovo milijardu litara. Kretanja vanjskotrgovinske razmjene u promatranom razdoblju se kreću u intervalima da je razlika uvoza i izvoza iznosila preko 900 milijuna €. Pokrivenost uvoza izvozom kreće se od 61% do 67%. Ono što je zabrinjavajuće jest da se sirovine poljoprivrednih proizvoda u velikoj mjeri izvoze na tržište EU-a, i to prema relativno niskim cijenama, a uvozimo gotove poljoprivredno-prehrambene proizvode, odnosno proizvode s dodanom vrijednošću. BiH je iznimno važna država za izvoz za RH, obzirom da do 70% izvoza u članice CEFTA-e odnosi se na izvoz u BiH, konkretno mlijeko i mliječne proizvode, zatim pekarske i mlinarske proizvode. Vrijednost vanjskotrgovinske razmjene poljoprivredno-prehrambenih proizvoda u 2018. godini čini 13,3% vrijednosti ukupne robne razmjene Republike Hrvatske, pri čemu je vrijednost izvoza poljoprivredno-prehrambenih proizvoda iznosila 13,7% ukupne vrijednosti izvoza robne razmjene, a vrijednost uvoza poljoprivredno-prehrambenih proizvoda je iznosila 13,5% od ukupne vrijednosti uvoza robne razmjene.

Ključne riječi: uvoz, izvoz, poljoprivredno-prehrambeni proizvodi, poljoprivreda, RH, EU

Uvod

Jedan od glavnih ciljeva makroekonomske politike suvremenih svjetskih gospodarstava je postići pozitivnu vanjskotrgovinsku razmjenu. Vanjskotrgovinska razmjena tumači se kao promet roba i usluga između različitih država. Glavne komponente vanjskotrgovinske razmjene su izvoz i uvoz, a njihov odnos jedan je od ključnih čimbenika za rast bruto domaćeg proizvoda, što se ogleda u bržem gospodarskom razvoju. Izvoz proizvoda s dodanom vrijednošću, za razliku od izvoza isključivo sirovina i polu-proizvoda, gospodarstvu donose dobit, održivu konkurentnost i punu zaposlenost. Vanjskotrgovinska bilanca iskazuje odnose izvoza i uvoza, a viša vrijednost izvoza od vrijednosti uvoza znači suficit, dok deficit označava višu vrijednost uvoza od vrijednosti izvoza. Međunarodna razmjena posljedica je unutrašnjih zbivanja u privredi, proizvodnji i potrošnji, odnosa ponude i potražnje, viškova i manjkova roba u gospodarstvu otvorenom prema svjetskom tržištu. Postoji snažna veza između vanjske trgovine i rasta društvenog proizvoda. Veće stope rasta ostvaruju države s uspješnim i konkurentnim izvoznim sektorima, razvijenim domaćim tržištem ili pristupom drugim tržištima. Imajući u vidu da države ovise jedne o drugima u vanjskotrgovinskoj

razmjeni, tako se razvila potreba za koordinacijom. U svrhu koordinacije, stvaranja institucionalnih okvira i zaštite zajedničkih interesa, osnovane su trgovinske organizacije kao što se za vanjskotrgovinsku razmjenu u Republici Hrvatskoj određuje članstvo u Svjetskoj trgovinskoj organizaciji (WTO) i Europskoj uniji (EU). Pristupanjem RH u EU, prestali su vrijediti svi bilateralni i multilateralni ugovori o slobodnoj trgovini koje je RH sklopila 2006, a od kojih je posebno važan Srednjoeuropski ugovor o slobodnoj trgovini (CEFTA). Na tržište CEFTA-e RH je prije pristupanja u EU prodavala gotovo petinu ukupnog izvoza (20%), a uvozila otprilike 6%. Važan je podatak i da s gotovo svim potpisnicama CEFTA-e RH je ostvarivala suficit u vanjskotrgovinskoj bilanci. Najvažniji trgovinski partneri CEFTA-e u koje je RH izvozila su Bosna i Hercegovina i Srbija.

Pristupanjem RH u punopravno članstvo EU (2013), trebalo je omogućiti poljoprivrednim proizvođačima sigurno tržište, te im se nastojalo omogućiti da posluju temeljem tržišnog mehanizma što, kako i za koga proizvoditi. Poslovanje prema ovom tržišnom mehanizmu svim proizvođačima bi se omogućilo osigurano tržište, dostatna ponuda za potražnju, što za posljedicu ima povećanje proizvodnje, te veliki utjecaj na vanjskotrgovinsku razmjenu. Vanjskotrgovinska razmjena poljoprivredno-prehrambenih proizvoda ovisna je o domaćoj proizvodnji, razvijenosti industrije prehrambenih proizvoda, stvarnoj i potencijalnoj domaćoj potražnji, promjenama na svjetskom tržištu i slično.

Stručnjaci i znanstvenici su predviđali pravne i ekonomske aspekte pristupanja RH u EU čitavo desetljeće prije samog pristupanja RH, te su tako Ćudina i Sušić (2013) predviđjeli da će se prihvaćanje trgovinskog režima EU, a napuštanje režima CEFTA-e negativno odraziti na vanjskotrgovinsku razmjenu sa BiH i Srbijom. Grgić i sur. (2011) smatrali su da će se pridruživanjem RH u EU povećati vanjskotrgovinska razmjena poljoprivredno-prehrambenih proizvoda, ali da će i negativno utjecati na čitav poljoprivredno-prehrambeni sektor obzirom na ne konkurentnost domaćih poljoprivrednih proizvođača u odnosu na proizvođače iz drugih država članica.

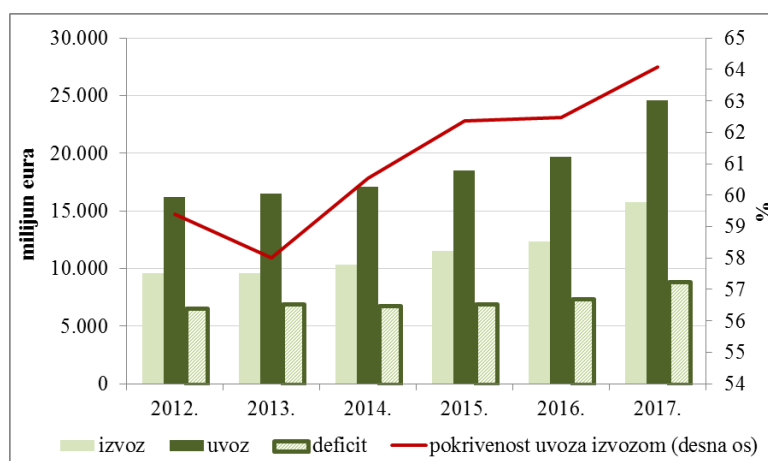
U radu se analizira ukupna vanjskotrgovinska razmjena RH nakon pristupanja u EU, vanjskotrgovinska razmjena u poljoprivrednom sektoru, s naglaskom na robnu razmjenu s Bosnom i Hercegovinom, u razdoblju od 2013. do 2018. godine.

Materijali i metode

U radu su korišteni sekundarni izvori podataka. Izvori sekundarnih podataka su bili znanstveni radovi, izvješća Hrvatske gospodarske komore, Državnog zavoda za statistiku, Ministarstva poljoprivrede, te internetski izvori koji aktualiziraju predmet rada. Svi analizirani podaci odnose se na razdoblje od pristupanja RH u EU 2013. godine do kraja 2018. godine. U ovom istraživanju su primijenjene deskriptivne metode, metode analize, metode sinteze, induktivne metode, metode komparacije, metode klasifikacije, generalizacije i sinteze za opisivanje pojedinih pojmova i komparativne metode za prikaz kretanja i strukture vanjskotrgovinske bilance.

Rezultati i rasprava

Prema podacima DZS-a ukupna vanjskotrgovinska razmjena RH s inozemstvom je od 2013. godine (26,1 milijarda €) u konstantnom porastu, tako je u 2018. godini ukupna vrijednost vanjskotrgovinske razmjene dobara i usluga iznosila 38,3 milijardi € (Grafikon 1).



Grafikon 1. Vanjskotrgovinska razmjena Republike Hrvatske od 2012. do 2017. godine
Izvor: DZS (2013-2019), obrada autora

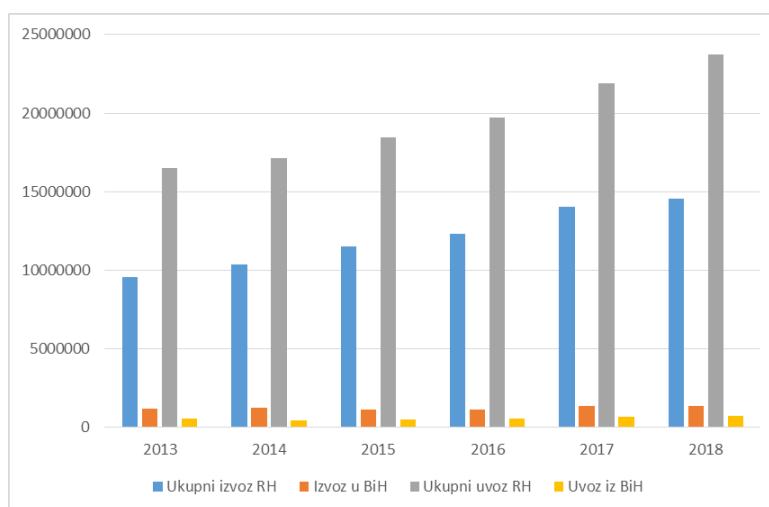
Za promatrano razdoblje, povećanje iznosi 31,85%. U 2017. godini vrijednost izvoza ukupne robne razmjene iznosi 14,0 milijardi €, dok vrijednost uvoza ukupne robne razmjene iznosi 21,9 milijarde € što je rezultiralo deficitom od 7,9 milijardi €. Deficit ukupne robne razmjene u 2017. godini povećan je za 479,4 milijuna € (za 6,5%) u odnosu na 2016. godinu. Pokrivenost uvoza sa izvozom ukupne robne razmjene u 2017. godini iznosila je 64,0%, odnosno razina pokrivenosti veća je za 1,6 postotna boda u odnosu na 2016. godinu. Vrijednost vanjskotrgovinske razmjene poljoprivredno-prehrambenih proizvoda od 2013. do 2018. godine čini od 13-14% vrijednosti ukupne robne razmjene RH, pri čemu vrijednost izvoza poljoprivredno-prehrambenih proizvoda također čini od 13-14% vrijednosti izvoza ukupne robne razmjene, a vrijednost uvoza poljoprivredno-prehrambenih proizvoda čini 13-14% vrijednosti uvoza ukupne robne razmjene (Tablica 1).

Tablica 1. Udio robne razmjene poljoprivrednih i prehrambenih proizvoda RH u ukupnoj robnoj razmjeni s inozemstvom

Godine	IZVOZ %	UVOZ%
2014.	13	14
2015.	14	13
2016.	13	13
2017.	14	13
2018.	14	13

Izvor: DZS (2014-2019), Obrada autora

Republika Hrvatska u promatranom razdoblju od poljoprivredno-prehrambenih proizvoda najviše je uvozila mesa i klaoničkih proizvoda, u iznosu od 310 milijuna € u 2018. godini, zatim proizvoda na bazi žitarica i škroba u iznosu od 208 milijuna €, te mlijeka, mliječnih proizvoda, jaja i meda u iznosu od 206 milijuna € u 2018. godini (HGK, 2019). Analizirajući izvoz poljoprivredno-prehrambenih proizvoda, RH najviše je u 2018. godini izvezla žitarica u iznosu od 144 milijuna €, zatim riba, ljuskara, mekušaca i beskralježnjaka u iznosu od 136 milijuna €, te 127 milijuna € pića, alkohola i octa (HGK, 2019). RH je i prije pristupanja u EU najviše izvozila u zemlje članice CEFTA-e, tako je i danas BiH najveći uvoznik poljoprivredno-prehrambenih proizvoda RH. Do 2013. godine, gotovo 70% proizvoda izvezenih u članice CEFTA-e, izvezeno je u BiH (Kovačićek i sur., 2018). I nakon pristupanja RH u EU, BiH je ostala najvažnije izvozno tržište za poljoprivredno-prehrambene proizvode iz RH, ali u manjem obujmu nego li prije. Tako je RH u promatranom razdoblju, izvozila gotovo 19% poljoprivredno-prehrambenih proizvoda od ukupnog izvoza u BiH. Najvažniji izvozni proizvodi RH u BiH su pića, žitarice, čokolada, kao i pekarski i slastičarski proizvodi (DZS, 2013-2019). Pristupanjem u EU, uvoz poljoprivredno-prehrambenih proizvoda iz BiH se smanjio, te sada uvoz iz BiH u RH iznosi 2,5% ukupnog uvoza RH. RH je u promatranom razdoblju najviše izvezla u BiH 2017. godine, i to 1.37 milijardi €, dok je rekordan uvoz iz BiH u RH iznosio 733 milijuna € 2018. godine.



Grafikon 2. Ukupna robna razmjena RH s inozemstvom i BiH od 2013. do 2018. godine

Izvor: DZS (2014-2019), obrada autora

Zaključak

Poljoprivreda, jedan od glavnih čimbenika kako nacionalne tako i međunarodne trgovine, doprinosi rastu i razvoju gospodarstva i društva na razini svake zemlje. Međunarodna trgovina ukazuje na važno mjesto poljoprivrede u razvoju RH, a poljoprivredni su proizvodi vrlo značajni za vanjskotrgovinsku razmjenu RH s inozemstvom. Sukladno predviđanjima stručnjaka, RH je pristupanjem u EU povećala uvoz poljoprivredno-prehrambenih proizvoda iz država članica EU, dok je smanjila izvoz, te se stvara sve veći vanjsko trgovinski deficit. Najvažniji partner za izvoz RH je i dalje BiH, ali postotni udjel izvoza u BiH se smanjio nakon ulaska u EU. Najvažniji izvozni proizvodi RH u BiH su pića, žitarice, čokolada, te pekarski i slastičarski proizvodi. Uvoz iz BiH u RH je u konstantnom porastu u promatranom razdoblju.

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External trade of agricultural and food products between the Republic of Croatia and Bosnia and Herzegovina in the period 2014-2018

Abstract

This paper analyzes the foreign trade of agricultural and food products between the Republic of Croatia and Bosnia and Herzegovina in the period of 2014-2018. Bosnia and Herzegovina remains the most important trading partner, although it is noticeable that Croatia's accession to the European Union has reduced the importance of foreign trade between these two countries. During the analyzed period, the Republic of Croatia imports the most pork meat (70.000 t) and imports the most at EU level. It is noticeable that the Republic of Croatia has problems in the production of milk and milk products, eggs and honey, where we also have a large import and a negative difference in relation to exports of 144 million €. Milk production has been reduced to 470 million liters and needs are almost one billion liters. Foreign trade trends in the observed period range at intervals that the difference between imports and exports amounted to over 900 million €. Export- import coverage ranges from 61% to 67%. What is worrying is that agricultural raw materials are largely exported to the EU market at relatively low prices, and we are importing finished agri-food and value-added products. Bosnia and Herzegovina is an extremely important export country for the Republic of Croatia, since up to 70% of exports to CEFTA member countries relate to exports to Bosnia and Herzegovina, specifically milk and dairy products, followed by bakery and mill products. The value of foreign trade in agri-food products in 2018 amounts to 13.3% of the value of total commodity exchange of the Republic of Croatia, with the value of exports of agri-food products amounting to 13.7% of the total value of exports of commodity, and the value of imports of agri-food products accounted for 13.5% of the total value of foreign trade imports.

Keywords: import, export, agri-food products, agriculture, Croatia, European Union

Analiza mišljenja domaćih proizvođača vina o konkurenciji

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Sažetak

Cilj rada je analizirati stavove proizvođača vina u Istočnoj Hrvatskoj, a koji se odnose na usporedbu s domaćim konkurentskim proizvođačima vina. Za prikupljanje podataka korištena je metoda ankete, a kao instrument korišten je anketni upitnik. Istraživanje je provedeno na uzorku od $n = 30$ proizvođača vina iz pet županija u Istočnoj Hrvatskoj (Osječko-baranjske, Vukovarsko-srijemske, Virovitičko-podravske, Požeško-slavonske i Brodsko-posavske županije). Rezultati istraživanja pokazuju da su anketirani vinari svjesni konkurencije u svome okruženju. Anketirani proizvođači vina smatraju da su po pitanju kvalitete vina, dobrog odnosa s dobavljačima i zaposlenicima bolji u odnosu na domaće konkurente, a da su slabiji od konkurencije u pogledu izvoza proizvedenog vina, ulaganja u istraživanje tržišta i razvoj nove linije proizvoda.

Ključne riječi: proizvođači vina, analiza konkurenata, učinkovitost i uspješnost vinara

Uvod

Tržište vina posljednjih godina je pod utjecajem brojnih promjena, a konkurencija je sve intenzivnija i proizvođačima je teže doći do potencijalnih potrošača te se javlja potreba za strateškim planiranjem proizvodnje i intenziviranjem marketinških aktivnosti (Marone i sur., 2017.). Za uspješno planiranje marketinških aktivnosti potrebno je poznavati vlastitu konkurenciju. Tvrtke da bi danas opstale na tržištu neophodno je da stalno uče i uspoređuju se s najboljima te se u skladu s tim prilagođavaju i mijenjaju kako bi mogle pratiti trendove iz okoline i ostvariti zacrtane ciljeve, a upravo to omogućava benchmarking. Stoga ga menadžeri smatraju alatom koji se isplati primjenjivati jer donosi značajnu korisnost za poslovanje (Tomić Rotim, 2005.). Benchmarking predstavlja alat taktičkog i strateškog upravljanja razvojem poduzeća njegova glavna prednost je održavanje postojeće učinkovitosti i identificiranje, razumijevanje i prilagođavanje proizvoda i usluga te usporedba vlastitih rezultata poslovanja s konkurentskim subjektima koji se smatraju najboljima u cilju poboljšanja vlastitog poslovanja (Velychko, 2019.; Kristić, 2016.; Neacșu Bleajă i Bobeică, 2013.). Prema istraživanju Meler i Horvat (2018.) gospodarski subjekti trebali bi međusobno surađivati i povezivati se kako bi održali stabilnost i smanjili konkurenciju unutar prostornog okruženja. Studija slučaja provedena na četiri proizvođača vina u Istočnoj Hrvatskoj pokazuje da proizvođači vina, iako imaju mogućnosti za provedbu istraživanja vezanih uz istraživanje tržišta, potrošača i konkurentnost, takva istraživanja ne provode ili ih ne provode u dovoljnoj mjeri (Jelić Milković, 2019.).

Materijal i metode

U istraživanju za prikupljanje podataka korištena je metoda ankete, a kao instrument korišten je anketni upitnik kojeg su ispunjavali proizvođači vina iz pet županija u Istočnoj Hrvatskoj (Osječko-baranjske, Vukovarsko-srijemske, Virovitičko-podravske, Požeško-slavonske i Brodsko-posavske županije). Istraživanje je provedeno u razdoblju od svibnja do rujna 2018. godine. Ciljana skupina ispitanika su proizvođači vina (mali, srednji i veliki) koji su upisani u Vinogradarski registar. U 2018. godini u Republici Hrvatskoj u Vinogradarskom registru registrirano je 188 proizvođača grožđa i vina koji se mogu klasificirati kao subjekti definirani primarnim istraživanjem. Anketni upitnik poslan je na adrese proizvođača vina kako bi se ispitala struktura proizvođača vina u Istočnoj Hrvatskoj i problemi s kojima se susreću u svome poslovanju. Anketni upitnik ispravno je popunilo 30 proizvođača vina, čime odaziv

istraživanja iznosi 15,96%. Cjelokupni upitnik sadrži 88 pitanja otvorenog i zatvorenog tipa podijeljenih u nekoliko skupina. Za potrebe ovoga rada radi velike opširnosti anketnog upitnika prikazani su samo dijelovi istraživanja vezani uz analizu stavova ispitanika koji se odnose na njihovu usporedbu s domaćim konkurentskim proizvođačima vina koja su mjerena korištenjem Likerove ljestvice od 5 stupnjeva (1 – najlošiji smo, 5 – najbolji smo).

Analiza podataka provedena je u statističkom programskom paketu SPSS Statistic V23. U analizi podataka korištena je deskriptivna statistika kako bi se opisao uzorak (postotci, frekvencije, aritmetička sredina, standardna devijacija). Od neparametrijskih testova korišten je Kruskal - Wallis test rangova vezanih uz analizu pitanja o usporedbi domaćih proizvođača vina s domaćim konkurentskim subjektima kako bi se provjerilo postoje li najmanje dvije skupine između više skupina koje se statistički značajno razlikuju.

Rezultati i rasprava

Istraživanjem je obuhvaćeno 30 proizvođača grožđa i vina iz 5 županija u Istočnoj Hrvatskoj. Nešto veći broj 43,3% ispitanika je iz Osječko-baranjske županije, 23,3% iz Vukovarsko-srijemske županije, 20,0% iz Požeško-slavonske, 10,0% Brodsko-posavske i 3,3% Virovitičko-podravске. Svi anketirani proizvođači vina ($n=30$) su uvedeni u Vinogradarski registar. Većina anketiranih proizvođača vina njih 52,2% posjeduje od 1 do 10 ha zasađenih pod vinovom lozom, 26,1% ih raspolaže s 10-50 ha, a njih 17,4% raspolaže s više od 100 ha zasađenih pod vinovom lozom. Najveći broj ispitanika njih 33,3% godišnje proizvodi 100-500 hl, a njih 26,7% više od 1 000 hl. Među anketiranim proizvođačima vina dominiraju proizvođači čiji poljoprivredni subjekt nije orijentiran prema izvozu (50,0%), 26,7% proizvođača vina je slabo orijentirano prema izvozu, a svega 6,7% njih se izjasnilo da je njihov gospodarski subjekt visoko orijentiran prema izvozu. 3,3% proizvođača vina izjasnilo se da uvozi grožđe za daljinu preradu u vino, ali nitko od anketiranih proizvođača ne izvozi grožđe.

U tablici 1. prikazani su stavovi ispitanika vezani za usporedbu poljoprivrednog subjekta s domaćim konkurentskim subjektom. Na temelju odgovora ispitanika izračunate su aritmetičke sredine (M) i standardna devijacija (SD) kako je prikazano u navedenoj tablici. Ispitanici su putem anketnog upitnika izrazili svoje mišljenje o navedenim tvrdnjama prikazanim u tablici 1. u usporedbi s domaćim konkurentskim proizvođačima vina, a odnose se na podatke o kvaliteti proizvoda, istraživanju, razvoju i dizajnu proizvoda, cijeni, asortimanu, odnosu s kupcima i dobavljačima i izvozu. Anketirani proizvođači vina smatraju da su kvaliteta vina ($M=3,90$; $SD=0,712$) i dobri odnosi s dobavljačima ($M=3,70$; $SD=1,022$) bolji u odnosu na domaće konkurente. Prema istraživanju Leko Šimić i Štimac (2013.) ispitivani gospodarski subjekti u Republici Hrvatskoj najvećom ocjenom na Likertovoj skali od 5 stupnjeva ocijenili su kao važno kvalitetu proizvoda, dobar odnos s dobavljačima, visoku kvalitetu pružanja usluge, dobar odnos s kupcima i ekonomičnu proizvodnju. Domaći proizvođači vina smatraju da su zadovoljstvo i plaće zaposlenika bolje u odnosu na konkurentске gospodarske subjekte ($M=3,63$; $SD=0,928$) te da je fluktuacija zaposlenika manja nego kod konkurencije ($M=3,57$; $SD=1,073$). Na temelju aritmetičkih sredina prikazanih u tablici 1. može se zaključiti kako ispitivani domaći proizvođači vina smatraju da su slabiji od konkurentskih gospodarskih subjekata u pogledu izvoza proizvedenog vina ($M=2,27$; $SD=1,311$), uvođenja nove linije proizvoda na tržište ($M=2,73$; $SD=0,944$) i ulaganja u istraživanje i razvoj proizvodnje ($M=2,63$; $SD=1,129$) što ne treba čuditi jer je većinom riječ o malim i srednjim poljoprivrednim subjektima koji nemaju dostatna financijska sredstva. Podatci dobiveni ovim istraživanjem poklapaju se s istraživanjem Kristić (2016.) gdje su se ispitivani gospodarski subjekti također izjasnili da su slabiji od konkurencije u pogledu izvoza, ulaganja istraživanje i razvoj, brendiranja proizvoda te sigurnosti zadržavanja radnog mjesta.

Tablica 1. Deskriptivni statistički pokazatelji za usporedbu s konkurentima domaćih proizvođača vina

Usporedba s konkurentima	N	M	SD
Redovito prikupljamo informacije o kupcima.	30	3,27	0,828
Razvoj novih proizvoda.	30	3,43	0,774
Kvaliteta vina.	30	3,90	0,712
Širok asortiman proizvoda.	30	3,37	1,066
Cijene vina.	30	3,10	0,995
Izvoz vina.	30	2,27	1,311
Brendiranje vina.	30	2,90	1,296
Ulaganje u istraživanje i razvoj.	30	2,63	1,129
Uvođenje na tržište nove linije proizvoda.	30	2,73	0,944
Dobri odnosi s dobavljačima.	30	3,70	1,022
Pristup tržištu preko strateških saveza i partnerstava.	30	3,07	1,112
Zadovoljstvo i plaće zaposlenika.	30	3,63	0,928
Fluktuacija zaposlenika manja je nego kod konkurencije.	30	3,57	1,073
Vještine upravljanja marketingom.	30	2,93	1,048
Vještine razvoja proizvoda i dizajna.	30	3,10	0,845

N = ukupan broj ispitanika; M = aritmetička sredina, SD = standardna devijacija

Rezultati Kruskal-Wallis testa prikazani u tablici 2. prikazuju kako u slučaju trinaest varijabli postoje najmanje dvije skupine ispitanika koje se statistički značajno razlikuju s obzirom na varijablu orijentacija poljoprivrednog subjekta prema izvozu. Najmanje prosječne ocijene imaju sve tvrdnje kod poljoprivrednih subjekata koji su se izjasnili da nisu orijentirani prema izvozu vina. Najveće prosječne ocijene vezane uz tvrdnje izvoz vina, ulaganje u istraživanje i razvoj, pristup tržištu preko strateških saveza i partnerstava, uvođenje nove linije proizvoda na tržište i cijene vina dali su ispitanici koji su se izjasnili da je njihov gospodarski subjekt visoko orijentiran prema izvozu, a poljoprivredni subjekti slabo orijentirani prema izvozu najveće prosječne ocijene dali su tvrdnjama vještine razvoja proizvoda i dizajna i vještine upravljanja marketingom (tablica 2.).

Tablica 2. Odabrani rezultati istraživanja i Kruskal-Wallis test kategorija orijentacija poljoprivrednog subjekta prema izvozu

Usporedba s konkurentima	Prosječni rangovi				H	Sig. p
	Nikakva	Slaba	Osrednja	Jaka		
Redovito prikupljamo informacije o kupcima.	11,20	16,81	24,10	21,00	13,296	0,004**
Razvoj novih proizvoda.	11,17	17,25	24,00	19,75	12,328	0,006**
Kvaliteta vina.	12,20	15,13	23,30	22,25	8,590	0,035*
Širok asortiman proizvoda.	9,37	19,06	24,60	24,50	17,360	0,001**
Cijene vina.	11,50	18,13	20,70	22,00	7,929	0,048*
Izvoz vina.	9,50	18,88	22,50	29,50	17,818	0,000**
Brendiranje vina.	10,47	18,69	22,80	22,25	11,128	0,011**

Ulaganje u istraživanje i razvoj.	10,27	20,31	19,80	24,75	12,096	0,007**
Uvođenje na tržište nove linije proizvoda.	11,40	19,63	18,20	23,00	7,904	0,048*
Dobri odnosi s dobavljačima.	10,07	19,00	23,40	22,50	13,458	0,004**
Pristup tržištu preko strateških saveza i partnerstava.	10,27	19,94	20,90	23,50	11,713	0,008**
Zadovoljstvo i plaće zaposlenika.	14,03	16,25	19,40	13,75	1,738	0,628
Fluktuacija zaposlenika manja je nego kod konkurencije.	14,77	16,19	15,23	19,00	0,530	0,912
Vještine upravljanja marketingom.	11,50	22,06	16,80	16,00	8,294	0,040*
Vještine razvoja proizvoda i dizajna.	10,43	22,13	18,60	19,25	11,916	0,008**

** $p \leq 0,01$, * $p \leq 0,05$

Prema podacima prikazanim u tablici 3. u slučaju šest varijabli postoje najmanje dvije skupine ispitanika koje se statistički značajno razlikuju s obzirom na varijablu veličina poljoprivrednog subjekta izražena u hektarima. Poljoprivredni subjekti koji obrađuju od 50-100 ha vinograda su oni s najvećim prosječnim rangovima osim kod tvrdnje izvoz vina gdje najveći prosječni rang imaju subjekti s više od 100 ha vinograda i kod tvrdnje zadovoljstvo i plaća zaposlenika gdje je najveća vrijednost zabilježena kod ispitanika s 10-50 ha vinograda. Prema istraživanju Medlin i sur. (2015.) konkurencija ne predstavlja pozadinsku varijablu jer svaka suradnja je proizašla iz konkurentskih razloga autori nadalje navode da su neki gospodarski subjekti više fokusirani na potrošače u odnosu na konkurente, a riječ je većinom o starijim gospodarskim subjektima s boljim resursima međutim, svaki gospodarski subjekt bi trebao vršiti analizu konkurenata i djelovati u skladu s njom. Ako poduzeće posjeduje konkurentsku prednost i ako bolje od konkurencije zadovoljava potrebe potrošača tada treba posvetiti posebnu pažnju identificiranju i praćenju aktivnosti konkurencije poduzeća (Stanić i sur., 2018.). Prema Čengić (2013.) konkurentnost vinogradarske i vinarske proizvodnje moguće je postići povećavanjem kvalitete vina, unaprjeđivanjem trženja vina, povezivanjem vinara, razvojem i očuvanjem proizvodnih potencijala, ulaganjem u nove tehnologije, edukacijom i informiranjem proizvođača.

Tablica 3. Odabrani rezultati istraživanja i Kruskal–Wallis test kategorija veličina poljoprivrednog subjekta (ha)

Usporedba s konkurentima	Prosječni rangovi				H	Sig. p
	1-10 ha	10-50 ha	50-100 ha	>100 ha		
Redovito prikupljamo informacije o kupcima.	9,29	16,00	21,50	11,75	9,082	0,028*
Razvoj novih proizvoda.	9,88	15,33	21,50	11,00	6,150	0,0105
Kvaliteta vina.	10,50	13,50	21,00	12,00	3,300	0,0348
Širok asortiman proizvoda.	8,29	16,00	21,00	14,88	9,153	0,027*
Cijene vina.	9,50	8,08	16,00	14,50	6,592	0,086
Izvoz vina.	8,08	16,00	14,50	17,13	8,955	0,030*
Brendiranje vina.	10,00	15,50	22,00	10,25	5,422	0,143
Ulaganje u istraživanje i razvoj.	9,75	15,42	19,00	11,88	4,182	0,242
Uvođenje na tržište nove linije proizvoda.	10,38	13,83	20,50	12,00	3,330	0,344
Dobri odnosi s dobavljačima.	8,00	16,00	20,00	16,00	9,964	0,019*
Pristup tržištu preko strateških saveza i partnerstava.	7,79	17,08	17,50	15,63	10,637	0,014*

Zadovoljstvo i plaće zaposlenika.	10,13	18,25	15,00	7,50	8,911	0,030*
Fluktuacija zaposlenika manja je nego kod konkurencije.	9,54	15,50	6,50	15,50	5,827	0,120
Vještine upravljanja marketingom.	10,33	16,33	18,50	8,88	5,526	0,137
Vještine razvoja proizvoda i dizajna.	10,08	16,75	18,50	9,00	6,923	0,074

** $p \leq 0,01$, * $p \leq 0,05$

Zaključak

Tržište vina posljednjih godina je pod utjecajem brojnih promjena te ga karakterizira velik broj konkurenata. Natjecanje između proizvođača vina danas je sve izraženije i važno je da proizvođači znaju kakvu poziciju zauzimaju na tržištu, ali i da budu svjesni konkurencije te da rezultate svoga poslovanja uspoređuju s konkurencijom. Istraživanje pokazuje da su anketirani vinari svjesni konkurencije u svome okruženju te smatraju da su po pitanju kvalitete vina, dobrog odnosa s dobavljačima i zaposlenicima bolji u odnosu na domaće konkurente, a da su slabiji od konkurencije u pogledu izvoza proizvedenog vina, ulaganje u istraživanje tržišta i razvoj nove linije proizvoda. Rezultati istraživanja mogu poslužiti kao okvir za buduća istraživanja ovakvog tipa, ali također mogu pružiti informacije i koristan alat vlasnicima i proizvođačima vina, potencijalnim investitorima, ali i nositeljima ekonomske politike kako poboljšati učinkovitost u sektoru proizvodnje vina.

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Analysis of domestic winemakers' opinions on competition

Abstract

The objective of this paper is to analyse the attitudes of winemakers in Eastern Croatia regarding the comparison with domestic competing winemakers. Survey was the method used to collect the data, and survey questionnaire was used as the instrument. The survey was performed on a sample of n=30 winemakers from five Eastern Croatian counties (Osijek-Baranja, Vukovar-Srijem, Virovitica-Podravina, Požega-Slavonija and Brod-Posavina Counties). Survey results show that the surveyed winemakers are aware of the competition in their business environment. The surveyed winemakers believe their wine quality, good contacts with suppliers and employees are the same as their competition and that they are weaker in terms of exporting produced wine, investing in market research and new product development.

Keywords: winemakers, competitor analysis, winemaker efficiency and success

Iskustva poljoprivrednika Krapinsko-zagorske županije s fondovima Europske Unije

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Sažetak

Ulaskom Hrvatske u Europsku uniju (EU) otvorile su se brojne mogućnosti korištenja sredstava iz europskih fondova s ciljem razvoja ruralnog prostora, zaštite okoliša te jačanja konkurentnosti poljoprivredne proizvodnje. Cilj rada je utvrditi dosadašnja iskustva poljoprivrednika iz Krapinsko-zagorske županije s fondovima EU. Rezultati istraživanja pokazali su da većina ispitanika smatra kako sredstvima iz EU fondova mogu unaprijediti poslovanje svog gospodarstva te doprinijeti razvoju županije, a nezadovoljni su kompliciranom dokumentacijom te dugotrajnim i zahtjevnim procesom prijave.

Ključne riječi: fondovi EU, Krapinsko-zagorska županija, poljoprivreda, ruralni razvoj

Uvod

Nakon što je 18. lipnja 2004. Republika Hrvatska stekla status zemlje kandidatkinje, postali su joj dostupni pretpristupni programi prve generacije (PHARE, ISPA i SAPARD) u ukupnom iznosu od 252 milijuna eura za razdoblje 2005.-2006. Program PHARE bio je usmjeren na projekte jačanja kapaciteta zemlje za članstvo u EU, iz programa ISPA financirani su infrastrukturni projekti u području prometa i zaštite okoliša, a program SAPARD koristio se za unaprjeđenje poljoprivrednih gospodarstava i preradu poljoprivrednih proizvoda. U drugoj generaciji (2007.-2013.) Hrvatskoj je na raspolaganju bio pretpristupni program IPA. Stjecanjem punopravnog članstva Europske unije, od 2013. godine Republika Hrvatska je postala korisnica sredstava iz europskih fondova. U financijskom razdoblju 2014.-2020. Hrvatskoj je iz Europskih strukturnih i investicijskih (ESI) fondova na raspolaganju ukupno 10.676 milijardi eura, od kojih 2.026 milijarde eura za poljoprivredu i ruralni razvoj.

Europski fondovi su zamišljeni kao financijski instrumenti koji obuhvaćaju sredstva poreznih obveznika, a alokacijom tih sredstava prema jasno određenim pravilima podupiru se i ostvaruju ključni ciljevi određenih javnih politika EU (Europski strukturni i investicijski fondovi, 2019.). Ti ciljevi uključuju regionalni i urbani razvoj, zapošljavanje, socijalnu uključenost, poljoprivredu, ruralni razvoj, humanitarnu pomoć, istraživanje i inovacije. U ovom je istraživanju naglasak stavljen na poljoprivredu i ruralni razvoj.

Jurić (2016.) istražuje funkciju EU fondova u razvoju poljoprivrede Vukovarsko-srijemske županije te navodi kako oni predstavljaju priliku za smanjenje nezaposlenosti i poboljšanje gospodarstva u županiji. Istraživanje je pokazalo kako prepreku tome uvjetuje nedostatak znanja i informacija o projektima, natjecajima te mogućnostima koje stoje na raspolaganju. Kao rješenje autorica navodi postojanje dobro organizirane i učinkovite poljoprivredne savjetodavne službe te povećanje broja edukacija i ulaganja.

Dušević (2018.) istražuje stavove gospodarskih subjekata u Zadarskoj županiji o korištenju sredstava iz fondova EU te zaključuje kako na bolju apsorpciju dostupnih sredstava utječe pozitivan stav o EU financiranju. Ispitanici su kao nedostatak naveli dugotrajan i kompliciran proces, no također smatraju kako pomoću EU fondova mogu unaprijediti poslovanje svog gospodarskog subjekta. Nadalje, istraživanje je pokazalo kako će se donositelji odluka češće prijaviti na natječaje ukoliko imaju višu razinu informiranosti i ranija pozitivna iskustva. Autorica zaključuje da su za bolju iskoristivost raspoloživih sredstava potrebne bolja komunikacija i edukacije o EU fondovima i programima.

Cilj ovog rada bio je utvrditi iskustvo poljoprivrednika sa fondovima EU, uočiti u kojoj mjeri razina informiranosti i ranija iskustva utječu na zainteresiranost za korištenje sredstava EU, te istražiti pretpostavke i mišljenja poljoprivrednika o sredstvima koja stoje na raspolaganju RH te korisnost dosadašnjih provedenih projekata, financiranih iz fondova EU, za Krapinsko-zagorsku županiju.

Materijal i metode

Istraživanje je provedeno u lipnju i srpnju 2019. godine na području Krapinsko-zagorske županije u svrhu izrade diplomskog rada. Empirijski podaci prikupljeni su pomoću anketnog upitnika koji se sastojao od 26 pitanja, a ispunilo ga je ukupno 64 članova OPG-a. Od toga, 43 anketna upitnika ispunjena su u područnim uredima Ministarstva poljoprivrede (Savjetodavna služba) te 21 on-line slanjem linka ankete na e-mail adrese članovima OPG-a. Uvjet za sudjelovanje u istraživanju bio je posjedovanje obiteljskog poljoprivrednog gospodarstva na području Krapinsko-zagorske županije.

Rezultati i rasprava

Krapinsko-zagorska županija nalazi se u sjeverozapadnom dijelu Hrvatske, upravno je podijeljena na 32 jedinice lokalne samouprave (7 gradova i 25 općina), a prema Popisu stanovništva iz 2011. godine u njoj živi 132.892 stanovnika. Poljoprivredna djelatnost na području županije uvjetovana je konfiguracijom terena, kvalitetom tla, razmještajem stanovnika i tradicionalnim načinom življenja na manjim posjedima. Prema kriteriju OECD-a na regionalnoj razini, Krapinsko-zagorska županija spada u pretežito ruralne regije, obzirom da u ruralnim područjima živi 67,22% stanovnika. U 2018. godini je zabilježen najveći broj OPG-a, ukupno 8.518. Prema podacima Agencije za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju, na području Krapinsko-zagorske županije 70% je obiteljskih poljoprivrednih gospodarstava čiji su nositelji muškarci, dok je samo na 30% žena nositeljica. Neke od prepreka učinkovitijoj i konkurentnijoj poljoprivrednoj proizvodnji su mala veličina zemljišnog posjeda po gospodarstvu i rascparceliranost poljoprivrednog zemljišta. Prema dostupnim podacima u 2014. godini 53% kućanstva je imalo veličinu posjeda 1-3 ha, dok je najmanji broj kućanstava imalo posjede veličine iznad 10 ha. Prema popisu stanovništva iz 2011. godine prosječna dob je 41,7 te je porasla u odnosu na popis stanovništva iz 2001. (39,6), a porastao je i udio starijih od 60 godina u ukupnom stanovništvu sa 44,5% na 49%. U razdoblju 2016.-2018. godine porastao je i broj poljoprivrednika starije dobi, a najveći porast zabilježen je u dobnoj skupini iznad 66 godina. Ovakav trend prijetnja je za budući ruralni razvoj jer je stanovništvo starije životne dobi skeptično prema promjenama i inovacijama u korištenju EU fondova (Strategija razvoja Krapinsko-zagorske županije do 2020. godine).

Prema spolnoj strukturi u uzorku prevladavaju muškarci (56,3%), a 43,8% čine žene, što nije reprezentativni prikaz stanja u županiji. Najzastupljenije dobne skupine u uzorku pripadaju kategoriji 26-40 godina starosti (30%), zatim slijedi dobna skupina 41-55 godina (28%) te kategorija 56-65 godina (17%). Mladih u dobi do 25 godina ima 14%, a najmanje ispitanika (11%) ima koji su stariji od 66 godina. Najveći je udio ispitanika sa završenim srednjoškolskim obrazovanjem (54,7 %). Ispitanika koji imaju završenu osnovnu školu je 17,2% u uzorku, dok onih koji su pohađali fakultet ima 15,6%, a visoko obrazovani čine udio od 12,5%. Ovakva distribucija posljedica je načina prikupljanja podataka (on-line), ali može biti dobar pokazatelj razmišljanja poljoprivrednika koji po dobi i obrazovanju spadaju u „razvojnu“ kategoriju.

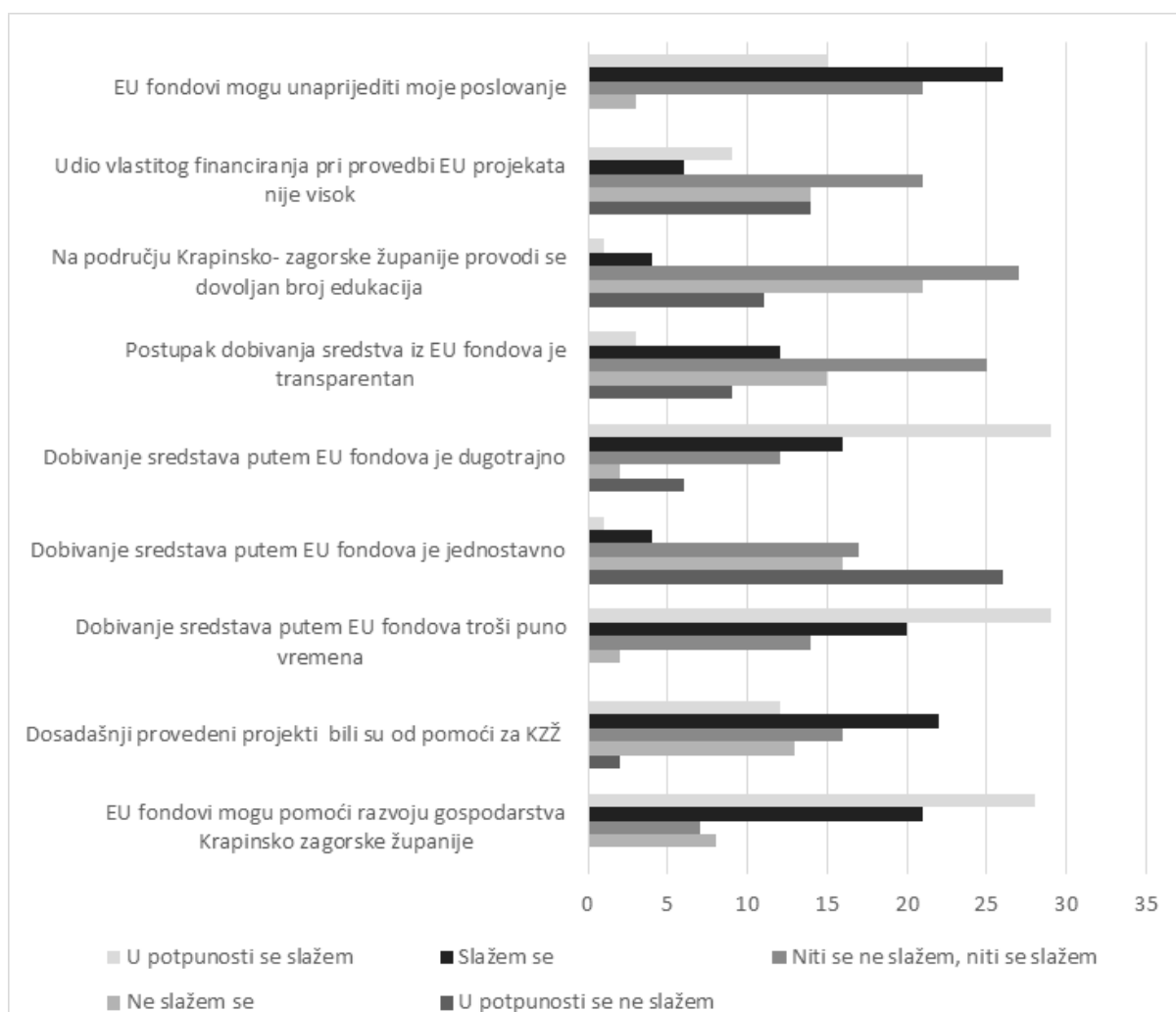
Najveći broj ispitanika ima dobru (31 ispitanik) i lošu (20 ispitanika) općenitu sliku o Europskoj uniji. Samo 5 ispitanika ima jako lošu, a 8 ispitanika ima vrlo dobru i odličnu sliku o EU. Upravo je pozitivan stav prema EU financiranju prepoznat kao pokretač jače apsorpcije sredstava. Na pitanje o asocijaciji na pojam EU fondovi najviše je bilo odgovora: novac, projekti, potpore. Kod procjene vlastitog znanja o EU fondovima 29 ispitanika je svoje znanje ocijenilo ocjenom „dobar“. Svoje znanje kao lošije procijenilo je 20 ispitanika, a kao vrlo dobro 14 ispitanika. Najzastupljeniji izvor informacija o EU fondovima su mediji (53%) dok su edukacije, tečajevi, radionice (23%) i redovito školovanje (24%) nešto slabije zastupljeni. Upravo su informiranost i znanje temelj za uspješno korištenje sredstava EU fondova, pa bi mogući razlog slabije informiranosti mogao biti izvor dobivanja informacija.

Na pitanje vezano za dosadašnje pohađanje edukacija samo 19 odgovora bilo je pozitivno, dok 45 ispitanika nikad nije bilo dodatno educirano o EU fondovima. Najviše ispitanika (6 od 19) edukaciju je polazilo u sklopu Ministarstva poljoprivrede (Savjetodavna služba), zatim u organizaciji fakulteta, pčelarskih udruga i sl. Kao najbolji način provođenja edukacija ispitanici navode individualno savjetovanje (49%), zatim slijede radionice (33%) te seminari (18%). Nadalje, ukazali su na potrebne promjene u načinu provođenja edukacija i to: da se detaljnije objasni procedura, individualniji pristup, konkretne edukacije s naglaskom na praktično.

U uzorku se samo 17 ispitanika prijavljivalo na natječaje za sredstva iz EU fondova, ostali (73,4%) se nikad nisu prijavljivali, a samim time nisu ni koristili sredstva EU. Kao razlog tome navode kompliciranost procesa prijave (23,9%), nedovoljnu informiranost (19,6%), nedostatak vremena za bavljenje pripremom projekata (17,4%) te da

nisu udovoljavali uvjetima natječaja (15,2%) ili nije bilo odgovarajućeg natječaja (13%). Od ovih 17 ispitanika, najviše prijavljenih je bilo na mjeru M11-Ekološki uzgoj (29,4%), M6-Razvoj poljoprivrednih gospodarstava i poslovanja (23,5%), M14-Dobrobit životinja (17,6%) te podjednako na mjere M10-Poljoprivreda, okoliš i klimatske promjene i M4-Ulaganje u fizičku imovinu (11,8%). Za pripremu projektne dokumentacije 58,8% ispitanika koristilo je i vlastite resurse i usluge konzultanata. Isključivo konzultantske usluge koristilo je 29,4%, a isključivo vlastite ljudske resurse 11,8% ispitanika.

Ispitanici su složni kako sredstva iz Europskih fondova mogu unaprijediti njihovo poslovanje, te pomoći razvoju gospodarstva Krapinsko-zagorske županije. No unatoč tome rezultati su pokazali (slika 1) kako se u potpunosti slažu da je dobivanje sredstava iz EU fondova dugotrajno (29 ispitanika), troši puno vremena (29 ispitanika) te nije jednostavno (43 ispitanika). Nadalje 59% ispitanika smatra da se na području Krapinsko-zagorske županije ne provodi dovoljan broj edukacija.



Slika 1. „Izrazite svoje slaganje ili neslaganje sa sljedećim izjavama!“
Izvor: Vlastita izrada na temelju provedenog istraživanja

Više od polovice ispitanika smatra kako se na području Krapinsko-zagorske županije ne provodi dovoljan broj edukacija, više od trećine ispitanika nikad nije sudjelovalo na edukacijama, radionicama ili seminarima, a to su izvori kvalitetnih informacija i mjesto gdje ispitanici mogu postaviti konkretna pitanja za rješavanje nedoumica i nejasnoća, te mogu naučiti jedni od drugih razmjenjujući svoja dosadašnja iskustva. Iz navedenog se može zaključiti kako poljoprivrednici uslijed nesigurnosti, nedovoljno znanja i iskustva imaju potrebu za iskazivanjem posebne pažnje njima kao pojedincu te preferiraju „gotova i servirana“ rješenja za svoje probleme i moguće uspjehe u budućem poslovanju.

Zaključak

Europska unija kroz fondove pruža brojne mogućnosti za razvoj, a za uspješno korištenje financijskih sredstava potrebne su razne upravljačke kompetencije. Istraživanje je pokazalo kako poljoprivrednici na području Krapinsko-zagorske županije imaju pozitivne stavove o fondovima EU, odnosno smatraju kako korištenje financijskih sredstava može unaprijediti njihovo poslovanje te pridonijeti razvoju županije. Unatoč pozitivnim stavovima, za bolju apsorpciju sredstava potrebno je više ulagati u edukacije kako bi se podigla razina informiranosti, znanja te poznavanje cjelokupnog procesa prijave.

Napomena

Rad je izvod iz diplomskog rada Nataše Šenjug, mag.ing.agr. naslova: „Stavovi i iskustva poljoprivrednika s fondovima Europske unije” obranjenog 30.09.2019. „Sveučilište u Zagrebu Agronomski fakultet.

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Experiences of farmers from Krapina-Zagorje County with EU funds

Abstract

With the accession of Croatia to the European Union, numerous possibilities have arisen for using the funds for developing rural area, protecting the environment and strengthening the competitiveness of agricultural production. The research aim is to find out the experiences of farmers from the Krapina-Zagorje County with EU funds. The results showed that most respondents believe that EU funds can improve their business and contribute to the development of the county but are dissatisfied with the complicated documentation and the lengthy and demanding application process.

Keywords: agriculture, EU funds, Krapina-Zagorje County, rural development

Stavovi proizvođača Brodsko-posavske županije o stanju poljoprivrede i poljoprivredne politike

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Sažetak

Cilj rada je anketnim istraživanjem procijeniti stanje poljoprivrede i poljoprivredne politike s aspekta proizvođača Brodsko-posavske županije. Brodsko-posavska županija je dio kontinentalnog prostora Hrvatske, pogodnog za poljoprivrednu proizvodnju i naseljenog s više od polovice ruralnog stanovništva. Polovica ispitanika stanje poljoprivrede u Županiji ocijenila je ocjenom dovoljan uz očekivanje stabilnijeg tržišta i plasmana poljoprivrednih proizvoda u narednom desetogodišnjem periodu. Anketnim istraživanjem je također utvrđena nedovoljna informiranost proizvođača o zakonskoj regulativi kao i njihovo dvojbeno razmišljanje o vlastitoj konkurentnosti na tržištu poljoprivrednih proizvoda Europske unije.

Ključne riječi: poljoprivredni proizvođači, Brodsko-posavska županija, poljoprivreda, poljoprivredna politika, anketno istraživanje

Uvod

Poljoprivredna proizvodnja je osnovica opskrbe stanovništva prehrambenim proizvodima i temelj gospodarskog razvoja države. Zahvaljujući raznolikim klimatskim i pedološkim uvjetima u Hrvatskoj, moguće je proizvoditi veliki broj poljoprivrednih proizvoda pri čemu se njezin kontinentalni dio smatra izuzetno pogodnim za poljoprivrednu proizvodnju (Državni zavod za statistiku RH, 2018.). Brodsko-posavska županija zauzima površinu od 2043 km² u južnom dijelu slavonske nizine. Omeđena je planinom Psunj, Požeškim i Diljskim gorjem sa sjeverne te rijekom Savom sa južne strane. Reljefno je raznolika i čine je tri cjeline: brdski, ravničarski i nizinski prostor pri čemu je najveći dio Županije ravničarski. Područje Županije pripada umjereno kontinentalnoj klimi (Društvo za oblikovanje održivog razvoja, 2011.). Više od polovice stanovništva Županije (53,74%) je ruralno s nejednakom distribucijom naseljenosti unutar Županije. U 2011. godini utvrđena je prosječna starost stanovništva 40,6 godina. Međutim, podaci u razdoblju 2001.- 2011. ukazuju na negativan trend kretanja broja stanovnika od 11%. Navedeni trend se nastavlja te je u 2016. godini saldo migracija s inozemstvom izrazito negativan (2751 osoba) (Brodsko-posavska županija, 2016.).

Ukupna površina poljoprivrednog zemljišta Županije 2016. godine iznosila je 92 092,99 ha (Državni ured za reviziju, 2017.). U Upisnik poljoprivrednih gospodarstava 2017. godine upisano je 7218 gospodarstava. Najviše gospodarstava (96,85%) su obiteljska poljoprivredna gospodarstva, a znatno manje obrti (1,78%), trgovačka društva (1,10%), zadruge (0,14%) i ostali oblici poljoprivrednih gospodarstava (0,13%) (Brodsko-posavska županija, 2018.).

Zajednička poljoprivredna politika EU nastala je s ciljem poticanja proizvodnje i stalne opskrbe hranom po pristupačnim cijenama. Zasniva se na načelima jedinstvenog tržišta, prednosti EU i financijskoj solidarnosti te na dva stupa: dohodovnoj i tržišnoj politici i politici ruralnog razvoja (Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju, 2011.). Prema istraživanju Eurobarometra iz listopada 2015. godine 62% hrvatskih ispitanika smatra da su ruralna područja i poljoprivreda važni za budućnost. Najvažnijim odgovornostima poljoprivrednika u društvu ispitanici smatraju opskrbu stanovništva kvalitetnim proizvodima i poticanje i poboljšanje života na selu. Glavnim ciljevima EU kada je u pitanju poljoprivreda i politika ruralnog razvoja ističu osiguranje razumnih cijena hrane za potrošače i razvoj ruralnih područja uz očuvanje okoliša. S izjavom da zajednička poljoprivredna politika pogoduje svim europskim građanima složilo se 75% ispitanika, a na pitanje o potporama 59% ispitanika odgovorilo je da zna za potpore, ali ne i pojedinosti o njima (Eurobarometar, 2015.).

Cilj istraživanja bio je ustanoviti kako poljoprivredni proizvođači s područja Brodsko-posavske županije ocjenjuju stanje poljoprivrede i poljoprivredne politike.

Materijal i metode

Podaci su prikupljeni metodom anketnog ispitivanja poljoprivrednih proizvođača u svibnju 2014. godine. Anketno ispitivanje provedeno je na uzorku od 100 ispitanika na području Brodsko-posavske županije. Anketni upitnik se sastojao od tri skupine pitanja. Prva skupina pitanja odnosila se na dob, mjesto prebivališta i završeno obrazovanje i poslužila je za opis uzorka. U sljedećoj skupini pitanja ispitanici su ocjenjivali stanje poljoprivrede u Brodsko-posavskoj županiji i odgovarali na pitanja o uzroku stanja, potencijalu za razvoj poljoprivrede u Brodsko-posavskoj županiji, prednostima života u ruralnim naseljima Županije i očekivanjima u sljedećih deset godina. U ovoj skupini pitanja ispitanicima je ponuđeno po pet odgovora od kojih su trebali izabrati onaj koji smatraju najtočnijim. Treća skupina pitanja zahtijevala je od ispitanika pozitivan ili negativan odgovor na pitanja o poznavanju regulative EU i poljoprivrednoj politici, izravnim plaćanjima i konkurentnosti hrvatskog poljoprivrednika u EU.

Rezultati

U istraživanju je sudjelovalo 100 ispitanika. Ispitanici u dobi iznad 55 godina zastupljeni su sa 12%, ispitanika starosti do 25 godina bilo je 46%, a ispitanika u rasponu starosne dobi od 25 do 55 godina bilo je 42%. Svi ispitanici imaju prebivalište na području Brodsko-posavske županije. Najveći broj ispitanika (71%) ima završeno srednješkolsko obrazovanje, a manje visokoškolsko (18%) i osnovnoškolsko (11%).

Rezultati

Ispitanici su ocjenama od 1 do 5 (1-loše...5-odlično) ocjenjivali stanje poljoprivrede u Brodsko-posavskoj županiji (prvo pitanje druge skupine pitanja). Ocjenu 2 stanju poljoprivrede u Županiji dodijelilo je 50% ispitanika, 29% ispitanika ocjenu 3, 17% ispitanika ocjenu 1, a ocjenama 4 i 5 trenutno stanje ocijenilo je samo po 2% ispitanika.

U tablici 1. navedena su ostala pitanja s ponuđenim odgovorima o ocjeni stanja poljoprivrede na području Brodsko-posavske županije te postotak ispitanika koji određeni odgovor smatraju najtočnijim.

Tablica 1. Ocjena stanja poljoprivrede u Brodsko-posavskoj županiji.

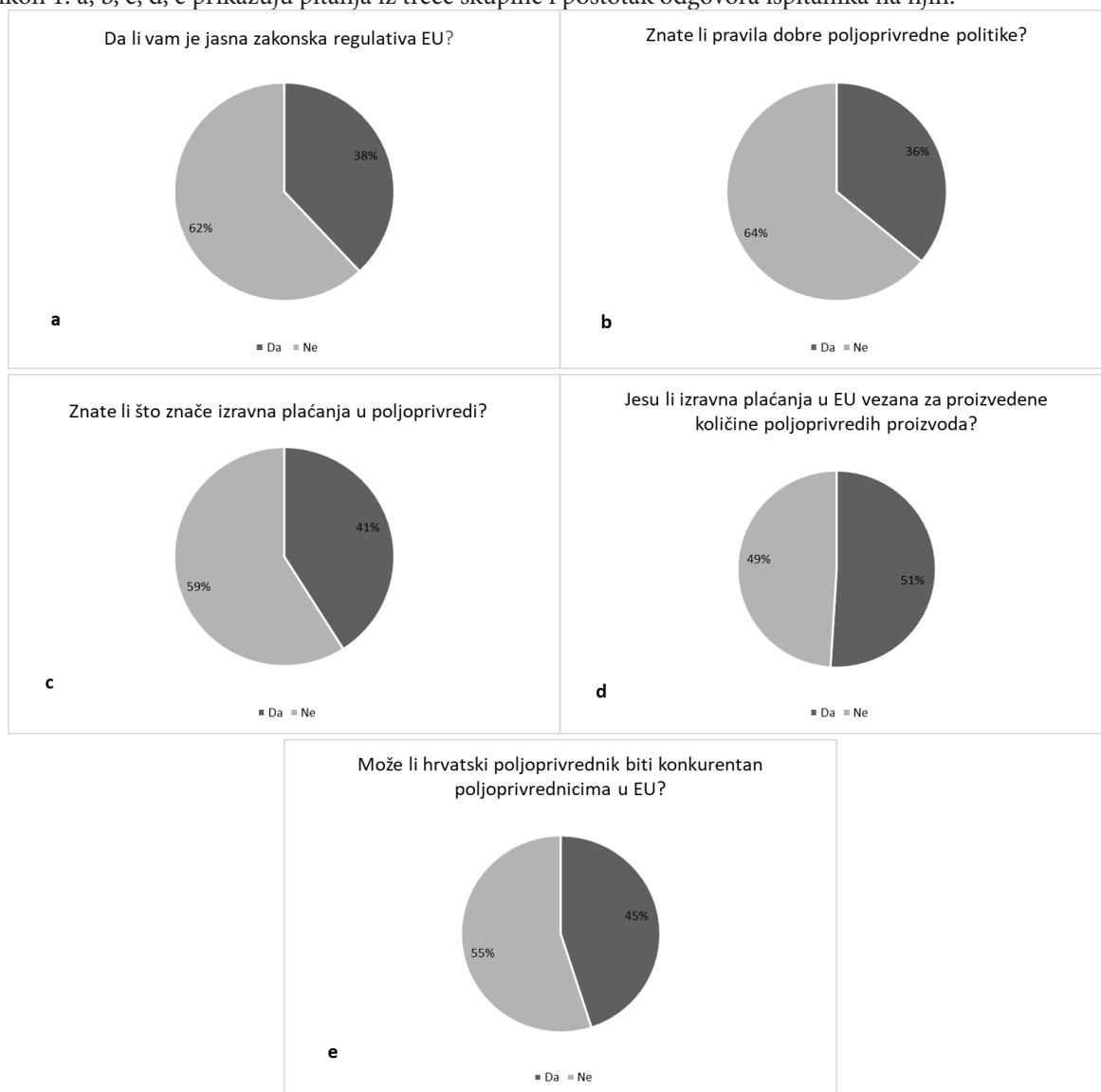
Što mislite koji je najveći razlog takvog trenutnog stanja u poljoprivredi u BPŽ-i?	
a) nekvalitetna organizacija poljoprivrednog tržišta i nekontrolirani uvoz polj. proizvoda	39%
b) visoka cijena proizvodnje	15%
c) neefikasna organizacija poljoprivrede politike	32%
d) nizak stupanj obrazovanja i visoka starosna dob polj. stanovništva	5%
e) komplicirana administracija i loša zemljišna politika	9%
Koji je najveći potencijal za razvoj poljoprivrede u BPŽ-i?	
a) mogućnosti razvoja u agroturizmu	9%
b) kvalitetni proizvodni resursi	22%
c) razvoj ekološke poljoprivrede	27%
d) mogućnost plasmana polj. proizvoda iz Slavonije na jadransku obalu tijekom turističke sezone	25%
e) izvoz na strana tržišta	17%
Koje su prednosti života u ruralnim naseljima BPŽ-e?	
a) smanjeni stres	24%
b) veća i jednostavnija uključenost pojedinca u život lokalne zajednice	10%
c) povezanost stanovništva s okolišem i mogućnost njegovog očuvanja	6%
d) prirodniji i zdraviji način života (prehrana, čistiji okoliš, aktivnosti, komunikacija...)	41%
e) mogućnosti proizvodnje na specifičan način i očuvanje tradicije	19%

Koje rezultate za poljoprivredno stanovništvo očekujete kroz period od 10 godina?

a) povećanje dohotka za polj. stanovništvo	14%
b) stabilnije tržište i plasman polj. proizvoda	55%
c) povećana produktivnost	12%
d) smanjene migracije stanovništva iz sela u grad	15%
e) veće mogućnosti za širenje polj. površina	4%

Najvažniji razlog za nisku ocjenu stanja poljoprivrede u Brodsko-posavskoj županiji prema mišljenju 39% ispitanika je loše organizirano poljoprivredno tržište i nekontrolirani uvoz poljoprivrednih proizvoda. Najmanji razlog je nizak stupanj obrazovanja i visoka starosna dob poljoprivrednog stanovništva što je za odgovor odabralo 5% ispitanika. Najveći potencijal za razvoj poljoprivrede u Županiji ispitanici vide u ekološkoj poljoprivredi (27%) i plasmanu poljoprivrednih proizvoda na jadransku obalu tijekom turističke sezone (25%). Prednosti života u ruralnim naseljima Županije za ispitanike su prirodni i zdraviji način života (41%), smanjeni stres (24%) i proizvodnja na specifičan način uz očuvanje tradicije (19%). U narednom desetogodišnjem razdoblju očekivanja ispitanika odnosila su se najviše na stabilnije tržište i plasman poljoprivrednih proizvoda (55%), a najmanje na širenje poljoprivrednih površina (4%).

Grafikon 1. a, b, c, d, e prikazuju pitanja iz treće skupine i postotak odgovora ispitanika na njih.



Grafikon 1. a, b, c, d, e. Rezultati odgovara na pitanja iz treće skupine pitanja ankete

Rezultati odgovora na anketna pitanja prikazani grafikonom 1. a, b, c i d pokazuju nedovoljnu informiranost proizvođača u vrijeme provođenja istraživanja. Grafikon 1. e pokazuje podijeljenost ispitanika u mišljenju o mogućnosti konkurentnosti hrvatskih poljoprivrednika s ostalim poljoprivrednicima u EU.

Zaključak

Anketnim istraživanjem poljoprivredni proizvođači na području Brodsko-posavske županije ocijenili su stanje poljoprivrede i poljoprivredne politike ocjenom dovoljan. S obzirom da se radi o županiji koja ima pogodne klimatske i pedološke karakteristike za poljoprivrednu proizvodnju te većinu ruralnog stanovništva, dobiveni rezultati su zabrinjavajući. Nezadovoljstvo je iskazano u pogledu organiziranosti tržišta poljoprivrednih proizvoda i nekontroliranom uvozu poljoprivrednih proizvoda. Pобољшanje postojećeg stanja iskazuju kroz razvoj ekološke poljoprivredne proizvodnje te plasman proizvoda kroz turističku ponudu na jadranskoj obali. Ipak, rezultati istraživanja ukazuju i na nedovoljnu informiranost navedene skupine o zakonskoj regulativi i zabrinutost o mogućnostima konkurentnosti njihovih proizvoda na tržištu ostalih zemalja EU. Anketni rezultati su u suglasju s općim stanjem agrara i agrarne politike u Hrvatskoj koji bi se mogli ublažiti kroz mjere ruralnog razvoja i financijska sredstva EU fondova.

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Attitudes of producers of Brod-Posavina county on the state of agriculture and agricultural policy

Abstract

The aim of this study was to evaluate the state of agriculture and agricultural policy from the perspective of producers of the Brod Posavina county. The Brod Posavina county is part of Croatian continental region suitable for agricultural production and inhabited with more than half of the rural population. Half of the respondents the state of agriculture in the county evaluated as sufficient, with the expectation of a more stable market and placement of agricultural products in the next ten-year period. This study also revealed that producers are not informed on legislation and have doubtful thinking about its own competitiveness in the EU agricultural market.

Keywords: agricultural producers, Brod-Posavina county, agriculture, agricultural policy, survey

Maslinarstvo kao dio turističke ponude

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Sažetak

Maslinarstvo je jedna od vodećih poljoprivrednih proizvodnji na području Jadranske Hrvatske. Varijabilnost prihoda potiče maslinare na diversifikaciju aktivnosti, vrlo često u segmentu turizma. Spoj maslinarstva i turizma čini novi selektivni oblik turizma - maslinarski turizam. Cilj rada je identificirati snage i slabosti maslinarskog turizma u Hrvatskoj, odnosno prilike i prijetnje iz okruženja, te na temelju njihovog međudjelovanja predložiti moguće strategije daljnjeg razvoja. Kao prognostički i dijagnostički instrument za utvrđivanje stanja maslinarskog turizma korištena je SWOT analiza. Najveću snagu maslinarskog turizma predstavljaju visoko kvalitetna, svjetski nagrađivana maslinova ulja.

Ključne riječi: maslinarstvo, turizam, diversifikacija, SWOT analiza

Uvod

Ostvarenje zadovoljavajućih prihoda poljoprivrednika važan je preduvjet za zadržavanja stanovništva i razvoja ruralnih sredina. Diversifikacija poljoprivrednih gospodarstava odnosi se na širenje osnovne poljoprivredne proizvodnje na aktivnosti koje se smatraju dopunskima, a mogu obuhvaćati proizvodnju poljoprivrednih i prehrambenih proizvoda, izradu neprehrambenih proizvoda i predmeta opće uporabe, pružanje usluga u poljoprivredi, te pružanje turističkih i ugostiteljskih usluga (Pravilnik o dopunskim djelatnostima na obiteljskim poljoprivrednim gospodarstvima NN 76/2014). Turističke aktivnosti u ruralnim područjima odnosno na obiteljskim poljoprivrednim gospodarstvima doprinose poboljšanju njihove ekonomske uspješnosti i efikasnosti poslovanja (Čurić, 2010). Diversifikacija u maslinarstvu, kao jednoj od vodećih grana poljoprivrede u Jadranskoj regiji, je važna zbog stabilnosti dohotka proizvođača koji može biti ugrožen uslijed neizvjesnih vremenskih prilika i/ili promjenjivih uvjeta na tržištu.

Širenje dijela poljoprivrednih gospodarstva ponudom turističkih usluga vrlo je očekivan smjer daljnjeg razvoja hrvatske poljoprivrede, osobito u Jadranskom dijelu zemlje gdje se ostvaruje preko 85% od ukupnih turističkih dolazaka godišnje.

Za spoj turizma i maslinarstva u stranoj literaturi koriste se nazivi kao: „*Olive tourism*“ (Alonso i Northcote 2010.), „*Olive oil tourism*“ (López i sur., 2013.) i „*Oleotourism*“ (Millán i sur., 2018.) iako se u većini studija koristi izraz „*Oleotourism*“ (Millán i sur., 2018.) odnosno maslinarski turizam (u nastavku teksta MT).

Millán i suradnici (2014.) definiraju MT kao oblik turizma posebno razvijen u ruralnim područjima, vezan uz gastronomiju i agroturizam, a koji posjetiteljima omogućava upoznavanje kulture uzgoja maslina uz produblivanje znanja o svemu što je povezano s maslinovim uljem, hranom, smještajem neposredno uz maslinike, korištenje kozmetičkih i zdravstvenih tretmana baziranih na maslinovom ulju, te jednostavno opuštanje u prirodnom okruženju. Aktivnosti vezane uz ovaj oblik turizam uglavnom obuhvaćaju posjete uljarama i maslinarskim gospodarstvima („*Olive farm*“, Tudisca i sur. 2015.), degustacije maslinova ulja, vođene obilaske maslinika, te ponudu ostalih lokalnih proizvoda (Millán i sur., 2014.). Također, sastavni dio ponude MT su i posjete oleotekama (specijaliziranim trgovinama za maslinova ulja), sajmovima i manifestacijama, te muzejima i interpretacijskim centrima posvećenim maslinama i maslinovom ulju.

Cilj ovoga rada je identificirati snage i slabosti MT u Hrvatskoj, odnosno prilike i prijetnje iz okruženja, te na temelju njihovog međudjelovanja predložiti moguće strategije daljnjeg razvoja MT.

Materijali i metode

U istraživanju se koristi SWOT analiza. SWOT analiza je često korištena kvalitativna analitička metoda za analizu i oslikavanje trenutne pozicije i razvojnih perspektiva određenog poduzeća, gospodarstva ili djelatnosti, a često se koristi i u razmatranju selektivnih oblika turizma, kao što je primjer kruzing turizam (Gračan i Zadel, 2013.).

Za izradu SWOT analize korišteni su primarni i sekundarni podaci. Primarni podaci prikupljeni su metodom ekspertnih intervju s dvoje stručnjaka iz područja maslinarstva i turizma (Institut za poljoprivredu i turizam i Fakultet za menadžment u turizmu i ugostiteljstvu), dok su sekundarni podaci prikupljeni iz znanstvenih istraživanja o MT (iz baza *Google scholar* i *Science direct*) te s ostalih relevantnih web stranica. Prikupljeni podaci obrađeni su metodama analize i sinteze, indukcije i dedukcije, te usporedbe i deskripcije. Na temelju SWOT analize predložene su četiri skupine strategija MT iz kojih proizlaze i moguće **buduće strategije**.

Rezultati i diskusija

Tablica 1. SWOT analiza maslinarskog turizma u Hrvatskoj

Snage	Slabosti
U N U T A R NJ E	<p>Pedoklimatski uvjeti sjevernog Mediterana pogoduju proizvodnji visokokvalitetnog maslinovog ulja</p> <p>Stoljetna tradicija i bogata baština maslinarstva i uljarstva</p> <p>Velik broj autohtonih sorti maslina</p> <p>Stari maslinici i očuvani okoliš pogodni za razvoj eko-agroturizma</p> <p>Trend povećanja broja modernih uljara i kušaonica maslinovog ulja</p> <p>Svjetski nagrađivana maslinova ulja</p> <p>Kompatibilnost s ostalim oblicima ruralnog turizma (eko, vinski, gastro)</p> <p>Sve prisutnija izravna prodaja maslinovog ulja turistima</p>
Prilike	Prijetnje
V A NJ S K E	<p>Višegodišnji trend rasta ukupnog turističkog prometa</p> <p>Porast broja turista u potrazi za lokalnim, autentičnim doživljajima</p> <p>Ponuda sadržaja MT u listopadu i studenom u fazi berbe i prerade plodova, produljuje glavnu turističku sezonu</p> <p>Blizina zemalja zapadnoeuropskog emitivnog turističkog tržišta</p> <p>Jačanje imidža i izvoznog potencijala kvalitetnog maslinovog ulja zahvaljujući promidžbenim aktivnostima tijekom turističke sezone</p> <p>Mogućnost financiranja ulaganja putem Programa ruralnog razvoja (PRR)</p> <p>Razvijena turistička infrastruktura</p> <p>Članstvo Hrvatske u „Kulturnim rutama, europskog vijeća“ – „Rute maslina“</p>

Izvor: obrada autora

Na osnovi SWOT analize predložene su četiri strategije daljnjeg razvoja sektora:

1) Maksi – maksii strategija (snage i prilike):

Najveći potencijal MT u Hrvatskoj predstavljaju kvalitetna, svjetski nagrađivana maslinova ulja koja su djelom rezultat pedoklimatskih uvjeta uzgoja – sjevernog Mediterana (Benčić, 2000; Aparacio i sur. 1994.). Zahvaljujući raznim poticajnim programima podignuti su mladi maslinici, izgrađene suvremene uljare i kušaonice što je osnova razvoja kvalitetnog MT. Ovakvom diversifikacijom maslinari imaju mogućnost promoviranja i izravne prodaje vlastitih proizvoda. Turistički sektor ima strateški interes poticanja i promoviranja MT budući da ponuda atraktivnih sadržaja, kao što su berba plodova i kušanje mladog maslinovog ulja, doprinosi produljenju turističke sezone. Uz bogatu maslinarsku i uljarsku baštinu te autohtoni sortiment potrebno je formirati jedinstvenu i autentičnu ponudu popraćenu individualnim pristupom posjetiteljima i edukacijom o maslinovim uljima. Ponudu treba usmjeriti prema sve većem broju turista koji traže lokalne, originalne i autentične doživljaje.

2) Mini – maksii strategija (slabosti i prilike):

Izostanak poslovne povezanosti maslinara ima za posljedicu oscilacije u proizvodnji ulja te neujednačenu i slabo promoviranu ponudu MT. U cilju izgradnje imidža Hrvatske kao maslinarske zemlje potrebno je da se dionici ponude MT uključe u za to specijalizirane međunarodne asocijacije. Nedostatna financijska sredstva za pokretanje nepoljoprivrednih djelatnosti ili za poslovno povezivanje maslinari trebaju osigurati iz Programa ruralnog razvoja (Mjere 4, 6, 9).

3) Maksi – minii strategija (snage i prijetnje):

MT se sve jače razvija i u drugim mediteranskim zemljama pa Hrvatska treba iskoristiti svoju glavnu konkurentsku prednost, a to su svjetski nagrađivana maslinova ulja, autohtone sorte i pedoklimatski uvjeti uzgoja. Treba inzistirati na visokoj kvaliteti ponude MT i očuvanju postignutog imidža maslinovog ulja organiziranjem sustava sljedivosti i tržišnog nadzora. Također, sinergija s ostalim oblicima ruralnog turizma, kao što su gastro, eno ili eko turizam, omogućuje cjelovitiju, stručniju i profesionalniju ponudu sadržaja MT.

4) Mini – minii strategija (slabosti i prijetnje):

Nepostojanje razvojne strategije MT na razini Hrvatske doprinosi slabom imidžu Hrvatske kao maslinarske destinacije na turističkom tržištu. Donošenje i provedba strategije rezultirat će boljom koordinacijom svih dionika u lancu ponude MT. Provedbom istraživanja o posjetiteljima uključenima u aktivnosti MT omogućit će se bolja prilagodba ponude potrebama tržišta.

Zaključak

Hrvatski turizam najrazvijeniji je u područjima maslinarske proizvodnje što otvara velike mogućnosti sinergije ovih dviju djelatnosti u cilju održivosti poljoprivrednih gospodarstva na mediteranskom prostoru.

Hrvatsko maslinarstvo karakterizira proizvodnja visokokvalitetnih maslinovih ulja koja uz bogatu prirodnu i kulturnu baštinu čine osnovu za planiranje novog specifičnog oblika turističke ponude. Maslinarski turizam kroz izravnu prodaju ulja, omogućava povećanje dohotka maslinarskih gospodarstava na mediteranskom prostoru.

Ponudu MT u Hrvatskoj karakterizira neujednačenost i slaba promidžba koja se uglavnom ogleda na sporadičnim primjerima maslinara koji otvaraju svoja gospodarstva za posjetitelje. Sve to, kao i nepostojanje strategije razvoja MT, utječe na to da Hrvatska nije izgradila zadovoljavajući imidž maslinarske zemlje.

Velike razvojne mogućnosti uvjetovane su uglavnom prirodnim položajem, ali i dugogodišnjom tradicijom ove grane poljoprivrede čiji uspjeh dokazuju svjetski nagrađivana maslinova ulja. MT ostvaruje i mnoštvo prilika koje se odnose na rast potražnje za lokalnim, autentičnim turističkim sadržajima, produljenje turističke sezone, infrastrukturne preduvjete i mogućnosti korištenja bespovratnih sredstava iz PRR. Najveću prijetnju predstavljaju razvijena konkurentska turistička tržišta na Mediteranu, slab sustav tržišnog nadzora prometa i kvalitete maslinovog ulja, te nedostatak sustavnih istraživanja o MT u Hrvatskoj.

Na osnovu SWOT analize predlažu se četiri strategije daljnjeg razvoja MT u Hrvatskoj: 1) Pružanje autentičnog

doživljaja posjetiteljima koristeći bogatu baštinu maslinarstva i uljarstva te svjetski nagrađivana maslinova ulja, 2) Poboljšanje imidža Hrvatske kao maslinarske zemlje putem promotivnih aktivnosti, ali i upotpunjavanja ponude MT iskorištenjem sredstava iz PRR, 3) Iskorištenje konkurentskih prednosti MT Hrvatske (svjetski nagrađivana maslinova ulja i mnoštvo autohtonih sorti) u svrhu promoviranja i pozicioniranja MT na inozemnom tržištu kroz promotivne kampanje i brendiranje proizvoda te ponudom izletničkih paketa u sklopu sveukupne turističke ponude Hrvatske, 4) Donošenje razvojne strategije MT na razini Hrvatske u svrhu razvoja i boljeg pozicioniranja MT na domaćem i inozemnom turističkom tržištu.

Dobiveni rezultati bit će podloga za daljnja istraživanja ponude i potražnje za MT u Hrvatskoj, a mogu poslužiti i za planiranje mjera strateškog razvoja ovog oblika turizma.

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Olive growing as a part of tourism supply

Abstract

Olive growing is one of the leading agricultural production in the Adriatic Croatia. A variability in income encourages olive growers to diversify they production, very often into the tourism sector. The combination of olive growing and tourism forms a new selective form of tourism - oleotourism. The aim of this paper is to identify strengths and weaknesses of oleotourism in Croatia, as well as opportunities and threats from the environment, and based on their interactions suggest possible strategies for further development of this type of tourism. SWOT analysis was used as a prognostic and diagnostic instrument for determining state-of-the-art of oleotourism. The most important strength of oleotourism are high quality, world-renowned olive oils.

Keywords: olive growing, tourism, diversification, SWOT analysis

Osnovni činitelji ekonomike proizvodnje mlijeka na farmama različitog kapaciteta

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Sažetak

Provedena je analiza osnovnih činitelja proizvodnje mlijeka 27 obiteljskih gospodarstava po skupinama od 30 do 150 krava u osnovnom stadu. Gospodarstva se značajno razlikuju prema veličini osnovnog stada, proizvedenom mlijeku, te cijeni koštanja i dohocima. Razlike su izražene i unutar pojedinih skupina s istim brojem grla, a na njih djeluju visina proizvodnje, ukupno angažirana osnovna sredstva, sustav hranidbe, utrošci rada na farmi. Najbolji rezultati zabilježeni su u populaciji s najviše grla u stadu, a najveći mogući pomaci u visini prinosa i dohotku proizvođača zabilježeni su na gospodarstvima do 50 muznih grla u stadu.

Ključne riječi: ekonomski pokazatelji, kapacitet, mlijeko, proizvodni pokazatelji

Uvod

U proizvodnji mlijeka u Hrvatskoj je nešto manje od 6.000 isporučitelja mlijeka. Govedarske farme su različite veličine stada, s različitim tehnologijama držanja krava i sustavima hranidbe, a postižu različite prinose mlijeka po grlu pri čemu visina prinosa nije uvijek ovisna o veličini farme. Ekonomija obujma samo djelomično utječe na ekonomsku efikasnost proizvodnje mlijeka, budući da su značajna ograničenja raspoloživo poljoprivredno zemljište koje određuje i cijenu koštanja hrane, te menadžment stada koji određuje upravljanje danima u mužnji i utjecajem držanja teladi radi dodatnog prihoda (Čačić 2018, Grgić 2013).

Materijal i metode

Analizom je obuhvaćeno ukupno 27 gospodarstava koja su grupirana prema veličini stada. Unutar svake skupine gospodarstva se razlikuju s obzirom na korištenje pašnog sustava, držanje krava na vezu i slobodno, te sustavima hranidbe u obliku suhe i zelene krme s dodatkom krepkih krmiva te hranidbe sjenažom i silažom. U okviru svake skupine gospodarstava provedene su usporedbe visine proizvodnje i cijene koštanja te odnosa prodajne cijene i cijene koštanja. Prema pokazateljima ekonomičnosti i rentabilnosti određena je ekonomska učinkovitost poslovanja farme, a na temelju razlike ostvarene i moguće dobiti izgubljena korist radi lošeg vođenja farme. Podaci su dobiveni anketnim ispitivanjem kapaciteta i proizvodnje u 2017. godini.

Rezultati

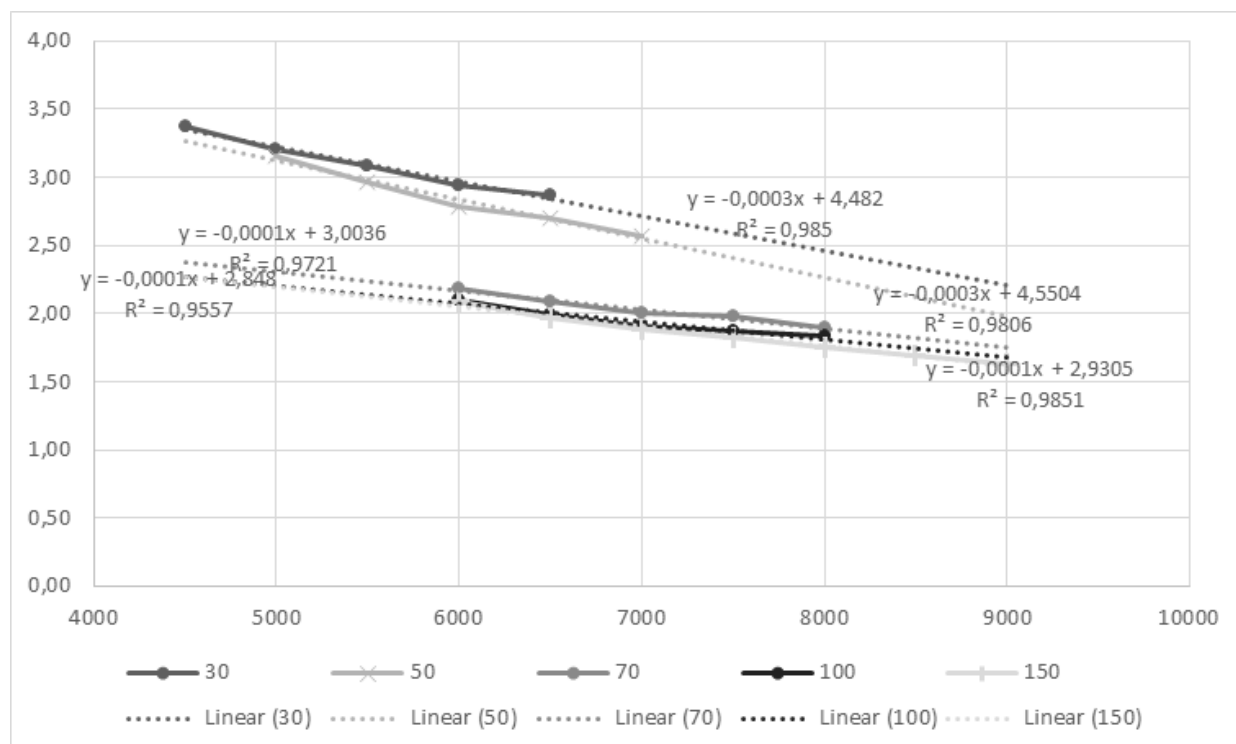
U skupinama proizvođača veličine 30 do 150 muznih grla postoji veće odstupanje u visini proizvodnje (od 4500 do 9000 kg po grlu) s gotovo pravilnim porastom mliječnosti od skupine s manjim brojem do skupine s većim brojem grla. Određena nepravilnost je uočena kod skupine s većim stadom (100 i 150 grla), gdje su ta odstupanja značajna toliko da neka gospodarstva imaju manju proizvodnju po grlu i ekonomske rezultate nego što je to kod gospodarstava s manjim stadom (30,50 i 70 grla). Gospodarstva koja koriste sjenažu i silažu u hranidbi imaju niže troškove proizvodnje po jedinici (kg mlijeka i kravi) i više prinose mlijeka nego što je to kod gospodarstava koja imaju manje intenzivne sustave. Slična su kretanja cijene koštanja, koja su značajno veća kod kategorije manjih proizvođača (30-50 grla) budući su im troškovi izravne proizvodnje u linearnom porastu s mliječnosti, ali ukupno gledano, na cijenu koštanja više djeluje opterećenost fiksnim troškovima, odnosno ukupnim osnovnim sredstvima na farmi.

Tablica 1 Osnovni proizvodni i ekonomski pokazatelji gospodarstava po skupinama

Veličina farme	Jed. mjere	30	50	70	100	150
N		5	5	5	5	7
Mliječnost po grlu	kg	4500-6500	5000-6500	6000-8000	6000-8000	6000-9000
Prosječna mliječnost	kg	5500	6000	7000	7000	7500
Proizvodnja mlijeka ukupno	kg	165.000	300.000	490.000	700.000	1.125.000
Ukupna imovina	kn	1.759.094	2.505.060	3.231.250	4.313.840	6.391.450
Prihodi	kn	516.450	930.500	1.497.825	2.166.000	3.433.125
Mlijeko	kn	398.850	734.125	1.221.325	1.771.000	2.840.625
Ostali prihodi	kn	55.500	92.500	129.500	185.000	277.500
Potpore	kn	62.100	103.875	147.000	210.000	315.000
Rashodi	kn	506.553	843.026	1.265.259	1.746.364	2.636.293
Varijabilni troškovi	kn	315.244	577.483	920.437	1.283.832	1.952.071
Fiksni troškovi	kn	191.309	265.543	344.823	462.532	684.223
Dohodak	kn	9.897	87.474	232.566	419.636	796.832
CK mlijeka	kn/kg	3,09	2,83	2,6	2,51	2,37
CK mlijeka, dodatni prihod	kn/kg	2,37	2,17	2,03	1,94	1,84
Dohodak po grlu	kn	329,9	1.749	3.322	4.196	5.312
Dohodak po kg mlijeka	kn	0,04	0,27	0,46	0,59	0,69
Pokazatelji						
Ekonomičnost		1,02	1,1	1,18	1,24	1,3
Rentabilnost		0,49%	3,45%	7,13%	9,67%	12,28%

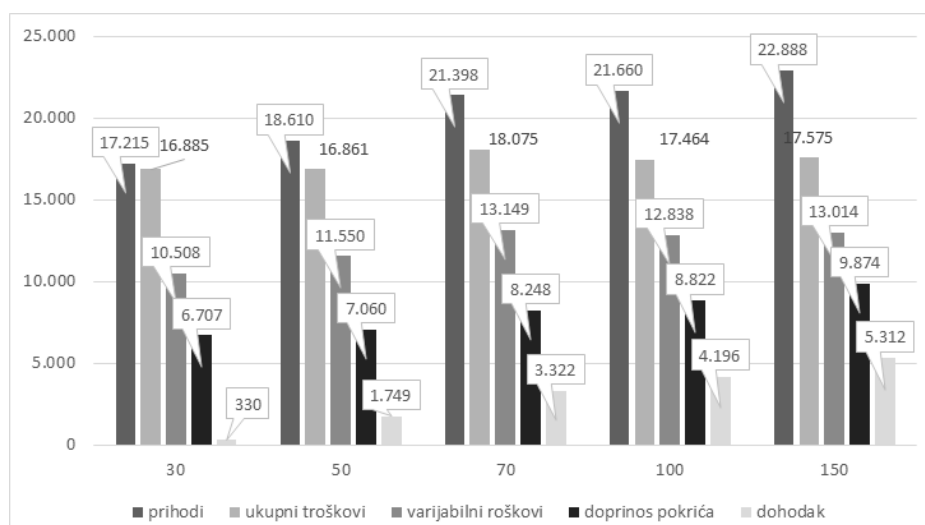
Izvor: Vlastita istraživanja

Kretanja ukupnih troškova s visinom prinosa su očekivano pravilna (S-krivulja prema teoriji kretanja troškova), s odstupanjima najslabijih i najboljih rezultata za 15-20%, što ukazuje na značajne neiskorištene mogućnosti unaprjeđenja tehnologije i organizacije proizvodnje. Odstupanja su u pravilu povezana s intenzivnošću proizvodnje, u korist intenzivne proizvodnje i većeg broja grla na farmi. U samoj tehnologiji najvažnijima se čine trošci i troškovi hranidbe te menadžment farme povezan s pravilnim razdobljima mužnje i teljenja tijekom godine, dok se u organizaciji proizvodnje može govoriti o trošcima rada i troškovima u staji. Prosječni prihodi analiziranih farmi su ujednačeni gledano po kilogramu mlijeka, ali se razlikuju ovisno o ostvarenim prinosima. Odstupanja troškova utječu na doprinos pokriva i dohodak, pri čemu su sva gospodarstva ostvarila zadovoljavajuće prihode s obzirom na izdatke proizvodnje, dok se u slučaju računanja ukupnih troškova značajno ugrožava dohodak kod proizvođača s najmanjim prinosima u svakoj izdvojenoj skupini.



Grafikon 1 Prikaz kretanja cijene koštanja s visinom prinosa po grlu, u kn/kg
Izvor: Preračunato prema tablici 1

Po skupinama je primjetna razlika u cijenama koštanja i dohotku u proizvodnji mlijeka. Skupine s većim brojem grla i relativno većim prinosima imaju manja odstupanja i neiskorištene mogućnosti postizanja dohotka. U cijeloj analiziranoj populaciji kretanja cijene koštanja i dohotka su korištena za ocjenu neiskorištenih mogućnosti, a najbolji rezultati zabilježeni su u populaciji s najviše grla u stadu. Iz podataka u grafikonu 1 i 2 te tablice 2 se može zaključiti kako su neiskorištene mogućnosti cijele analizirane skupine proizvođača u obliku dohotka od 0,46 do 1,01 kn po kg mlijeka, od 2.664,09 do 5.574,47 kn po grlu i od 167.234 do čak 266.409 kn po gospodarstvu. Najveći mogući pomaci u visini prinosa i dohotku proizvođača po muznom grlu zabilježeni su kod kategorija do 50 muznih grla u stadu. Prema podacima iz ankete ova populacija nije spremna unaprijediti svoju proizvodnju. Najčešće smatraju da su već postigli vršni stupanj svoje organizacije i proizvodnje, a da bi svako intenziviranje proizvodnje značilo puno veći rizik i troškove koji se ne bi opravdali dodatnim prihodima i dohotkom. Najveći „gubici“ odnosno neiskorištene mogućnosti u apsolutnim iznosima gledano po skupinama farmi su kod drugog najvećeg stada od 100 grla.



Grafikon 2 Prikaz prosječnih prihoda, troškova, doprinosa pokrića i dohotka, po grlu
Izvor: Preračunato prema tablici 1

Tablica 2 Odstupanja cijene koštanja i dohotka od najboljeg rezultata po skupinama, u kn/kg mlijeka

	30	50	70	100	150
Cijena koštanja					
Najveća	2,52	2,39	2,18	2,39	2,09
Najmanja	2,25	2,00	1,89	1,83	1,63
Odstupanje	0,28	0,38	0,29	0,56	0,45
Doprinos pokrića					
Najmanji	1,15	1,08	1,05	1,08	1,14
Najveći	1,29	1,25	1,27	1,31	1,44
Odstupanje	0,13	0,18	0,22	0,23	0,30
Dohodak					
Najmanji	-0,12	0,04	0,27	0,43	0,44
Najveći	0,20	0,47	0,63	0,70	0,89
Odstupanje	0,33	0,43	0,37	0,26	0,45

Izvor: Preračunato prema tablici 1

Zaključak

Analizirana gospodarstva s proizvodnjom mlijeka značajno se razlikuju prema veličini osnovnog stada, količini proizvodnje mlijeka, te cijenom koštanja i dohocima. Razlike su izražene i unutar pojedinih skupina s istim brojem grla, a na njih djeluju visina proizvodnje, ukupno angažirana osnovna sredstva, sustav hranidbe, utrošci rada na farmi. Najbolji rezultati s obzirom na cijenu koštanja, doprinos pokrića i dohodak zabilježeni su u populaciji s najviše grla u stadu. Neiskorištene mogućnosti u obliku razlike dohotka između najbolje proizvodnje i najslabijih rezultata po skupinama su od 0,46 do 1,01 kn po kg mlijeka, ili od 2.664,09 do 5.574,47 kn po grlu i od 167.234 do čak 266.409 kn po gospodarstvu. Najveći mogući pomaci u visini prinosa i dohotku proizvođača zabilježeni su kod najbrojnije kategorije u hrvatskom mljekarstvu – gospodarstvima do 50 muznih grla u stadu, ali upravo su ovi proizvođači najmanje spremni na unaprjeđenje proizvodnje.

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Basic factors of economics of milk production on farms of different capacity

Abstract

The analysis of the basic factors of milk production of 27 family farms by groups of 30 to 150 cows in the herd was carried out. The analyzed farms differ significantly in size of the herd, the amount of milk production, the cost and income. Differences are also expressed within individual groups with the same number of heads, and they are affected by the amount of production, the total fixed assets engaged, the feeding system and the farm labor costs. The best results were recorded in the population with the highest number of animals in the herd, and the largest possible shifts in the level of yield and income of producers were recorded at farms up to 50 milking cows in the herd.

Keywords: capacity, economic indicators, milk, production indicators

Introducing QSPM Analysis to Agribusiness Firms

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Abstract

In formulating strategies, companies are increasingly using QSPM (Quantitative Strategic Planning Matrix) (David, David and David, 2016) analysis to determine the relative attractiveness of alternative actions being considered. Conceptually, QSPM analysis is an extension of the SWOT (Croman and Ronen, 2009) (David, Creek, and David, 2020) and determines the relative attractiveness of various strategies being considered for implementation based on the extent that key external and internal factors are capitalized on or improved upon. The aim of this paper is to propose and exemplify how QSPM analysis can enable agribusinesses to be more effective in their strategic planning decision-making process. Specificity in using QSPM analysis is important, because in the turbulent agribusiness industry, there are high stakes associated with strategic planning; QSPM analysis can be a valuable decision-making tool for agribusinesses, as explained in this paper.

Keywords: Strategic planning, QSPM, SWOT, Agribusiness

Introduction

The purpose behind utilizing QSPM (Quantitative Strategic Planning Matrix) analysis is to enable companies to more effectively select among or decide between competing alternative strategies. The first step in QSPM analysis is to perform external and internal assessments of the firm that provide, respectively, a finite list of key external opportunities/threats and internal strengths/weaknesses (Simon, et. al, 2010) that can provide a foundation for strategic decision-making (David, et al., 2016). Specifically, firms should take advantage of opportunities, avoid or mitigate the impact of external threats (Koo, et al., 2011), capitalize upon internal strengths, and improve upon weaknesses (Capps III and Glissmeyer, 2012).

Steps in Performing QSPM Analysis

QSPM analysis can be summarized in six steps as indicated below:

Step 1 - List the firm's key external opportunities and threats and internal strengths and weaknesses in the left column of the QSPM.

Step 2 - Assign weights to each key external and internal factor. These weights indicate the relative importance of each factor to being successful in the industry. The weights for the external factors must sum to 1.0 and similarly must sum to 1.0 for the internal factors.

Step 3 - Identify alternative strategies that the organization should consider implementing. Record these strategies in the top row of the QSPM.

Step 4 - Determine the Attractiveness Scores (AS), defined as numerical values that indicate the relative attractiveness of each strategy considering a single external or internal factor. Attractiveness Scores (AS) are determined by examining each key external or internal factor, one at a time, and asking the question, "Does this factor affect the choice of strategies being made?" If the answer to this question is yes, then the strategies should be compared relative to that key factor. The range for AS is 1 = not attractive, 2 = somewhat attractive, 3 = reasonably attractive, and 4 = highly attractive. If the answer to the previous question is no, indicating the respective key factor has no effect on the specific choice being made, then do not assign AS to the strategies in that set. Use a 0 to indicate that the key factor

Introducing QSPM Analysis to Agribusiness Firms

does not affect the choice being made for both strategies. Note: If you assign an AS score to one strategy, then assign an AS score(s) to the other.

Step 5 - Compute the Total Attractiveness Scores (TAS). TAS's are defined as the product of multiplying the weights (Step 2) by the AS (Step 4) in each row. The TAS's indicate the relative attractiveness of each alternative strategy.

Step 6 - Compute the Sum Total Attractiveness Score (STAS). Add TAS in each strategy column of the QSPM. STAS's reveal which strategy is most attractive in each set of alternatives.

QSPM Analysis for an Agribusiness: A Hypothetical Example

Exhibit 1 provides a hypothetical example for how QSPM analysis can be utilized in the agribusiness industry. In Exhibit 1, note that two alternative strategies are being considered by a family run agribusiness specializing in meat and fruit/grain production. Note by the STAS's of 2.85 versus 2.49 that the analysis indicates that the particular agribusiness should buy two new 18-wheel trucks. Note the use of 0s to indicate which external and internal factors do not affect the strategy choice being considered. Note also in Exhibit 1 that there are no consecutive 1s, 2s, 3s, or 4s across any row in a QSPM; never assign the same AS score across a row. Always prepare a QSPM working row by row. Also, if you have more than four strategies in the QSPM, then let the AS scores range from 1 to "the number of strategies being evaluated." This will enable assignment of a different AS score for each strategy. These are all important guidelines to follow in developing a QSPM.

Exhibit 1 – An Example QSPM for a Hypothetical Agribusiness Firm in a Hypothetical Country

Strategy 1 – Purchase two new 18-wheel trucks to transport our product to markets regionally

Strategy 2 – Acquire a poultry packaging operation in neighboring country GHI

		Strategy 1		Strategy 2	
Strengths	Weight	AS	TAS	AS	TAS
1 New irrigation systems give us 40% better insurance against possible droughts.	0.10	0	0.00	0	0.00
2 Our vendors are asking for more of our product, up 14% annually the last two years.	0.09	4	0.36	2	0.18
3 The pooling of 25% of our agricultural output has enabled us to increase our downstream bargaining power with prospective buyers in addition to our upstream bargaining power with suppliers.	0.08	3	0.24	4	0.32
4 Our profit margin for grain production increased from 6% to 10% in 2019 vs 2018.	0.05	3	0.15	1	0.05
5 We increased our # of vineyards by 10% in 2019 vs 2018.	0.04	2	0.08	1	0.04
6 Our waste and pollution control systems were improved 30% in 2019.	0.04	0	0.00	0	0.00
7 Our two new combines have enabled our labor costs to decrease 31%.	0.03	3	0.09	1	0.03
8 In June 2019, we secured a new line of credit for \$500,000 from XYZ Bank.	0.03	2	0.06	4	0.12
9 We social marketing and website effectiveness improved 40% in the last twelve months.	0.03	0	0.00	0	0.00
10 We hired a new accountant and technology officer in 2019.	0.02	0	0.00	0	0.00

		Strategy 1		Strategy 2		
Weaknesses	Weight	AS	TAS	AS	TAS	
1	Our exports to other countries declined 7% from 2108 to 2019.	0.08	1	0.08	4	0.32
2	Our labor costs related to apple harvesting grew 8% from 2018 to 2019.	0.07	0	0.00	0	0.00
3	We have no clear vision, mission, or long-range objectives.	0.07	0	0.00	0	0.00
4	We only obtain 35% of customers from online versus rival firms that average 50%.	0.05	0	0.00	0	0.00
5	Our employee morale has declined 10% in past twelve months due to large farms encroaching on our business.	0.05	0	0.00	0	0.00
6	Our fruit tree operations yield only a 4% annual return versus 12% for our key rival firm.	0.05	0	0.00	0	0.00
7	Our fertilizer suppliers are merging and increasing prices to us by 5% annually.	0.04	0	0.00	0	0.00
8	Our available cleared land is 25% of what we need given our 10% annual growth.	0.04	1	0.04	3	0.12
9	The average age of our equipment is 14 years versus 8 years for our key rival firm.	0.03	4	0.12	2	0.06
10	Our return on investment for turkey operations is 6% compared to our 11% average overall.	0.01	1	0.01	3	0.03

		Strategy 1		Strategy 2		
Opportunities	Weight	AS	TAS	AS	TAS	
1	A close rival agribusiness, ABC Company, is liquidating and seeking a buyer.	0.10	1	0.10	4	0.40
2	China has increased its imports of fruit from our country by 20% from a year ago.	0.09	0	0.00	0	0.00
3	Russia has reduced its exports of grain by 25%, so prices for our grain has increased 15%.	0.06	4	0.24	1	0.06
4	Interest rates have declined to 5% from 7% two years prior.	0.05	4	0.20	3	0.15
5	Political support for small agribusinesses is growing 10% annually.	0.04	4	0.16	3	0.12
6	A neighboring country, DEF, to the north has a 5% GDP.	0.04	3	0.12	4	0.16
7	Our country's political stability has improved 25% in two years.	0.04	0	0.00	0	0.00
8	The demand for poultry products is increasing 10% annually.	0.04	2	0.08	4	0.16
9	Our country's GDP increased from 3% to 4% in the last two years.	0.03	3	0.09	2	0.06
10	The market price for eggs is growing 8% annually.	0.02	2	0.04	4	0.08

		Strategy 1		Strategy 2		
Threats	Weight	AS	TAS	AS	TAS	
1	The population of our country is declining 3% annually.	0.09	2	0.18	1	0.09
2	Consumption of red meat is declining nationally by 5% annually.	0.07	2	0.14	4	0.28
3	The market share of large cooperatives has grown to 60%, while small farms' market share has declined to 30% in neighboring country RST; small farm MNO in RST is available for sale.	0.06	2	0.12	3	0.18
4	Government financial support for small farms is 33% less than for large farms.	0.06	0	0.00	0	0.00
5	Increased world wide awareness to eating high sugar diets has increased 15%.	0.05	0	0.00	0	0.00
6	Large companies have 20% higher economies of scale than our small farm.	0.04	1	0.04	2	0.08
7	Country GHI has political unrest that curtails our exports 40% to that region.	0.04	0	0.00	0	0.00
8	Local banks have reduced their lending to small firms by 33% in the prior two years.	0.03	0	0.00	0	0.00
9	Demand for packaged meats is growing 8% annually yet we do not package meats.	0.03	1	0.03	3	0.09
10	Transportation (trucking) costs are increasing 10% annually yet we own no 18-wheel trucks.	0.02	4	0.08	1	0.02
TOTALS				2.85		2.49

As competitiveness becomes more intense in the agribusiness of eastern Europe, many firms, especially small farmers, are struggling to make effective strategic decisions and manage their resources (Barney, 1991). Large cooperatives that have greater economies of scale and resources are putting more and more pressure on small firms. The good news however is that even small agribusinesses can be just as effective, or even more effective, than larger enterprises in doing strategic planning. As explained and exemplified in this paper, QSPM analysis can be used to determine the relative attractiveness of alternative strategies, before large capital investments are made implementing a particular strategy or decision. Having an excellent strategy or game plan before going into battle oftentimes makes the difference between being successful and unsuccessful. Thus, QSPM analysis should be utilized, we contend, always in making important strategy decisions.

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Mogućnost uvođenja jednokratne ambalaže za ajvar na domaće tržište

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Sažetak

Na hrvatskom tržištu ne postoje jednokratna pakiranja za ajvar. Temeljno istraživačko pitanje ovog rada je postoji li potencijalna potražnja za jednokratnim pakiranjima ajvara te koje su tehničke mogućnosti i ograničenja za uvođenje tih pakovina na domaće tržište. Cilj je rada bio utvrditi stavove potrošača i mišljenje stručnjaka o mogućnosti uvođenja na tržište jednokratnog pakiranja za ajvar. Za potrebe izrade rada provedena su tri terenska istraživanja, intervju s tehnologom za izradu pakovina, intervju s ugostiteljima te anketno istraživanje potrošača ($n = 114$). S tehničke strane ne postoje ograničenja za proizvodnju i korištenje jednokratne ambalaže za ajvar. U ugostiteljstvu, posebice restoranima brze prehrane, postoji potreba uvođenja takve pakovine. Većina potrošača ima pozitivan stav o uvođenju jednokratne ambalaže za ajvar i kupovali bi ga za kućnu upotrebu odnosno naručivalo u ugostiteljskim objektima. Dio potrošača upozorio je na negativan ekološki aspekt uvođenja jednokratne ambalaže.

Ključne riječi: novi proizvod, ajvar, ambalaža

Uvod

Novi proizvod, u marketinškom smislu, je svaki proizvod koji kupci percipiraju kao novi (usp. Homburg i Krohmer, 2009). To može biti apsolutna inovacija, proizvod koji nikad prije i nigdje nije bio ponuđen na tržištu. Međutim, puno češće od stvarnih inovacija su poboljšice postojećih proizvoda bilo da se dodaju novi okusi, mijenja dizajn odnosno ambalaža proizvoda itd. Novi proizvod je također kad se postojeći proizvod s određenog tržišta po prvi put pojavi na novom tržištu (usp. Decker i sur., 2015; Kotler i sur., 2014; Previšić, Ozretić Došen, 2007). Razvoj novih proizvoda i njihovo uvođenje na tržište je rizična strategija, a uspješnost njihovog uvođenja razmjerno mala. Primjerice, vjerojatnost neuspjeha novog proizvoda u njemačkoj prehrambenoj industriji procjenjuje se na oko 65% (usp. Rohwetter, 2004., Halaszovich, 2011). U velikom broju studija ali i u medijima spominje se stopa neuspjeha kod uvođenja novog proizvoda između 80 – 95 %. Nagorny (2019) opovrgava taj podatak i navodi da se radi o tržišnom mitu te da je prosječna stopa neuspjeha u stvarnosti znatno niža, te da iznosi oko 40% u zadnjih 50 - 60 godina, ona varira o zemlji i industriji.

Pogreške pri uvođenju novog proizvoda uzrokuju značajne financijske gubitke i nanose štete imidžu poduzeća. Stoga se taj proces mora sustavno planirati. Jedna od najvažnijih faza u tom procesu je razvoj i testiranje koncepta proizvoda. U ovoj fazi treba utvrditi rješava li novi proizvod određeni problem, koji su konkurentski proizvodi, je li cijena razumna u odnosu na vrijednost proizvoda, treba li unaprijediti koncept te namjeravaju li potrošači kupovati proizvod (usp. Kotler, 2014, Marušić i Vranešević, 2001).

Predmet ovog rada je uvođenje inovativnog pakiranja za ajvar na domaće tržište.

Ajvar je dodatak jelima koji se može poslužiti u obliku namaza, priloga, umaka i dr. Prisutan je na policama većine trgovina i u ugostiteljskim objektima. Dosadašnje pakiranje ajvara se temeljilo na staklenkama, dok su primjerice, majoneza i kečap dostupni već dugi niz godina u manjim, jednokratnim pakiranjima - paketićima ili čašicama. Snimkom tržišta je utvrđeno da slična pakiranja ne postoje za ajvar. Temeljno istraživačko pitanje ovog rada je postoji li potreba za jednokratnim pakiranjima ajvara te koja su tehničke mogućnostima i ograničenja za uvođenje tih pakiranja na tržište. Cilj rada je utvrditi mišljenja i namjere potrošača i stručnjaka o mogućnosti uvođenju na tržište jednokratnog pakiranja za ajvar.

Rezultati istraživanja dat će odgovor je li koncept jednokratnog pakiranja za ajvar tržišno prihvatljiv i postoje li tehnološka ograničenja za njegovu proizvodnju.

Materijal i metode

U okviru istraživanja provedena su tri polustrukturirana intervjua i anketa potrošača. Intervjui su provedeni s ekspertom za izradu pakovina, kuharom te dva ugostitelja. Intervju su poslužili za izradu koncepta jednokratnog pakiranja za ajvar te za procjenu mogućnosti uvođenja novog proizvoda u ugostiteljske objekte. Anketno istraživanje provedeno je u razdoblju srpanj – kolovoz 2019. godine na uzorku od 114 ispitanika. Ispitani su mišljenja, stavovi i namjere potrošača ajvara o korištenju jednokratnih pakiranja ajvara. Anketiranje je provedeno u elektroničkom obliku. Anketni upitnik je izrađen putem Google Forms obrasca. Ispitanici su kontaktirani putem društvene mreže Facebook. Prikupljeni podatci su analizirani i obrađeni u SPSS programu, a pri tome je korištena jednovarijatna analiza.

Rezultati i rasprava

Intervju s tehnologom i kuharom

Kako bi se osvijetlile tehničke mogućnosti i ograničenja za uvođenje jednokratnog pakiranja za ajvar provedeni su intervjui s tehnologom i kuharom.

Mišljenje eksperata je da postoji potreba za uvođenjem jednokratne ambalaže za ajvar u ugostiteljske objekte i maloprodaju. Za odabir ambalaže ključna je tekstura ajvara pa stoga tube ne dolaze u obzir. Primjereni oblici su čašice i vrećice. Od materijala preporuča se korištenje polietilena i aluminij odnosno višeslojni materijali. U slučaju korištenja plastične ambalaže preporučuje se visokotlačna pasterizacija koja bi sterilizirala proizvod i zadržala sva nutritivna svojstva. Upotreba čašica mogla bi biti problematična zbog ostatka zraka pri punjenju. Međutim, taj problem je moguće riješiti s kvalitetnijim punilicama. Vrećice imaju prednost u odnosu na čašice i zbog transporta jer zauzimaju puno manji volumen.

Predloženi volumen pakiranja ajvara je 30 ml.

Cijene jednokratne ambalaže ne bi trebale značajnije povećati cijenu ajvara.

Intervju s ugostiteljima

Provedena su dva intervjua s ugostiteljima kako bi se procijenilo prihvaćanja jednokratne ambalaže za ajvar.

Ajvar se u ugostiteljskim objektima nudi kao dodatak pojedinim jelima, kao što su primjerice jela sa žara, ali se može naručiti i kao poseban prilog.

Ugostitelji su do sada imali dosta problema sa serviranjem ajvara. Prilikom serviranja ajvara s pečenim mesom problem je što ajvar dotiče meso, a to mnogi gosti ne vole. Drugi veliki problem je taj što dio gostiju ne konzumira ajvar ili ga konzumira jako malo pa se dio ajvara baca. Uvođenjem jednokratne ambalaže tanjur s jelom bi vizualno ljepše izgledao i ajvar se ne bi bacao u slučaju da ga gosti ne žele konzumirati. Osim toga, jednokratna ambalaža bi olakšala posluživanje ajvara. Preferirani oblik su jednokratne čašice ili posudice, dok bi vrećice bile praktičnije. Preferirani volumen je 60 grama. Moguće ograničenje je povećanje nabavne cijene ajvara.

Anketa potrošača

U anketi je sudjelovalo 62,3% ženskih i 37,7 % muških osoba. Najviše ispitanika je u dobi od 21 do 30 godina (70,2 %), slijede oni od 31 do 40 godina (10,5%), od 41 – 50 godina (7,9 %) te osobe mlađe 20 godina i starije od 50 godina (4,4 %). Prema obrazovanju najviše ispitanika ima višu stručnu spremu (47,4%), zatim srednju stručnu spremu (27,2 %) te magisterij ili doktorski studij (25,4%). Prema visini mjesečnih primanja najbrojnija su kućanstva koja imaju od 4.000 do 8.000 kuna (37,7%), zatim ona od 8.001 do 12.000 kuna (28,9%), dok prihode veće od 12.000 kuna ima 23,7 % kućanstava. Prihod manji od 4.000 kuna ima 9,6% kućanstava.

Većina ispitanih konzumira ajvar 2 do 3 puta mjesečno (35,1 %), daljnjih 25% jednom mjesečno, 23,7% konzumira ajvar 2 do 3 puta godišnje te 13,2% njih najmanje jednom tjedno. Svega 2,7% ispitanika konzumira ajvar jednom godišnje odnosno rjeđe. Ajvar se najčešće konzumira kao hladni dodatak uz meso s roštilja, a puno rjeđe kao namaz i dodatak jelima.

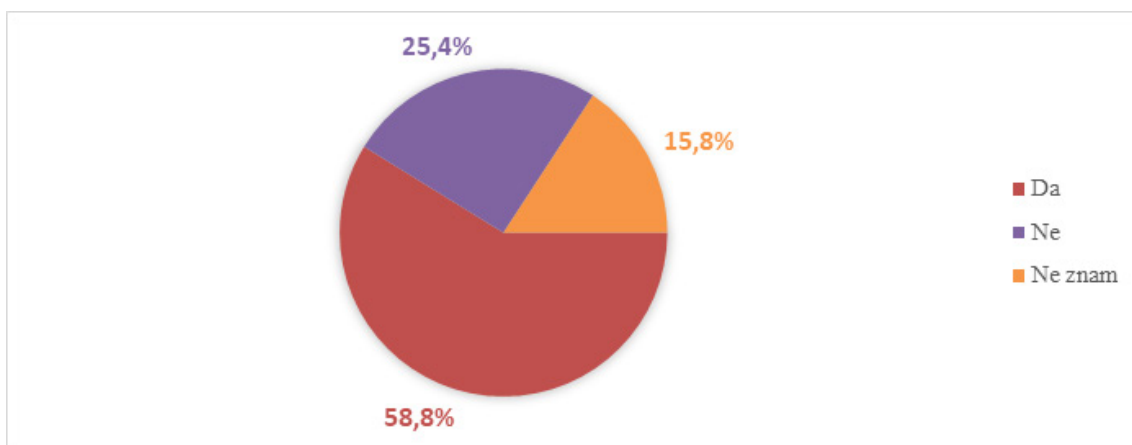
Većina ispitanika kupuje ajvar u supermarketima ili lokalnim trgovinama, ali ga značajan broj ispitanika sam proizvodi.

Preferencije potrošača prema jednokratnoj ambalaži umaka (ketchup, majoneza, senf) su podijeljene. Gotovo polovica ispitanika preferira jednokratnu ambalažu (45,6%), 39,5 % ju ne preferira, a 14,9% nema izražene preferencije. Čak tri četvrtine ispitanika konzumira umake iz jednokratnih ambalaža, dok 23,7 % ne konzumira. Razlozi za korištenje jednokratne ambalaže su: jednostavno korištenje i skladištenje, veća svježina i bolji okus umaka, te manji rizik od kvarljivosti proizvoda. Razlozi protiv korištenja jednokratne ambalaže su: veće količine otpada što dodatno onečišćuje okoliš te viša cijena umaka. Dio ispitanika drži da su jednokratna pakiranja bolja za ugostiteljske objekte, a veća pakiranja za kućanstva.

Veličina pakiranja ajvar važna je odnosno jako važna za trećinu ispitanih (35,1%), nevažna za 30,7% ispitanika, a niti važna niti nevažna za njih 34,2%

Nešto više od polovice ispitanih (58,8%) namjerava kupovati ajvar u jednokratnoj ambalaži, četvrtina ispitanih ne namjerava, a 15,8% nema izražene namjere. Gotovo $\frac{3}{4}$ ispitanika (71,1%) koristilo bi jednokratna pakiranja ajvara u ugostiteljskim objektima, 17,5% ne bi, a 11,4% ne zna.

Prema mišljenju ispitanika prednosti jednokratnog pakiranja za ajvar su slijedeće: smanjilo bi se bacanje hrane, higijenski su sigurnija te se lakše transportiraju i skladište. Dodatno, takva ambalaža sprječava mogućnost manipulacije s količinama u ugostiteljskim objektima. Najveća barijera za korištenje jednokratne ambalaže su veće količine otpada koje će takva ambalaža izazvati. Većina ispitanika misli da bi jednokratna pakiranja ajvara povećalo cijene objeda u ugostiteljskim objektima. Isto tako, većina ispitanih ne želi platiti veću cijenu objeda zbog jednokratne ambalaže.



Graf 1. Biste li kupili ajvar u jednokratnoj ambalaži?

Zaključak

Na domaćem tržištu ne postoje jednokratna pakiranja za ajvar. S tehničke strane ne postoje ograničenja za proizvodnju i korištenje ajvara u jednokratnoj ambalaži. Primjereni oblici za pakiranje su čašice i vrećice, a od materijala preporuča se korištenje polietilena i aluminij odnosno višeslojni materijali. Upotreba čašica mogla bi biti problematična zbog ostatka zraka pri punjenju što je moguće riješiti s kvalitetnijim punilicama. Predložena masa pakiranja ajvara je 30 g. Ugostitelji navode da postoji potreba za uvođenjem jednokratnog pakiranja ajvara u ugostiteljske objekte. Pri tome oni preferiraju oblik čašice i pakiranja od 60 g. Većina potrošača ima pozitivan stav o uvođenju jednokratne ambalaže za ajvar i kupovali bi ga za kućnu upotrebu odnosno naručivalo u ugostiteljskim objektima. Dio potrošača upozorio je na negativan ekološki aspekt uvođenja jednokratne ambalaže te moguće poskupljenje objeda u ugostiteljstvu. Nedoumice vezane za oblik i masu jednokratne ambalaže za ajvar treba razriješiti u fazi razvoja i testiranja proizvoda.

Napomena

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The possibility of introducing a disposable ajvar packaging on the domestic market

Abstract

There is no ajvar packet in the Croatian market. The basic research question of this paper is whether there is a potential demand for ajvar packet and what are the technical possibilities and limitations for introducing these packets in the domestic market. The aim of the paper was to determine consumers' attitudes and experts' opinions on the possibility of introducing ajvar packet in the market. Therefore, three field surveys were conducted, an interview with a packaging technologist, interviews with caterers, and a consumer survey (n = 114). Technically, there are no restrictions on the production and use of ajvar packets. In the catering industry, especially in fast food restaurants, there is a need to introduce such small packaging. Most consumers have a positive attitude about ajvar packets; they would buy it for home use or order at catering establishments. Some consumers pointed to the negative environmental aspect of introducing condiment packets.

Keywords: novel product, ajvar, packaging

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Utjecaj osvjetljenja različitih spektralnih karakteristika na formiranje puči kapara (*Capparis orientalis* Veill.) u *in vitro* i *in vivo* uvjetima

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Sažetak

Cilj istraživanja bio je utvrditi promjene u broju puči po jedinici lisne površine pod utjecajem osvjetljenja različitih spektralnih karakteristika na izdancima kapara u fazi *in vitro* zakorjenjivanja te na zakorjenjenim i uspješno aklimatiziranim biljkama *in vivo*. Testirana su tri tipa osvjetljenja: fluorescentne lampe, LED osvjetljenja s omjerom crvenog i plavog spektra 70:30 i LED osvjetljenje širokog spektra: 53% crveni, 4% plavi, 16% zeleni spektar i 17% tamno crvena svjetlost.

Broj puči po jedinici lisne površine bio je značajno veći u *in vitro* uvjetima, dok je u oba tipa uzgoja najveći broj puči s abaksijalne i adaksijalne strane izmjeren pri osvjetljenju LED rasvjetom širokog spektra. Ovisno o vrsti svjetla uspješnost zakorjenjivanja se kretala od 59 do 83 %, a uspješnost aklimatizacije od 65 do 69%.

Ključne riječi: kapar, puči, fluorescentno svjetlo, LED

Uvod

Kapar (*Capparis orientalis* Veill.) je grmolika trajnica koja raste uglavnom u mediteranskim zemljama. Dio je mediteranske prehrane više od 5000 godina (Güleryüz i sur., 2009). Ima važnu ulogu u prehrambenoj industriji, ali i farmaceutskoj i kozmetičkoj industriji radi čega je komercijalno vrijedan proizvod. Obalno područje Republike Hrvatske je prirodno stanište istočnjačkog kapara (*Capparis orientalis* Veill.).

Usprkos tome nema niti jednog komercijalnog nasada, a kompletna ponuda ukiseljenih i usoljenih cvjetnih pupova dostupnih na OPG-ima temelji se na sakupljanju pupova samoniklih biljaka. Štoviše, kapar je na popisu tradicijskih povrtnih sorti Dalmacije (Ozimeci sur., 2015), ali na tržištu nema sadnica tradicijskih genotipova, kao niti kultivara oplemenjivanih u našim uvjetima.

Uzrok tome može biti nedovoljno poznavanje nutritivnog i farmaceutskog potencijala kapara, nepostojanje istraživanja o utjecaju okolišnih uvjeta na proizvodnju bioaktivnih spojeva i slaboj uspješnosti razmnožavanja kapara bilo generativno ili vegetativno. Rješenje za ekspanziju komercijalnog uzgoja ove kulture je mikropropagacija.

Opisano je uspješno *in vitro* umnažanje kapara (Al-Mahmood i sur., 2011; Carra i sur., 2012; Kereša i sur., 2019), ali u dostupnoj literaturi nema podataka o utjecaju različitih spektralnih karakteristika svjetlosti na razvoj kapara. Poznato je da spektralne karakteristike svjetlosti, intenzitet osvjetljenja i fotoperiod imaju središnju ulogu u morfogenezi, rastu i diferencijaciji biljnih stanica (Vieira i sur., 2015) te snažno utječu na fotosintezu (Tripathy i Brown, 1995; Vieira et al. 2015). S obzirom da ne postoji konsenzus o univerzalnom spektru svjetla koji bi bio optimalan za razvoj u *in vitro* uvjetima, već ovisi o biljnoj vrsti, takva istraživanja su trenutno vrlo aktualna (Gupta i Jatothu, 2013; Vieira i sur., 2015; Cioć i sur., 2018).

Aklimatizacija predstavlja kritičnu točku *in vitro* razmnožavanja jer se biljke moraju prilagoditi na nove okolinske uvjete kao što su niža relativna vlažnost, veći intenzitet svjetlosti, variranje temperatur i konstantan infektivni pritisak zbog nesterilnih uvjeta *ex vitro*. Osim toga istraživanja (Moyo i sur., 2015; Pospišilova i sur., 1999; Sahay i Varma 2000) upućuju na razliku morfologije listova, osobito broja i veličine puči u *in vitro* i *ex vitro* uvjetima.

Stoga je razumijevanje promjena folijarne mikromorfologije koje nastaju kao posljedica *in vitro* uvjeta od velike važnosti. Stoga je cilj ovog rada bio praćenje promjena u broju puči po jedinici lisne površine između biljaka u *in vitro* uvjetima i nakon aklimatizacije pod osvjetljenjem različitih spektralnih karakteristika.

Materijal i metode

Uniformni izdanci kapara (*Capparis orientalis* Veill.) mikropropagiranih na MS mediju (Murashige i Skoog, 1962) s dodatkom 0,6 mg/l meta-topolina (mT) korišteni su kao početni biljni materijal. Ujednačeni izdanci dužine 1-1,5 cm postavljeni su na medij za ukorjenjivanje sastava: MS makro- i mikroelementi (Murashige i Skoog, 1962), MS vitamini, 0,1 g/L inozitola, 30 g/L saharoze i 2 mg/L indolil-3 octene kiseline (IAA), Bacto™ Agar 8 g/L, pH 5,8. Medij, Magenta posudice i pribor su sterilizirani u autoklavu na 121°C, pri tlaku od 1 bara i trajanju od 25 min.

Magenta posudice s eksplantatima smještene su u komoru rasta namijenjenu za rast kulture tkiva. Temperatura u komori bila je 23,5±1°C i fotoperiod 16 sati dan/ 8 sati noć. Izvori umjetnog osvjetljenja bile su fluorescentne lampe Osram L 36W/77 FLUORA, LED osvjetljenje proizvođača Philips, model GP LED DR/B 120 HB LO s omjerom crvenog i plavog spektra 70%:30% i LED osvjetljenje proizvođača Valoya, model NS12 širokog spektra 53% crveni, 4% plavi, 16% zeleni spektar i 17% tamno crvena svjetlost. Kod sva tri tipa osvjetljenja intenzitet je bio jednak, 40 µE m⁻²s⁻¹.

Na svaki tretman svjetlom postavljeno je 8 Magenta posuda s 9 izdanaka u svakoj; ukupno 72 biljke po tretmanu. Nakon 70 dana procijenjen je udio zakorjenjenih biljaka. Prije sadnje i aklimatizacije zakorjenjenih izdanaka kapara, po 6 izdanaka sa svakog tretmana poslužilo je za određivanje broja puči po jedinici lisne površine u *in vitro* uvjetima. Zakorjenjene biljke su posađene u smjesu komposta (2/3) i perlita (1/3), prekrivene plastičnom prozirnom folijom te aklimatizirane u komori rasta pri istim uvjetima i tretmanima svjetlom na kojima su biljke i zakorijenile. Uspješnost aklimatizacije procijenjena je 60 dana nakon sadnje, a 120 dana nakon sadnje uzeti su uzorci lisnog tkiva za određivanje broja puči po jedinici lisne površine u *in vivo* uvjetima.

Utjecaj tipa osvjetljenja na broj puči po jedinici lisne površine zakorjenjenih biljaka u *in vitro* uvjetima i aklimatiziranih biljaka (u *in vivo* uvjetima) testirani su analizom varijance. Za usporedbu prosjeka tretmana osvjetljenja različitih spektralnih karakteristika i različitih uvjeta uzgoja (*in vitro* ili *in vivo*) korišten je Duncan test za višestruke usporedbe. Statistička analiza podataka provedena je programskim paketom SAS (SAS, 2010). Radi testiranja uspješnosti ukorjenjivanja i aklimatizacije biljaka s obzirom na tretmane svjetlom, proveden je χ^2 -test u excelu.

Rezultati i rasprava

Različiti tretmani svjetlom i različiti uvjeti uzgoja značajano su utjecali na broj puči po mm² lisne površine. I u *in vitro* i u *in vivo* uvjetima, najveći broj puči razvijen je kod biljaka uzgajanih pod LED osvjetljenjem širokog spektra dok je najmanji broj puči razvijen kod biljaka uzgajanih pod fluorescentnim lampama (Tablica 1, Tablica 2, Slika 1). O pozitivnom učinku tamno crvene svjetlosti na formiranje i aktivnost puči izdanaka banana u odnosu na kontrolno osvjetljenje fluorescentnim lampama izvjestili su Vieira i sur. (2015). Pozitivan utjecaj kombinacije tamno crvene svjetlosti i plavog LED spektra opisali su i Kim i sur. (2004) na krizantemi. S obzirom da je LED osvjetljenje širokog spektra Valoya (NS12) rezultiralo najvećim brojem puči po jedinici lisne površine, možemo pretpostaviti da je tamno crvena svjetlost, koju ovaj tip osvjetljenja sadrži, pozitivno utjecala na razvoj puči kapara.

Tablica 1: Prosječan broj puči po mm² lisne površine kod različitih vrsta osvjetljenja

	Broj puči/mm ² lista (abaksijalna strana lista)	Broj puči/mm ² lista (adaksijalna strana lista)
Fluorescentne lampe	158,9 C	187,7 C
Tip osvjetljenja		
LED široki spektar	287,1 A	299,5 A
LED 70:30	215,2 B	239,4 B

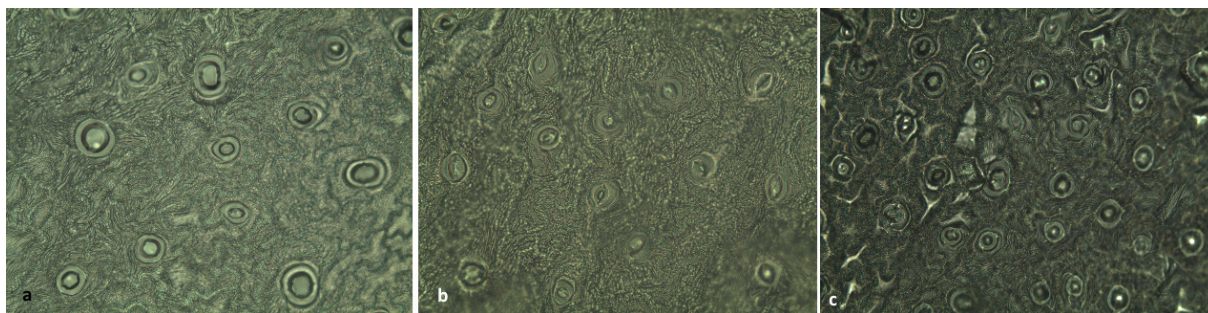
*Vrijednosti označene istim slovom ne razlikuju se značajno ($p < 0,01$) prema Duncan testu. Vrijednosti broja puči po lisnoj površini su izračunati kao prosječne vrijednosti za oba tipa uzgoja (u *in vitro* i *in vivo* uvjetima)

Utjecaj osvjetljenja različitih spektralnih karakteristika na formiranje puči kapara (*Capparis orientalis* Veill.) u *in vitro* i *in vivo* uvjetima

Tablica 2: Prosječan broj puči/mm² lisne površine kod različitih uvjeta uzgoja (*in vitro* i *in vivo*) kroz sve tipove osvjetljenja

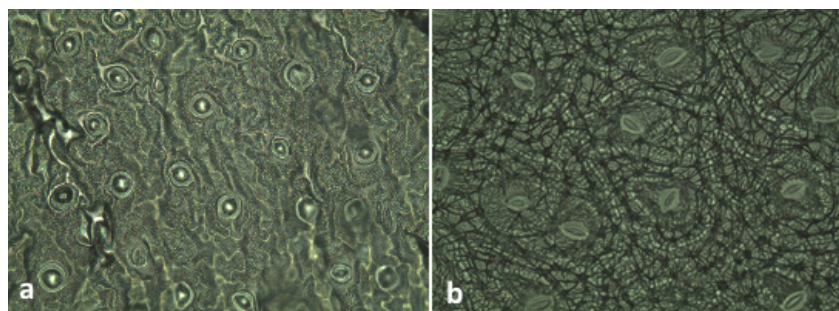
Tip uzgoja	Broj puči/mm ² lista (abaksijalna strana lista)		Broj puči/mm ² lista (adaksijalna strana lista)	
	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>
			317 A	328,6 A
			135,8 B	182,7 B

*Vrijednosti označene istim slovom ne razlikuju se značajno ($p < 0,01$) prema Duncan testu. Vrijednosti broja puči po lisnoj površini su izračunati kao prosječne vrijednosti za sve tri vrste osvjetljenja.



Slika 1: Puči s abaksijalne strane lista razvijene pod: a) fluorescentnim osvjetljenjem, b) LED osvjetljenjem crvenog i plavog spektra 70:30, c) LED osvjetljenjem širokog spektra Valoya (NS12)

Karakteristika puči razvijenih u *in vitro* uvjetima je okrugli oblik i potpuna otvorenost (Slika 1, Slika 2a), dok su puči razvijene *in vivo* eliptičnog oblika i manje otvorene (Slika 2b). Okrugli oblik puči povezuje se s odsutnošću ili jako narušenom funkcijom puči u *in vitro* uvjetima, dok je eliptičan oblik karakteristika funkcionalnih puči u *in vivo* uvjetima (Zacchani i sur., 1997). Smatra se da visoka vlaga u *in vitro* uvjetima utječe na tijek razvoja stanica zapornica, kao i na mehanizam zatvaranja puči (Marin i Gella, 1988).



Slika 2: Puči s adaksijalne strane lista razvijene pod LED osvjetljenjem širokog spektra u: a) *in vitro* uvjetima, u b) *in vivo* uvjetima

Uspješnost zakorjenjivanja iznosila je u prosjeku 74%, a uspješnost aklimatizacije 67% pri čemu tip osvjetljenja nije imao statistički značajnog utjecaja. Ovaj rezultat je u skladu s dostupnim istraživanjima prema kojima se uspjeh aklimatizacije kapara razvijenih u *in vitro* uvjetima, kreće se od 60% do 70% (Musallam i sur., 2011; Carra i sur., 2012; Mehrabani 2016; Kereša i sur., 2019).

Zaključak

Puči omogućavaju biljci da se prilagodi promjenama u okolišu i kroz kontrolu otvorenosti pora održavaju ravnotežu utječući na fizičku razmjenu između biljke i njezine okoline. Zbog ovih su osobina puči važne u aklimatizaciji i preživljavanju sadnica nakon prenošenja iz *in vitro* uvjeta kada je regulacija vode u biljci izuzetno bitna da bi se izbjegla dehidracija. Ovo istraživanje je pokazalo da tip osvjetljenja značajno utječe na formiranje puči kapara te da

postoje znatne razlike u broju, obliku i otvorenosti puči kapara u *in vitro* i *in vivo* uvjetima. Iz toga zaključujemo da biljka u fazi aklimatizacije mora značajno smanjiti velik broj uglavnom slabo funkcionalnih puči razvijenih u *in vitro* uvjetima na manji broj funkcionalnih puči kakav smo zabilježili u *in vivo* uvjetima kod aklimatiziranih biljaka kapara te da niti jedan od testiranih tipova osvjetljenja nije značajno utjecao na taj proces.

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Effect of different light spectra on stomata formation of caper (*Capparis orientalis* Veill.) *in vitro* and *in vivo*

Abstract

The aim of this study was to examine stomata formation of caper plantlets rooted *in vitro* as well as on plants successfully acclimatised *in vivo* under different light spectra. Three types of lights were tested: fluorescent lamps, LED with the ratio of red and blue light 70% : 30% and LED wide spectrum: 53% red, 4% blue, 16% green and 17% deep red light. Stomata density was significantly higher in *in vitro* conditions. Across both types of growth (*in vitro* and *in vivo*), the highest stomata density was recorded under LED wide spectrum. Depending on type of light, the success of rooting varied between 59 and 83 %, while 65 to 69% of rooted plantlets were successfully acclimatised.

Keywords: caper, stomata, fluorescent light, LED

Genetic diversity of some important wheat (*Triticum aestivum* L.) genotypes in Albania based on morphological traits and RAPD markers

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Abstract

The aim of the study was to assess the genetic variability among three Albanian bread wheat (*Triticum aestivum* L.) cultivars using morphological traits and RAPD markers.

Strong correlations were observed between various traits, as tillering capacity and seed size or seed size and spike length. Based on 14 morphological traits cultivars 'Progresi' and 'LVS' showed higher similarity levels. Nine polymorphic RAPD markers that gave reproducible results were used to assess genetic diversity among three bread wheat cultivars. There were generated in total 31 fragments with a mean of 3.4 fragments per primer. Cluster analysis based on molecular data revealed two groups, however no correlation was observed between clustering obtained with molecular markers and morphological characters. The highest variability among genotypes was observed for number of seeds per spike, stem length, and number of seeds per spikelet, and they could be used to select favourable traits for crosses in the future genetic improvement programs.

Keywords: *Triticum aestivum* L. RAPD, morphology, genetic variability

Introduction

Albania is an area rich in crop biodiversity, it is characterized by a high diversity of climatic and agronomic conditions. Therefore, this genetic diversity needs to be characterized and measured. Genetic variability evaluation based on morphological characters of economic interest might be used to choose suitable materials for crop improvement (Dos Santos et al., 2009). As previously reported (Al Khanjari et al., 2008), quantitative traits are often used to assess and describe the wheat characters due to their role in the estimation of genetic diversity and discrimination of closely related types. Genetic diversity assessment based on RAPD molecular markers has been widely implemented in wheat due to their lack of environmental conditions influences (Cao et al., 2000; Kutka et al., 2016; Gurcan et al., 2017). Genetic diversity studies in Albanian wheat cultivars based on molecular markers system has not been done to date. Therefore, three important bread wheat cultivars were evaluated using the morphological traits and RAPD markers to gain better understanding of their genetic diversity.

Materials and methods

Plant material: Three Albanian cultivars of bread wheat (*Triticum aestivum* L.) were included in the present study. Seeds of 'Progresi', 'Dajti' and 'LVS' cultivars were germinated and analysed at the Laboratory of Molecular Biology of the Biotechnology Department of the Natural Science Faculty of Tirana.

Method: After seedling emergence morphological measurements were made for root length/cm (RL), stem length/cm (SL), leaf length/cm (LL), stem weight/g (SW) and fresh root weight/g (RW). Other traits such as tillering capacity (TC), plant height/cm (PH), spike length/cm (SpL), number of spikelet per spike (NSpklSp), number of seeds per spikelet (NSeSpkl), number of seeds per spike (NSeSp), weight of seeds per spike/g (WSeSp) and weight of 1000 seeds/g (W1000S), were measured on the new set of plants of the three cultivars. Two weeks old seedlings were used for genomic DNA extraction following cetyltrimethylammonium bromide (CTAB) method according to Kump

Genetic diversity of some important wheat (*Triticum aestivum* L.) genotypes in Albania based on morphological traits and RAPD markers

and Javornik (2006). The amplification was done by means of 11 RAPD markers (OPA01, OPA08, OPA13, OPA15, OPA16, OPA17, OPB01, OPAG04, OPJ04, OPJ07 and OPJ12). The PCR mixture in a volume of 15 µl contained 20ng DNA, 1xPCR buffer, 2mM MgCl₂, 0.2mM dNTPs, 0.2µl primer and 0.3U of *Taq* polymerase. Amplification were carried out in PCRG-400 Gradient Thermal Cycler, using the following conditions: 94°C for 1.5 min, 36 cycles of 94°C for 30s, 36°C for 45s, 72°C for 1 min and final step on 72°C for 5 min. PCR amplified fragments were separated by electrophoresis in 1.5% agarose gel.

Data analysis: Descriptive statistics for 14 morphological traits as well as Pearson's correlation coefficients among them were calculated in the Statistical Package for Social Sciences (SPSS version 21). A binary matrix was obtained based on scoring the fragments generated by RAPD based analysis in the gel. These data were used to calculate Jaccard's coefficient and the dendrogram was generated using similarity matrix based on Neighbour joining cluster analysis, implemented in NTSYS v. 2.2 software (Rohlf, 2000).

Results and discussion

The morphological data findings (Table 1) could be the result of genetic differences among the three cultivars. Cultivar 'Progresi' had the highest values for root length, root fresh weight and seed size, which are higher than those reported by other authors (Peltonen et al., 2007; Xhulaj et al., 2017). All three wheat cultivars had high tillering capacity (Table 1). The estimated coefficient of variation was high for traits such as number of seeds per spike, stem length, and number of seeds per spike, similar to reports by other authors (Ali et al., 2008; Sabaghina et al., 2014; Xhulaj et al., 2019).

Table 1. Descriptive statistics of 14 morphological traits in three cultivars of bread wheat

Trait	Cultivar			Mean	cv (%)
	Progresi	Dajti	LVS		
RL/cm	13.87	10.88	12.89	12.5	12.1
SL/cm	14.23	19	12.12	15.1	23.3
LL/cm	7.27	9.35	6.68	7.8	18.1
SW/g	0.07	0.08	0.072	0.1	7.2
RW/g	0.05	0.04	0.043	0.0	11.6
TC	3.1	3.3	3.4	3.3	4.7
PH/cm	98.3	90.5	105.5	98.1	7.6
SpL/cm	9.76	10.1	10.26	10.0	2.5
NSpklSp	20.5	24.2	21.4	22.0	8.8
NSeSpkl	2.33	3.01	2.98	2.8	13.9
NSeSp	35.9	56.2	38.4	43.5	25.4
SS/mm	6.23	6.13	6.06	6.1	1.4
WSeSp/g	2.67	3.18	2.84	2.9	9.0
W1000S/g	45.3	50.04	49.7	48.3	5.5

RL –root length/cm; *SL*- stem length/cm; *LL*-leaf length/cm; *SW*-Stem weight/g; *RW*- fresh root weight/g; *TC* - tillering capacity; *PH* - plant height/cm; *SpL* - spike length/cm; *NSpklS* - number of spikelet per spike; *NSeSpkl* - number of seeds per spikelet; *NSeSp* - number of seeds per spike; *SS* - seed size/mm; *WSeSp* – weight of seeds per spike/g and *W1000S*- weight of thousand seeds.

Cultivar 'Dajti' had the highest values for number of seeds per spikelet, number of seeds per spike and weight of thousand seeds. Seed weight is an important trait in wheat, which could increase seed germination capacity, seedling emergence, tillering capacity, spike density and yield (Bellatreche et al., 2017). Cultivar LVS showed the highest value for plant height (105.5 cm), which is higher than those reported by Aliu et al., (2010), and goes within the limits of plant heights reported by Sabaghina et al., (2014) and Mahmood et al., (2006).

Correlation Coefficient Analysis: Pearsons' correlation coefficients among morphological traits are shown in table 2.

The highest positive correlations ($r=1$) were observed between tillering capacity and spike length, as well as between weight of thousand seeds and number of seeds per spikelet, whereas the highest negative correlation ($r=-1$) was found between weight of seeds per spike and root length. A number of very strong correlations (>0.9 or >-0.9) were also observed between various traits, e.g. tillering capacity and seed size or seed size and spike length.

Table 2. Correlation matrix among 14 morphological traits

Variables	RL	SL	LL	SW	RW	TC	PH	SpL	NSpkl Sp	NSe Spkl	NSeSp	SS	WSe Sp
SL	-0.80												
LL	-0.85	0.99											
SW	-0.95	0.95	0.97										
RW	0.93	-0.54	-0.62	-0.77									
TC	-0.49	-0.11	-0.02	0.20	-0.77								
PH	0.67	-0.98	-0.95	-0.87	0.37	0.30							
SpL	-0.50	-0.09	-0.01	0.21	-0.78	1.00	0.29						
NSpklSp	-0.99	0.85	0.90	0.97	-0.89	0.41	-0.74	0.42					
NSeSpkl	-0.77	0.25	0.34	0.54	-0.95	0.93	-0.06	0.93	0.71				
NSeSp	-0.97	0.91	0.94	0.99	-0.83	0.29	-0.81	0.31	0.99	0.62			
SS	0.41	0.20	0.11	-0.11	0.71	-0.99	-0.38	-0.99	-0.33	-0.89	-0.21		
WSeSp	-1.00	0.80	0.85	0.94	-0.93	0.50	-0.67	0.51	0.99	0.78	0.97	-0.42	
W1000	-0.79	0.28	0.36	0.56	-0.95	0.92	-0.08	0.92	0.73	1.00	0.64	-0.88	0.79

Cluster analyses: Genetic diversity among wheat cultivars based on the 14 morphological traits measured, was calculated after Agglomerative Hierarchical Clustering (AHC, Wards method). The tree (Figure 2) based on the variance decomposition for the optimal classification was divided in three clusters. The two genotypes with major similarity level were cultivar 'Progresi' and 'LVS'. This low distance among the wheat genotypes was basically for fully similarity existence in trait as SW, RW, SpL, NSpklSp. Cultivar 'Dajti' is different from two other genotypes for higher values in yield contributing traits as NSpklSp, NSeSpkl, NSeSp, WSeSp and W1000S significant at the probability $F < P_{0.05}$.

Genetic diversity based on RAPD markers: Eleven RAPD markers were used to assess genetic diversity among three bread wheat cultivar. Out of these markers two were monomorphic (OPA01 and OPJ04), while nine RAPD markers were polymorphic and gave reproducible results. The nine polymorphic RAPD markers amplified in total 31 fragments with the size range 300-1500 bp from which 54.8% were polymorphic. The dendrogram based on the Jaccard's similarity coefficient matrix showed two clusters (Figure 3).

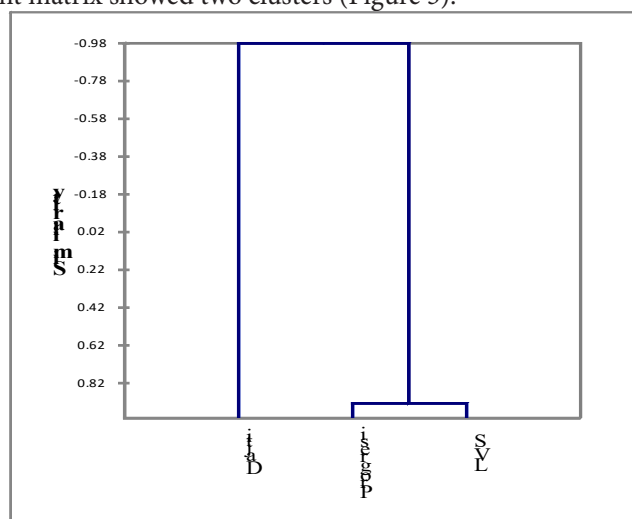


Figure 2. Dendrogram (Euclidean distance) of three bread wheat cultivars based on 14 morphological traits

Genetic diversity of some important wheat (*Triticum aestivum* L.) genotypes in Albania based on morphological traits and RAPD markers

The lowest similarity was observed between 'Progresi' and 'LVS'. The observed differences in obtained clusters of wheat cultivars based on RAPD and on morphological data in this study might be due to the genomic regions examined by RAPD markers, which might be different from the regions where genes controlling morphological traits, are located. The obtained results provide better understanding of genetic diversity of these important wheat bread cultivars.

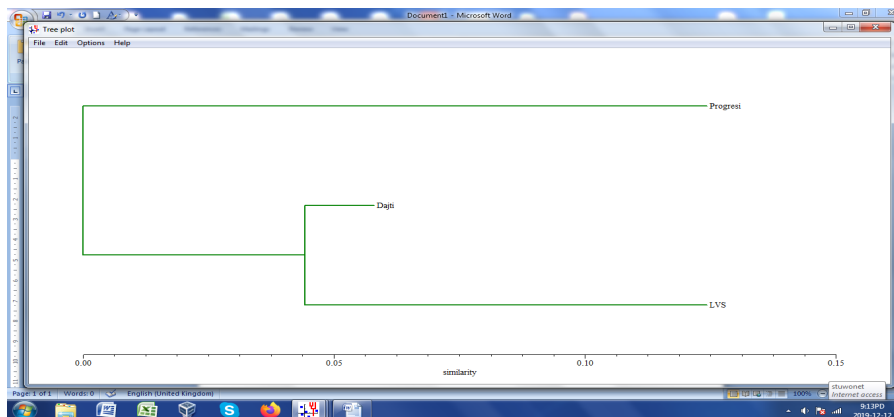


Figure 3. Dendrogram of genetic relationships among three bread wheat cultivars based on RAPD markers

Conclusions

In this study we analysed the variability of three bread wheat cultivars, on the basis of the morphological traits and RAPD markers. The estimation of genetic variation of morphological traits showed high variability, which provides a good basis for future studies of quantitative traits. The highest variability among genotypes was observed for number of seeds per spike, stem length, and number of seeds per spikelet, and they could be used to select favourable traits for crosses in the genetic improvement programs. No correlation was observed between clustering obtained with molecular markers and morphological characters. Further research based on biochemical or other categories of molecular markers should be performed in the future to grasp the genetic variation in our wheat genotypes.

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Reakcija genotipova pšenice na različite gustoće sjetve

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Sažetak

Gustoća sjetve direktno utječe na broj klasova po m^2 , a posljedično i na druge komponente uroda pšenice. Stoga je važno utvrditi optimalnu gustoću sjetve za određeni genotip koja će rezultirati maksimalnim urodom. Cilj ovog istraživanja je bio utvrditi utjecaj gustoće sjetve na formiranje komponenti uroda i urod različitih genotipova ozime pšenice. U istraživanje je bilo uključeno deset genotipova ozime pšenice: sorte AFZG Karla, Kaja, Mara i Antonija te šest oplemenjivačkih linija uzgajanih u dvije gustoće sjetve, 400 zrna/ m^2 i 650 zrna/ m^2 . Analizom je utvrđeno da gustoća sjetve značajno utječe na broj klasova po m^2 , urod zrna po klasu i žetveni indeks, dok je interakcija genotip \times gustoća bila značajna jedino za urod zrna po klasu. Masa 1000 zrna kao i hektolitarska masa najstabilnije su komponente na koje gustoća sjetve nije značajno utjecala.

Ključne riječi: gustoća sjetve, komponente uroda, urod, ozima pšenica

Uvod

U poljoprivrednoj proizvodnji ostvareni urod usjeva rezultat je produktivnosti polja (cenoze) u kojem je produktivnost pojedine biljke manja od stvarno moguće genetske (Barić i sur., 1994). Za proizvođača je najvažniji ostvareni urod koji je uvijek manji od stvarno mogućeg, a rezultat je interakcije genotipa i ostalih okolišnih uvjeta. Kako bi ostvareni urod bio što bliže stvarno mogućem urodu potrebno je osigurati okolišne uvjete u kojima će genotip moći ostvariti maksimalni urod. Gustoća sjetve predstavlja jedan od važnih čimbenika koji utječu na produktivnost polja (raspoloživost vode, svjetla, hranjiva). Produktivnost usjeva može se do određene mjere povećati povećanjem gustoće sjetve do određene, za genotip specifične gustoće, a nakon tog limita urod se smanjuje (Barić, 1993). Komponente uroda pšenice, koje pridonose formiranju uroda zrna, su broj fertilnih vlati po m^2 , broj klasova po biljci, broj zrna po klasu i masa zrna po klasu. Navedena svojstva mogu se kompenzirati jedno za drugo u svrhu stabilnosti uroda prilikom promjene okolinskih uvjeta (Reynolds i sur., 1996). To je u direktnoj vezi s plastičnošću genotipa. Stoga je vrlo važno utvrditi gustoću sjetve koja će rezultirati maksimalnim urodom pojedinog genotipa. Posljednjih godina troškovi proizvodnje rastu. Proizvođači žele ekonomičniju proizvodnju, tj. da se uz što manja ulaganja na istoj površini ostvare veći i stabilniji urod. Sjetvom genotipova velikog potencijala busanja za istu proizvodnu površinu potrebna je manja količina sjemena za sjetvu, što smanjuje troškove sjetve. S druge strane, povećavaju se poljoprivredne površine na kojima se primjenjuje organska i integrirana proizvodnja, a koje daju prednost genotipovima koji se mogu sijati u gušćem sklopu. Naime, u organskoj i integriranoj proizvodnji, zbog minimalnog korištenja pesticida, utvrđeno je da povećana gustoća sjetve ne utječe samo na urod zrna, već i na suzbijanje korova (Kristensen i sur., 2008). Cilj ovog istraživanja bio je utvrditi utjecaj gustoće sjetve, genotipa i njihove interakcije na formiranje komponenti uroda i urod 10 genotipova ozime pšenice.

Rad je izvod iz završnog rada Anje Gotić, univ. bacc. agr. naslova „Reakcije genotipova pšenice na različite gustoće sjetve”

Materijal i metode

U istraživanje je bilo uključeno deset genotipova ozime pšenice i to četiri sorte (AFZG Antonija, AFZG Mara, AFZG Kaja i AFZG Karla) te šest linija iz oplemenjivačkog programa Zavoda za oplemenjivanje bilja, genetiku i biometriku Agronomskog fakulteta u Zagrebu. Pokus je bio postavljen tijekom vegetacijske godine 2013/2014. na eksperimentalnom polju Sveučilišta u Zagrebu Agronomskog fakulteta, prema split-plot dizajnu u dva ponavljanja i dvije gustoće sjetve (Gu1-400 i Gu2-650 zrna/m²). Pokusna parcelica bila je površine 5 m². U žetvenoj zriobi ručno su požeti uzorci od 1 m² (iz svakog genotipa i svake repeticije), a ostatak parcelice požet je kombajnom. Na uzorku od 1 m² utvrđen je žetveni indeks, broj klasova po m², masa zrna po klasu (g) i masa 1000 zrna (g), dok je na osnovi parcelice utvrđena hektolitarska masa i prinos (t/ha).

Analiza varijance deset genotipova provedena je za urod (t/ha), urod zrna po klasu (UZKL-g), masu 1000 zrna (MTZ-g), broj klasova po m² (BRKLM), hektolitarsku masu (HL-kg/hl) i žetveni indeks (ŽI-%). Za usporedbu genotipskih prosjeka korišten je Bonferronijev test za višestruke usporedbe. Sve statističke analize izvedene su pomoću statističkog programa SAS (SAS Institute, 2013).

Rezultati i rasprava

Analiza varijance komponenti uroda kod dvije gustoće sjetve (Gu1, Gu2) ukazuje na postojanje statistički značajne razlike između genotipova i gustoća za broj klasova po m², urod zrna po klasu, žetveni indeks i urod, dok je značajna razlika među genotipovima utvrđena još i za masu 1000 zrna te hektolitarsku masu (Tablica 1). Interakcija genotip × gustoća bila je značajna jedino za urod zrna po klasu. Ovi rezultati u suglasnosti su s rezultatima Valerio i sur. (2013) koji su utvrdili da urod po klasu kod pojedinog genotipa ovisi o dostupnim okolišnim čimbenicima. Kod veće gustoće sjetve pojačava se kompeticija među biljkama za svjetlost, vodu, hranjiva, a to rezultira smanjenom produkcijom asimilata potrebnih kod nalijevanja zrna.

Tablica 1. Rezultati analize varijance za komponente uroda i urod

Izvor	Komponente uroda						
	n-1	BRKLM	UZKL	MTZ	HL	ŽI	UROD
Genotip (G)	9	5,46**	9,44***	7,28**	3,77**	5,93**	12,00***
Gustoća (Gu)	1	112,57***	95,42***	2,54	0,84	73,03***	0,31
G x Gu	9	1,90	3,88*	0,91	1,39	2,11	0,40

Kod testiranih genotipova utvrđeno je značajno povećanje vrijednosti svojstava u Gu2 u odnosu na G1 za broj klasova po m² i žetveni indeks (Tablica 2). Jedino svojstvo kod kojeg je utvrđena značajno veća vrijednost u Gu1 u odnosu na Gu2 je urod zrna po klasu (g). Kod svih testiranih genotipova broj klasova po m² bio je veći u gušćoj sjetvi (Gu2), što je u suglasnosti s rezultatima drugih autora (Barić i sur., 2008; Lloveras i sur., 2012; Valerio i sur., 2013; Lazzaro i sur., 2017). Povećanje gustoće sjetve je, gotovo uvijek, povezano s povećanjem broja klasova po jedinici površine, ali i istovremenim progresivnim smanjenjem broja fertilnih vlati po biljci (Gate, 1995). Prosječan broj klasova po m² varirao je ovisno o gustoći sjetve i genotipu. U gušćoj sjetvi (Gu2) broj klasova po m² bio je za 30% veći nego kod rjeđe sjetve (Gu1). Genotipovi kod kojih je utvrđen značajno veći broj klasova u gušćoj sjetvi (Gu2) su sorta Mara (44%) i linija L4 (53%). Sorte kod kojih je utvrđen najveći broj klasova po m² u obje gustoće sjetve su Kaja (Gu1=633, Gu2=787) i Karla (Gu1=570, Gu2=721), stoga za njih možemo reći da su to genotipovi visokog potencijala busanja.

Urod zrna po klasu kod svih testiranih genotipova bio je značajnije veći u rjeđoj sjetvi (Gu1). Ozturk i sur. (2006) također su utvrdili smanjenje uroda zrna po klasu s povećanjem gustoće sjetve. Urod zrna po klasu varirao je ovisno o gustoći sjetve i genotipu. U rjeđoj sjetvi (Gu1) urod zrna po klasu bio je za 22% veći od gušće sjetve (Gu2). Genotipovi kod kojih je utvrđen značajno veći urod zrna po klasu u rjeđoj sjetvi (Gu1) su sorta Mara (34%) te linije L3 (48%), L4 (30%) i L6 (22%). Najveći urod zrna po klasu u Gu1 utvrđen je kod genotipova koji su u toj gustoći sjetve imali najmanji broj klasova po m² (Mara (1,97 g), L4 (1,63 g) i L6 (1,83 g)). Ovi rezultati u suglasnosti su s tvrdnjom Valerio i sur., (2013) koji zaključuje da žitarice posjeduju plastičnost koja dopušta da se povećanjem mase zrna i produkcije klasa kompenzira manji broj klasova. Najmanja razlika u urodu zrna po klasu utvrđena je kod sorti

Antonija (Gu1=1,54 g, Gu2=1,45 g) i Karla (Gu1=1,55 g, Gu2=1,44 g).

Tablica 2. Srednje vrijednosti genotipova za navedena svojstva u dvije gustoće sjetve

GENOTIP	BRKLM		UZKL (g)		MTZ (g)		HL (kg hl ⁻¹)		ŽI (%)		UROD (t ha ⁻¹)	
	Gu1	Gu2	Gu1	Gu2	Gu1	Gu2	Gu1	Gu2	Gu1	Gu2	Gu1	Gu2
Karla	570	721	1,55	1,44	48,5	47,5	82,55	83,65	55,4	48,9	8,25	8,72
Kaja	633	787	1,36	1,21	45,5	46,5	80,65	81,65	55,4	46,7	8,23	8,25
Mara	408	730**	1,97	1,31**	52,0	52,5	80,95	81,90	53,6	45,9	7,19	7,78
Antonija	483	664	1,54	1,45	38,5	39,5	79,95	79,35	52,5	48,6	7,20	7,63
L1	482	616	1,56	1,31	45,0	42,5	79,20	79,95	52,5	47,3	5,83	6,23
L2	493	582	1,61	1,32	48,5	42,5	77,45	77,05	53,3	48,2	6,46	6,98
L3	459	685	1,47	0,76**	54,5	48,0	78,20	78,35	51,8	35,1**	4,81	5,43
L4	395	629**	1,63	1,15**	44,5	44,5	74,95	80,85	48,3	43,4	5,18	5,60
L5	466	715	1,35	1,08	44,0	41,5	84,15	80,45	54,8	50,3	5,87	5,93
L6	431	579	1,83	1,43*	42,5	43,5	80,40	81,05	56,9	50,7	6,47	6,52
Gu2-Gu1	198,8***		-0,34***		-1,50		0,58		6,92***		0,12	

Gustoća sjetve nije imala utjecaj na masu 1000 zrna i hektolitarsku masu, međutim utvrđena je značajna razlika među genotipovima. Ovi rezultati u suglasnosti su s rezultatima Hobbs i Sayre (2001), koji su utvrdili da je masa 1000 zrna u najvećoj mjeri karakteristika genotipa. U istoj gustoći sjetve za masu 1000 zrna utvrdili su veću varijabilnost između genotipova, nego sjetvom istog genotipa u dvije različite gustoće.

Žetveni indeks kod svih testiranih genotipova bio je značajnije veći u rjeđoj sjetvi (Gu1). Smanjenje žetvenog indeksa utvrdili su Farina i sur. (2014) i zaključili da veća produkcija biomase smanjuje nalijevanje zrna. Rezultat smanjenja u nalijevanju zrna je isti ili smanjen urod kod veće gustoće. Žetveni indeks varirao je ovisno o gustoći sjetve i genotipu. U rjeđoj sjetvi (Gu1) žetveni indeks bio je za 13% veći nego u gušćoj sjetvi (Gu2). Genotip kod kojeg je utvrđen značajno veći žetveni indeks u rjeđoj sjetvi je linija L3 (32%). Visok žetveni indeks u obje gustoće sjetve utvrđen je kod sorte Karle (Gu1=55,4%, Gu2=48,9%) i linije L6 (Gu1=56,9%, Gu2=50,7%).

Urod zrna značajno je varirao između genotipova, međutim gustoća sjetve na urod nije značajno utjecala. Prema Hecht i sur. (2016) ovakvi rezultati posljedica su smanjenja očekivanog žetvenog indeksa kod povećanja gustoće sjetve. Kod svih genotipova utvrđen je viši urod kod veće gustoće sjetve (Gu2). Genotipovi kod kojih je utvrđen najveći urod u obje gustoće sjetve su sorte Karla (Gu1=8,25 t/ha, Gu2=8,72 t/ha) i Kaja (Gu1=8,23 t/ha, Gu2=8,25 t/ha).

Zaključak

Povećanjem gustoće sjetve značajno povećanje uroda nije utvrđeno niti kod jednog genotipa. Prosječni urod (prosječno svih genotipova) u gušćoj sjetvi povećan je za svega 2%. Gustoća sjetve utjecala je na broj klasova po jedinici površine, urod zrna po klasu i žetveni indeks. U gušćoj sjetvi signifikantno se povećao broj klasova/m² za 30%, a smanjila se produkcija klasa za 22%, dok se masa 1000 zrna nije značajno smanjila (3,3%). Vrlo mala razlika u urodu između dviju gustoća sjetve rezultat je dobre kompenzacijske sposobnosti testiranih genotipova. Manji broj klasova po jedinici površine kod rjeđe sjetve (G1) genotipovi su kompenzirali povećanjem uroda po klasu. Sorte kod kojih je utvrđen najveći broj klasova po m² u obje gustoće sjetve, a i najveći urod su Kaja i Karla, stoga za njih možemo reći da su to visokorodni genotipovi s velikim potencijalom busanja.

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Response of wheat genotypes to different sowing densities

Abstract

The sowing density directly affects the number of spikes per m², and consequently other yield components in wheat. Therefore, it is important to determine the appropriate sowing density for a particular genotype that will result in maximum yield. The aim of this study was to determine the influence of sowing density on yield components and yield of different winter wheat genotypes. In this study ten winter wheat genotypes: the varieties AFZG Karla, Kaja, Mara and Antoni and six breeding lines were grown at two sowing densities, 400 grains/m² and 650 grains/m². The results of analysis found that sowing density significantly influenced the number of spikes per m², grain yield per spike and harvest index, while the genotype × density interaction was significant only for grain yield per spike. Thousand grain weight and test weight, are the most stable yield components that were not significantly affected by sowing density.

Keywords: sowing density, yield components, yield, winter wheat.

Otpornost na priježetveno proklijavanje u potomstvima dvaju križanja ozime pšenice

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Sažetak

Dormantnost zrna je glavna komponenta otpornosti na priježetveno proklijavanje kod pšenice. Cilj ovoga rada je bio kod F_7 rekombinantnih inbred linija (RIL) dvaju biparentalnih križanja pšenice procijeniti otpornost na priježetveno proklijavanje, kao i utvrditi učinak selekcije na otpornost na priježetveno proklijavanje provedene u F_3 generaciji, na otpornost, visinu biljke i duljinu vegetacije proizvedenih RIL potomstava. Za procjenu otpornosti na priježetveno proklijavanje korišten je test klijavosti s ovršenim zrnima. Rezultati su pokazali da je u RIL potomstvima relativno visoki udio linija s vrijednošću indeksa klijanja signifikantno manjom od dormantnijeg roditelja. Selekcija na povećanu dormantnost se pokazala relativno uspješnijom kod brkulja nego kod golica. Selekcija na povećanu dormantnost zrna dovela je do signifikantnog smanjenja visine biljke i kasnijeg klasanja kod RIL potomstava.

Ključne riječi: pšenica, dormantnost zrna, selekcija, visina, datum klasanja

Uvod

U oplemenjivanju pšenice osim već dobro poznatih ciljeva poput visokog potencijala rodosti, visoke kvalitete zrna, cilj je imati sorte koje su otporne na priježetveno proklijavanje. Otpornost na priježetveno proklijavanje kod pšenice nasljeđuje se kao kvantitativno svojstvo i pod izrazitim je utjecajem okolinskih čimbenika i interakcije genotip \times okolina (Mares, 1993; Biddulph i sur., 2005.). Glavne komponente otpornosti na priježetveno proklijavanje kod pšenice su: razina dormantnosti zrna u žetvenoj zriobi, brzina gubitka dormantnosti tijekom posliježetvenog dozrijevanja, morfologija klasa te sadržaj vodotopivih inhibitora klijanja u vegetativnim dijelovima cvijeta (Derera, 1990).

Uobičajno je otpornost na proklijavanje testirati kod već homozigotnih linija u kasnijim generacijama nakon križanja. Pri tome je od velike važnosti i poznavanje korelacije među agronomskim svojstvima, posebice ako se selekcija provodi simultano na više svojstava (Hucl, 1995; Dvojković, 2010).

Cilj rada je bio kod F_7 rekombinantnih inbred linija (RIL) dvaju biparentalnih križanja pšenice procijeniti otpornost na priježetveno proklijavanje, kao i utvrditi učinak selekcije na otpornost na priježetveno proklijavanje provedene u F_3 generaciji, na otpornost, visinu biljke i duljinu vegetacije proizvedenih RIL potomstava.

Materijal i metode

U istraživanje su bile uključene F_7 rekombinantne inbred linije (RIL) kao i F_7 rekombinantne inbred linije u čijem je razvoju provedena selekcija na povećanu dormantnost zrna u F_3 generaciji (RILSel), iz križanja linije ozime pšenice brkulje (Hu1) s dvjema linijama ozime pšenice golice (Bc1 i Bc2). Majčinska linija Hu1 ima nisku razinu otpornosti na priježetveno proklijavanje, dok očinske linije imaju nisku (Bc1) odnosno umjerenu do nisku (Bc2) razinu otpornosti na priježetveno proklijavanje u žetvenoj zriobi. Potomstva RIL i RILSel iz obje kombinacije križanja, zajedno s roditeljima Hu1, Bc1 i Bc2 uzgajani su na lokaciji Botinec u 2016/2017. vegetacijskoj sezoni u poljskom

pokusu postavljenom prema slučajnom bloknom rasporedu u dvije repeticije. Ukupno je sijano 225 RIL potomstava i 109 RILSel potomstava iz križanja Hu1×Bc1 te 176 RIL potomstava i 49 RILSel potomstava iz križanja Hu1×Bc2. U oba tipa potomstava bio je podjednak broj potomstava golica (glc) odnosno brkulja (brk).

U pokusu su korišteni agrotehnički postupci uobičajeni u proizvodnji pšenice. U vegetaciji je za svaku parcelicu ocjenjen datum klasanja (50% biljaka u parceli izbacilo bazu klasa iznad zastavice) i izmjerena prosječna visina biljke (od tla do baze klasa). Broj dana do klasanja izražen je kao broj dana od 1. siječnja do prosječnog datuma klasanja. Otpornost na priježetveno prokljivanje procijenjena je stupnjem dormantnosti zrna. U tu svrhu su u žetvenoj zriobi provedeni testovi klijavosti s ovršenim zrnima. Testovi su provedeni u komorama rasta na 20°C u trajanju od 6 dana. Na osnovi broja prokljalih zrna nakon 3 odnosno 6 dana izračunat je indeks klijanja (GI), prema Ikić i sur. (2012). GI je obrnuto povezan sa stupnjem dormantnosti zrna, što znači da manji GI ukazuje na veću dormantnost zrna, a time i na veću otpornost genotipa na priježetveno prokljivanje. Analiza varijance koja je uključivala sva potomstva i roditelje provedena je posebno za svako križanje. Razlike između srednjih vrijednosti genotipova (roditelji i potomstva) testirane su pomoću Fisherovog LSD testa. Razlike prosjeka svojstava između potomstava glc i brk kao i razlike prosjeka svojstava između potomstava RIL i RILSel za pojedinačna križanja testirane su pomoću dvosmjernog odnosno jednosmjernog t-testa. Između proučavanih svojstava unutar svakog križanja izračunati su Pearsonovi korelacijski koeficijenti. Statističke analize provedene su u programskom paketu SAS/STAT (SAS Institute Inc., 2009).

Rezultati i rasprava

Analiza varijance pokazala je postojanje signifikantnih razlika između genotipova (roditelji i potomstva) za sva proučavana svojstva. U tablici 1. prikazane su prosječne vrijednosti i raspon za indeks klijanja (GI) roditelja i potomstava golica (glc) i brkulja (brk) iz križanja Hu1×Bc1 te Hu1×Bc2 prije (RIL) i nakon selekcije (RILSel).

Tablica 1. Prosječne vrijednosti i raspon (u zagradi) za indeks klijanja roditelja i potomstava iz križanja Hu1×Bc1 te Hu1×Bc2 prije selekcije (RIL) i nakon selekcije (RILSel)

	glc+brk	glc	brk	Sig. ^b
	Hu1×Bc1			
Roditelji		Bc1 (33,1)	Hu1 (32,9)	
RIL	29,7 (12,3-33,3)	27,8 (12,3-33,3)	31,5 (12,5-33,3)	**
RILSel	29,3 (11,3-33,3)	29,3 (11,3-33,3)	29,1 (12,5-33,3)	ns
Sig ^a	ns	*	**	
	Hu1×Bc2			
Roditelji		Bc2 (31,3)	Hu1 (32,9)	
RIL	31,2 (18,4-33,3)	29,8 (18,4-33,3)	32,3 (20,2-33,3)	**
RILSel	25,1 (8,1-33,3)	23,8 (8,1-33,3)	26,7 (15,4-33,3)	ns
Sig ^a	**	**	**	

glc-golice; brk-brkulje; ^aSig (RIL vs. RILSel); ^bSig. (glc vs. brk);

*, ** i ns – t test signifikantan kod $P < 0,05$ i $P < 0,01$ odnosno t test nesignifikantan

Prosječna vrijednost GI iznosila je 32,9, 33,1 i 31,3 za roditeljske linije Hu1, Bc1 odnosno Bc2. Kod oba križanja prije selekcije prosječna vrijednost za GI bila je signifikantno niža kod potomstava glc nego brk. Selekcija na povećanu dormantnost zrna je bila uspješna kod oba križanja rezultirajući u signifikantno nižim prosječnim vrijednostima za GI osim za potomstva glc kod križanja Hu1×Bc1, gdje su signifikantno veće vrijednosti za GI utvrđene nakon selekcije. Nakon selekcije niti kod jednog križanja nisu utvrđene signifikantne razlike između glc i brk. U skladu s našim rezultatima (otpornost potomstava glc i brk prije selekcije), Thomason i sur. (2009) su u testovima s intaktnim klasovima utvrdili, da linije pšenice bez osja te linije s kraćim osjem upijaju manju količinu vode, čineći ih otpornijim na priježetveno prokljivanje. Nasuprot ovim rezultatima, Cao i sur. (2016.) utvrdili su umjerenu pozitivnu korelaciju

otpornosti na priježetveno proklijavanje s duljinom osja. Smještaj lokusa za kvantitativna svojstva *QDor-4A* i *QAwn-4A* na kromosomu 4A, koji kontroliraju dormantnost zrna odnosno razvoj osja kod pšenice, mogla bi uslijed vezanosti ili plejotropnog učinka na oba svojstva dati objašnjenje opažene povezanosti između navedenih svojstava Cao i sur. (2016.).

Utvrđene srednje vrijednosti za GI proporcionalne su i udjelu linija s vrijednošću GI značajno manjom od dormantnijeg roditelja (tablica 2). Prije selekcije je udio linija sa GI značajno manjim od dormantnijeg roditelja kod oba križanja bio veći kod linija glc, dok je nakon selekcije udio takvih linija bio podjednak za obje skupine linija pokazujući veću uspješnost selekcije između linija brk.

Tablica 2. Udio (%) linija prije selekcije (RIL) i nakon selekcije (RILSel) s vrijednošću GI značajno manjom od dormantnijeg roditelja (Bc1 i Bc2) za kombinacije križanja Hu1×Bc1 te Hu1×Bc2

Genotip	Hu1×Bc1			Hu1×Bc2		
	glc+brk	glc	brk	glc+brk	glc	brk
	%					
RIL	39	45	10	12	26	3
RILSel	27	27	26	51	50	52

glc-golice; brk-brkulje

Prosječne vrijednosti za broj dana do klasanja i visinu biljke u potomstvima križanja Hu1×Bc1 te Hu1×Bc2 prije (RIL) i nakon selekcije (RILSel) prikazane su u tablici 3. Kod niti jednog križanja nisu utvrđene značajne razlike između golica i brkulja za prosječnu vrijednost broja dana do klasanja i visinu biljke prije selekcije (RIL). Nakon selekcije (RILSel) kod oba križanja i kod golica i kod brkulja došlo je do povećanja prosječne vrijednosti za broj dana do klasanja i smanjenja visine biljke. Za oba svojstva su nakon selekcije utvrđene značajne razlike između golica i brkulja.

U tablici 4. prikazane su korelacije između indeksa klijanja, broja dana do klasanja i visine biljke u potomstvima križanja Hu1×Bc1 te Hu1×Bc2 prije (RIL) i nakon (RILSel) selekcije. Prije selekcije (RIL) kod križanja Hu1×Bc1 korelacije između navedenih svojstava bile su slabe i nesigurne, osim kod brkulja, kod kojih je utvrđena slaba pozitivna korelacija između GI i broja dana do klasanja, te slaba negativna korelacija između broja dana do klasanja i visine biljke. Nakon selekcije (RILSel) kod brkulja utvrđena je slaba pozitivna korelacija između GI i broja dana do klasanja. Za sve linije (glc+brk), golice (glc) i brkulje (brk) utvrđena je slaba do umjerena negativna korelacija između broja dana do klasanja i visine biljke koja se kretala u rasponu od -0,32** do -0,69**.

Tablica 3. Prosječne vrijednosti za broj dana do klasanja i visinu biljke u RIL potomstvima križanja Hu1×Bc1 te Hu1×Bc2 prije (RIL) i nakon selekcije (RILSel)

Genotip	Hu1×Bc1				Hu1×Bc2			
	glc+brk	glc	brk	Sig. ^b	glc+brk	glc	brk	Sig. ^b
	Broj dana do klasanja							
RIL	128	128	128	ns	129	129	129	ns
RILSel2	130	129	131	**	131	130	132	**
Sig. ^a	**	**	**		**	**	**	
	Visina biljke (cm)							
RIL	87	87	86	ns	85	85	86	ns
RILSel2	84	85	82	**	80	77	83	**
Sig. ^a	**	**	**		**	**	**	

glc-golice; brk-brkulje; ^ausporedba RILvs. RILSel; ^busporedba glc vs. brk;

**, ** i ns – t test signifikantan kod P<0,05 i P<0,01 odnosno t test nesigurn*

Kod križanja Hu1×Bc 2 prije selekcije (RIL) utvrđena je slaba pozitivna korelacija između GI i visine biljke kod svih linija (glc+brk) i golica (glc), dok je kod golica (glc) također utvrđena slaba negativna korelacija između GI i broja dana do klasanja ($r=-0,39^{**}$).

Tablica 4. Korelacije između indeksa klijanja (GI), broja dana do klasanja (BDK) i visine biljke (VIS) u potomstvima križanja Hu1×Bc1 te Hu1×Bc2 prije (RIL) i nakon selekcije (RILSel) za sve linije (glc+brk), golice (glc) i brkulje (brk)

	glc+brk		glc		brk				
	BDK	VIS	BDK	VIS	BDK	VIS			
Hu1×Bc1 (RIL)									
GI	0,08	0,05	-0,07	0,06	0,23	**	0,10		
BDK		-0,12		0,05			-0,27	**	
Hu1×Bc1 (RILSel)									
GI	0,00	0,00	-0,17	-0,06	0,20	*	0,01		
BDK		-0,58	**	-0,32	**		-0,69	**	
Hu1×Bc2 (RIL)									
GI	-0,19*	0,26	**	-0,11	0,33	**	-0,39	**	0,03
BDK		-0,24	**		-0,28	**		-0,21	**
Hu1×Bc2 (RILSel)									
GI	0,03	0,23		-0,08	0,40	**	-0,19		-0,23
BDK		0,35	*		0,24				0,10

*, ** i ns – t test signifikantan kod $P<0,05$ i $P<0,01$ odnosno t test nesignifikantan

Za sve linije (glc+brk), golice (glc) i brkulje (brk) utvrđena je slaba negativna korelacija između broja dana do klasanja i visine biljke u rasponu od $-0,21^{**}$ do $-0,28^{**}$. Nakon selekcije (RILSel) samo kod golica (glc) utvrđena je umjerena pozitivna korelacija između GI i visine biljke ($r=0,40^{**}$). Slaba pozitivna korelacija između broja dana do klasanja i visine biljke ($r=0,35^*$) utvrđena je kod svih linija (glc+brk). Slaba pozitivna korelacija između broja dana do klasanja i visine ($r=0,35^*$), utvrđena je kod svih linija (glc+brk). Utvrđene korelacije u skladu su s izvještajima iz literature koji ukazuju na ovisnost korelacija između otpornosti na priježetveno proklijavanje i visine odnosno broja dana do klasanja o proučavanom genetskom materijalu. Tako je u istraživanju Wang i sur. (2018) visina bila u negativnoj korelaciji s otpornošću na priježetveno proklijavanje, dok s druge strane Kulwal i sur. (2012) nisu utvrdili signifikantne korelacije između visine biljke i dormantnosti. Slično je i korelacija između otpornosti na priježetveno proklijavanje i broja dana do klasanja bila pozitivna u istraživanju koje je proveo Hucl (1995.), odnosno negativna u radu Cao i sur. (2016). S druge strane Kulwal i sur. (2012) te Graybosch i sur. (2013) nisu utvrdili signifikantnu korelaciju između broja dana do klasanja i otpornosti na priježetveno proklijavanje.

Zaključak

Relativno visoki udio RIL potomstava s vrijednošću GI signifikantno manjom od dormantnijeg roditelja ukazuje da i u potomstvima relativno neotpornih roditelja možemo očekivati pozitivne transgresije za otpornost na priježetveno proklijavanje. Kod oba križanja prije selekcije prosječna vrijednost za GI bila je signifikantno niža kod potomstava glc nego brk. Nakon selekcije kod oba križanja nisu utvrđene značajne razlike za GI ukazujući na veću uspješnost selekcije u povećanju dormantnosti zrna između potomstava brk nego glc. Procijenjene korelacije između indeksa klijanja i broja dana do klasanja kao i visine biljke bile su uglavnom slabe u RIL potomstvima kako prije tako i nakon selekcije. Ipak selekcija je imala signifikantan učinak na ova svojstva rezultirajući kasnozreljim i nižim potomstvima.

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Resistance to pre-harvest sprouting in progenies of two winter wheat crosses

Abstract

Grain dormancy is a major component of pre-harvest sprouting resistance in wheat. The aim of this study was to evaluate the resistance pre-harvest sprouting in F_7 recombinant inbred lines (RIL) of two biparental wheat crosses, as well as to determine the effect of selection on pre-harvest sprouting resistance carried out in F_3 generation, on the resistance, plant height and days to heading of the produced RIL progeny. To estimate pre-harvest sprouting resistance the germination test with threshed grains was used. The results showed a relatively high proportion of lines with germination index significantly lower than in more dormant parent in the RIL progeny. Selection for increased dormancy has proven to be relatively more successful in awned than in awnless progeny. Selection for increased grain dormancy led to a significant decrease in plant height and increase in number of days to heading.

Keywords: wheat, grain dormancy, selection, plant height, number of days to heading

Chlorophyll fluorescence as a method for the prediction of germination success in common bean (*Phaseolus vulgaris* L.)

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Abstract

To explore the utility of chlorophyll fluorescence (CF) as a tool for the prediction of germination success we tested Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') differing in seed traits (length, width, height, weight) for seed germination. The significant differences in germination time were observed, landrace 'Biser' being the fastest to germinate. The univariate model including CF parameter F_v/F_m was not significant while the multivariate Cox model (seed length+ F_v/F_m) had the highest concordance index. The utility of CF parameter F_v/F_m as a tool for the prediction of germination success is limited especially when other factors have a clear impact.

Keywords: common bean, landraces, germination, chlorophyll fluorescence (CF)

Introduction

Common bean (*Phaseolus vulgaris* L.) is of great agronomic interest worldwide and the most important grain legume for direct human consumption (Angioi et al., 2011). In Croatia, this crop is neglected and in danger of genetic erosion (Carović-Stanko et al., 2017).

Seed from different sources and with different weight may result in similarly high levels of germination under optimal conditions, but the same seed under the more stressful conditions in the field may have very contrasting abilities to establish plants due to differences in their vigour (Lima et al., 2005; Finch-Savage and Bassel, 2015). Seed viability is the ability of the embryo to germinate and is affected by a number of different conditions. Being able to predict seed viability is an important part of the planning process in agriculture (Shaban, 2013). Chlorophyll fluorescence (CF) is a rapid, non-destructive and inexpensive technique that has been used successfully in the evaluation of plant photosynthetic activity and it is seed sorting technique relying on measuring the amplitude of the CF signals of seeds (Gorbe and Calatayud, 2012; Kenanoğlu et al., 2016). It has been used in researches in common bean in leaves infected with bean rust (Peterson and Aylon, 1995), field screening for heat tolerant common bean cultivars (Petkova et al., 2001), as a marker for seed maturity and seed performance of *Brassica oleracea* seeds (Jalink et al., 1998), to assess seedling emergence potential and vigour of commercial tomato and cucumber seed lots (Demir et al., 2013) and to check seed germination performance of stored pepper seeds (Kenanoğlu et al., 2016). There are many different available Chl fluorometers and some of CF parameters that were used through last few decades are F_0 (minimum fluorescence), F_m (maximum fluorescence), F_v/F_m (maximum quantum yield of photosystem II) etc. (Roháček, 2002; Gorbe and Calatayud, 2012).

The aim of this study was to test the seed germination of the three Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') differing in basic seed traits (length, width, height, weight), and to explore the utility of chlorophyll fluorescence as a tool for the prediction of germination success.

Material and methods

The research material included one hundred seeds of each of the three Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') multiplied and collected in a field trial at the University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology during the year 2018. The experiment was conducted in 2019. The seed length (mm), width (mm) and height (mm) of each seed was measured by Caliper and the weight (g) of each seed was measured by analytical balance.

The determination of seed chlorophyll fluorescence (parameters F_0 (minimum fluorescence) and F_v/F_m (maximum quantum yield of photosystem II), and chlorophyll fluorescence signal (Chl signal) was done using CropReporter[®] (PhenoVation, Wageningen, the Netherlands). Seeds were placed in a germination chamber under controlled conditions for germination following the recommendations of the ISTA (1993). Seeds were counted at regular time intervals every day to assess the number of germinated and nongerminated seeds.

The differences among landraces in seed germination were tested by survival analysis using Kaplan-Meier method with log-rank test (Kaplan and Meier, 1958) as implemented in R package 'survival' (Therneau and Grambsch, 2000; Therneau, 2015). The impact of landraces and seed traits (length, width, height, weight) on germination success were tested by Cox proportional-hazards model (Cox, 1972) using 'coxph' function from R package 'survival' (Therneau and Grambsch, 2000; Therneau, 2015). The same procedure was used to test the prediction accuracy of the chlorophyll fluorescence parameter F_v/F_m .

Results and discussion

Results of Kaplan-Meier analysis with log-rank test indicated that there were significant differences ($P < 0.05$) among three Croatian common bean landraces ('Trešnjevac', 'Zelenčec', 'Biser') in germination success. Landrace 'Trešnjevac' with the largest seeds had the maximum mean germination time (2.19 days), whereas 'Zelenčec' had medium seeds and mean germination time 1.82 days, while 'Biser' with the smallest seeds had the shortest mean germination time (1.41 days). Similarly, a Cox regression model for germination success that included landraces as a source of variation was significant ($\chi^2 = 20.92$; $P < 0.05$). In comparison to landrace 'Biser' used as a reference (Hazard Ratio = 1), 'Trešnjevac' reduced the germination success by a factor of HR = 0.43 (or 57%) while 'Zelenčec' by HR = 0.59 (41%) (Table 1).

Table 1. Germination success expressed in terms of regression coefficients (β), Hazard Ratios (HR) with 95% confidence intervals (CI) and P values (Wald test), as estimated using a Cox regression model with time to germination as time scale. The model compares germination of seeds of the landrace 'Trešnjevac' and 'Zelenčec' to seeds of the landrace 'Biser' as reference (HR = 1.00).

Landrace	β	HR	CI (95%)	P_{Wald}
'Trešnjevac'	-0.836	0.43	0.33-0.58	***
'Zelenčec'	-0.530	0.59	0.44-0.78	***

ns – non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$

A series of separate univariate Cox regression models that included four seed traits size (length, width, height, weight) and chlorophyll fluorescence parameters revealed that only parameter F_v/F_m was significant while all the seed traits had a significant impact on germination success. In all cases, the negative β coefficients indicated that all the variables had a negative effect of germination success. For every 1-unit increase (mm) of seed length, width and height, the germination success decreased by the factor of HR = 0.89 (11%), HR = 0.73 (27%) and HR = 0.76 (24%) in relation to overall germination success while for every 1-unit increase (g) of seed weight germination success was reduced by the factor of HR = 0.13 (87%). However, the values of F_v/F_m were from 0.16 to 0.33 and these values failed to predict germination success (Table 2.)

Chlorophyll fluorescence as a method for the prediction of germination success in common bean (Phaseolus vulgaris L.)

Table 2. Germination success as influenced by seed size (length, width, height, weight) or predicted by chlorophyll fluorescence parameter F_v/F_m , as estimated using separate univariate Cox regression models with time to germination as time scale.

Variable	β	HR	CI (95%)	P_{Wald}
Seed length	-0.121	0.89	0.85-0.92	***
Seed width	-0.322	0.73	0.62-0.85	***
Seed height	-0.277	0.76	0.68-0.84	***
Seed weight	-2.028	0.13	0.06-0.28	***
F_v/F_m	-3.433	0.03	0.00-1.12	ns

β - regression coefficient; HR - Hazard Radion; CI (95%) - confidence intervals; P_{Wald} - significance of the Wald test (ns - non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$)

The use of F_v/F_m in prediction of germination success was further explored by multivariate Cox regression models that included F_v/F_m values with the four seed traits. Two out of four models gave significant results for F_v/F_m (Table 3) and had a higher concordance index (defined as a fraction of correct predictions) in comparison to the same models without F_v/F_m (Table 4). The best model included seed length and F_v/F_m and it had a concordance index of 0.750. However, there were no significant differences in concordance indices of the six best models presented in Table 4.

Table 3. Multivariate Cox regression models for germination success in which chlorophyll fluorescence parameter F_v/F_m gave significant P values

Model	Variable	β	HR	CI (95%)	P_{Wald}
1	Seed length	-0.124	0.88	0.85-0.92	***
	F_v/F_m	-3.720	0.02	0.00-0.87	*
2	Seed weight	-2.059	0.13	0.06-0.27	***
	F_v/F_m	-3.631	0.03	0.00-0.97	*

β - regression coefficient; HR - Hazard Radion; CI (95%) - confidence intervals; P_{Wald} - significance of the Wald test (ns - non-significant; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$)

Table 4. Goodness-of-fit of the six best Cox regression models for germination success as compared using concordance index (C-index; standard error: SE) defined as a fraction of correct predictions. P values were obtained by comparing models to the best one (Seed length + F_v/F_m) using Z-test.

No.	Model variables	C-index	SE	P_{Z-test}
1	Seed length + F_v/F_m	0.750	0.025	-
2	Seed height	0.745	0.024	ns
3	Seed weight + F_v/F_m	0.742	0.025	ns
4	Landrace	0.736	0.023	ns
5	Seed length	0.736	0.026	ns
6	Seed weight	0.728	0.026	ns

In concordance with previous studies (Borji et al., 2007; De Ron et al., 2016), the results indicate that landraces with larger seeds take more time to germinate probably because it need more time for water absorption. The utility of F_v/F_m in prediction of overall germination success is generally limited although it could improve the concordance of the models that include some other seed traits.

Conclusion

There are significant differences among Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') in germination time most likely due to differences in basic seed traits (length, width, height, weight) as all the traits have a significant impact *per se* on germination success. It seems that the utility of chlorophyll fluorescence parameter F_v/F_m as a tool for the prediction of germination success is limited especially in cases in which other factors have a clear impact on germination time.

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Fluorescencija klorofila kao metoda za određivanje klijavosti sjemena graha (*Phaseolus vulgaris* L.)

Sažetak

Kako bi se utvrdila korisnost fluorescencije klorofila (CF) kao alata za predviđanje klijavosti sjemena, testirana je klijavost hrvatskih tradicijskih kultivara graha ('Trešnjevac', 'Biser', 'Zelenčec') koji se razlikuju u svojstvima sjemena (dužina, širina, debljina, težina). Uočene su značajne razlike u vremenu klijanja pri čemu je 'Biser' najbrže klijao. Univarijantni model, koji je uključivao parametar fluorescencije klorofila F_v/F_m , nije bio značajan, dok je multivarijantni Cox-ov model (duljina sjemena + F_v/F_m) imao najviši indeks podudaranja. Korisnost parametra fluorescencije klorofila F_v/F_m kao alata za predviđanje klijanja je ograničena, posebno u slučajevima kada i drugi čimbenici imaju utjecaj.

Ključne riječi: grah, tradicijski kultivari, klijanje, fluorescencija klorofila

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Morfološka svojstava kultivara mini lubenice

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Sažetak

Cilj rada bio je utvrditi prikladnost triploidnih kultivara lubenice 'WDL 5571', 'WDL 5574' i 'WDL 5575' za hidroponski uzgoj, određujući porast i broj novonastalih listova primarnog izboja, kao i morfološka svojstva i kakvoću plodova. Testirani kultivari su se razlikovali u porastu i broju novonastalih listova primarnog izboja između 14. i 18. dana nakon presađivanja (DNP) te između 22. i 26. DNP. Kultivar 'WDL 5575' imalo je najveću masu, dužinu i opseg plodova, debljinu mezokarpa te promjer endokarpa. Imao je također i najveću količinu ukupne suhe tvari i najvišu EC vrijednost soka endokarpa.

Ključne riječi: *Citrullus lanatus*, hidroponski uzgoj, triploidni kultivari

Uvod

Poliploidne biljke imaju važnu ulogu u poljoprivrednoj proizvodnji (Lewis, 1980) te se smatraju superiornijima u odnosu na diploidne biljke obzirom na genetsku prilagodljivost te toleranciju na okolišne stresove (Estilai i Shannon, 1993). Lubenica je jednodomna biljka s pojedinačnim jednospolnim (muškim i ženskim) cvjetovima. Po biljci se najčešće razvije 30 do 40 ženskih cvjetova, ali se samo tri do četiri cvijeta oplode i formiraju plod. Po Nikoliću (2017) plod lubenice je boba. Plod kod diploidnih lubenica može težiti od 1 do 15 kg.

Oplemenjivanje lubenice ima cilj poboljšanje jednog ili više svojstava. Tetraploidne lubenice se dobivaju tretiranjem diploidnog sjemena ili presadnica sa kolhicinom (Kirhara, 1951) te se one križaju sa diploidnim biljkama za dobivanje triploida (Jaskani i sur., 2005). Upotreba triploidnih kultivara lubenica se povećava zbog bezsjemenskih plodova visoke kakvoće kao i prinosa koji se može usporediti sa diploidnim kultivarima (Jaskani i sur., 2005). Plodovi triploidnih kultivara imaju veću količinu ukupnih šećera, izvrstan okus te duže zadržavaju svježinu, obično su okruglog oblika, težine 5 do 10 kg (Troidahl i Napier, 2009). Po nutritivnoj vrijednosti plodovi triploidnih kultivara jednaki su diploidnim kultivarima. Unatoč prednosti u kakvoći plodova, zbog sterilnosti polena triploidnih kultivara u uzgoju su potrebni diploidni oprašivači, koji cvjetaju u isto vrijeme kada i triploidni kultivari (Camacho and Fernandez, 1997). To zahtjeva njihovu zajedničku sadnju (Nunez i sur., 2008), odnosno na tri triploida sadi se jedan diploidni oprašivač (Troidahl i Napier, 2009). Odabir diploidnog kultivara za oprašivanje može utjecati na poboljšanje kakvoće plodova i prinosa. Važno je da se plodovi diploidnih i triploidnih kultivara međusobno vizualno razlikuju kako se njihovi plodovi prilikom berbe ne bi pomiješali (Camacho and Fernandez, 2000).

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Oprašivanje triploidnih kultivara provodi se pčelama, gdje je za razvoj ploda potrebno šest pčelinjih posjeta cvijetu. Preporučuje se jedna pčela na 100 cvjetova, što se postiže sa dvije košnice po jednom hektaru (Troidahl i Napier, 2009). Uzgoj triploidnih kultivara provodi se u zaštićenom prostoru, ali i na otvorenom. Uzgoj na otvorenom kod triploidnih kultivara gotovo je isti kao kod diploidnih kultivara. U proizvodnji, u zaštićenom prostoru, kod nekih triploidnih kultivara koristi se armatura koja ima svojih prednosti, ali i nedostataka. Nedostaci korištenja armature umanjuju se smanjenim brojem izboja kojim se postiže bolje korištenje prostora, poboljšanje osvjetljenja biljaka te njihovo prozračivanje, odnosno, bolje zdrastveno stanje (Nunez i sur., 2008).

Proizvodnja plodova triploidnih kultivara s ekonomskog aspekta je skuplja od proizvodnje 'tradicionalnih' diploidnih kultivara te je stoga potrebno postići zadovoljavajući prinos i kakvoću plodova.

Cilj ovog istraživanja bio je utvrditi morfološka svojstva i kakvoću tri triploidna kultivara lubenice.

Materijal i metode

Pokus je proveden u eksperimentalnom stakleniku Instituta za jadranske kulture i melioraciju krša u Splitu. Prozračivanje staklenika osigurano je pomoću krovne i bočne ventilacije. Sjeme lubenice triploidnih kultivara 'WDL 5571', 'WDL 5574' i 'WDL 5575' nabavljeno je od komercijalnog proizvođača (Syngenta Seeds B.V., Nizozemska).

Sjetva sjemena u čepove kamene vune, prethodno saturirane vodom iz vodovoda, obavljena je u drugom tjednu lipnja, nakon čega su stavljene na klijanje u vegetacijsku komoru pri temperaturi 22 °C i relativnoj vlažnosti zraka 95 %. Klijanje biljaka lubenice uslijedilo je 7 dana nakon sjetve (DNS), a pikiranje u kocke kamene vune (65 x 65 x 75 mm; Grodan A/S, Hedenhusene, Denmark) obavljeno je 17 DNS. Kocke su prethodno bile natopljene hranjivom otopinom (EC 1,1 dS/m i pH 5,5 do 5,7), sljedećeg kemijskog sastava (mg l^{-1}): NO_3^- 233; NH_4^+ 23; P 52; K 299; Ca 164; Mg 45; Fe 2,7; Mn 1,2; B 0,5 (Watanabe i sur., 2001). Za korekciju pH vrijednosti hranjive otopine korištena je 0,1 M sulfatna kiselina. Tijekom pokusa električna vodljivost i pH vrijednost hranjive otopine mjerene su prijenosnim pH metrom i konduktometrom (Mettler Toledo, Columbus, OH, USA).

Dvadesetog dana nakon sjetve kocke su prebačene u ploče kamene vune (1000 x 150 x 80 mm; Grodan B.V., Roermond, Nizozemska) prethodno saturirane hranjivom otopinom (EC 2,2 dS/m, pH 5,5 do 5,7). Ploče kamene vune stavljene su na uzgojne stolove visine 60 cm, širine 110 cm i dužine 33 m. Pokus je bio postavljen po shemi slučajnog blokno rasporeda s ukupno 36 biljaka po kultivaru, podijeljenih u tri ponavljanja. Osnovnu parcelu činilo je 6 ploča kamene vune s ukupno 12 biljaka.

Dva dana nakon prebacivanja (DNP) kocaka u ploče kamene vune, folija na pločama je bila prorezana na 2/3 visine od dna ploče, na dva mjesta, kako bi korijenje biljaka moglo prorasti u cijeli volumen ploče. Folija na pločama prerezana je pri dnu 4 DNP kako bi se osiguralo procjeđivanje viška hranjive otopine. Biljke su navodnjavane sustavom navodnjavanja 'kapanjem' u količini od 0,5 do 4,0 l po biljci dnevno, raspoređeno u 4 do 10 obroka ovisno o fazi razvoja biljke i temperaturi zraka u stakleniku. Dnevna količina procijeđene hranjive otopine iznosila je od 25 do 35 % ukupno dodane količine. Uzgoj lubenica bio je vertikalalan, na mrežnoj konstrukciji s tri izboja (jedan primarni i dva sekundarna). Cvatnja je započela 20. DNP te su biljke početkom kolovoza bile ručno oprašivane dva tjedna tijekom jutarnjih sati (07:00 do 09:00). U nasadu je bio prisutan diploidni kultivar 'Fantasy' čije su biljke služile za oprašivanje. Vegetativni porast i broj novonastalih listova primarnog izboja određeni su mjerenjem 14., 18., 22., 26. i 30. DNP, na 12 biljaka svakog triploidnog kultivara.

Berba plodova započela je 56. DNP i trajala je 10 dana u ukupno tri roka berbe.

Na 15 plodova (5 plodova po berbi) svakog triploidnog kultivara izmjerena je masa, dužina i opseg ploda. Kakvoća plodova lubenice određena je mjerenjem sljedećih parametara: masa ploda, longitudinalna dužina ploda, opseg ploda, debljina mezokarpa, promjer endokarpa, količina ukupne suhe tvari, pH i EC soka ploda.

Podatci su statistički analizirani, analizom varijance (ANOVA) te su nakon signifikantnog F-testa srednje vrijednosti uspoređene s odgovarajućim testovima na razini signifikantnosti $P \leq 0,05$ pomoću računalnog programa StatView (SAS Institute Inc. Version 5.0.1).

Rezultati i rasprava

Porast primarnog izboja u razdoblju između 14. i 18. DNP te između 22. i 26. DNP bio je isti kod kultivara 'WDL 5571' i 'WDL 5574' te znatno veći nego kod kultivara 'WDL 5575' (tablica 1). Između kultivara nije bilo razlike u porastu primarnog izboja u razdoblju između 18. i 22. DNS i između 26. i 30. DNS.

Kultivar 'WDL 5575' imao je znatno manje listova u razdoblju između 14. i 18. DNS te između 22. i 26. DNS u odnosu na kultivara 'WDL 5571' i 'WDL 5574' (tablica 1). U razdoblju između 18. i 22. DNS te između 26. i 30. DNS kultivari se međusobno nisu razlikovali po broju listova.

Tablica 1. Dužina porasta i broj novonastalih listova primarnog izboja kod tri triploidna kultivara lubenice.

Kultivar oznake	Porast primarnog izboja (cm)				Broj novonastalih listova na primarnom izboju			
	14 - 18 DNP	18 - 22 DNP	22 - 26 DNP	26 - 30 DNP	14 - 18 DNP	18 - 22 DNP	22 - 26 DNP	26 - 30 DNP
WDL 5571	29,5 a	36,1	54,4 a	37,8	5,0 a	4,8	6,1 a	4,2
WDL 5574	29,7 a	35,8	54,1 a	32,0	4,9 a	4,3	6,0 ab	3,5
WDL 5575	23,5 b	32,1	44,3 b	31,3	4,0 b	4,5	5,3 b	3,8
<i>Signifikantnost</i>	*	NS	**	NS	**	NS	*	NS

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$.

Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05, 0,01$ i $0,001$.

Značajno manje vrijednosti mase, dužine kao i opsega plodova imao je kultivar 'WDL 5571' u odnosu na kultivare 'WDL 5574' i 'WDL 5575' čije se vrijednosti međusobno nisu razlikovale (tablica 2).

Debljina mezokarpa (kore) plodova bila je najveća kod kultivara 'WDL 5574' u odnosu na kultivare 'WDL 5571' i 'WDL 5575' koje se međusobno nisu razlikovale. Promjer endokarpa (placente) bio je najmanji kod kultivara 'WDL 5571' u odnosu na kultivare 'WDL 5574' i 'WDL 5575' koji se međusobno nisu razlikovali. Istraživani triploidni kultivari imali su izmjerenu relativno malu masu prema kojoj spadaju u kategoriju 'icebox' (<5,44 kg) kultivara (Barnes i sur., 1994).

Količina ukupne suhe tvari bila je značajno veća kod kultivara 'WDL 5575' u odnosu na kultivare 'WDL 5571' i 'WDL 5574' koji se međusobno nisu statistički razlikovali (tablica 3). Dobivene vrijednosti suhe tvari podudarale se sa dobivenim vrijednostima suhe tvari kod mini kultivara Ingrid cijepljenog na tikvu (PS 1313; *Cucurbita moschata* Duchesne \times *Cucurbita maxima* Duchesne) u istraživanju Roupael i sur. (2008).

Između kultivara nije bilo značajne razlike u pH vrijednosti soka endokarpa, dok je statistički značajno veća EC vrijednost soka endokarpa u odnosu na druge kultivare utvrđena kod kultivara 'WDL 5575'. Određivanje pH vrijednosti soka endokarpa kao i ukupne količine suhe tvari spada u jednu od metoda koje ukazuju na zrelost plodova kod mini lubenica. Vinson III i sur. (2010) u istraživanju na mini lubenicama kultivara 'Valdoria' i 'Vanessa' utvrdili su pH vrijednost soka endokarpa zrelih plodova između 6 i 6,5. Nižu dobivenu vrijednost pH soka endokarpa utvrđenu u ovom istraživanju možemo pripisati razlici istraživanih kultivara i načinu uzgoja. S obzirom da triploidni kultivari lubenice imaju čvršće meso (endokarp), veću količinu suhe tvari, veću koncentraciju likopena (Perkins-Veazie i sur., 2006) te duže zadržavanje svježine kao i manju masu plodova u odnosu na diploidne kultivare, vrlo su interesantne potrošačima i za pretpostaviti je da će se njihov uzgoj širiti.

Tablica 2. Morfološka svojstva plodova triploidnih kultivara lubenice.

Kultivar oznake	Masa ploda (g)	Longitudinalna dužina ploda (cm)	Opseg ploda (cm)	Debljina mezokarpa (cm)	Promjer endokarpa (cm)
WDL 5571	752,3 b	12,9 b	34,2 b	4,8 b	9,3 b
WDL 5574	1168,7 a	15,4 a	39,4 a	6,7 a	11,0 a
WDL 5575	996,9 a	14,4 a	37,8 a	4,8 b	11,6 a
<i>Signifikantnost</i>	***	***	***	***	***

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$. Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05, 0,01$ i $0,001$.

Tablica 3. Kakvoća soka plodova triploidnih kultivara lubenice.

Kultivar oznake	Količina topive suhe tvari (°brix)	pH vrijednost soka endokarpa	EC vrijednost soka endokarpa
WDL 5571	8,5 b	5,3	3,5 b
WDL 5574	8,6 b	5,4	3,9 b
WDL 5575	9,2 a	5,3	4,0 a
<i>Signifikantnost</i>	***	NS	***

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$.

Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05, 0,01$ i $0,001$.

Zaključak

Temeljem rezultata jednogodišnjeg istraživanja triploidnih kultivara lubenice 'WDL 5571', 'WDL 5574' i 'WDL 5575' može se zaključiti:

- najmanji vegetativni porast primarnog izboja i broj novonastalih listova među testiranim kultivarima ostvario je kultivar 'WDL 5575', što je isto je bilo utvrđeno i u drugim razdobljima, samo što nije utvrđena statistički značajna razlika
- najveću masu, dužinu i opseg plodova kao i debljinu mezokarpa imao je kultivar 'WDL 5574', a utvrđene vrijednosti bile su podjednake sa kultivarom 'WDL 5575' koji je imao najveći promjer endokarpa,
- najveću količinu ukupne suhe tvari te najvišu EC vrijednost soka endokarpa imao je kultivar 'WDL 5575'.

Iz navedenog može se zaključiti kako se sva tri testirana kultivara mogu preporučiti za hidroponski uzgoj od kojih se naročito izdvaja, svojim kvalitativnim svojstvima, kultivar 'WDL 5575'.

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Morphological traits of mini watermelon cultivars

Abstract

The aim of the study was to determine the suitability of triploid watermelon cultivars 'WDL 5571', 'WDL 5574' and 'WDL 5575' for hydroponic cultivation, determining growth and number of primary shoot leaves, as well as morphological characteristics and fruit quality. The cultivars tested differed in the primary shoot growth as well as in the number of newly emerged leaves on it only between the 14th and 18th days after transplantation (DNP) and between the 22nd and 26th DNPs. The cultivar 'WDL 5575' had the highest mass, length, and range of fruits, mesocarp width, and endocarp diameter. It also had the highest amount of total dry matter and the highest EC value of endocarp juice.

Keywords: *Citrullus lanatus*, hydroponic cultivation, triploid cultivars

Morfometrijska svojstva ploda i prinos krastavca tretiranog biljnim biostimulatorima

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Sažetak

Zbog sve većeg značaja koji stimulatori biljnoga rasta dobivaju u proizvodnji povrća, provedeno je istraživanje s ciljem testiranja njihovog utjecaja na morfometrijska svojstva ploda i sastavnice prinosa krastavca u hidroponskom uzgoju. U dvofaktorijalnom pokusu s biostimulatorima ('AminoVital', 'Phylgreen') i kultivarima krastavca ('Q Smart', 'Touareg', 'Paraiso') nije zabilježen utjecaj pojedinačnih faktora, no značajan je, ali neujednačen utjecaj njihove interakcije. Najveći prinos tržnih plodova ostvaren je u kombinaciji 'Touareg'×'AminoVital' i 'Q Smart' bez tretiranja biostimulatorom (13,61 i 13,55 kg/m²).

Ključne riječi: *Cucumis sativus*, hidroponski uzgoj, kultivari

Uvod

Krastavac se uz papriku i rajčicu najviše proizvodi u zaštićenim prostorima, a povećanje zahtjeva tržišta za krastavcem izvan sezone utjecalo je na širenje hidroponske proizvodnje u zaštićenim prostorima. Hidroponi su tehnike uzgoja bilja bez tla, odnosno uzgoj bilja na inertnim supstratima ili bez njih, s kontinuiranom opskrbom hranivom otopinom. Glavni je cilj hidroponske tehnologije osigurati svakoj biljnoj vrsti u određenoj fazi rasta potrebnu količinu hraniva i optimalne mikroklimatske uvjete, radi postizanja maksimalnih prinosa i kvalitete (Benko i Fabek, 2009; Borošić i sur., 2011).

Biljni biostimulatori su fiziološki aktivne tvari i mikroorganizmi izvedeni iz prirodnih ili bioloških izvora koje, primijenjene u malim količinama, potiču rast i razvoj biljaka tijekom cijelog životnog ciklusa. Kada se primjenjuju na biljke ili u rizosferu, utječu na poboljšanje asimilacije, uporabu i translokaciju hranivih tvari, povećavaju prinos i kvalitetu usjeva, povećavaju tolerantnost na abiotičke stresove i učinkovitost korištenja vode. Biostimulatori se razlikuju prema djelatnim tvarima koje sadrže, a to mogu biti humusne tvari, mikrobiološki inokulanti, proteinski hidrolizati, aminokiseline te ekstrakt algi (Bulgari i sur., 2015; Calvo i sur., 2014; Du Jardin, 2015; Gluhić, 2017).

Cilj istraživanja provedenog tijekom proljetno-ljetnog roka hidroponskog uzgoja salatnog krastavca u negrijanom zaštićenom prostoru bio je utvrditi utjecaj biostimulatora na morfometrijska svojstva ploda (masa, dužina i promjer) i sastavnice prinosa (broj i masa tržnih plodova, tržni prinos, udio netržnih plodova).

Rad je izvod iz završnog rada Mislava Đurića, mag. ing. agr. naslova 'Utjecaj biostimulatora na sastavnice prinosa krastavca u hidroponskom uzgoju'.

Materijali i metode

Istraživanje je provedeno tijekom 2018. godine u negrijanom zaštićenom prostoru na pokušalištu Maksimir Zavoda za povrćarstvo Agronomskog fakulteta Sveučilišta u Zagrebu. Dvofaktorijalni pokus s biljnim biostimulatorima 'AminoVital' (Biofa AG, Njemačka) i 'Phylgreen' (Tradecorp Ltd., Španjolska) te kultivarima (cv.) salatnog krastavca 'Q Smart' (Nunhems, Nizozemska), 'Touareg' (Rijk Zwaan, Nizozemska) i 'Paraiso' (Enza Zaden, Nizozemska) postavljen je po metodi slučajnog blokno rasporeda u tri ponavljanja.

Sjetva je obavljena 3. travnja u čepove kamene vune smještene u polistirenske kontejnere s 240 sjetvenih mjesta. Prilikom sjetve čepovi su navlaženi i prekriveni vermikulitom kako bi se očuvala vlaga i toplina tijekom klijanja. Posijano je po 40 sjemenki svakog kutivara. U fazi razvijenih kotiledona i početka razvoja prvog pravog lista, 12. travnja obavljeno je pikiranje u kocke kamene vune (10 cm × 10 cm × 7,5 cm). Kocke su prethodno natopljene 1 %-tnom hranivom otopinom pH-vrijednosti 5,8, pripremljenom otapanjem kompleksnog vodotopivog gnojiva Poly-Feed 11-44-11 + 1 MgO + ME. Tijekom razdoblja uzgoja presadnica obavljeno je jedno razmicanje biljaka, kako bi se izbjeglo zasjenjivanje i nepoželjno izduživanje. Sadnja presadnica s tri do četiri razvijena lista obavljena je 3. svibnja na ploče kamene vune. Na svaku ploču posađene su tri biljke na razmak od 33 cm, dok je razmak između redova bio 150 cm. Ostvaren je sklop od 2,02 biljke/m². Jedna ploča kamene vune s tri biljke predstavljala je obračunsku parcelu. Tijekom vegetacije svakodnevno su praćeni mikroklimatski uvjeti zaštićenog prostora (minimalna i maksimalna temperatura i relativna vlaga zraka). Periodički su prilikom pripreme otopine mjerene pH- i EC-vrijednosti hranive otopine u spremnicima i zoni korijena. Jednom tjedno biljke su usmjeravane u okca potporne mreže te su prema potrebi uklanjani zaperci i suhi listovi.

Prvo tretiranje biostimulatorima obavljeno je 5 dana nakon sadnje, nakon toga svakih 10 dana, a zadnje je tretiranje obavljeno 12. srpnja. Biostimulatori su primijenjeni prskanjem biljaka prema uputama proizvođača: 'AminoVital' u koncentraciji 3 mL/L, 'Phylgreen' u koncentraciji 1,5 mL/L. Utrošak pripravka po tretiranju bio je u rasponu od 0,5 do 1 litre, ovisno o fazi razvoja biljaka. Netretirane biljke su predstavljale kontrolnu varijantu.

Dnevno je bilo 8 obroka fertirigacije hranivom otopinom sastava planiranog prema Enzo i sur. (2001). Tijekom prijepodneva fertirigacija se odvijala u 7, 9 i 11 sati, a u poslije-podnevnim satima, u razdoblju od 13 do 17 sati, svakog sata. Pojedini obrok trajao je od 3 do 8 minuta, ovisno o razvojnoj fazi biljaka i uvjetima u plasteniku. Po biljci je dnevno primijenjeno od 0,8 do 2,13 litara hranive otopine.

U periodu od 21. svibnja do 30. srpnja obavljene su dvadeset i tri berbe. Pri svakoj berbi su utvrđeni broj i masa tržnih i netržnih plodova, dok su morfometrijska svojstva (masa, duljina i promjer) tržnih plodova mjerena jednom tjedno.

Statistička obrada rezultata provedena je statističkim programom SAS Software v. 9.3 (2010). Razlike između prosječnih vrijednosti promatranih svojstava analizirane su analizom varijance (ANOVA), a utvrđene značajne razlike između prosječnih vrijednosti testirane su LSD testom, na razini značajnosti $p \leq 0,05$.

Rezultati i rasprava

Tijekom vegetacije krastavaca u negrijanom zaštićenom prostoru zabilježeno je učestalo variranje temperature i relativne vlage zraka te pH- i EC-vrijednosti hranjive otopine (rezultati nisu prikazani), što je moglo negativno utjecati na samu biljku, ali i smanjiti pozitivan utjecaj biostimulatora.

Nije utvrđen statistički opravdan učinak istraživanih faktora (biostimulator, kultivar) na morfometrijska svojstva tržnih plodova krastavca (tablica 1). Ovisno o primijenjenom biostimulatoru, duljina i promjer ploda bili su između 187 i 199 mm, odnosno 40 i 41 mm, dok je masa ploda bila u minimalnom rasponu od 234 do 237 g. Bez obzira na korišteni biostimulator, kultivar je također rezultirao malim rasponom mase ploda (232 do 238 g), duljine (190 do 197 mm) i promjera (40 do 41 mm).

Interakcija promatranih faktora imala je značajan, ali neujednačen utjecaj na masu i dužinu ploda krastavaca, a nije imala utjecaj na promjer. Najveća masa ploda razvijena je kod cv. 'Touareg' bez primjene biostimulatora (250 g), a značajno manja uz primjenu oba biostimulatora (232 i 230 g). Suprotno, masa ploda cv. 'Paraiso' bila je značajno veća uz primjenu 'Phylgreen' (243 g) u odnosu na kontrolni tretman (221 g), dok je kod 'Q Smart' bila podjednaka uz/bez primjene biostimulatora (236 do 239 g). Najdulja dužina ploda zabilježena je kod cv. 'Paraiso' u kombinaciji s 'Phylgreen' (207 mm), a najkraća kod istog cv., ali u kontrolnom tretmanu (165 mm). Kod svih ostalih kombinacija, dužina ploda bila je statistički podjednaka (187 – 203 mm).

Tablica 1. Utjecaj biostimulatora, kultivara i njihove interakcije na morfolometrijska svojstva tržnih plodova krastavca

Faktori	Tretmani	Masa, g	Duljina, mm	Promjer, mm
Biostimulator	Kontrola (K)	235	187	41
	AminoVital (A)	234	196	40
	Phylgreen (P)	237	199	41
LSD _{p≤0,05}		7,4 n.s.	23,4 n.s.	3,5 n.s.
Kultivar	Q smart (QS)	238	197	41
	Touareg (TOU)	238	195	40
	Paraiso (PAR)	232	190	41
LSD _{p≤0,05}		9,9 n.s.	25,6 n.s.	4,2 n.s.
Interakcija	KQS	236 ^{abc}	199 ^{ab}	41
	KTOU	250 ^a	196 ^{ab}	40
	KPAR	221 ^c	165 ^b	41
	AQS	239 ^{ab}	189 ^{ab}	39
	ATOU	232 ^{bc}	203 ^{ab}	42
	APAR	231 ^{bc}	197 ^{ab}	39
	PQS	238 ^{abc}	202 ^{ab}	41
	PTOU	230 ^{bc}	187 ^{ab}	39
	PPAR	243 ^{ab}	207 ^a	43
	LSD _{p≤0,05}		17,2	40,7

Ostvareni rezultati djelomično su u skladu s navodima Raeisi i sur. (2013) koji tvrde da biostimulator na bazi aminokiselina značajno utječe na dužinu ploda, ali ne i na broj, promjer i masu plodova te dužinu internodija i promjer stabljike.

Nije utvrđen značajan utjecaj istraživanih faktora na sastavnice prinosa (Tablica 2). Ovisno o primijenjenom biostimulatoru i testiranom kultivaru krastavca, po četvornom je metru ubrano između 49,3 i 52,7 tržnih plodova, ostvaren je tržni prinos u rasponu od 11,43 do 12,52 kg/m², dok je udio netržnih plodova bio od 12,5 do 15,3 %.

Između testiranih kombinacija faktora utvrđene su značajne razlike u broju tržnih plodova i ostvarenom tržnom prinosu, dok je udio netržnih plodova bio statistički podjednak (tablica 2). Najveći broj tržnih plodova po jedinici površine (58,6 kom./m²) imao je cv. 'Touareg' tretiran biostimulatorom 'AminoVital', što je uz prosječnu masu ploda od 232 g rezultiralo najvećim tržnim prinosom (13,61 kg/m²). Ista sorta na kontrolnim parcelama imala je značajno najmanje tržnih plodova (45,9 kom./m²), ali je zbog najveće prosječne mase ploda (250 g) ova kombinacija s prinosom od 11,45 kg/m² ipak pripadala rangu najvećih, statistički podjednakih vrijednosti, a kojem je pripadala većina kombinacija s prinosom većim od 11 kg/m² (11,45 do 13,61 kg/m²). Statistički najmanja masa tržnih plodova (221 g), kao i najmanji tržni prinos (10,69 kg/m²) te relativno najveći udio netržnih plodova (16,3%) utvrđeni su kod cv. 'Paraiso' bez primjene biostimulatora. Prema Hamail i sur. (2014) folijarna primjena biostimulatora značajno povećava broj ženskih cvjetova, zametanje plodova te rani i ukupni prinos krastavca u usporedbi s netretiranom kontrolom. Raeisi i sur. (2013) su utvrdili značajan utjecaj biostimulatora na broj tržnih plodova, a Papadopoulos i sur. (2006) navode značajno povećanje broja plodova po biljci i tržnog prinosa pri tretiranju kinetinom (2,5 ppm) u odnosu na kontrolu tijekom proljetno-ljetnog uzgoja, dok u jesenکو-zimskom uzgoju ne bilježe značajne razlike. Isti su autori utvrdili kako se prosječna masa tržnih plodova statistički ne razlikuje za vrijeme proljetno-ljetnog uzgoja, dok za vrijeme jesenکو-zimskog uzgoja postoji značajna razlika u korist primjene kinetina. Prema Farrag i sur. (2015) folijarno tretiranje biostimulatorima, mikroorganizmima i/ili njihovim smjesama značajno povećava masu ploda te rani i ukupni prinos krastavca u odnosu na kontrolu. Bayoumi i sur. (2009) navode kako primjena suhih kvasaca, H₂O₂, huminske kiseline i aminokiselina najčešće pozitivno utječe na rani i ukupni prinos, dok tretiranje salicilnom kiselinom rezultira najmanjim vrijednostima i bez značajne je razlike u odnosu na kontrolu.

Tablica 2. Utjecaj biostimulatora, kultivara i njihove interakcije na sastavnice prinosa krastavca

Faktori	Tretmani	Broj tržnih plodova po m ²	Tržni prinos, kg/ m ²	Udio netržnih, %
Biostimulator	Kontrola (K)	50,5	11,90	14,3
	AminoVital (A)	51,7	12,08	14,5
	Phylgreen (P)	50,3	11,96	13,8
LSD _{p≤0,05}		3,018 n.s.	0,714 n.s.	3,379 n.s.
Kultivar	Q smart (QS)	52,7	12,52	14,9
	Touareg (TOU)	50,5	11,98	12,5
	Paraiso (PAR)	49,3	11,43	15,3
LSD _{p≤0,05}		4,846 n.s.	0,926 n.s.	4,456 n.s.
Interakcija	KQS	57,8 ^{ab}	13,55 ^a	15,1
	KTOU	45,9 ^c	11,45 ^{ab}	11,5
	KPAR	48,1 ^{abc}	10,69 ^b	16,3
	AQS	48,7 ^{abc}	11,66 ^{ab}	13,7
	ATOU	58,6 ^a	13,61 ^a	14,3
	APAR	47,7 ^{bc}	10,97 ^b	15,4
	PQS	51,9 ^{abc}	12,36 ^{ab}	15,7
	PTOU	47,1 ^c	10,85 ^b	11,6
	PPAR	52,1 ^{abc}	12,65 ^{ab}	14,1
	LSD _{p≤0,05}		5,227	2,498

Zaključci

Vrijednosti morfometrijskih svojstava tržnih plodova, kao i sastavnice prinosa krastavca u hidroponskom uzgoju na kamenoj vuni nisu se značajno razlikovale pod utjecajem testiranih faktora (biostimulator, kultivar), no, značajan, ali neujednačen utjecaj utvrđen je kroz njihovu interakciju. Najveći prinos tržnih plodova ostvaren je u kombinaciji ‘Touareg’×‘AminoVital’ i ‘Q Smart’ bez tretiranja biostimulatorom. Ostvareni rezultati sa sigurnošću ne omogućavaju izdvajanje najbolje kombinacije biostimulatora i kultivara.

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Fruit morphometric properties and yield of cucumber treated with plant biostimulants

Abstract

Due to the increasing importance of plant biostimulants in vegetable crops production, a study was conducted to test their effects on the fruit morphometric properties and the yield components of cucumber in soilless culture. Two-factorial trial with biostimulants ('AminoVital', 'Phylgreen') and cucumber cultivars ('Q Smart', 'Touareg', 'Paraiso') did not show the effect of individual factors, but significant and uneven impact of their interaction was observed. The highest yield of marketable fruits was achieved in combination of 'Touareg'×'AminoVital' and 'Q Smart' without treatment with biostimulants (13.61 i 13.55 kg m⁻²).

Keywords: *Cucumis sativus*, soilless culture, cultivar

Vermicompost influence on seedlings quality of *Kniphofia uvaria* species

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Abstract

Producing of planting material of floral species is one of the technological components which could determine the success of a crop. Various factors could influence seedlings' production, and one of those ones being the substrate utilized for sowing. In the current article are presented the results regarding the influence of vermicompost on seedlings' producing for *Kniphofia uvaria* floral species. The rate of vermicompost into substrate was of 10% (V₁), 20% (V₂), 30% (V₃) and 0% (V₄). Vermicompost caused a slightly increase of germination process and obtaining of more vigorous seedlings in comparison with control variant (V₄) and the best results being recorded at V₃ with a rate of 30% vermicompost. Vermicompost caused a vigorous growth of seedlings, being efficient in root's forming.

Keywords: *Kniphofia uvaria*, propagation, vermicompost

Introduction

Genus *Kniphofia* Moench belongs to Asphodelaceae family and includes over 70 species with origins in Central (Ramdhani et al., 2008; Fischer and Ackermann, 2019) and South part of Africa (Stern, 2002; Hlongwane et al., 2015; Fischer and Ackermann, 2019; www.missouribotanicalgarden.org), from which over 48 species are coming from South Africa (Hlongwane et al., 2015).

The genus name was given in the honour of Johannes Hieronymus Kniphof (1704-1763), who was a professor at Erfurt University, Germany. As common name the species belonging to this genus are known under the name of red-hot poker or torch lily (Stern, 2002; www.missouribotanicalgarden.org; www.rhsplants.co.uk).

Kniphofia uvaria is a perennial species, generally hardy to semi-hardy (USDA zones 5 to 9) (Stern, 2002; Hlongwane et al., 2015; Fischer and Ackermann, 2019; www.missouribotanicalgarden.org).

This species could be multiplied also by seeds as well as through scission. Multiplication by seeds involves a longer period till flowers are obtained in comparison with multiplication through scission.

Seeds germination duration, germination dynamics, germination rate could be influenced by various factors, from which we mention the cropping substrate (Mathivanan et al., 2012; Chelariu and Ghiorghe, 2017; Chelariu et al., 2018). The aim of the current research was to observe the influence of substrate composition, by the presence of vermicompost, on seeds germination.

More and more researchers recommend utilization of vermicompost in different stages of plants' cropping technology (Atiyeh et al., 2000; McGinnis et al., 2004; Ascittol et al., 2006; Kalra et al., 2010; Mathivanan et al., 2012; Chelariu and Ghiorghe, 2017; Chelariu et al., 2018). Vermicompost has a fine structure of particles and contains nutritive substances which are easily affordable for plants' absorption. Atiyeh et al. (2000) observed that growth of tomato and marigold seedlings was significantly improved at adding into substrate of a rate of 10% or 20% vermicompost. This substrate led to an increasing of seeds germination rate as well as seedlings' quality, due to its physical, biological and nutritional qualities, because of intensified nutrient absorption, initial development of roots and seedlings development capacity (McGinnis et al., 2004; Kalra et al., 2010; Mathivanan et al., 2012).

At different plant species was observed that vermicompost had a positive effect on seeds germination and seedlings quality: *Impatiens wallerana* (Asciuttol et al., 2006), *Arachis hypogaea* L. (Kalra et al., 2010; Mathivanan et al., 2012); *Ocimum basilicum* (McGinnis et al., 2004; Chelariu et al., 2018); ornamental grasses (Chelariu and Ghiorghe, 2017).

In the current paper are presented the results regarding the influence of vermicompost on red-hot poker or torch lily seeds germination and seedlings quality before being planted in field.

Material and methods

Research was carried out in the didactical glasshouses belonging to Floriculture discipline from UASVM from Iași, Romania, during March-May 2018. The utilised material in this research was species *Kniphofia uvaria*.

For research were organized four experimental variants represented by the rate in which vermicompost participated into substrate together with garden soil, respectively 10% (V_1), 20% (V_2), 30% (V_3) and 0% (V_4 control) (Table 1). As basic substrate, for all those four experimental variants, was utilised a mixture formed by 2 parts peat and 1 part garden soil.

The utilised vermicompost is a substrate obtained at SC SUPERPĂMÂNT SRL, Iași, Romania. As feed for earthworms was utilized a pre-composed mixture, formed by cattle, horse, swine manure, cereals straws, alfalfa, vegetal remains after greenswards cutting and water.

For each variant were utilised 50 seeds, sowed in alveolar trays with round cells (5.5 x 4 cm), and transplanting was realised in squared pots VQB series (9 x 9 x 9.5 cm)

Table 1. Experimental design

Species	Variant	Substrate type
Kniphofia uvaria	V_1	basic substrate + 10% vermicompost
	V_2	basic substrate + 20% vermicompost
	V_3	basic substrate + 30% vermicompost
	V_4	basic substrate + 0% vermicompost (control)

During our research we aimed to observe the aspects regarding seeds germination and characterization of seedlings.

At seedlings of red-hot poker or torch lily were made also observations upon morphological characters such as: mean height of seedlings, mean number of leaves, mean number of roots and mean diameter of main roots.

Characterization of seedlings was done in the moment in which were planted in field (end of August), using the entire obtained seedlings for each experimental variant.

The obtained results were centralized in tables and graphs and were statistically analysed. The data was processed using analysis of variance, by testing the difference between variants with LSD test.

The symbols utilised to indicate the differences' significance from the control are: ns = insignificant; * = significant positive difference; ** = distinct significant positive difference; *** = very significant positive difference.

Results and discussion

The germination rate of *Kniphofia uvaria* seeds was between 83% at control variant (V_4) and 92% at variant with 30% vermicompost (V_3). At variant V_1 (10% vermicompost) seeds germinated in a rate of 88%, and at variant V_2 (20% vermicompost) the rate was 90%.

Statistically speaking can be observed that for the obtained results the differences face to control were very significant positive at variants 30% and 20% rate of vermicompost into substrate, and distinct significant positive differences at variant with 10 % rate of vermicompost (Table 2).

Table 2. Results regarding influence of substrate on *Kniphofia uvaria* seeds germination rate

Variant	Germinated seeds (%)	% face to control	Difference	Signification
V ₁ – 10% vermicompost	88	106.02	+5	**
V ₂ – 20% vermicompost	90	108.43	+7	***
V ₃ – 30% vermicompost	92	110.84	+9	***
V ₄ – 0% vermicompost (control)	83	100.00	0.0	control
LD _{5%} = 2.2 LD _{1%} = 3.3 LD _{0.1%} = 5.3				

The input of vermicompost into substrate determined an improving of red-hot poker or torch lily seedlings quality. So, the mean height of seedlings was of 38.4 cm at control variant (V₁) and between 47.7 cm and 59.7 cm at variants with vermicompost. Mean number of leaves was of 5.4-5.9 pieces/plant at variants with vermicompost in substrate, face to 3.52 pieces/plant at control variant without vermicompost. Mean number of main roots was 4.1 roots/plant at control variant and 5.3-6.8 roots/plant at variants with vermicompost (Table 3). Since from seedling stage could be observed a thickening of main roots, so at control variant the mean diameter of roots was of 0.28 cm, and at variants with vermicompost was between 0.49 cm (V₁) and 0.62 cm (V₃) (Table 3, Figure 1).

From statistically point of view was noticed that at all variants with vermicompost input, the analysed characters recorded very significant positive differences face to control (Table 3).

Table 3 Characterization of seedlings at in field planting

Variant	After 30 days from sowing			
	Mean height of seedlings (cm)	Mean number of leaves (pcs.)	Mean number of main roots (pcs.)	Mean Ø of main roots (cm)
V ₁ – 10% vermicompost	47.7***	5.4***	5.3***	0.49***
V ₂ – 20% vermicompost	52.9***	5.7***	6.1***	0.56***
V ₃ – 30% vermicompost	59.7***	5.9***	6.8***	0.62***
V ₄ – 0% vermicompost (control)	38.4	3.5	4.1	0.28
	LD _{5%} = 0.8 cm	LD _{5%} = 0.6 pcs.	LD _{5%} = 0.2 pcs.	LD _{5%} = 0.1 cm
	LD _{1%} = 1.2 cm	LD _{1%} = 0.9 pcs.	LD _{1%} = 0.3 pcs.	LD _{1%} = 0.1 cm
	LD _{0.1%} = 1.9 cm	LD _{0.1%} = 1.4 pcs.	LD _{0.1%} = 0.6 pcs.	LD _{0.1%} = 0.2 cm

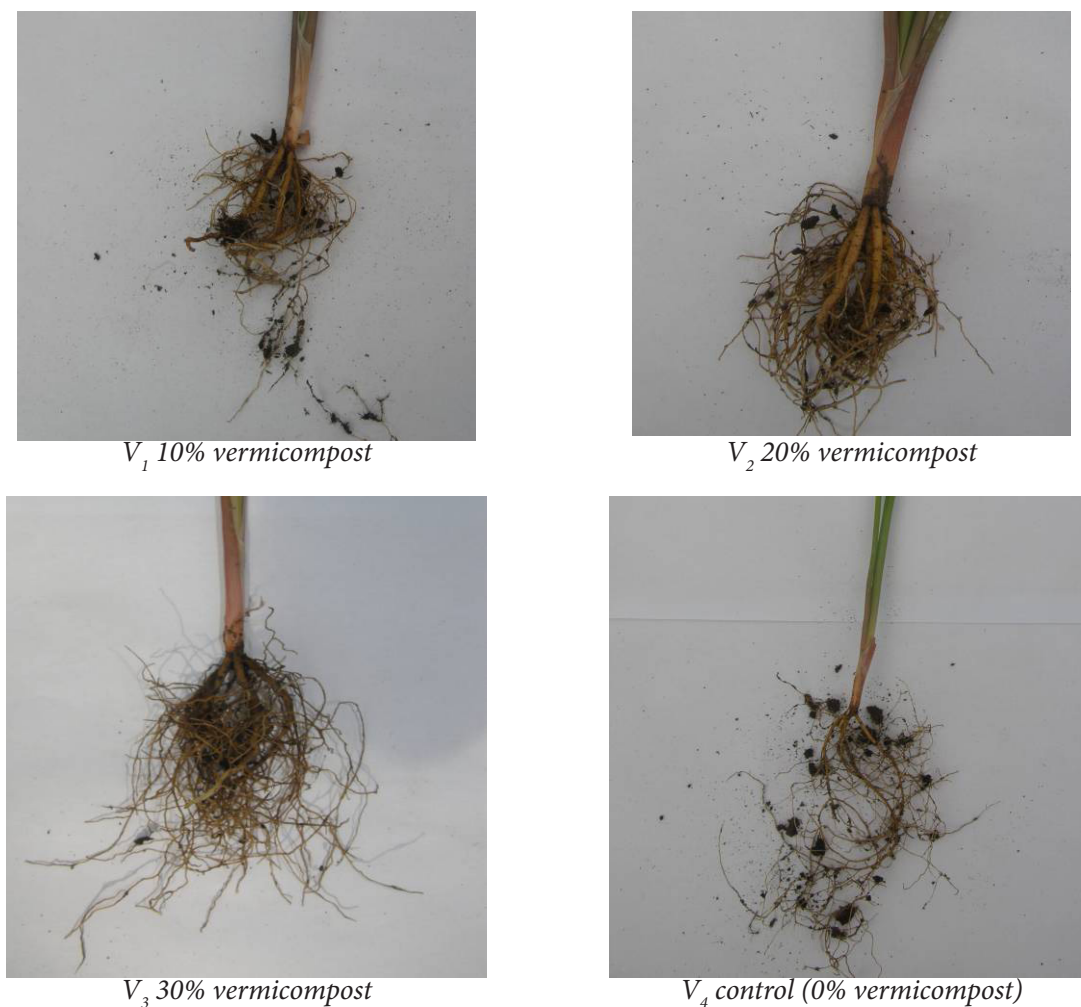


Figure 1. Influence of vermicompost on radicular system

Conclusions

At *Kniphofia uvaria*, vermicompost caused an increase of germination rate from 83% to 92%. Vermicompost had a positive influence on development of plant's roots.

The presence of vermicompost in substrate influence the characteristics of *Kniphofia uvaria* seedlings, leading to increasing of diameter and number on main roots on plant, aspects which conducted to increasing of leaves number and height of seedling.

Rates of 30% vermicompost into substrate determine obtaining of seedlings with a high quality.

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The impact of planting date and cultivar on yield and morphological traits of garlic (*Allium sativum* L.) bulbs: data from a small-scale experiment

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Abstract

Planting date and cultivars are among the critical factors determining the yield and quality of garlic. The aim of this small-scale experiment, conducted in the period of autumn – summer 2018-2019 was to assess the influence of planting date (autumn, spring) and cultivar (‘Gardos’, ‘Haloški’, ‘Ptujski spomladanski’) on garlic bulb morphological parameters and yield. Experiment was designed as a two-factorial experiment in four randomized blocks. Autumn planting date increased garlic yield only with Spanish cv. ‘Gardos’. Values of bulb morphological characteristics were higher for autumn planting date compared to spring planting: higher bulb mass and diameter were obtained for all three cultivars, while the number of cloves per bulb was higher only for cv. ‘Ptujski spomladanski’.

Keywords: *Allium sativum* L., cultural practice, bulb characteristics

Introduction

Garlic is the second most important *Allium* species beside onion. It is grown worldwide, in all temperate to subtropical areas as an important spice and medicinal plant (Fritsch and Friesen, 2002). In recent years, there has been a significant increase in the productivity of garlic bulbs, because of the many health benefits attributed to garlic consumption. China is the largest producer of garlic in the world, with over 22 million tons of garlic, represents 80% of the global supply (Faostat, 2015). Growth and development of garlic are mainly influenced by environmental conditions, among all by soil, photoperiod and temperature (Youssef and Tony, 2014). Agricultural practices such as planting date, cultivar, fertilization and irrigation also have an effect on the growth, yield and the quality of garlic bulbs (El-Zohiri and Farag, 2014). The daylength and temperature to which dormant cloves and growing plants are exposed, control the beginning of bulbing, which requires long photoperiods and warm temperature (Takagi, 1990). Therefore, earlier planting of garlic cloves will have a longer growth period before bulb initiation, resulting in larger plants with larger bulbs.

During the growing period, garlic plants are exposed to a great seasonal fluctuation of temperature and photoperiod, both having a strong effect on the growth and development of garlic (Pooler and Simone, 1993). Garlic cloves as a reproductive material, require moderate temperature for germination, while vegetative growth stage requires short days with moderate temperatures. However, the bulbing stage of garlic is enhanced as soon as daylength exceeds a certain minimum number of hours, although some of the specific garlic cultivar in one specific area will reach maturity at more or less the same time, regardless of planting date (Qaryouti and Kasrawi, 1995). According to Qaryouti and Kasrawi (1995) a delay in planting date from October to January led to the small diameter of bulbs and reducing their fresh and dry weight and finally to reducing yield. Youssef and Tony, (2014) also confirmed positive effect of early planting date on yield and some morphologic traits of garlic bulbs, compared to the delayed planting date, but the influence seems to be cultivar specific.

The aim of research was therefore to evaluate the influence of planting date and cultivars on garlic bulb morphological parameters and yield.

Material and methods

The study was conducted on the experimental field of the Biotechnical Faculty, University of Ljubljana, (Ljubljana, Slovenia, 46°03' N and 14°31' E, 298 m a. s. l.), in the period of autumn – summer 2018-2019. The soil of the experimental site is classified as gleyic fluvisol and endogenic fluvisol containing 22 g kg⁻¹ soil organic matter in the 0-0.3 m soil layer. Fourteen days before the sowing date, the average initial soil nitrate content was 5.2 mg kg⁻¹ for the same depth, soil assimilable P and K was 24 mg kg⁻¹ and 26 mg kg⁻¹, respectively, on the basis of which application rates of macronutrients were calculated according to the Regulations on Integrated Production of Vegetables (IOBC, 1997). One day before transplanting, granulated mineral fertilizers were incorporated on the plots at a rate of 90 kg N ha⁻¹, 90 kg P ha⁻¹ and 150 kg K ha⁻¹, as calcium ammonium-nitrate, super phosphate and potassium sulfate, respectively. Nitrogen was applied during the growing period, in two equal halves, the first half, when plants reached the growth stage of 3-4 leaves and the second half, when plants developed 7 to 8 leaves.

For each planting date (autumn date – 23/10/2018 and spring date – 6/3/2019), the experimental field was divided into four blocks, each of which had three plots, where garlic cultivars were randomly allocated. The garlic cloves (*Allium sativum* L.) were planted on plots of 1.5 m × 1.0 m, with a spacing of 0.25 m between rows and 0.15 m between plants within a row. Each plot comprised 40 plants (4 rows, 10 plants per row). Three garlic cultivars were included. 'Haloški' (autochthonous Slovenian cultivar, Semenarna Ljubljana, Slovenia) is a „white“ type, spring cultivar. 'Gardos' (Plantas de Navarra S.A. Planasa, Spain) and 'Ptujski spomladanski' (autochthonous Slovenian cultivar, Semenarna Ljubljana, Slovenia) are „purple“ garlic cultivars. 'Gardos' and 'Haloški' can also produce scape, while 'Ptujski spomladanski' does not produce a flower stalk. The plants were managed according to the integrated pest management guidelines (Eur-Lex, 2014).

Plants were manually harvested (during July 2019) from the 2 external rows of each plot (20 plants from the 0.9 m²), when approximately 60% of the leaves were senesced. The garlic plants were bunched and after a drying period in an open space for 12 days, leaves and roots were trimmed and bulb samples were transported to the laboratory for further morphological analyses. After harvest, garlic bulbs were arranged into 3 classes, according to the bulb diameter: first class (> 5×5 cm), second class (> 3×3 cm) and third class (< 3×3 cm). Variation of garlic bulb characteristics (mass, diameter and the number of cloves per bulb) were studied on samples (n = 8 to 20), taken from the second sized class. The diameter of the bulbs was measured using a Powerfix Profi Electronic Vernier Caliper, and bulb fresh weight was weighed on high-precision Kern EW 600-2M scales. The yield was determined by weighing the fresh bulb mass, multiplying by the number of plants per square meter, calculated on the basis of inter and intra-rows spaces. Yield was expressed in tons per hectare. Total yield was reduced by 30% due to consideration for the tractor wheel paths, where plants would not have been planted under normal field production technology. The mean bulb mass (g) is calculated as a ratio of total bulb mass harvested per plot and the number of bulbs per plot.

The data were analysed by analysis of variance (ANOVA) for mixed models. When necessary, logarithmic transformation was used to fulfil the assumptions of ANOVA. Three comparisons were analysed: for each cultivar, the difference between autumn and spring planting date. The significance level of 0.05 was used. Statistical analysis of the data was performed using the statistical program R with *nlme* package for mixed models and *multcomp* package for multiple comparison analyses (Team, 2011 #51).

Results and discussion

The data for average mean, maximum and minimum monthly air temperatures and the amount of precipitation were taken from the Environmental Agency of the Republic of Slovenia (Manual, 2003) and are presented in Figure 1.

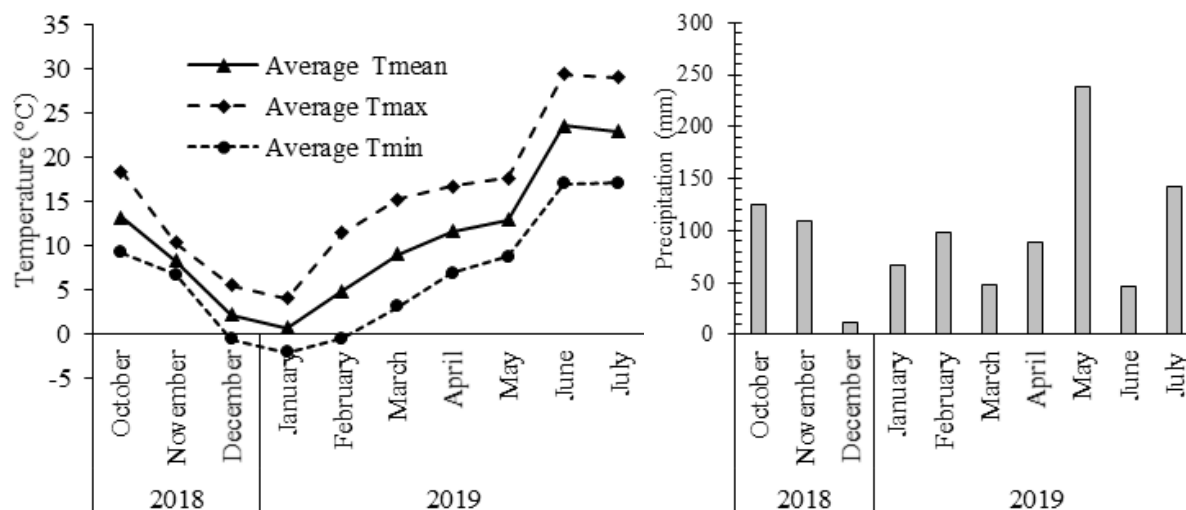


Figure 1 Average mean, minimum and maximum monthly air temperatures (left) and amount of precipitation (right) during the growing period of garlic, EARS (Manual, 2003).

During the experimental period from October 2018 to July 2019, the lowest average mean monthly temperature was measured in January (0.7 °C) and the highest in June (23.5 °C). The amount of precipitation was the lowest in December (12.3 mm) and the highest in May (238.6 mm).

Data for garlic yield and bulb mass are presented in Table 1. Significant effect of planting date on yield of garlic bulbs was recorded at cultivar ‘Gardos’ only. Yield of autumn planting plants is for 4.7 t ha⁻¹ higher (approximately 25%) than the yield of spring planting plants.

Differences between the yield of autumn and spring planted plants of other two cultivars are much lower (1.4 and 2.2 t ha⁻¹ for cultivars ‘Haloski’ and ‘Ptujski’) and not significant. The yield results are in agreement with those of El-Zohiri and Farag (2014), Youssef and Tony (2014) and El-Shabasi et al. (2018) who found that the yield decreases gradually with the late planting.

The highest ratio is detected for cv. ‘Ptujski’: the ratio in bulb mass for autumn and spring planting date is 2.7 with 95% confidence interval (1.5, 4.5). The ratio for cv. ‘Gardos’ is 2.0 with 95% CI (1.2, 3.8) and is also significant, while for cv. ‘Haloski’ the ratio of 1.7 with 95% CI (0.9, 2.9) is not significant.

Table 1. Mean of yield (with confidence interval (CI) for mean differences) and mean of bulb mass (with CI for mean ratio), for three garlic cultivars, planted in autumn and spring, 2018-2019.

Variable	‘Gardos’		‘Haloski’		‘Ptujski’		
	Autumn	Spring	Autumn	Spring	Autumn	Spring	
Yield (t/ha)	Mean	12.9	8.2	10.3	8.9	6.3	4.1
	Significance	*		ns		ns	
	CI for mean diff.	(0.8, 8.9)		(-2.8, 5.5)		(-1.6, 6.0)	
Mean bulb mass (g)	Mean	48.6	24.0	43.4	25.6	46.2	17.3
	Significance	**		ns		***	
	CI for mean ratio	(1.2, 3.8)		(0.9, 2.9)		(1.5, 4.5)	

CI – confidence interval; ns – non significant; * - significant at 0.05; ** - significant at 0.01; *** - significant at 0.001

The results in table 2 show significant difference in mean logarithmic values for bulb mass for all three cultivars. The mean bulb mass of cv. ‘Gardos’ planting in autumn is 1.7-fold higher than in spring planting bulbs, with 95% confidence interval (1.4, 2.2). The mean bulb mass of cv. ‘Haloski’ planted in autumn is 2.0-fold higher than in spring with 95% confidence interval (1.5, 2.6). The highest ratio is detected for cv. ‘Ptujski’: the ratio in mean bulb mass for autumn and spring is 2.4 with 95% confidence interval (1.7, 3.2).

The impact of planting date and cultivar on yield and morphological traits of garlic (*Allium sativum* L.) bulbs: data from a small-scale experiment

Table 2. Bulb mass, bulb diameter and number of cloves per bulb (with confidence interval (CI) for mean ratio or differences), for three garlic cultivars, planted in autumn and spring, 2018-2019

Variable		'Gardos'		'Haloski'		'Ptujski'	
		Autumn	Spring	Autumn	Spring	Autumn	Spring
Bulb mass (g)	Mean	51.1	30.1	56.0	28.5	45.3	18.8
	Significance	***		***		***	
	CI for mean ratio	(1.4, 2.2)		(1.5, 2.6)		(1.7, 3.2)	
Bulb diameter (mm)	Mean	50.4	40.7	52.0	40.6	47.6	33.4
	Significance	***		***		***	
	CI for mean differences	(5.7, 13.8)		(6.8, 16.1)		(8.8, 19.5)	
Cloves per bulb	Mean	12.4	11.4	9.5	7.8	17.9	7.4
	Significance	ns		ns		***	
	CI for mean differences	(5.7, 13.8)		(-0.9, 4.2)		(7.6, 13.0)	

CI – confidence interval with 95%; ns – non significant; * - significant at 0.05; ** - significant at 0.01; *** - significant at 0.001

The results in table 2 also show significant differences in mean values for bulb diameter for all three cultivars. For cv. 'Gardos', the mean bulb diameter is 9.7 mm higher for autumn planting compared to spring planting, with 95% confidence interval (5.7, 13.8). The highest differences are detected at bulbs of cv. 'Ptujski': the autumn planting bulbs are 14.2 mm higher than the spring planting bulbs, with 95% confidence interval (8.8, 19.5). For cv. 'Haloski', the mean bulb diameter for autumn planting bulbs is 11.4 mm higher than for spring planting bulbs, with 95% confidence interval (6.8, 16.1).

The number of cloves per bulb at cv. 'Gardos' ranged from 9 to 17 (autumn) and 8 to 15 (spring), at cv. 'Haloski' from 5 to 12 (autumn) and 1 to 15 (spring) and at cv. 'Ptujski' from 12 to 24 (autumn) and 6 to 11 (spring). Differences are significant for bulbs of cv. 'Ptujski' only: the autumn planting bulbs has 10 cloves per bulb more than spring planting bulbs, with 95% confidence interval (7.6, 13.0). Poldma et al. (2004) also reported that garlic planted in spring had lower yield with lower bulb mass. They pointed out, that 16% of bulbs formed only one round clove. Similar effect of later planting date on one clove bulb appeared also in this study, mostly at cv. 'Haloski' from spring planting date.

Conclusions

The results obtained in this small-scale study revealed that planting date is a critical factor determining the yield and quality parameters of garlic bulbs, but the influence is cultivar specific. Autumn planting date significantly increased garlic yield only with Spanish cv. 'Gardos'. Values of bulb characteristics are higher at autumn planting date compared to spring planting: higher bulb mass and diameter were obtained at all three cultivars, while the number of cloves per bulb was higher only at cv. 'Ptujski'. Due to longer growing period before bulb initiation, autumn planted garlic results in larger plants, producing larger bulbs and yield compared to spring planting plants.

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Chemical composition and antimicrobial activity of essential oils from Albanian conifer plants

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Abstract

Commercial essential oils from Albanian coniferous plants have been investigated for their chemical composition and biological activity on different target organisms. Main volatiles of the essential oils were α -pinene (41.99%) and c-verbenol (11.26%) in black pine; β -pinene (24.78%), α -fenchone (12.93%) and α -pinene (10.8%) in Douglas fir; limonene (20.24%), β -pinene (18.39%), α -pinene (13.33%) and camphene (11.13%) in Silver fir; α -pinene (24.34%), sabinene (13.86%), β -myrcene (11.46%) in Juniperus berry. All oils showed low antioxidant activity. The essential oil of black pine showed good antifungal and antibacterial activity comparable with synthetic antibiotic.

Keywords: *Abies alba*, *Juniperus communis*, *Pinus nigra*, *Pseudotsuga menziesii*, antioxidant activity

Introduction

Some synthetic antioxidants and antimicrobials used in food industry are thought to be toxic and carcinogenic (Bauer et al., 2005; Eichholzer and Gutzwiller, 1998). There is a negative consumer perception regarding the use of synthetic chemicals used to control insects and fungi in agricultural crops and commodities. Some of these synthetic chemicals are reported to be carcinogenic (Research Council, Board of Agriculture, 1987), not effective due to pathogen resistance (Kinay et al., 2007), and toxic for the environment (Wightwick et al., 2010). All the above mentioned problems related to synthetic chemicals have attracted the attention to natural alternatives. Essential oils (EOs) from woody and herbaceous plants are known to produce different volatile compounds. Due to the presence of these compounds many EOs are used in cosmetics and food manufacturing for different purposes. Many of these compounds are biologically active and could be used as antioxidant, antimicrobial and insecticidal agents. EOs from woody coniferous plants are rich in terpenes such as α -pinene, β -pinene, β -myrcene, camphene, limonene etc (Angioni et al., 2003; Jirovetz et al., 2000; Tumen et al., 2010; Wajs et al., 2010). Many of these compounds have different biological activities. However, few reports are available on the chemical composition of EOs from Albanian conifers. Additionally, the coniferous wood industry generates large amounts of residues. Therefore it is important to study the chemical constituents of coniferous wood essential oils and their biological activity to provide knowledge-based waste valorization tools. The aim of the present work was to study the chemical composition and screening for biological activities (antimicrobial and antioxidant) of commercial EOs from Albanian coniferous plants.

Material and methods

Essential oils (EOs) of *Abies alba* Mill., *Juniperus communis* L., *Pseudotsuga menziesii* (Mirbel) Franco, and *Pinus nigra* J. F. Arnold of wild origin were provided from Albanian company "Mediterranean Spices & Imports" Tiranë. *A. alba*, *P. menziesii*, and *Pinus nigra* young shoots collected in August-October were steam distilled for four hours while berries of *J. communis* collected in August-November were steam distilled for 36 hours with intervals. The extracted EOs were analyzed by gas chromatography mass spectrometry (GC-MS) using Shimadzu GC-2010 coupled to Shimadzu GCMS-QP2010 Ultra mass detector (electron ionization, 70 eV) and equipped with Teknokroma

TRB-5 (95%) Dimetil-(5%) diphenylpolisiloxane, 30 m × 0.25 mm i.d. capillary column (0.25 µm film thickness). Working conditions were as follows: split ratio (20:1), injector temperature 300°C, temperature of the transfer line connected to the mass spectrometer 250°C, initial column temperature 70°C, then heated to 290°C at 6°C/min. Electron ionisation mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley 229 Mass Spectral Database. The free radical scavenging activity of the EOs was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay according to Saeed et al. (2012). The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: Scavenging effect % = {(control absorbance - sample absorbance) / (control absorbance)} x 100.

Antibacterial activity of the EOs was investigated by disc diffusion method as already described by Sfeir et al. (2013). The bacterial suspension of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 was adjusted to a density of bacterial cells of 1.0×10^8 CFU/mL. The antifungal activity of EOs were tested on colony growth using the methods given by Shao et al. (2013) and Soylyu et al. (2010) with some modifications. The efficacy of the treatment was evaluated by measuring the average of two perpendicular diameters of each colony. Percentage mycelial inhibition = $[(dc - dt)/dc] \times 100$, where *dc* is the mean colony diameter for the control sets (distilled water) and *dt* is the mean colony diameter for the treatment sets was calculated. All tests were repeated two times.

Results and discussion

GC-MS analysis of the Black pine EO allowed the identification of different compounds. Main components of each EO are presented in table 1.

Table 1. Major compounds indentified in the essential oils of Albanian coniferous plants

Name of the compound	Percentage (%) of the compounds in each essential oil			
	Black pine	Douglas fir	Silver fir	Juniperus berry
α-pinene	41.99	10.8	13.33	24.34
α-fenchone		12.93		
β-pinene		24.78	18.39	
β-myrcene				11.46
verbenol	11.26			
limonene			20.24	5.75
champene			11.13	
endobornyl acetate			9.94	
sabinene				13.86

The scavenging effect of the EOs on DPPH radical was measured after 30 minutes. The scavenging effect was low at the concentrations tested (Table 2). Other authors who tested the scavenging effect of the EOs from these coniferous plants found low antioxidant activity (Emami et al., 2007; Politeo et al., 2011a; Wajs-Bonikowska et al., 2015), as well.

Table 2. DPPH scavenging activity of the essential oils of Albanian coniferous plants

	Essential oils			
	Black pine	Juniperus berry	Silver fir	Douglas fir
DPPH % Scavenging	11.5±0.7	12.6±0.4	11.7±0.4	11.4±0.7

Table 3 shows the antibacterial effects of the test oils. The inhibition zone diameters are given in mm. The EOs of Juniperus berry, Silver fir and Douglas fir showed low activity against the three bacterial species at the concentration tested, with inhibition zone diameters ranging from 2.3 to 5 mm. The essential oil of Black pine showed good antibacterial activity. In the case of *E. coli* and *S. typhimurium* the inhibition zone was 27 mm, higher than the positive control (19.3 and 18.3 mm respectively). The inhibition zone against *S. aureus* was high (30 mm) but lower than the positive control (33.3 mm). Similar results were obtained in previous studies from different authors (Angioni et al., 2003; Wajs-Bonikowska et al., 2015; Yang et al., 2009). In a study conducted by Politeo et al. (2011b), the EO of black pine (Dalmatian black pine; *Pinus nigra* Arnold ssp. *Dalmatica* (Vis.) Franco), at a dose of 5 µL per disc, gave inhibition zones of 22.5 mm on *S. aureus* while on *E. coli* showed no activity. In our experiments this EO was active on these two pathogens and also on *S. typhimurium*.

Table 3. Inhibition zone diameters (mm) on three bacterial species and percentages of fungal mycelial inhibition of coniferous essential oils

Pathogens	Black pine	Douglas fir	Silver fir	Juniperus berry	Control	Cefazolin
Bacterial species						
<i>E. coli</i>	27.0±1	3.0±1	3.3±0.6	5.0±1	0.0	19.3±0.6
<i>S. typhimurium</i>	27.0±1	3.0±1	3.3±0.6	4.7±2.1	0.0	18.3±0.6
<i>S. aureus</i>	30.0±1	5.0±1	2.3±0.6	2.3±0.6	0.0	33.3±0.6
Fungal species						
<i>B. cinerea</i>	81.3±6.8	7.6±7.6	9.6±0.9	19.2±3.8		
<i>P. digitatum</i>	-11.4±2.9	-7.1±1.4	-16.7±2.2	-28.6±0		
<i>P. expansum</i>	22.7±3.9	-1.5±3.5	-3.0±2.6	-7.6±3.5		
<i>P. italicum</i>	16.3±3.2	-3.7±3.6	-6.3±0.9	7.4±1.6		

Values in the table represent the mean value of three replicates (± standard deviation). The negative values represent a growth higher than control (distilled water). For antifungal bioassays the EOs were tested at the amount of 10 µL (0.14 g/L) per disc (Petri dish). For antibacterial bioassays the EOs were tested at the amount of 5 µL per disc while Cefazolin 30 µg/disc.

Table 3, shows the antifungal effects of the test oils. Since the EOs were not able to inhibit the spore germination of the three *Penicillium* species, the same Petri dishes were used to calculate the percentage mycelial inhibition. The EOs showed different effects on mycelial growth. All the tested oils promoted the growth of *P. digitatum*. The EOs of Juniperus berry, Silver fir and Douglas fir promoted the growth of *P. expansum*, while the EOs of Juniperus berry and Silver fir promoted the growth of *P. italicum*. A moderate antifungal activity was given by the EO of black pine on *P. expansum* and *P. italicum*. Also the EOs of Juniperus berry, Silver fir and Douglas fir gave a moderate antifungal activity against *B. cinerea*. The highest antifungal activity was found for the EO of black pine against *B. cinerea* (81.3% mycelial inhibition). In previous studies, Douglas fir and Silver fir EOs gave none or low *in vitro* antifungal activity (Bağcı et al., 1996; Johnston et al., 2001; Pattnaik et al., 1996). Some authors reported the antifungal activity of Juniperus berry EO in the contact phase (Cabral et al., 2012; Cavaleiro et al., 2006) but showed no antifungal activity in volatile phase on postharvest pathogens (Lee et al., 2007). Similarly, in our experiments this oil gave low or none antifungal activity. The oil of black pine has been shown to be active against *Candida albicans* and *Penicillium* sp. but not against *Aspergillus niger* (Politeo et al., 2011b). In our experiment this oil was active against *P. expansum*, *P. italicum* and *B. cinerea* but not against *P. digitatum*.

Conclusion

The major compound found in EO of Black pine and Juniperus berry was α-pinene while the major compounds in Silver fir and Douglas fir EO were limonene and β-pinene, respectively. The commercial EOs from Albanian coniferous plants showed varying degrees of biological activity. Overall, the major activity was observed from the Black pine EO.

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Količina makroelemenata u mladim izdancima cikle i brokule

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Sažetak

Mladi izdanci (eng. *microgreens*) su nepotpuno razvijene biljke izražene nutritivne vrijednosti koje se konzumiraju u fazi kotiledona s ili bez prvog para pravih listova. Cilj istraživanja bio je utvrditi utjecaj tri podloge ('supstrat', 'supstrat+perlit', 'jute') na količinu suhe tvari, sirovih proteina i makroelemenata u mladim izdancima cikle i brokule. Uzgoj na podlozi 'supstrat' rezultirao je najvećom količinom fosfora u mladim izdancima brokule te kalcija u mladim izdancima cikle. Najveće vrijednosti sirovih proteina i kalija utvrđene su kod uzgoja obje promatrane vrste mladih izdanaka na podlozi 'supstrat+perlit' dok je primjena jute pozitivno utjecala na količinu suhe tvari i magnezija obje vrste.

Ključne riječi: *Brassica oleracea* var. *italica*, *Beta vulgaris* var. *conditiva*, microgreens, minerali, suha tvar

Uvod

Različite vrste povrća, aromatičnog bilja i žitarica u fazi mladih izdanaka ('microgreens'), odnosno kotiledona i djelomično ili potpuno razvijenih prvih pravih listova, dakle, u razvojnem stadiju između klijanaca i mlade biljke, novija je kategorija namirnica biljnog porijekla (Di Gioia i Santamaria, 2015; Pinto i sur., 2015). Mladi izdanci su sve popularniji među potrošačima zbog izražene nutritivne vrijednosti, atraktivnog izgleda te široke palete tekstura i okusa (Franks i Richardson, 2016; Xiao i sur., 2015; Ebert i sur., 2014), a među proizvođačima zbog jednostavnog i kratkog ciklusa uzgoja (Ebert, 2012). Recentna istraživanja ukazuju da mladi izdanci sadrže više fitonutrijenata i minerala te manje nitrata u odnosu na biljke u kasnijoj fenološkoj fazi, zbog čega imaju izražen pozitivan utjecaj na ljudsko zdravlje (Pinto i sur., 2015; Xiao i sur., 2012). Mladi se izdanci konzumiraju u svježem stanju, pa nema gubitka hranjivih tvari uobičajenih za termičku obradu, što ih čini odličnim dodatkom svakodnevnoj prehrani (Ebert i sur., 2014). Jedan od čimbenika koji utječe na prinos i nutritivnu vrijednost mladih izdanaka je podloga za uzgoj (Franks i Richardson, 2009). Uz vrtno tlo, najčešće korištene podloge za uzgoj mladih izdanaka su treset i mješavine komercijalnih supstrata s tresetom. Iako treset ima optimalna fizikalno-kemijska svojstva, zbog visoke cijene istražuju se zamjenske podloge za uzgoj, poput vlakana kokosa, pamuka, konoplje i jute, pamučnog ili vunenog filca, perlita i kamene vune (Di Gioia i sur., 2016). Cilj ovog rada bio je utvrditi prikladnost zamjenskih podloga za uzgoj mladih izdanaka cikle i brokule te njihov učinak na količinu suhe tvari, sirovih proteina i minerala.

Rad je izvod iz diplomskog rada Tamare Brlek, mag. ing. agr. naslova 'Utjecaj supstrata na prinos i nutritivnu vrijednost mladih izdanaka povrća i suncokreta'.

Materijal i metode

Istraživanje je postavljeno tijekom proljeća 2019. godine u Zavodu za povrčarstvo Sveučilišta u Zagrebu

Agronomskog fakulteta (SuZAF) po metodi slučajnog bloknoeg rasporeda u 3 ponavljanja. Pokus je uključivao mlade izdanke brokule (*Brassica oleracea* L. var. *italica* Plenck) sorte 'Calabrese' i cikle (*Beta vulgaris* var. *conditiva* Alef.) sorte 'Red lady' te 3 podloge za uzgoj: komercijalni supstrat za uzgoj presadnica 'Klasman Potgrond H' ('supstrat'), mješavina supstrata 'Klasman Potgrond H' i perlita 'Europerl Agroperl' u omjeru 5:1 ('supstrat+perlit'), četveroslojna juta ('juta') rezana na odgovarajuće dimenzije za uzgojne posude (26 cm × 36 cm × 6,3 cm). Sjetva sjemena ekološkog porijekla proizvođača 'Rem sprout' provedena je 30. svibnja u količini od 262 g/m² (cikla) i 3. lipnja u količini 354 g/m² (brokula). U svrhu poboljšanja klijavosti, sjeme cikle namakano je u vodi 24 sata prije sjetve. Posijano sjeme cikle i brokule prekriveno je papirnatim ubrusima koji su redovito prskani vodom te su uklonjeni 3 (brokula), odnosno, 5 (cikla) dana nakon sjetve. Zbog održavanja optimalnih uvjeta (tama, visoka vlaga) uzgojne posude su od sjetve sjemena do faze nicanja bile pokrivene poklopcima. Nakon uklanjanja ubrusa svakodnevno se provodilo zalijevanje količinom od 100 do 200 ml vode po uzgojnoj posudi, prema subjektivnoj procjeni potreba mladih izdanaka za vodom. Tijekom uzgoja abiotski čimbenici održavani su u optimalnom rasponu: srednja temperatura zraka iznosila je 24,8 °C, a srednja relativna vlaga bila je 57,8 %.

Berba mladih izdanaka brokule na svim podlogama obavljena je 10. lipnja dok su berbe mladih izdanaka cikle bile 12. lipnja ('supstrat' i 'supstrat+perlit') i 14. lipnja ('juta'). Neposredno nakon berbe, na reprezentativnim uzorcima biljnog materijala u laboratoriju Zavoda za ishranu bilja SuZAF-a utvrđena je količina suhe tvari i makroelemenata (dušik, fosfor, kalij, kalcij i magnezij). Biljni materijal je usitnjen i osušen pri temperaturi od 105 °C, a zatim samljeven, homogeniziran te analiziran u triplikatu. Određivanje suhe tvari (%) provedeno je gravimetrijskom metodom prema normi HRN ISO 11465:2004 (HZN, 2004.). Za utvrđivanje ukupnog dušika korištena je metoda po Kjeldahlu (AOAC, 1995.), dok su sirovi proteini (SP) preračunati prema formuli: % N×6,25 (Vajić, 1964.). Fosfor je određen spektrofotometrijski, kalij plamenfotometrijom, a kalcij i magnezij atomskom apsorpcijskom spektrofotometrijom, nakon digestije s koncentriranom HNO₃ i HClO₄ (AOAC, 1995.), a rezultati su izraženi kao postotak navedenih minerala u suhoj tvari (% P/ST, % K/ST, % Ca/ST, % Mg/ST). Statistička obrada podataka provedena je u programu SAS Software v. 9.3 (2010), procedura PROC GLM (opći linearni model). Rezultati su podvrgnuti jednosmjernoj analizi varijance (ANOVA), a srednje vrijednosti uspoređene su t-testom (LSD).

Rezultati i rasprava

U tablici 1 prikazani su podaci za količinu suhe tvari, sirovih proteina i makroelemenata u mladim izdancima cikle i brokule. Vidljiv je opravdan utjecaj primijenjenih podloga na istraživana svojstva mladih izdanaka obje vrste povrća. Uzgoj na podlozi 'juta' rezultirao je opravdano najvišim vrijednostima suhe tvari u mladim izdancima cikle i brokule (6,86 i 6,98 % ST), dok je podloga 'supstrat+perlit' utjecala na smanjenje količine suhe tvari kod obje vrste (5,35 i 4,93 % ST), što je u skladu s istraživanjem Di Gioia i sur. (2016). Vrijednosti suhe tvari ostvarene uzgojem brokule na podlogama 'supstrat' i 'supstrat+perlit' u skladu su s količinom od 5,08 % ST koju su u mladim izdancima brokule uzgajanim na supstratu 'Klasman Potgrond H' utvrdili Opačić i sur. (2016). Neovisno o podlozi, prosječna količina suhe tvari u mladim izdancima brokule bila je 5,66 % ST, a cikle 6,09 % ST, što je za 30 % više u odnosu na količinu koju u mladim izdancima cikle navodi Xiao (2013).

Tablica 1. Količina suhe tvari, sirovih proteina i makroelemenata u mladim izdancima

Vrsta	Varijanta	Suha tvar	Sirovi proteini	P	K	Ca	Mg
Cikla	Supstrat	6,06 ^B	35,90 ^B	1,39 ^B	5,17 ^B	0,95 ^A	0,77 ^C
	Supstrat + perlit	5,35 ^C	40,83 ^A	1,64 ^A	7,37 ^A	0,67 ^B	0,86 ^B
	Juta	6,86 ^A	/	1,65 ^A	3,29 ^C	0,60 ^C	0,94 ^A
Prosjeck		6,09	38,37	1,56	5,28	0,74	0,86
Brokula	Supstrat	5,07 ^B	48,06 ^A	1,39 ^A	3,32 ^A	1,12 ^B	0,46 ^B
	Supstrat + perlit	4,93 ^B	48,42 ^A	1,26 ^B	3,39 ^A	1,18 ^A	0,43 ^C
	Juta	6,98 ^A	42,98 ^B	1,15 ^C	1,03 ^B	1,16 ^A	0,49 ^A
Prosjeck		5,66	46,49	1,27	2,58	1,15	0,46

Različita slova pridodana vrijednostima unutar stupca za svaku vrstu predstavljaju značajno različite vrijednosti prema LSD testu, $p \leq 0,01$

Najveća količina sirovih proteina u mladim izdancima cikla i brokule (40,83 i 48,42 % SP/ST) utvrđena je pri uzgoju na podlozi 'supstrat+perlit', a kod brokule se nije statistički razlikovala od količine ostvarene na podlozi 'supstrat' (48,06 % SP/ST), dok je kod cikla ta razlika bila opravdana obzirom na ostvarenu značajno manju količinu od 35,90 % SP/ST. Podloga 'juta' je rezultirala statistički najmanjom količinom sirovih proteina u mladim izdancima brokule (42,98 % SP/ST), dok na ciklu zbog premalog uzorka nije bilo moguće provesti kemijsku analizu dušika, pa posljedično ni odrediti količinu sirovih proteina. Prosječna vrijednost sirovih proteina u mladim izdancima, bez obzira na podlogu za uzgoj, kod cikla je bila manja (38,37 % SP/ST) nego kod brokule (46,49 % SP/ST), a što je približno 7 puta veća količina u odnosu na utvrđenu u tehnološki zreloom cvatu brokule koju navodi Fabek (2012). Podloga 'supstrat' u uzgoju mladih izdanaka cikla imala je pozitivan učinak jedino na količinu kalcija obzirom na ostvarenu značajno veću količinu (0,95 % Ca/ST) u odnosu na ostale istraživane podloge. Na količinu fosfora i magnezija (1,39 % P/ST i 0,77 % Mg/ST), a također i kalija (5,17 % K/ST) u mladim izdancima cikla, ova je podloga imala negativan učinak zbog opravdano manjih vrijednosti u odnosu na ostale istraživane podloge, odnosno na podlogu 'supstrat+perlit'. Podloga 'supstrat' u uzgoju mladih izdanaka brokule rezultirala je značajno većom količinom fosfora (1,39 % P/ST) u odnosu na ostale podloge te kalija (3,32 % K/ST) u odnosu na podlogu 'juta', dok je količina kalcija bila opravdano manja u odnosu na najviše ostvarene vrijednosti na ostalim podlogama.

Utjecaj podloge 'supstrat+perlit' bio je opravdan na količinu kalija u mladim izdancima cikla (7,37 % K/ST) i brokule (3,39 % K/ST) kod koje je količina kalija bila statistički jednaka ostvarenoj na podlozi 'supstrat' (3,32 % K/ST). Također, pozitivan učinak podloge 'supstrat+perlit' utvrđen je na količinu fosfora kod cikla (1,64 % P/ST) i kalcija kod brokule (1,18 % Ca/ST) čije se vrijednosti nisu značajno razlikovale od količina ostvarenih na podlozi 'juta' (1,65 % P/ST i 1,16 % Ca/ST).

U uzgoju mladih izdanaka cikla i brokule na podlozi 'juta' ostvarena je najveća količina magnezija (0,94 i 0,49 % Mg/ST), značajno veća nego na ostalim testiranim podlogama. Također, pozitivan učinak podloge 'juta' utvrđen je i na količinu fosfora kod cikla (1,65 % P/ST) i količinu kalcija kod brokule (1,16 % Ca/ST). Međutim, primjena iste podloge negativno je utjecala na količinu kalija kod cikla i brokule (3,29 % i 1,03 K/ST), zatim kalcija kod cikla (0,60 % Ca/ST) i fosfora kod brokule (1,15 % P/ST) jer su navedene vrijednosti bile opravdano manje od ostvarenih na ostalim podlogama.

Prosječne vrijednosti kalija i magnezija u mladim izdancima cikla bile su 2 puta više u odnosu na mlade izdanke brokule. Suprotno tome, u mladim izdancima brokule prosječna vrijednost kalcija bila je 1,6 puta veća u odnosu na mlade izdanke cikla. Prosječna količina kalcija u mladim izdancima brokule u skladu je s rezultatima istraživanja Xiao i sur. (2016). Isti autori prikazuju nešto više vrijednosti kalija i magnezija (4,18 % K/ST i 0,65 % Mg/ST) te manju količinu fosfora (0,87 % P/ST) u odnosu na ovo istraživanje. Prema Lešić i sur. (2016) cikla u tehnološkoj zrelosti sadrži višestruko manje vrijednosti makroelemenata u usporedbi s mladim izdancima cikla u ovom radu.

Zaključak

Na podlozi 'supstrat' ostvarene su više vrijednosti Ca u ciklu te sirovih proteina i P u brokuli. Podloga 'supstrat+perlit' rezultirala je značajno višim vrijednostima sirovih proteina i K u mladim izdancima cikla i brokule, kao i P u ciklu te Ca u brokuli. U obje istraživane vrste podloga 'juta' pozitivno je utjecala na količinu suhe tvari i Mg te P u ciklu, odnosno Ca u brokuli. Bez obzira na korištenu podlogu pri uzgoju, cikla se pokazala kao bolji izvor fosfora, kalija i magnezija, a brokula sirovih proteina i kalcija.

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Macroelements content of red beet and broccoli microgreens

Abstract

Microgreens are not fully developed plants of marked nutritional value that are consumed in the cotyledon phase with or without first pair of true leaves. The aim of the research was to establish the impact of three tested growing media ('substrate', 'substrate+perlite', 'burlap') on the quantity of dry matter, crude proteins and macroelements in red beet and broccoli microgreens. The cultivation on 'substrate' medium resulted in the highest amount of phosphorus in the broccoli microgreens and potassium in the red beet microgreens. The highest values of crude proteins and potassium were identified in the cultivation of microgreens on 'substrate+perlite' medium. The use of 'burlap' medium had a positive effect on the quantity of dry matter and magnesium for both types of microgreens that were taken into account.

Keywords: *Brassica oleracea* var. *italica*, *Beta vulgaris* var. *conditiva*, microgreens, minerals, dry matter

Determination of caffeine content by UV-VIS spectrophotometer in coffee and tea samples available on Albanian market

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Abstract

The aim of this study was the determination of caffeine content in 6 coffee samples (three Arabica varieties and three Robusta varieties) and 3 tea samples (green tea, oolong tea and black tea) that are most commonly consumed in Albania. The method used for measuring caffeine content in coffee and tea samples was by using UV/vis spectrophotometer. Caffeine extraction for both matrices was conducted by using water as solvent at temperature 75°C for 20 min with ultrasound (US) mixer. The results show that caffeine content in coffee samples varied from 0.59% to 0.69% and from 0.36% to 0.4% for tea samples. Caffeine content in Arabica's samples were higher than those in Robusta samples and there were no significant changes in caffeine content between tea samples.

Keywords: Caffeine, physiological effects, Arabica and Robusta coffee, tea UV-VIS

Introduction

Caffeine is a natural alkaloid, differently known as methylxanthines, found in the leaves, seeds and fruits worldwide, i.e. tea, coffee, cocoa also in a number of foods like: beverages, soft drinks and candy bars. The content of caffeine in mentioned food categories ranges from 71–220 mg/150 mL for coffee, 32–42 mg/150 mL for tea and 32–70 mg/330 mL for cola (Debry, 1994). Caffeine consumption in different countries varies and the main source usually 80–100% of the caffeine intake comes only from coffee. In the United States and Canada the caffeine consumption from all sources is 210–238 mg/ and 400 mg/person/day in Sweden and Finland (Barone and Roberts, 1996; Viani, 1996). UK is an exception as the main source of caffeine intake, respectively 72% comes from the tea and the caffeine consumption from all sources is higher than the other countries mentioned above (Debry, 1994). Moderate caffeine consumption leads very rarely to health risks (James and Stirling, 1983). But in caffeine-sensitive individuals moderate to higher doses of caffeine induce negative effects such as anxiety, restlessness, insomnia and tachycardia (Benowitz, 1990). Starting exactly from wide range of physiological effects of caffeine on the human body and for quality controls reason the scientific community works continuously to establish rapid and cheap analytical methods for the determination of caffeine in coffee beans. Spectrophotometer is a fast, simple and relatively easy method used worldwide but with the drawback that it is not possible to determine caffeine directly in coffee beans by conventional UV absorption measurement due to the spectral overlap (Zhang et al., 2005), requiring in this way a lot of work in sample preparation. The aim of this study was the determination of caffeine content on coffee and tea samples in Albania by using UV-Vis absorption measurement.

Material and methods

Chemicals and instrument

Six different coffee sample (three Arabica and three Robusta) and three tea sample were taken from Albanian supermarket. All the coffee samples were roasting at 240°C for 14 min and ground in way to increase the contact surface with the water. The caffeine pure powder and solvents used for extraction were provided from Sigma Aldrich.

Table 1. Sample codification and description

Sample code	Sample	Sample description
A K1	Coffee Etiopia	Arabica
A K 2	Coffee Santos	Arabica
A K 3	Coffee Columbia	Arabica
R K 4	Coffee AAA	Robusta
R K 5	Coffee Vietnam	Robusta
R K 6	Coffee Uganda	Robusta
C1	Black tea	Fully oxidized, origin: China
C2	Green tea	Unoxidized, origin: China
C3	Oolong tea	Partially oxidized , origin: China

Standard solution preparation and calibration curve

The stock solution of caffeine was prepared by dissolving 10 mg of pure caffeine in 1000 mL of distilled water to obtain 10 ppm caffeine solution. Standard solution was prepared by pipetting 0, 1, 2 and 4 mL of stock solution into 10 mL volumetric flask and then filled with distilled water up to the mark. The calibration curve was done by plotting the absorbance of each standard solution, measured at maximum absorption wavelength for caffeine, 270 nm (in triplicate) using 10 mm quartz cuvettes.

Extraction of caffeine from coffee and tea

The extraction of caffeine was as follows: 100 mg of coffee/tea was placed to an Erlenmeyer flask where 100 mL water at temperature 75 °C was added. The mixture was left under stirring 20 min by using ultrasound (US) and then was filtered. Liquid–liquid extraction with dichloromethane (Belay et al., 2008) was performed in order to extract caffeine from solution due to the fact that water is a polar solvent and except caffeine, other compounds present in coffee and tea are extracted forming a matrix that interfere with caffeine at the same wavelength. Liquid – liquid extraction was repeated four time until the presence of caffeine in water solution was negligible. The absorbance of the combined dichloromethane solution was measured by UV/vis spectrophotometer (Biochrom Libra S22 UV/Vis) at 270 nm, against the corresponding reagent blank. The caffeine content in the solution was determined through the equation of standard solution. Caffeine content in coffee and tea samples was calculated from the equation:

$$X = \frac{C \cdot V}{1000 \cdot p} \cdot 100 \quad (1)$$

Where **X** - the concentration of caffeine, expressed as %; **C** - the concentration of caffeine in solution; **V** - the final volume of solution (100 mL), **L**; and **p** is the weight of coffee/tea in g.

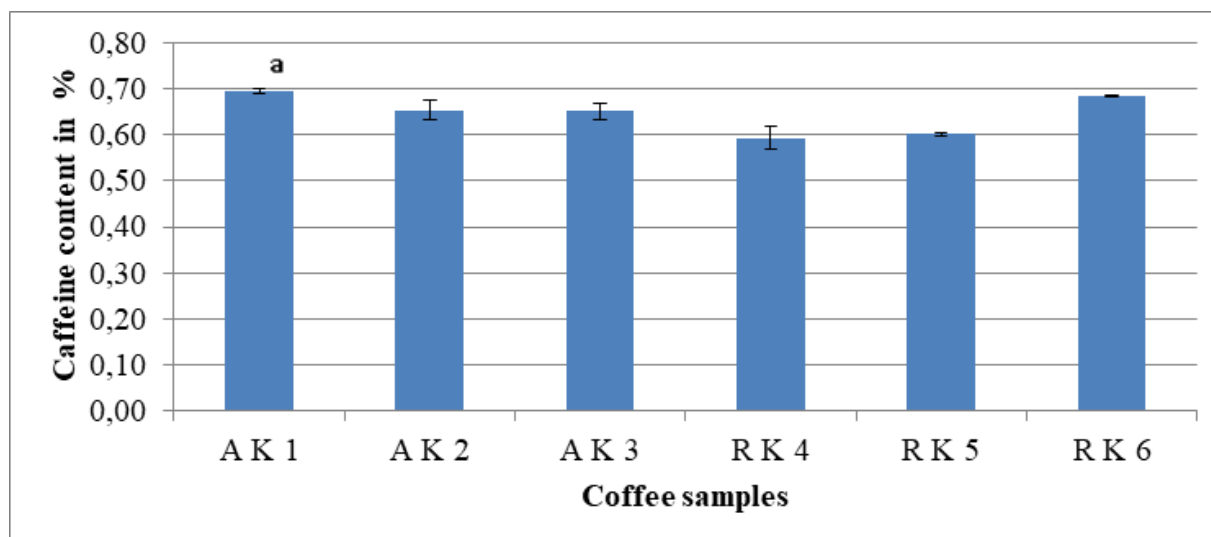
Statistical analysis

Data were analyzed using the one-way ANOVA with SPSS software (IBM SPSS Statistics V25.0). All data are expressed as means ± SD. Means were separated using Duncan test and considered significant at P<0.05.

Results and discussion

Caffeine content in six coffee samples (three Arabica varieties and three Robusta varieties) and three tea samples (green tea, oolong tea and black tea) are presented Graph 1 and 2.

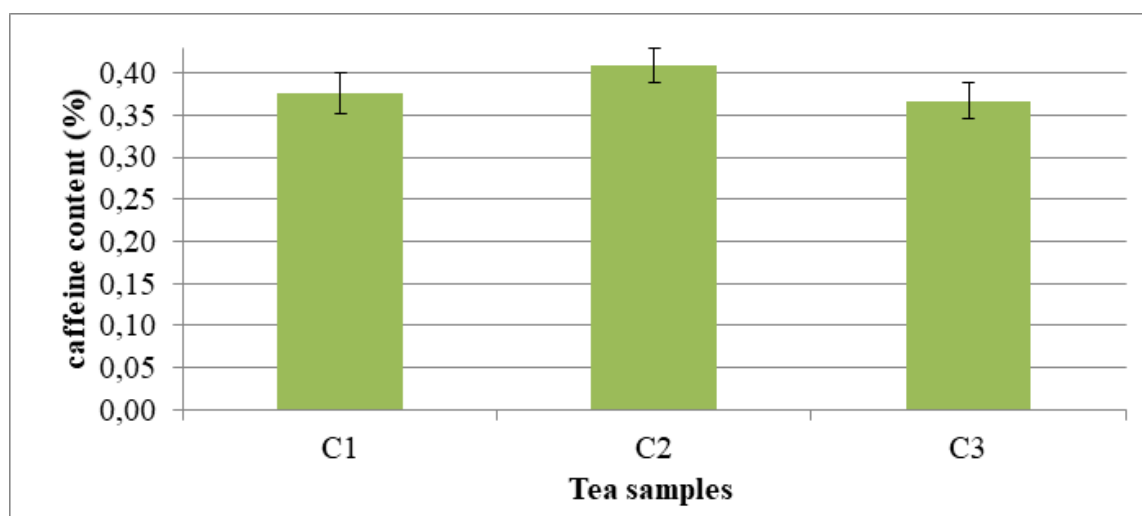
The caffeine content in coffee samples (Figure 1) ranged from 0.59% (RK5) to 0.69% (AK1). According to Duncan Test, means of caffeine content with different letters are significantly different at $P \leq 0.05$. The obtained results were lower than those reported by Belay et al. (2008) which showed the caffeine content in coffee beans ranged from 1% to 1.19%.



Means with different letters are significantly different at $P \leq 0.05$

Figure 1. Caffeine content in analyzed coffee samples

According to the results, the quantity of caffeine extracted from different varieties (Arabica and Robusta) are significantly different (Fig 1), with exception of samples AK1 and RK6 among which no significant differences in the caffeine content were not determined. Arabica varieties showed a higher caffeine content compared to the Robusta but still results were lower compared to those reported by other literature data (Farah et al., 2006) which indicated that the highest caffeine content observed in Arabic green coffee beans was 1.23% while the lowest 0.96%. Farah et al. (2008) also concluded that the roasting did not affect the caffeine content other than causing a slight relative increase due to the loss of other component.



Means with different letters are significantly different at $P \leq 0.05$

Figure 2. Caffeine content in analyzed tea samples

In tea samples the caffeine content ranged from 0.36% (C3) to 0.4% (C2) (Fig 2). Regarding the Duncan Test no significant changes in the caffeine content were determined between analyzed tee samples. But still the highest caffeine content was determined in green tea (C2), followed by black tea (C1) and oolong tea (C3). The obtained results were lower than those reported from other studies (Komes et al., 2009) which reported that the content of caffeine in green, black and oolong tea ranged from 2.04% (green tea) to 3.86% (black tea). These differences are expected as studies have revealed that caffeine content is associated to origin, genetic and environmental variability, harvest time and processing manner of plant material (Athayde et al. 2000).

Conclusion

Results shows that in coffee samples the higher content of caffeine was present in Arabica varieties compared to Robusta varieties. Coffee Arabica from Ethiopia (AK1) has the highest amount of caffeine (0.69%) and the Coffee Robusta from Vietnam (RK5) the lowest amount (0.59%). The order of caffeine content in tea samples was as follows green tea (C2) > black tea (C1) > oolong tea (C3).

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Utjecaj predsjetvenih tretmana na klijavost sjemena mrkve, peršina i kopra

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Sažetak

Cilj istraživanja bio je utvrditi utjecaj predsjetvenih tretmana ispiranjem na energiju klijanja i ukupnu klijavost sjemena mrkve, peršina i kopra. Tretmani ispiranja uključivali su destiliranu vodu, otopine kalijevog permanganata (KMnO_4) i preparata 'Eko-rast' u usporedbi s netretiranim sjemenom. Preparat 'Eko-rast' pokazao se najboljim za ispiranje inhibitora klijanja prisutnih u sjemenoj ovojnici peršina, rezultirajući opravdano najvećom ukupnom klijavašću (80,0 %). Predsjetveni tretman ispiranjem nije opravdan kod mrkve i kopra, obzirom da je najveća ukupna klijavost utvrđena u kontrolnom tretmanu (90,0 i 96,7%).

Ključne riječi: *Anethum graveolens*, *Daucus carota*, ispiranje sjemena, *Petroselinum crispum*, ukupna klijavost

Uvod

Klijanje sjemena je fiziološki proces koji počinje ako su osigurani osnovni abiotički čimbenici koji uključuju dovoljnu količinu vode, topline ili svjetlosti. Također, na klijavost utječu i biološka svojstva sjemena, poput krupnoće, dozrelosti, debljine i propusnosti sjemene ovojnice. Sjeme koje neće proklijati ni u povoljnim uvjetima smatra se dormantnim (Lešić i sur., 1993). Dormantnost je oblik biološkog prilagođavanja sjemena koji omogućuje njegovo mirovanje sve dok se ne steknu uvjeti za prekid mirovanja (Čmelik i Perica, 2007). Baskin i Baskin (2004) razlikuju fizikalnu, morfološku, morfofiziološku i fiziološku dormantnost sjemena, no molekularni i biokemijski mehanizmi postojanja i otklanjanja dormantnosti nisu još uvijek potpuno razjašnjeni (Nonogaki, 2006). Ovo svojstvo sjemena posebno je problematično u poljskim uvjetima, gdje može rezultirati manjim i nejednolikim sklopom te u konačnici manjim prinosom (Borošić i sur., 2006). U svrhu ujednačavanja klijavosti i prekidanja dormantnosti provode se postupci predsjetvenog tretiranja sjemena, poput temperaturnih tretmana (stratifikacija), oštećivanja sjemene ovojnice mehaničkim ili kemijskim putem (skarifikacija), primjene regulatora rasta te ispiranja sjemena (Žutić i Dudai, 2008; Palfi, 2007; Shakila i Rajeswari, 2006; Yildirim i sur., 2002). Slaba klijavost te dulji period klijanja utvrđeni su kod vrsta iz porodice štitarki, posebice peršina, a pripisuju se prisutnosti kemijskih inhibitora klijanja u sjemenoj ovojnici. Oni uvjetuju fiziološku dormantnost sjemena, a moguće ih je otkloniti predsjetvenim tretmanima ispiranja sjemena (Grzesik i Janas, 2014; Hassell i Kretchman, 1997). Cilj rada bio je utvrditi utjecaj predsjetvenih tretmana ispiranjem na energiju klijanja i ukupnu klijavost sjemena mrkve, peršina i kopra.

Rad je izvod iz diplomskog rada Antonie Skomrak, mag. ing. agr. naslova „Inhibitori klijanja u sjemenu mrkve, peršina i kopra i njihovo uklanjanje ispiranjem”

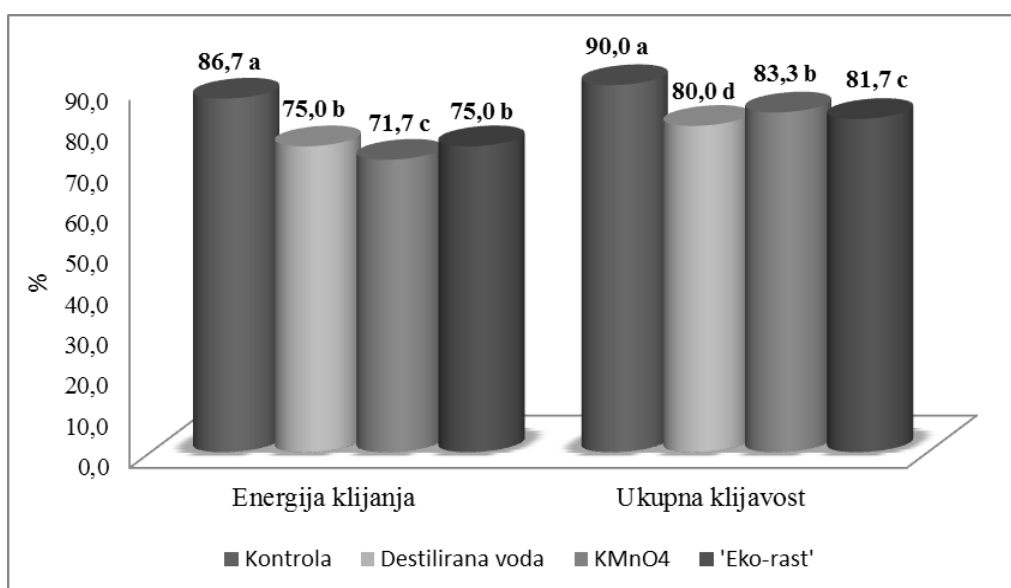
Materijal i metode

Istraživanje je provedeno u proljeće 2016. godine u Zavodu za povrćarstvo Sveučilišta u Zagrebu Agronomskog fakulteta. U pokusu je korišteno ekološko sjeme vrsta mrkve (*Daucus carota* L.), peršina (*Petroselinum crispum* L.) i kopra (*Anethum graveolens* L.) tvrtke Vilmorin. Po 100 sjemenki svake vrste močeno je u 100 ml destilirane vode, 2 %-tnoj otopini kalijevog permanganata (KMnO_4) te 0,5 %-tnoj otopini komercijalnog preparata 'Eko-rast' na bazi silicija (56 %) tvrtke MultiNatura. Ostatak sjemena bio je kontrola te nije korišten niti jedan tretman ispiranja. Nakon močenja u trajanju od 30 minuta uz povremeno miješanje, otopine su profiltrirane kroz filter papir, a sjeme svake vrste stavljeno je na sušenje na sobnu temperaturu tijekom 24 sata te potom posijano u Petrijeve posude 31. ožujka. Sjeme je stavljeno na naklijavanje u kontrolirane uvijete rasta (20 °C i 80 % RV) u klima komoru (BTESe-frigomat). Pokus je postavljen u 3 ponavljanja, pri čemu je svako tretiranje u ponavljanju bilo zastupljeno 1 Petrijevom posudom sa 20 sjemenki. Energija klijanja (vitalnost sjemena) te ukupna klijavost definirani su Pravilnikom o metodama uzorkovanja i ispitivanja kvalitete sjemena (Narodne novine 99/2008). Energija klijanja za mrkvu i kopar definirana je 4. dan nakon sjetve, a za peršin 8. dan nakon sjetve. Ukupna klijavost za sve tri vrste definirana je 15. dan iza sjetve. Statistička obrada podataka provedena je u programu SAS Software v. 9.3 (2010), procedura PROC GLM (opći linearni model). Podaci za energiju klijanja te ukupnu klijavost obrađeni su analizom varijance (ANOVA), a značajnost razlika među srednjim vrijednostima testirana je LSD testom na razini značajnosti $p \leq 0,01$.

Rezultati i rasprava

U grafikonima 1-3 prikazan je utjecaj predstjetvenog tretiranja ispiranjem na energiju klijanja (EK) i ukupnu klijavost (UK) sjemena mrkve, peršina i kopra. Iz grafikona je vidljivo da je utjecaj tretmana bio statistički opravdan ($p \leq 0,01$) za energiju klijanja i ukupnu klijavost sjemena navedenih vrsta. Kod mrkve (grafikon 1), najveći postotak klijanja (EK 86,7 %; UK 90,0 %) utvrđen je u kontroli, što znači da su primijenjeni tretmani inhibirali klijanje sjemena mrkve. Predstjetveno ispiranje destiliranom vodom i preparatom 'Eko-rast' rezultiralo je statistički jednakom EK (75,0 %), opravdano većom u usporedbi s KMnO_4 (71,7 %) koji je negativno utjecao na vitalnost sjemena. Suprotno tome, KMnO_4 je rezultirao opravdano većom ukupnom klijavašću (83,3 %) u odnosu na preparat 'Eko-rast' (81,7 %) i destiliranu vodu (80,0 %). Slabi učinak destilirane vode kao predstjetvenog tretmana također su utvrdili Žutić i Dudai (2008) proučavajući klijavost kadulje.

Suprotno mrkvi, najveća energija klijanja za peršin (Grafikon 2) utvrđena je pri ispiranju s KMnO_4 (35,0 %), a najmanja kod sjemena bez ispiranja (8,3 %). Preostala dva predstjetvena tretmana imala su pozitivan učinak na vitalnost sjemenu, pri čemu je ispiranje destiliranom vodom bilo djelotvornije (25,0 %) u odnosu na 'Eko-rast' (21,7 %). Suprotno tome, Borošić i sur. (1995) navode da razlika u energiji klijanja kod peršina ispiranog destiliranom vodom i kontrolnog sjemena nije bila statistički opravdana.

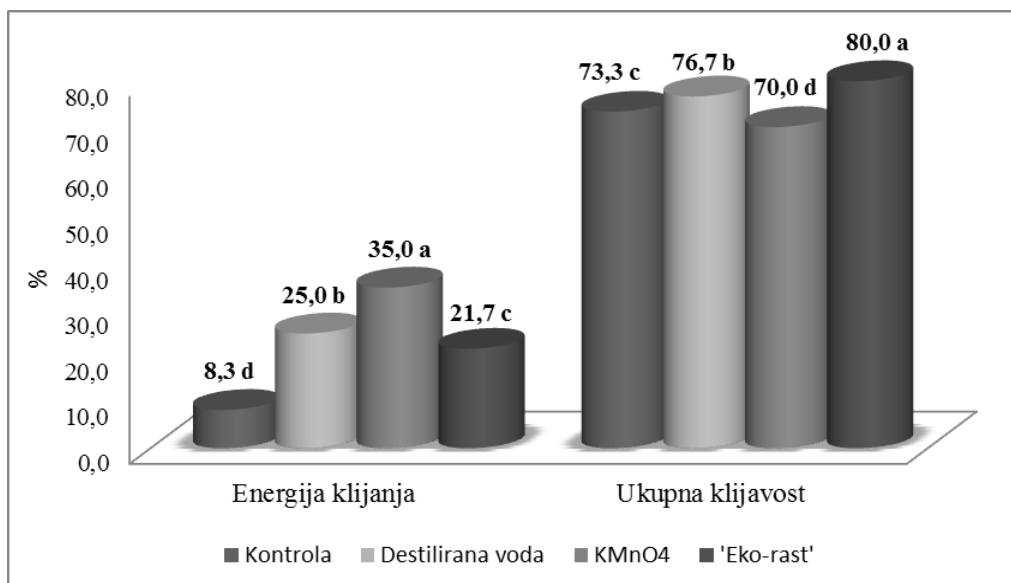


Različita slova pridodana vrijednostima energije klijanja i ukupne klijavost označavaju razlike između tretmana, $p \leq 0,01$

Grafikon 1. Utjecaj predstjetvenih tretmana na energiju klijanja i ukupnu klijavost sjemena mrkve

Utjecaj predsjetvenih tretmana na klijavost sjemena mrkve, peršina i kopra

Kod ukupne klijavosti trend je nešto drugačiji te je najveću klijavost imalo sjeme močeno u otopini preparata 'Eko-rast' (80,0 %), a najmanju u otopini $KMnO_4$ (70,0 %).

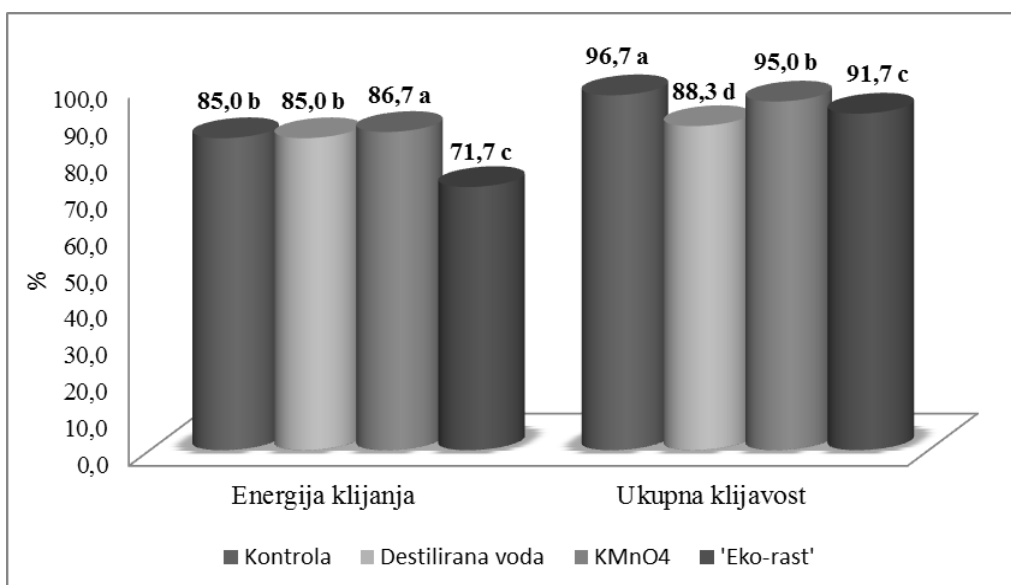


Različita slova pridodana vrijednostima energije klijanja i ukupne klijavost označavaju razlike između tretmana, $p \leq 0,01$

Grafikon 2. Utjecaj predsjetvenih tretmana na energiju klijanja i ukupnu klijavost sjemena peršina

Pozitivno djelovanje navedenog preparata također potvrđuju Samobor i sur. (2010) na pšenici, dok Megla (2015) zaključuje da njegova primjena u uzgoju presadnica buhača nije opravdana. Učinak destilirane vode i dalje je bio pozitivan (76,7 %), što potvrđuju Grzesik i Janas (2014) u istraživanju na peršinu.

Kod kopra (Grafikon 3) je, kao i kod peršina, opravdano najveća vitalnost sjemena utvrđena je primjenom otopine $KMnO_4$ (86,7 %). Kontrolno sjeme te močeno u destiliranoj vodi imalo je jednaku energiju klijanja (85,0 %), ali statistički veću od preparata 'Eko-rast' (71,7 %) koji je djelovao inhibitorno na početno klijanje kopra. Najveća ukupna klijavost kopra utvrđena je bez primjene tretmana ispiranja (96,7 %), što je bio slučaj i kod mrkve. Destilirana voda inhibirala je klijanje kopra (88,3 %), dok su pozitivan učinak imale otopine $KMnO_4$ i preparata 'Eko-rast' (95,0 i 91,7 %).



Različita slova pridodana vrijednostima energije klijanja i ukupne klijavost označavaju razlike između tretmana, $p \leq 0,01$

Grafikon 3. Utjecaj predsjetvenih tretmana na energiju klijanja i ukupnu klijavost sjemena kopra

Zaključci

Ovim istraživanjem potvrđena je opravdanost primjene predstjetvenih tretmana na peršinu, pri čemu je najbolji učinak na povećanje klijavosti imalo ispiranje sjemena destiliranom vodom i preparatom 'Eko-rast'. Predstjetveno tretiranje sjemena ispiranjem nije opravdano kod vrsta mrkve i kopra, obzirom na utvrđenu najveću ukupnu klijavost u netretiranom sjemenu.

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Effect of pre-sowing treatment on seed germination of carrot, parsley and dill

Abstract

The aim of the study was to determine the effect of pre-sowing treatments on germination energy and total germination of carrot, parsley and dill seeds. The soaking treatments included distilled water, KMnO_4 and 'Eko-rast' compared to untreated seeds. The 'Eko-rast' preparation proved to be the best for flushing out the inhibitors present in the parsley seed coat, resulting in justifiably the highest total germination (80.0%). It proved not economically justifiable to treat carrot and dill seeds, since the highest percentage of germination rate was recorded in control variants (90.0 and 96.7%).

Keywords: *Anethum graveolens*, *Daucus carota*, seed soaking, seed germination, *Petroselinum crispum*

Ispitivanje klijavosti sjemena pustenaste divizme (*Verbascum phlomoides* L.) na različitim podlogama

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Sažetak

Cilj rada bio je utvrditi utjecaj predtretmana grijanja i hlađenja na klijavost sjemena pustenaste divizme (*Verbascum phlomoides* L.) na različitim podlogama. Sjeme je podvrgnuto predtretmanima grijanja u sušioniku na 35 °C i hlađenja u hladnjaku na 4 °C tijekom sedam dana prije postavljanja pokusa. Klijavost sjemena ispitana je na 3 podloge u Petrijevim zdjelicama: filter papir, supstrat te pijesak. Kako bi se utvrdio utjecaj predtretmana i podloga po završetku istraživanja uz ukupnu klijavost zabilježeni su i morfološki parametri. Općenito, predtretmani grijanja i hlađenja sjemena značajno su utjecali na klijavost, dok su podloge utjecale značajno na ostale ispitivane parametre.

Ključne riječi: hlađenje, grijanje, supstrat, pijesak

Uvod

Pustenasta divizma (*Verbascum phlomoides* L.) jedna je od 21 vrste roda *Verbascum* zabilježene u flori Republike Hrvatske (RH) (Nikolić, 2015.). Rasprostranjena je u gotovo svim dijelovima RH, a najveća koncentracija staništa zabilježena je u centralnoj Hrvatskoj. U svijetu je poznato najmanje 250 vrsta ove dvogodišnje biljke iz porodice *Scrophulariaceae*. Pustenasta divizma je biljka koja u prvoj godini stvara lisnu rozetu karakterističnu po mnoštvu dlačica, a u drugoj godini cvjetnu stabljiku sastavljenim od 2 do 9 cvjetova (Marian i sur., 2018.). Cvjeta od srpnja do rujna kada se i sakupljaju žuti cvjetovi cijenjeni kao prirodni lijek za rješavanje respiratornih tegoba. Ekspektoransko djelovanje saponina uz umirujući efekt služi čine cvjetove divizme jednom od najkorisnijih ljekovitih droga za liječenje promuklosti, kašlja, bronhitisa i astme (Turker i Gurel, 2005.). Također, neka od istraživanja potvrđuju protuupalno i antimikrobno djelovanje (Grigore i sur., 2013.; Speranza i sur., 2009.). Prema Tatli i Akdemir (2004.) vrste roda *Verbascum* koriste se u liječenju hemeroida, reumatske boli, površinskih gljivičnih infekcija, proljeva pa čak i virusa gripe. Ljekovita svojstva ove biljke pripisuju se sadržaju biološki aktivnih spojeva poput iridoida, saponina, flavonoida, feniletanoida i neolignanskih glikozida (Armatu i sur., 2011.). Općenito, sadržaj biološki aktivnih komponenti je pod utjecajem mnogih čimbenika od kojih su, posebice za samoniklo bilje, ključni okolišni čimbenici (Ncube i sur., 2012.). Preduvjet za uspješan razvoj zdravih biljaka te prevladavanje nepovoljnog utjecaja okolišnih čimbenika je dobra klijavost sjemena i pravilan razvoj klijanaca. Predtretmani sjemena mogu povoljno utjecati na poboljšanje klijavosti pa je i cilj ovog istraživanja bio ispitati utjecaj predtretmana grijanjem i hlađenjem na klijavost sjemena pustenaste divizme na različitim podlogama.

Materijal i metode

Istraživanje je provedeno tijekom 2019. godine u laboratoriju za Povrčarstvo, cvjećarstvo i ljekovito bilje Fakulteta agrobiotehničkih znanosti Osijek. Sjeme pustenaste divizme prikupljeno je u Jarmini kod hobi proizvođača 2018. godine te spremljeno u papirnate vrećice na suho mjesto do ispitivanja klijavosti. Prije postavljanja pokusa odvojen je dio sjemena za predtretman hlađenje (H) pri kojem su sjemenke držane u hladnjaku 7 dana na temperaturi 4 °C i predtretman grijanje (G) pri kojem su sjemenke držane 7 dana u sušioniku na temperaturi 35 °C. Nakon provedenih predtretmana sjemenke pustenaste divizme stavljene su na naklijavanje na 3 različite podloge u Petrijeve zdjelice:

filter papir, pijesak i supstrat. Filter papir je postavljen na dno Petrijeve zdjelice kako bi sjeme klijalo na papiru, pijesak je prethodno prosijan i steriliziran, a kao supstrat je korišten Klasmann potgrond H. Na svaku podlogu postavljeno je u 4 ponavljanja po 100 sjemenki oba predtretmana (H i G) te kontrolni tretmana (K) odnosno netretirano sjeme. Sve Petrijeve zdjelice zalivene su s 5 mL destilirane vode pri postavljanju pokusa i po potrebi tijekom 12 dana koliko je trajalo istraživanje. Pokus je postavljen u klima komoru na temperaturu 20 ± 1 °C i fotoperiod 12h dan/12h noć. Po završetku istraživanja zabilježene su ukupna klijavost, dužina hipokotila, dužina korijena klijanaca te svježa i suha masa klijanaca. Dobiveni rezultati statistički su obrađeni analizom varijance pomoću statističkog paketa SAS 9.3 ($p < 0,05$, Fisher test).

Rezultati i rasprava

U ovom istraživanju ispitan je utjecaj predtretmana sjemena i različitih podloga na klijavost sjemena pustenaste divizme. Predtretmani grijanja i hlađenja sjemena značajno su utjecali na klijavost sjemena, dok ali nisu imali utjecaj na vrijednosti ostalih ispitivanih parametara. Najveća klijavost zabilježena je kod predtretmana H te je iznosila 89,75 %, dok je najmanja zabilježena na kontrolnom tretmanu K 79,25 % (tablica 1.).

Tablica 1. Utjecaj predtretmana sjemena pustenaste divizme na ispitivane parametre

Predtretman	Klijavost (%)	Dužina korijena (cm)	Dužina hipokotila (cm)	Svježa masa klijanca (mg)	Suha masa klijanca (mg)
Grijanje (G)	83,58 ^b	1,05 ^a	0,76 ^a	0,0901 ^a	0,0110 ^a
Hlađenje (H)	89,75 ^a	1,03 ^a	0,76 ^a	0,0863 ^a	0,0286 ^a
Bez tretmana (K)	79,25 ^c	1,06 ^a	0,75 ^a	0,0836 ^a	0,0116 ^a

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički se značajno se razlikuju pri razini ($P < 0,05$)

Općenito je predtretman hlađenjem na svim ispitivanim podlogama utjecao na povećanje klijavosti pustenaste divizme (tablica 1 i 3). Slično, u istraživanju Hilooğlu i sur. (2018.) predtretman hlađenjem na 4 °C utjecao je na povećanje klijavosti *Verbascum calycosum* gotovo 50 % u odnosu na kontrolni tretman. U ovom istraživanju neovisno o podlozi zabilježeno je povećanje klijavosti od 11 % kod predtretmana hlađenjem, odnosno 18 % na filter papiru.

Tablica 2. Utjecaj podloge na ispitivane parametre pustenaste divizme

Podloga	Klijavost (%)	Dužina korijena (cm)	Dužina hipokotila (cm)	Svježa masa klijanca (mg)	Suha masa klijanca (mg)
Filter papir (NP)	85,00 ^a	1,49 ^a	0,40 ^c	0,062 ^c	0,0070 ^b
Supstrat (S)	82,17 ^a	1,21 ^b	1,22 ^a	0,112 ^a	0,0113 ^{ab}
Pijesak (P)	85,41 ^a	0,44 ^c	0,66 ^b	0,087 ^b	0,0330 ^a

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju pri razini ($P < 0,05$)

Suprotno predtretmanima, među ispitivanim podlogama nije zabilježena razlika u klijavosti, no zabilježena je u svim ostalim parametrima (tablica 2). Na filter papiru najveća je dužina korijena (1,49 cm), na supstratu najveće dužina hipokotila (1,22 cm) i svježa masa klijanca (0,112 mg) te na pijesku najveća suha masa klijanca (0,0330 mg).

Na podlozi filter papir u kombinaciji s predtretmanom hlađenja utvrđena je značajno najveća klijavost sjemena pustenaste divizme (95 %), što je ujedno i najveća postignuta klijavost u pokusu (tablica 3). Predtretman grijanjem također je pozitivno utjecao na klijavost u odnosu na kontrolni tretman no nije zabilježena statistički značajna razlika. Među ostalim ispitivanim parametrima na filter papiru nije zabilježena razlika u odnosu na predtretmane, što je pokazatelj kako ne postoji negativan utjecaj predtretmana na daljnji razvoj biljaka.

Tablica 3. Utjecaj predtretmana na ispitivane parametre pustenaste divizme ovisno o podlozi

Predtretman	Klijavost (%)	Dužina korijena (cm)	Dužina hipokotila (cm)	Svježa masa klijanca (mg)	Suha masa klijanca (mg)
Filter papir					
Grijanje (G)	82,25 ^b	1,40 ^a	0,39 ^a	0,0612 ^a	0,0065 ^a
Hlađenje (H)	95,00 ^a	1,59 ^a	0,39 ^a	0,0583 ^a	0,0073 ^a
Bez tretmana (K)	77,75 ^b	1,47 ^a	0,41 ^a	0,0661 ^a	0,0070 ^a
Supstrat					
Grijanje (G)	81,75 ^a	1,21 ^a	1,22 ^a	0,1163 ^a	0,0115 ^a
Hlađenje (H)	85,75 ^a	1,11 ^a	1,23 ^a	0,0999 ^a	0,0108 ^a
Bez tretmana (K)	79,00 ^a	1,31 ^a	1,20 ^a	0,1185 ^a	0,0116 ^a
Pijesak					
Grijanje (G)	86,75 ^{ab}	0,52 ^a	0,66 ^a	0,0928 ^a	0,0148 ^b
Hlađenje (H)	88,50 ^a	0,40 ^b	0,66 ^a	0,1009 ^{ab}	0,0677 ^a
Bez tretmana (K)	81,00 ^b	0,41 ^b	0,65 ^a	0,0663 ^b	0,0163 ^b

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju pri razini ($P < 0,05$)

Na podlozi supstrat nije utvrđena značajna razlika u vrijednostima niti jednog ispitivanog parametra. Kod podloge pijesak utvrđena je značajno slabija klijavost netretiranog sjemena (K) u odnosu na predtretman hlađenjem. Također, kod dužine korijena utvrđena je značajno veća vrijednost kod predtretmana grijanjem u odnosu na ostale predtretmane. Isik i sur. (2017.) navode kako hladno-vlažni predtretman djeluje stimulirajuće na klijavost nekih od *Verbascum* vrsta, no i kako je biogeografska regija određenih vrsta povezana s potrebom za takvim predtretmanom.

Kod predtretmana hlađenjem utvrđen je značajan utjecaj podloge na sve ispitivane parametre (tablica 4). Najslabija klijavost zabilježena je na supstratu (85,75 %). Kod dužine korijena i hipokotila zabilježene su značajne razlike između sve tri podloge. Najveća dužina korijena zabilježena je kod klijanaca na filter papiru (1,59 cm), dok je najveća dužina hipokotila kod klijanaca na supstratu (1,23 cm). Značajno manja vrijednost svježe mase klijanaca zabilježena je na filter papiru u odnosu na ostale podloge. Nadalje, značajno veća vrijednost suhe mase klijanaca zabilježena je na pijesku u odnosu na druge ispitivane podloge.

Tablica 4. Utjecaj podloge na ispitivane parametre pustenaste divizme ovisno o predtretmanu

Podloga	Klijavost (%)	Dužina korijena (cm)	Dužina hipokotila (cm)	Svježa masa klijanca (mg)	Suha masa klijanca (mg)
Hlađenje (H)					
Filter papir (NP)	95,00 ^a	1,59 ^a	0,39 ^c	0,0583 ^b	0,0073 ^b
Supstrat (S)	85,75 ^b	1,11 ^b	1,23 ^a	0,0999 ^a	0,0108 ^b
Pijesak (P)	88,50 ^{ab}	0,40 ^c	0,66 ^b	0,1009 ^a	0,0677 ^a
Grijanje (G)					
Filter papir (NP)	82,25 ^a	1,40 ^a	0,39 ^c	0,0612 ^b	0,0065 ^c
Supstrat (S)	81,75 ^a	1,21 ^a	1,22 ^a	0,1163 ^a	0,0115 ^b
Pijesak (P)	86,75 ^a	0,52 ^b	0,66 ^b	0,0928 ^a	0,0148 ^a
Bez tretmana (K)					
Filter papir (NP)	77,75 ^a	1,47 ^a	0,41 ^c	0,0661 ^b	0,0070 ^b
Supstrat (S)	79,00 ^a	1,31 ^a	1,20 ^a	0,1185 ^a	0,0116 ^{ab}
Pijesak (P)	81,00 ^a	0,41 ^b	0,65 ^b	0,0663 ^b	0,0163 ^a

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju pri razini ($P < 0,05$)

Kod predtretmana grijanjem utvrđen je značajan utjecaj podloge na dužinu korijena i hipokotila te na svježiu i suhu masu klijanaca. Na podlozi pijesak značajno je kraći korijen u odnosu na ostale podloge. Dužina hipokotila i suha masa klijanaca značajno su se razlikovale među sve tri podloge. Značajno manja svježia masa klijanaca zabilježena je na filter papiru u odnosu na ostale podloge. Najbolji pokazatelj utjecaja podloga vidljiv je kod kontrolnog tretmana K jer nema utjecaja predtretmana. Značajan utjecaj podloge utvrđen je kod svih ispitivanih parametara osim klijavosti. Suprotno, u istraživanju Lekić i sur. (2011) zabilježen je negativan utjecaj pijeska kao podloge za klijavost divlje artičoke (*Cynara cardunculus*). Najveća dužina korijena zabilježena je na filter papiru (1,47 cm), a najmanja na pijesku (0,41 cm). Značajno duži hipokotil i veća svježia masa klijanaca zabilježene su na podlozi supstrat u odnosu na ostale podloge. U istraživanju Güleriyüz i sur. (2016) utvrđena dužina korijena klijanaca endemskih *Verbascum* spp. na filter papiru je $13,0 \pm 2,6$ mm, a dužina hipokotila $2,5 \pm 0,1$ mm, što je u skladu s rezultatima ovog istraživanja.

Zaključak

Na osnovi dobivenih podataka može se zaključiti kako je jedino predtretman hlađenjem imao pozitivan učinak na klijavost pustenaste divizme na svim podlogama. Različite podloge utjecale su više na razvoj klijanaca te je na supstratu zabilježena najveća dužina hipokotila klijanaca, a na pijesku, unatoč manjoj dužini hipokotila i korijena klijanca, najveća suha masa klijanca. Iako je klijavost sjemena pustenaste divizme zadovoljavajuća i bez predtretmana, ovo istraživanje potvrdilo je mogućnost bolje iskoristivosti sjemena pri sjetvi uz fizički i financijski nezahtjevan predtretman sjemena hlađenjem.

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Germination test of woolly mullein (*Verbascum phlomoides* L.) on different growth media

Abstract

The aim of the study was to determine the influence of heating and cooling pretreatment on germination of the woolly mullein (*Verbascum phlomoides* L.) on different growth media. The seeds were subjected to pre-treatment at 35 °C in the oven or cooled at 4 °C in the refrigerator for seven days prior the experiment set up. The germination test was conducted on 3 different growth media in Petri dishes: filter paper, substrate and sand. In order to determine the effect of pre-treatment and growth media the values of total germination and morphological parameters were recorded. In general, pre-treatments of heating and cooling seeds significantly influenced germination, while the substrates significantly influenced the other parameters tested.

Keywords: cooling, heating, substrate, sand

Učinak vermikomposta na morfološka svojstva, prinos i mineralni sastav ploda paprike

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Sažetak

Cilj istraživanja bio je utvrditi učinak gnojidbe vermikompostom na morfološka svojstva, prinos i mineralni sastav ploda paprike te kemijske karakteristike tla. Poljski pokus s paprikom sorte 'Slonovo uho' imao je četiri gnojidbena tretmana: mineralna gnojiva (MG); peletirani pileći gnoj 'Orgevit' i MG (PPG+MG); vermikompost 'Lumbri humus' i MG (VK+MG); VK+MG i vodeni ekstrakt vermikomposta (VK+MG+VEVK). Relativno najveći udio tržnih plodova i tržni prinos imao je tretman VK+MG+VEVK, odnosno VK+MG. Značajno najveću količinu suhe tvari i dušika ostvario je tretman PPG+MG, a kalcija tretman VK+MG. Tretmani s vermikompostom najviše su povećali sadržaj humusa i količinu dušika u tlu, a najmanje su utjecali na promjenu pH vrijednosti tla.

Ključne riječi: *Capsicum annuum*, makroelementi, mineralna gnojiva, peletirani pileći gnoj, vodeni ekstrakt vermikomposta

Uvod

Degradacija fizikalno-kemijskih karakteristika tla posljedica je intenzivne poljoprivredne proizvodnje, posebice pri korištenju mineralnih gnojiva kao jedinog izvora biljnih hranjiva. Sa svrhom veće dostupnosti, jednostavnijeg transporta i primjene, životinjski gnoj se dehidriranjem prerađuje u stabilan proizvod u obliku peleta ili granula. Među alternativnim organskim gnojivima ističe se vermikompost (glisnjak, lumbrihumus) kao stabilan, netermofilan proizvod nastao biološkom razgradnjom organskog materijala interakcijom glista i mikroorganizama. Castellanos i sur. (2017) navode da vermikompost može imati veću hranidbenu vrijednost od klasičnog komposta kod kojeg tijekom dužeg procesa termofilnog kompostiranja dolazi do gubitka biljnih hranjiva, posebice dušika. Prema Sinha i sur. (2009) vermikompost prosječno sadrži 2-3 % N, 1,55-2,25 % P i 1,85-2,25 % K, a mineralni sastav najviše ovisi o polaznom materijalu. Isti autori ističu da su pozitivne reakcije biljaka na gnojidbu vermikompostom posljedica viših razina biljnih hormona i regulatora rasta povezanih s većom količinom huminskih kiselina i humata, a ne samo biljnih hranjiva iz ovog gnojiva. Prema Edwards i sur. (2004) gnojidba vermikompostom doprinosi suzbijanju nematoda i zaustavlja razvoj biljnih bolesti uzrokovanih patogenima iz tla, a Marin i sur. (2013) navode da i vodeni ekstrakt vermikomposta primijenjen folijarno ili fertirigacijom također sprečava razvoj ovih bolesti. Aminifard i Bayat (2016) navode da se najveći prinos paprike postiže dozom vermikomposta od 5 t/ha, dok povećanje na 10 i 15 t/ha ne rezultira daljnjim povećanjem prinosa. Jamir i sur. (2017) su utvrdili da gnojidbena kombinacija koja osigurava dušik iz vermikomposta i mineralnog NPK gnojiva (1:1), ostvaruje značajno veće vrijednosti pokazatelja rasta i sastavnica prinosa paprike u odnosu na gnojidbu isključivo mineralnim gnojivom te različitim odnosom mineralnog gnojiva i zrelog goveđeg ili pilećeg gnoja. Stoga je istraživanje imalo za cilj utvrditi učinak gnojidbe vermikompostom i vodenim ekstraktom vermikomposta na morfološka svojstva, prinos i mineralni sastav ploda paprike te kemijske karakteristike tla.

Rad je izvod iz diplomskog rada Leopolda Prlića, mag. ing. agr. naslova 'Utjecaj vermikomposta i peletiranog pilećeg gnoja na prinos i kvalitetu ploda paprike'.

Materijal i metode

Istraživanje s vermikompostom provedeno je 2018. godine na pokušalištu Maksimir Zavoda za povrćarstvo Sveučilišta u Zagrebu Agronomskog fakulteta (SuZAF). Monofaktorijelni gnojidbeni poljski pokus sa sortom paprike 'Slonovo uho' tvrtke Superior Seeds postavljen po metodi slučajnog blokno rasporeda u tri ponavljanja uključivao je četiri gnojidbena tretmana. Tretmani su imali jednaku količinu (kg/ha) glavnih makrohranjiva (220 N, 150 P₂O₅ i 320 K₂O), ostvarenu kombinacijama gnojiva u osnovnoj gnojidbi (mineralna gnojiva /MG/; peletirano pileće gnojivo 'Orgevit' i MG /PPG+MG/; vermikompost 'Lumbri humus' i MG /VK+MG/; VK, MG i vodeni ekstrakt vermikomposta /VK+MG+VEVK/) te jednakom prihranom fertirigacijom (Poly-feed gnojiva i Ca(NO₃)₂) kojom je osigurano 50 % potrebnog dušika, 32 % P₂O₅ i 57 % K₂O. Peletirano pileće gnojivo 'Orgevit' (pH 7 i 65 % organske tvari) nizozemske tvrtke 'MeMon', korišteno je u dozi od 250 g/m². Vermikompost 'Lumbri humus' (pH 7,4 i 47 % organske tvari), proizvod OPG-a Marko Pejić dobiven preradom govedeg stajskog gnoja aktivnošću kalifornijskih glista, primijenjen je u dozi od 250 g/biljci, odnosno 625 g/m². MG i PPG inkorporirani su u tlo prije postavljanja sustava za navodnjavanje kapanjem i crnog PE-malča, dok je vermikompost dodan prilikom sadnje uz svaku presadnicu. Vodeni ekstrakt vermikomposta (10:1) primijenjivan je nakon sadnje svakih 10 dana (0,5 l/biljci) kao folijarna prihrana u tretmanu VK+MG+VEVK. Sadnja presadnica paprike s grudom supstrata obavljena je 21. svibnja u dvoredne trake (razmak redova 0,5 m, biljaka u redu 0,5 m i traka 1,0 m; sklop 2,5 biljke/m²). Osnovna parcela imala je površinu 7,2 m² i 18 biljaka, a obračunska parcela 4 m² i 10 biljaka. Za potrebe kemijske analize tla provedene u Zavodu za ishranu bilja SuZAF-a, uzeti su uzorci tla (dubina 0 do 30 cm) prije postavljanja pokusa te po završetku berbe sa svake osnovne parcele. Provedene su dvije berbe plodova paprike u fiziološkoj zrelosti (17. kolovoz i 17. rujan) tijekom kojih je analizirano: broj tržnih i netržnih plodova te morfološka svojstva tržnih plodova (dužina, širina, masa, debljina perikarpa). Utvrđen je udio tržnih i netržnih plodova te prinos tržnih plodova. U Analitičkom laboratoriju Zavoda za ishranu bilja (SuZAF) na reprezentativnim uzorcima plodova svakog tretmana utvrđena je ukupna količina dušika i glavnih minerala u suhoj tvari (ST): dušik metodom po Kjeldahlu, fosfor spektrofotometrijski, kalij plamenfotometrijom te kalcij i magnezij atomskom apsorpcijskom spektrometrijom (AOAC, 1995). Podaci za varijable kemijskih analiza i morfoloških svojstava su statistički obrađeni analizom varijance (ANOVA). Značajnost razlika između srednjih vrijednosti testirana je primjenom LSD testa. Statistička analiza podataka provedena je programom SAS verzija 9.3.

Rezultati i rasprava

Prema podacima Državnog hidrometeorološkog zavoda RH za mjernu postaju Maksimir (podaci nisu prikazani), meteorološki uvjeti za uzgoj paprike nisu bili optimalni. Visoke temperature odmah nakon sadnje bile su stresne, a 12. lipnja bila je jaka tuča nakon koje je uslijedilo kišno i hladnije razdoblje koje je pogodovalo razvoju bolesti i odgodilo početak plodonošenja. Veći dio srpnja i cijeli kolovoz bili su izrazito sušni s prevladavajućom maksimalnom temperaturom iznad 30 °C, uslijed čega je došlo do slabije cvatnje i jačeg abortiranja plodova.

Gnojidbeni tretmani u obje berbe nisu značajno utjecali na promatrana morfološka svojstva ploda (Tablica 1), što je u skladu s rezultatima koje navodi Alhrout (2017). Neovisno o gnojidbi, u prvoj berbi plodovi su bili krupniji, odnosno prosječne vrijednosti bile su veće za: 2,2 cm (dužina), 1,0 cm (širina), 55 g (masa) i 1 mm (debljina perikarpa).

Tablica 1. Učinak gnojidbe na morfološka svojstva ploda paprike

Gnojidbeni tretmani	Dužina (cm)		Širina (cm)		Masa (g)		Debljina perikarpa (mm)	
	Berba							
	1.	2.	1.	2.	1.	2.	1.	2.
MG	12,7	10,8	6,6	5,5	150	100	6,3	5,4
PPG+MG	12,2	10,9	6,2	5,4	130	80	6,2	5,3
VK+MG	13,3	10,2	6,5	5,3	150	90	6,5	5,3
VK+MG+VEVK	13,2	10,7	6,5	5,5	140	80	6,3	5,5

Legenda: MG – mineralna gnojiva; PPG+MG – peletirano pileće gnojivo i mineralna gnojiva; VK+MG – vermikompost i mineralna gnojiva; VK+MG+VEVK – vermikompost, mineralna gnojiva i vodeni ekstrakt vermikomposta

Gnojidbeni tretmani nisu imali opravdan utjecaj na broj tržnih i netržnih plodova kao niti na tržni prinos paprike (Tablica 2). Relativno najviše tržnih plodova u prvoj i drugoj berbi ubrano je pri tretmanu VK+MG+VEVK (7,5 i 5,1), a najmanje pri tretmanu MG (6,4 i 3,9), što odgovara navodima Ghnomae i Shafeek (2005) o većem broju tržnih plodova pri organskoj i organomineralnoj gnojidbi u odnosu na isključivo mineralnu. Gnojidbeni tretman VK+MG, iako s nešto manjim brojem tržnih plodova, zbog veće mase ploda u obje berbe, ostvario je relativno najveći tržni prinos u prvoj i drugoj berbi kao i ukupni (1,50, 1,18 i 2,68 kg/m²). Sukladno istraživanju Adhikari i sur. (2016) utvrđen je pozitivan učinak vermikomposta obzirom na veće vrijednosti tržnog prinosa pri tretmanima VK+MG i VE+MG+VEVK, za 21 i 17 % u odnosu na tretman PPG+MG te za 11 i 6 % u odnosu na tretman MG. Prosječno, u prvoj berbi tržni prinos bio je 21,4 % veći nego u drugoj berbi.

Tablica 2. Učinak gnojidbe na broj tržnih i netržnih plodova po m² i tržni prinos paprike

Gnojidbeni tretmani	Broj tržnih plodova		Broj netržnih plodova		Tržni prinos (kg/m ²)		Ukupni
	Berba						
	1.	2.	1.	2.	1.	2.	
MG	6,4	3,9	1,7	1,0	1,38	1,01	2,39
PPG+MG	7,4	4,1	1,9	1,1	1,24	0,88	2,12
VK+MG	7,1	4,6	1,5	1,4	1,50	1,18	2,68
VK+MG+VEVK	7,5	5,1	1,2	1,3	1,42	1,13	2,55

Legenda: MG – mineralna gnojiva; PPG+MG – peletirano pileći gnojivo i mineralna gnojiva; VK+MG – vermikompost i mineralna gnojiva; VK+MG+VEVK – vermikompost, mineralna gnojiva i vodeni ekstrakt vermikomposta

Na količinu suhe tvari (ST) u plodu paprike u prvoj i drugoj berbi, gnojidbeni tretmani nisu imali opravdan učinak (Tablica 3). U obje berbe pri tretmanu PPG+MG plodovi su imali relativno najveću količinu ST (7,79 i 8,80 %), dok je gnojidba samo mineralnim gnojivima rezultirala većom količinom ST u odnosu na gnojidbene tretmane s vermikompostom. Plodovi paprike u drugoj berbi imali su prosječno 0,75 % više ST nego u prvoj berbi. Iz tablice 3. vidljiv je opravdan učinak gnojidbenih tretmana i na količinu dušika, kalcija i magnezija u plodovima iz prve berbe te dušika, fosfora, kalija i kalcija iz druge berbe. U prvoj berbi značajno veća količina dušika utvrđena je pri tretmanima PPG+MG i VK+MG+VEVK, kalcija pri tretmanu VK+MG te magnezija pri svim tretmanima izuzev MG. Relativno najveću količinu fosfora i kalija u prvoj berbi imali su plodovi iz gnojidbenog tretmana PPG+MG. U drugoj berbi, jedino je gnojidbeni tretman VK+MG pripadao rangovima sa značajno najvećom količinom četiri (dušik, fosfor, kalij i kalcij) od pet testiranih makroelemenata.

Tablica 3. Učinak gnojidbe na količinu suhe tvari (ST) i makroelemenata u plodu paprike

Gnojidbeni tretmani	ST (%)	Makroelementi (% ST)				
		N	P	K	Ca	Mg
1. berba						
MG	7,56	1,75 B*	0,307	2,90	4,14 C	0,086 B
PPG+MG	7,79	1,97 A	0,310	2,96	3,70 D	0,097 A
VK+MG	7,40	1,84 B	0,287	2,89	4,59 A	0,093 A
VK+MG+VEVK	7,55	1,91 A	0,297	2,89	4,31 B	0,098 A
2. berba						
MG	8,28	1,51 B	0,287 A	2,77 A	3,05 B	0,099
PPG+MG	8,80	1,74 A	0,257 B	2,78 A	3,03 B	0,094
VK+MG	8,12	1,69 A	0,280 A	2,83 A	3,45 A	0,098
VK+MG+VEVK	8,10	1,48 B	0,267 B	2,64 B	3,01 B	0,095

Legenda: MG – mineralna gnojiva; PPG+MG – peletirano pileći gnojivo i mineralna gnojiva; VK+MG – vermikompost i mineralna gnojiva; VK+MG+VEVK – vermikompost, mineralna gnojiva i vodeni ekstrakt vermikomposta

**Različita slova unutar pojedine berbe predstavljaju značajno različite prosječne vrijednosti varijabli prema LSD testu, $P \geq 1\%$*

Svi gnojidbeni tretmani utjecali su na povećanje pH vrijednosti tla, najviše MG, a najmanje tretmani s vermikompostom (Tablica 4). Gnojidba vermikompostom imala je najveći pozitivan učinak na udio dušika i humusa obzirom na povećanje $>1\%$ u odnosu na tlo prije postavljanja pokusa. Po završetku pokusa količina fosfora kod svih gnojidbenih tretmana bila je neznatno manja, a kalija gotovo nepromijenjena, izuzev kod tretmana MG.

Tablica 4. Kemijska analiza tla prije postavljanja pokusa (kontrola) i nakon druge berbe

Gnojidbeni tretmani	pH		%		AL-mg/100 g	
	H ₂ O	nKCl	humus	N	P ₂ O ₅	K ₂ O
Kontrola	7,50	6,86	2,22	0,20	41,1	25,5
MG	7,81	7,06	2,33	0,18	40,0	19,5
PPG+MG	7,77	7,01	2,85	0,19	38,5	25,5
VK+MG	7,91	6,98	3,26	0,23	39,0	25,5
VK+MG+VEVK	7,83	6,96	3,29	0,23	40,9	24,5

Legenda: MG – mineralna gnojiva; PPG+MG – peletirani pileći gnojivo i mineralna gnojiva; VK+MG – vermikompost i mineralna gnojiva; VK+MG+VEVK – vermikompost, mineralna gnojiva i vodeni ekstrakt vermikomposta

Zaključci

U prvoj berbi plodovi paprike bili su krupniji, veće mase i debljeg perikarpa, dok su u drugoj berbi imali veću količinu suhe tvari te manju količinu dušika, kalija i kalcija. Gnojidbeni tretmani nisu značajno utjecali na morfološka svojstva i sastavnice prinosa, međutim, pozitivan učinak vermikomposta potvrđen je relativno većim brojem tržnih plodova i većim tržnim prinosom u odnosu na gnojidbu peletiranim pilećim gnojivom te isključivo mineralnim gnojivima. Kombinacijom vermikomposta i mineralnih gnojiva ostvarena je najveća količina kalcija i magnezija u plodovima paprike iz prve berbe te dušika, fosfora, kalija i kalcija iz druge berbe. Primjena vermikomposta rezultirala je najmanjim povećanjem kiselosti tla i najvećim povećanjem sadržaja humusa u tlu.

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Effect of vermicompost on morphological characteristics, yield and mineral content of pepper fruit

Abstract

The aim of the study was to determine the effect of vermicompost fertilization on the morphological characteristics, yield and mineral composition of the pepper fruit and the chemical characteristics of the soil. The field experiment with the pepper cultivar 'Elephant's ear' had four fertilization treatments: mineral fertilizers (MG); pelleted chicken manure 'Orgevit' and MG (PPG+MG); vermicompost 'Lumbri humus' and MG (VK+ MG); VK+MG and water extract of vermicompost (VK+MG+VEVK). Relatively highest share of market fruits and market yield had the treatment VK+MG+VEVK and VK+MG, respectively. Significantly the highest amount of dry matter and nitrogen was achieved by PPG+MG treatment, and calcium by VK+MG. Treatments with vermicompost most increased humus and nitrogen content in the soil, and least affected the change of soil pH.

Keywords: *Capsicum annuum*, macroelements, mineral fertilizer, pelletized chicken manure, vermicompost tea

Optimization of the extraction of caffeine from coffee and tea samples

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Abstract

The aim of the study was to optimize the extraction of caffeine from coffee and tea by two extracting methods with different solvents (distilled water, ethyl acetate, chloroform and ethanol): classic solid/liquid extracting method in periods (10', 20', 30', 40' and 50') and ultrasound assisted extraction method (UAE) in 20'. For both, coffee and tea, the optimal extraction method was UAE in 20'. Extending extraction period in classic extracting method didn't have significant effect on caffeine extraction. The optimal solvent for the caffeine extraction regardless of the method for tea and coffee was water, followed by ethanol, chloroform and ethyl acetate.

Keywords: Caffeine, classic solid/liquid extraction method, ultrasound assisted extraction method, solvents, UV-VIS

Introduction

Coffee and tea are most common beverages consumed in world due to beneficial role on human body (Grigg, 2002). This is related to the components present in coffee and tea. Among the other natural compounds found in coffee and tea, caffeine known as methylxanthines is one of the alkaloids found in abundance in this two species (Nathanson, 1984). It has been reported that caffeine is found in seeds and fruits of sixty different plants (Palatini et al., 2009). Furthermore, caffeine is frequently added as antioxidant in many soft and energy drinks making it addictive (Nathanson et al., 1984; Xhaferaj et al., 2019). German chemist Ferdinand Runge made possible isolation of caffeine in synthetic manner in 1819 (Maidon et al., 2012). Caffeine has drawn more attention in the past decades related to its physiological effects on major organ systems, including the nervous, cardiovascular, digestive, and respiratory system (Nehlig et al., 1992; Spiller, 1998). As a result, various agencies like Food and Drug Administration (FDA) in USA and European Food Safety Authority (EFSA) stated that single doses of caffeine up to 200 mg (about 3 mg/kg bw) from all sources do not raise safety concerns for the general adult population (EFSA, 2015). In light of this, consuming decaffeinated coffee and tea in order to avoid caffeine for the problem mentioned above, is becoming more widespread among consumers around the world (McCusker et al., 2006). There are several analytical methods to determine the caffeine concentration present in coffee, tea and other drinks, like HPLC, UV-VIS, HPTLC and FT-IR (Armenta et al., 2006; Tzanavaras and Themelis, 2007; Aranda and Morlock, 2006; Souto et al., 2010). Due to fast answer and accurate determination, UV-VIS is a technique frequently used in different laboratories. The purpose of this research was to test various methods of extracting caffeine from coffee and tea with the aim to define combination of solvent and extraction period which results with the largest caffeine quantity.

Material and methods

Six coffee and three tea samples were taken from different Albanian supermarkets. The caffeine pure powder and solvents used for extraction were provided from Sigma Aldrich (Milano, Italy). All analyses were made spectrophotometrically (Biochrom Libra S22, Cambridge, United Kingdom).

The stock solution of caffeine was prepared by dissolving 10 mg of pure caffeine in 1000 mL of distilled water to obtain 10 ppm caffeine solution. Standard solution was prepared by pipetting 0; 1; 2; 4 mL stock solution into 10 mL volumetric flask and then filled with distilled water up to the mark. The calibration curve was done by plotting

the absorbance of each standard solution, measured at maximum absorption wavelength for caffeine, 270 nm (in triplicate) using distilled water as blank probe.

The extraction of caffeine from coffee and tea was done as follows: 100 mg of coffee/tea was placed to an Erlenmeyer flask where 100 mL solvent (water, ethanol, ethyl acetate, chloroform) was added and the solution was left under stirring in different time respectively 10, 20, 30, 40 and 50 min for each solvent (classic solid/liquid extraction). The ultrasound assisted extraction (UAE) procedure (Kunshan Ultrasonic, China, output power 150 W) was conducted only for 20 min (100 mg coffee/tea) with different solvents (100 mL of water, ethanol, ethyl acetate, chloroform). Then the suspension was filtered off and the caffeine concentration was measured spectrophotometrically at 270 nm using distilled water as blank test. Caffeine extracted content was calculated from the equation:

$$X = \frac{C \cdot V}{1000 \cdot p} \cdot 100$$

X - caffeine content (%); *C* - concentration of caffeine in solution; *V* - final volume of solution (100 mL); *p* - coffee/tea mass (g).

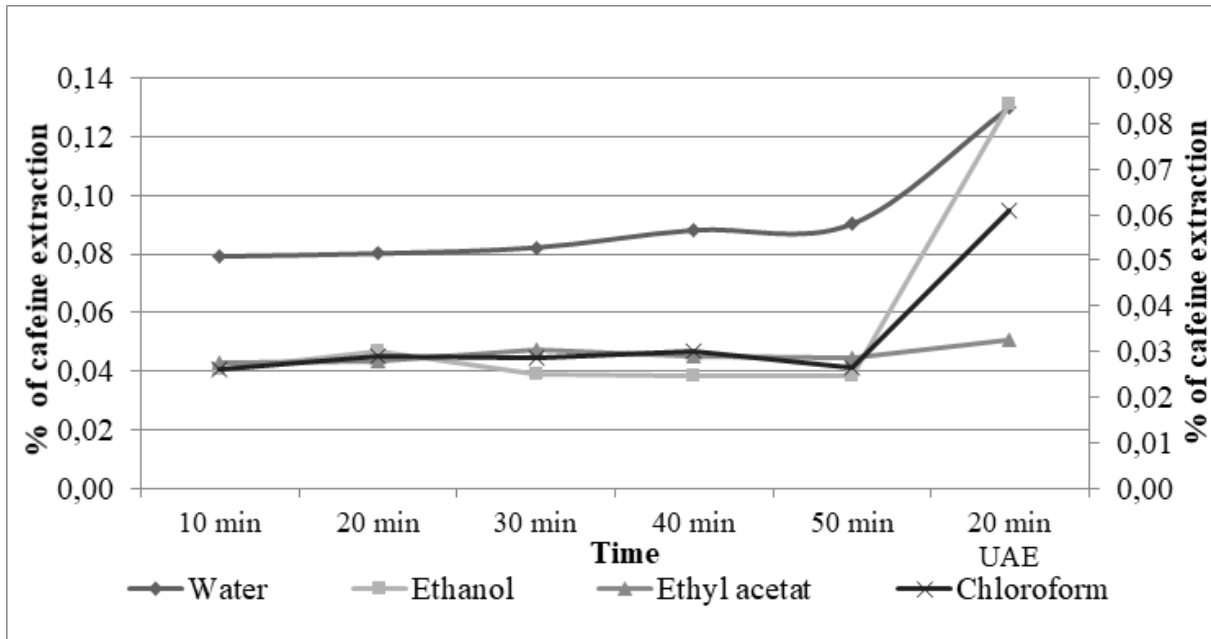
Result and Discussion

Besides the water as one of the most reported solvent for caffeine extraction, few organic solvents are suitable for application in this process, their characteristics are presented in Table 1 (Shinde and Shinde, 2017; Demir et al., 2015; Khalik and Abdullah, 2017). In order to extract maximum quantity of caffeine, physical/chemical parameters of solvent play an important role (Table 1). According to these facts, a good solvent for extraction needs to meet several characteristics like low boiling point, high solubility of caffeine, non-toxicity and non-reactivity (Shinde and Shinde, 2017).

Table 1. Characteristics of solvents for caffeine extraction

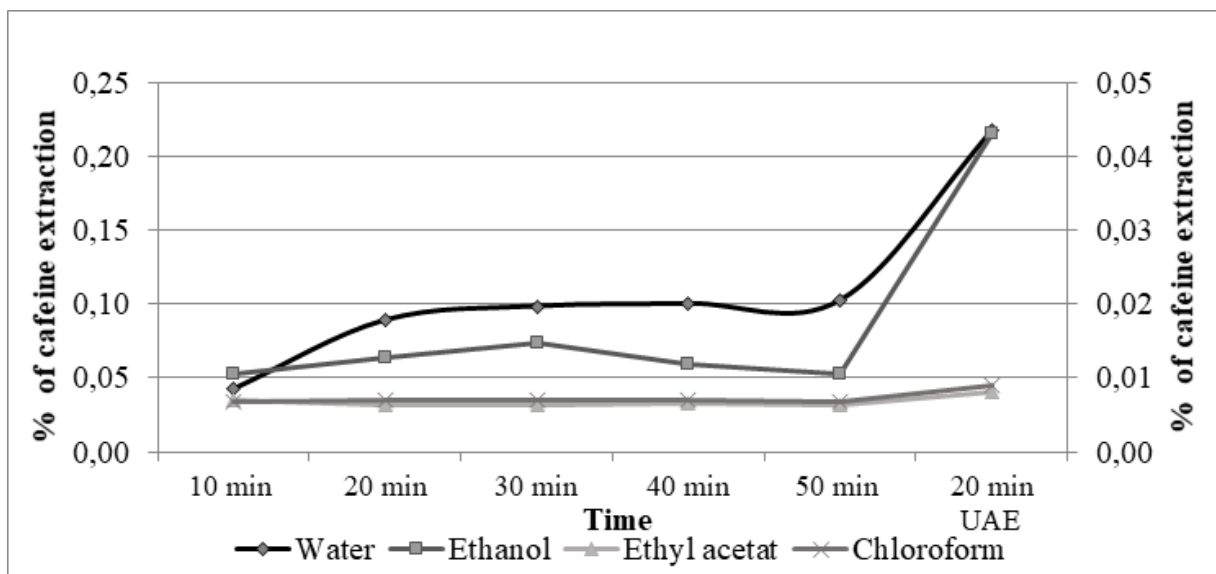
Solvent	Temperature/Pressure	Relative Permittivity	Viscosity, dynamic	Polarity
Water	20 °C/1 atm	80	0.9	
Ethanol	20 °C/1 atm	24	1.5	
Ethyl Acetate	20 °C/1 atm	16	2.4	
Chloroform	20 °C/1 atm	4.81	0.536	

It is worth to mention the difference existing between two methods selected for this studies. Classic solid/liquid extraction offer simple way but is time consuming and usually provide low yield of extraction. From the other side, UAE is faster kinetic rate and more effective. In addition to, UAE can be performed even in low temperature offering good extraction results (Schnor et al., 2004). As it can be seen from the Figure 1, water was the best solvent for caffeine extraction from coffee and the best time for classic solid/liquid extraction method was 40 min. Water was followed by ethanol, chloroform and ethyl acetate, nearly at the same rate.



Legend: Left vertical axis is for water and right vertical axis is for other solvents.

Figure 1. Effect of extraction method, solvent and extraction period on caffeine extraction from coffee



Legend: Left vertical axis is for water and right vertical axis is for other solvents.

Figure 2. Effect of extraction method, solvent and extraction period on caffeine extraction from tea

In contrast with classic solid/liquid extraction, UAE gave a remarkable results for all solvents and the rate of increasing yield of extraction when UAE was applied compared with classic solid/liquid extraction for 10 minutes were as follow: ethanol 223%, chloroform 133%, water 64% and ethyl acetate 17%.

Same situation was observed even in tea samples (Figure 2), where water was again the best solvent for caffeine extraction by classic solid/liquid extraction method in 30 minutes. Unlikely from the others tested solvents, in case of ethanol, increasing time of classic extraction reduced the extraction of caffeine from tea. As was expected, UAE method also enhanced the yield of extracted caffeine from tea and the rate of increasing yield of extraction compared with classic solid/liquid extraction for 10 minutes was as follows: water 413%, ethanol 305%, chloroform 35% and ethyl acetate 17%. As such, confirming the superiority of UAE against classical solid/liquid extraction method.

Conclusion

Regardless of the studied extraction methods, water proved to be the best solvent of caffeine from coffee and tea. The presence of caffeine obtained by other solvents (ethanol, ethyl acetate, chloroform) was significantly lower compared with water. Time, as a parameter for classic solid/liquid extraction method, didn't show any significant role in final quantity of caffeine in coffee and tea solutions. From the other side, using ultrasound assisted extraction (UAE) method gave a remarkable result, because the extraction of caffeine was increased for all solvents used. The caffeine yield obtained by using water and ethanol as solvent by the UAE extraction method was higher 2 and 3 times in coffee and 4 and 5 times in tea, respectively, than the values obtained by the classic solid/liquid extraction method.

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Klijavost sjemena kapare (*Capparis orientalis* Veill.) pod utjecajem različitih kemijskih tvari

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Sažetak

Kapara je višegodišnja grmolika biljka koja samoniklo raste na području Mediterana, a u Hrvatskoj nije zabilježen plantažni uzgoj. Uglavnom se razmnožava vegetativno (stabljičnim reznicama), budući da pri sjetvi sjemena problem predstavlja dormantnost sjemena. Cilj ovog rada bio je ispitati utjecaj različitih predstjetvenih tretmana sjemena (močenje u destiliranoj vodi, ekstraktu kamilice te otopinama giberelinske kiseline GA_3 , kalijeva nitrata i preparata 'EkoBooster 1', uz ili bez skarifikacije sjemena sumpornom kiselinom) na povećanje klijavosti sjemena kapare. Dokazano je da GA_3 može djelomično doprinijeti povećanju klijavosti neskarificiranog sjemena, dok ostali primijenjeni tretmani nisu bili učinkoviti.

Ključne riječi: dormantnost sjemena, GA_3 , predstjetveni tretmani, skarifikacija

Uvod

Kapara, *Capparis orientalis* Veill. (syn. *Capparis spinosa* L. ssp. *orientalis* Veill.), višegodišnja je grmolika biljna vrsta s prilegnutim ili visećim ograncima i s korijenovim sustavom koji je gust i djelomično odrvenjen, a dopire i do 10 m dubine (Dursun i Dursun, 2005). Dijelovi biljke koji se konzumiraju kao začini su čvrsti cvjetni pupoljci konzervirani u octu ili soli i imaju oštar, pikantan okus i osebujan miris (Güleryüz et al., 2009), dok se znatno rjeđe koriste mladi izbojci i poluzreli plodovi. Kapara sadrži brojne bioaktivne spojeve (Maldini et al., 2016), značajna je s etnobotaničkog aspekta, a ima i veliku estetsko-pejsažnu vrijednost (Manikandaselvi et al., 2016). Kod nas nisu zabilježeni proizvodni nasadi, a cvjetni se pupovi sakupljaju sa samoniklih biljaka koje rastu u pukotinama stijena uz jadransku obalu i na otocima. Tolerantnost na nepovoljne uvjete okoline, mali zahtjevi tijekom uzgoja, kao i sve veći značaj u prehrani daju ovoj vrsti veliki potencijal za introdukciju u plantažni uzgoj, što može pridonijeti razvoju poljoprivredne proizvodnje (Al-Safadi i Elias, 2011).

Kaparu je moguće razmnožavati vegetativno i generativno, no kod oba je načina izražen niski potencijal umnožavanja. Pri vegetativnom razmnožavanju stabljičnim reznicama problem predstavlja slaba mogućnost rizogeneze, dok je kod generativnog izrazito niska klijavost uzrokovana dormantnošću sjemena. Prema Pascual et al. (2008) više je mogućih razloga mirovanja sjemena kapare: tvrda sjemena ovojnica koja sprječava upijanje vode, lučenje sluzi nakon vlaženja sjemenke, koja onemogućava pristup kisika embriju te prisutnost inhibitora klijanja. Da bi sjeme kapare proklijalo, treba ga izložiti fizikalnim postupcima i/ili kemijskim tretmanima koji omogućuju oštećivanje sjemenne ovojnice te stimulatorima rasta i drugim tvarima, u različitim koncentracijama i ekspozicijama (Basbag et al., 2009; Ölmez et al. 2004). Bhojar et al. (2010) navode da su stratifikacija, skarifikacija i tretmani s GA_3 i kalijevim nitratom (KNO_3) uspješni postupci poboljšanja klijavosti dormantnih sjemenki kapare, čemu Suleiman et al. (2009) priključuju i hlađenje tijekom tjedan dana. Cilj ovog rada bio je utvrditi mogućnost poboljšanja klijavosti sjemena kapare primjenom skarifikacije sumpornom kiselinom u kombinaciji s tretmanima močenja sjemena u različitim kemijskim tvarima.

Materijali i metode

U Zavodu za povrćarstvo Agronomskog fakulteta Sveučilišta u Zagrebu proveden je pokus s predstjetvenim tretmanima svježeg sjemena kapare sakupljenog u mjestima Zavala i Jelsa na otoku Hvaru. Dvofaktorijalni pokus postavljen je 5. srpnja 2016. godine po slučajnom bloknom rasporedu u tri ponavljanja. Glavni faktor, kemijska

skarifikacija sjemena, zastupljen je s dvije stepenice (sa i bez primjene sumporne kiseline). Sporedni faktor, močenje sjemena u otopinama kemijskih tvari, zastupljen je s pet stepenica: destilirana voda, ekstrakt kamilice, komercijalni preparat 'EkoBooster 1' (Ekopatent d.o.o., Vrbas), kalijev nitrat i hormon rasta GA₃. Koncentracije korištenih tvari i trajanje tretmana prikazani su u tablici 1. Nakon tretmana sumpornom kiselinom, sjeme kapara je isprano tekućom vodom i osušeno, a potom je slijedila primjena ostalih tretmana. Ekstrakt kamilice pripremljen je prelijevanjem svježih cvjetova kamilice (vrhom puna velika žlica) kipućom vodom (1 L) te procijeđivanjem pripravka nakon 24 sata.

Tablica 1. Tretmani primijenjeni u pokusu poboljšanja klijavosti sjemena kapare

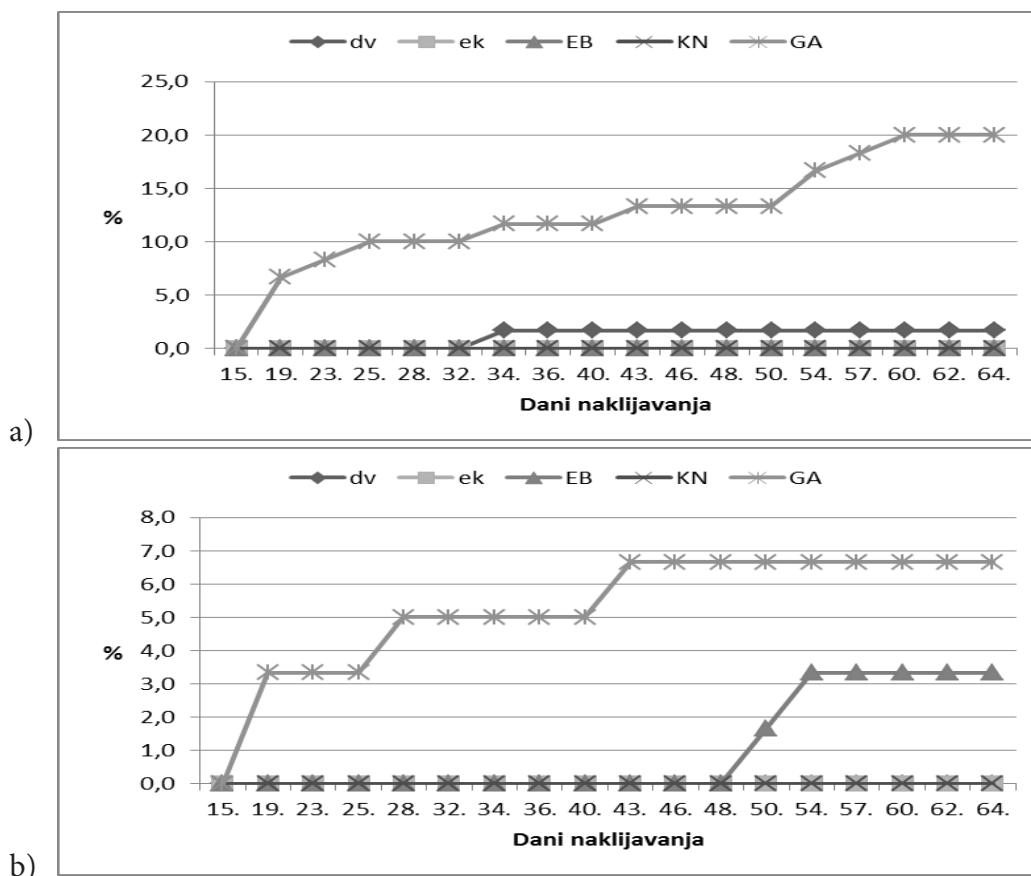
Faktori	Tretmani, koncentracija	Oznaka	Trajanje tretmana
Glavni	Sumporna kiselina (H ₂ SO ₄), 70 %	SK	5 minuta
	Bez primjene	0	-
Sporedni	Destilirana voda	dv	12 sati
	Ekstrakt kamilice	ek	12 sati
	'EkoBooster 1', 1 %	EB	20 min
	Kalijev nitrat (KNO ₃), 4000 ppm	KN	12 sati
	Giberelinska kiselina (GA ₃), 2000 ppm	GA	12 sati

Svaki tretman u ponavljanju zastupljen je s jednom Petrijevom posudom u koju je pravilno raspoređeno 20 prethodno tretiranih sjemenki. Kao podloga za naklijavanje služio je filter papir natopljen destiliranom vodom. Petrijeve posude stavljene su u klima komoru s kontroliranim konstantnim uvjetima (temperatura zraka 25 °C, relativna vlaga zraka 70 %, mrak). Tijekom procesa klijanja prema potrebi je provođeno vlaženje sjemena destiliranom vodom kao i prskanje 5 %-tnom otopinom klora kada je uočen razvoj inokuluma plijesni.

Budući da u 'Pravilniku o metodama uzorkovanja i ispitivanja kvalitete sjemena' (Narodne novine, 2008) nema podataka o danima za utvrđivanje energije klijanja (EK) i ukupne klijavosti (UK) sjemena kapare, dani su određeni temeljem promatranja intenziteta klijanja. Za utvrđivanje EK određen je 40. dan od stavljanja sjemena na naklijavanje, jer je tada klijanje bilo intenzivnije i vidljivo u više tretmana, dok je za UK određen 60. dan jer nakon toga više nije bilo znakova klijanja. Proces klijanja promatran je u dinamici tri puta tjedno od 21. srpnja do 30. rujna 2016. godine. Za statističku analizu podataka korišten je statistički program SAS[®] Software v. 9.3 (2010), procedura PROC GLM (opći linearni model). Podaci za energiju klijanja i ukupnu klijavost statistički su obrađeni analizom varijance (ANOVA), a značajnost razlika između srednjih vrijednosti testirana je LSD testom na razini signifikantnosti p≤0,05.

Rezultati istraživanja

Dinamika klijanja sjemena kapare prikazana je u grafikonu 1. Kod neskarificiranog sjemena klijanje je zabilježeno samo kod dva tretmana. U tretmanu s giberelinskom kiselinom (GA) klijanje sjemena započelo je 19. dana od početka naklijavanja. Početna klijavost od 6,7 %, povećavala se do 25. dana te 34., 43., 54. i 60. dana, do maksimalno 20,0 %. Početak procesa klijanja zabilježen je i kod sjemena močenog u destiliranoj vodi no samo 34. dana (1,7 %), nakon čega se klijanje nije nastavilo.



dv - destilirana voda, ek - ekstrakt kamilice, EB - 'EkoBooster 1', KN - KNO_3 ; GA - giberelinska kiselina

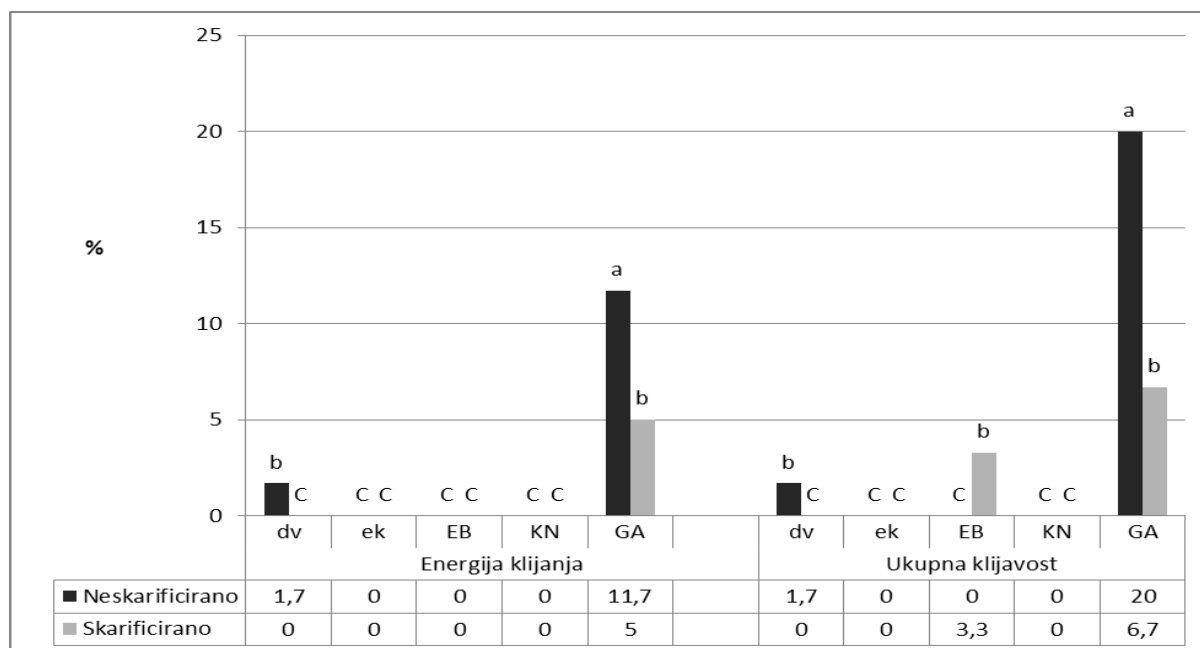
Grafikon 1. Dinamika klijanja neskarifificiranog (a) i skarificiranog (b) sjemena kapare tretiranog otopinama različitih kemijskih tvari

Kod skarificiranog sjemena klijanje je zabilježeno također samo kod dva tretmana. Uz GA_3 klijanje je započelo također 19. dana od stavljanja na naklijavanje, bilo je dvostruko slabije (3,3 %) nego kod neskarifificiranog sjemena te je, uz jedno povećanje (na 5 %), završilo 43. dana sa konačnih 6,7 % prokljalih sjemenki. Uz primjenu komercijalnog preparata 'EkoBooster 1' klijanje skarificiranog sjemena započelo je 50. dana od početka naklijavanja (1,7 %), a završilo je 54. dana s 3,3 % prokljalih sjemenki. U preostalim tretmanima nije zabilježeno klijanje sjemena.

Energija klijanja (EK) sjemena kapare (grafikon 2) očitana je 40. dana od početka naklijavanja. Opravdano najveću EK imalo je neskarifificirano sjeme tretirano s GA_3 (11,7 %), dok su značajno niže, ali podjednake EK imali skarificirano sjeme također u tretmanu s GA_3 (5,0 %) i neskarifificirano sjeme tretirano destiliranom vodom (1,7 %). U ostalim tretmanima EK bila je 0 %.

Ukupna klijavost (UK) sjemena kapare utvrđena je 60. dana od početka naklijavanja (grafikon 2). Statistički najbolju UK imalo je neskarifificirano sjeme tretirano s GA_3 (20 %), dok je značajno manja, no podjednaka UK zabilježena kod dva tretmana skarificiranog sjemena (GA_3 6,7 % i 'EkoBooster 1' 3,3 %) te kod samo jednog tretmana neskarifificiranog sjemena (destilirana voda 1,7 %). Primjena ekstrakta kamilice i otopine KNO_3 nije potaknula klijanje sjemena kapare pa je i ukupna klijavost bila 0 %.

Promatrajući faktor skarifikacije neovisno o tretmanima s različitim kemijskim tvarima, kod neskarifificiranog sjemena utvrđena je vrlo niska prosječna klijavost (4,3 %), a dvostruko niža zabilježena je kod skarificiranog sjemena (2,0 %).



dv - destilirana voda, ek - ekstrakt kamilice, EB - 'EkoBooster 1', KN - KNO_3 ; GA - giberelinska kiselina; a,b,c - srednje vrijednosti unutar setova podataka označene s različitim slovima razlikuju se temeljem LSD testa na razini signifikantnosti $p \leq 0,05$.

Grafikon 2. Energija klijanja i ukupna klijavost nekarificiranog i skarificiranog sjemena kapare tretiranog otopinama različitih kemijskih tvari

Promatrajući EK i UK (grafikon 2) vidi se da je najveće povećanje klijavosti zabilježeno u tretmanu s GA_3 kod nekarificiranog sjemena (EK 11,7 %, UK 20,0 %), dok je u tretmanu vodom kod nekarificiranog sjemena ukupna klijavost bila jednaka energiji klijavosti.

Tretman s GA_3 u ovom se istraživanju pokazao kao jedini opravdan. No, iako je ukupna klijavost kod primjene GA_3 bila najviša u odnosu na ostale tretmane, od malog je praktičkog značaja. Farhoudi i Tafti (2013) ispitivali su klijavost skarificiranog sjemena kapara močenog tijekom 12 sati u GA_3 i KNO_3 uz uključen tretman ispiranja sjemena tekućom vodom tijekom narednih 12 sati. Najveću klijavost (98 i 75 %) utvrdili su u tretmanima u kojima je kombinirano ispiranje vodom i močenje sjemena u 1000 mg/l i 500 mg/l GA_3 , dok je u tretmanu s 3 % KNO_3 klijavost bila vrlo niskih 6 %, uz i bez prethodnog ispiranja sjemena tekućom vodom. No, pozitivan učinak KNO_3 na klijavost sjemena kapare dokazali su Arefi et al. (2012), utvrdivši najvišu klijavost (72 %) kod tretmana s 8000 mg/l KNO_3 u trajanju od 24 sata, uz stavljanje sjemena na naklijavanje na filter papir močen s 250 mg/l GA_3 .

Zaključak

Različiti predstjetveni tretmani imali su vrlo neujednačene učinke na klijanje sjemena kapare. Skarifkacija sjemena sumpornom kiselinom u trajanju 5 minuta nije imala značajan učinak na poticanje klijavosti sjemena. Močenje sjemena u otopini giberelinske kiseline (GA_3 , 2000 ppm) tijekom 12 sati potaknulo je proces klijanja, no učinak je intenzivnije bio izražen na nekarificiranom sjemenu. Močenje sjemena u destiliranoj vodi i otopini komercijalnog pripravka 'EkoBooster 1' (1 %) nije rezultiralo značajnim povećanjem klijavosti, dok su tretiranja ekstraktom kamilice i KNO_3 (4000 ppm) u potpunosti bila bez učinka na klijanje sjemena kapare.

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Germination of caper (*Capparis orientalis* Veill.) seeds affected by different chemical treatments

Abstract

Caper is a perennial shrub that grows wild in the Mediterranean region, while in Croatia cultivation is not recorded. Vegetative propagation (stem cuttings) is mainly used, since in generative reproduction the largest issue is low percentage of germinated seeds due to seed dormancy. The aim of this study was to research the effect of different pre-seeding treatments (soaking in distilled water, chamomile extract, and in solutions of gibberellic acid GA₃, potassium nitrate and 'EkoBooster 1', along with or without chemical scarification with sulfuric acid) on elimination of seed dormancy and increase of the seed germination. It was proved that the GA₃ treatment can partly eliminate dormancy of non-scarified caper seed, while other treatments didn't show any significant effect on seed germination.

Keywords: GA₃, pre-seeding treatments, scarification, seed dormancy

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Učinak pokrovne kulture na kontrolu korova na strništu

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Sažetak

Na šest obiteljskih poljoprivrednih gospodarstava (OPG 1-6) istraživana je učinkovitost pokrovne kulture (smjese sjemena pet biljnih vrsta) na korove. Učinak pokrovne kulture u odnosu na tretman bez agrotehničkih zahvata (konvencionalni tretman), utvrđivan je brojem jedinki korova po m⁻², svježom masom pokrovne kulture te pokrovnošću tla masom korova i pokrovne kulture. Osim broja jedinki, učinak pokrovne kulture je iskazao pozitivan učinak na smanjenje zakorovljenosti. Smanjenje pokrovnosti tla korovima iznosilo je od 65,7 do 88,6 % u odnosu na konvencionalni tretman što potvrđuje da istraživana smjesa pokrovne kulture ima potencijal za smanjenje zakorovljenosti usjeva u integriranoj proizvodnji.

Ključne riječi: integrirano suzbijanje korova, broj korova, pokrovnost korova, pokrovnost pokrovne kulture

Uvod

Otkako je utvrđen negativan učinak intenzivne poljoprivrede na okoliš, bioraznolikost i zdravlje ljudi, znanstvenici istražuju mogućnosti „redizajniranja“ uzgoja bilja koje bi bile prihvatljive u održivom sustavu proizvodnje (Thiessen Martens et al. 2015). Postoji više alternativnih mogućnosti a primjenjivost im ovisi o načinu korištenja poljoprivrednog zemljišta, tehničkoj opremljenosti i znanju poljoprivrednika. Pokrovne kulture su multifunkcionalna mogućnost u „redizajniranju“ poljoprivredne proizvodnje. Pieters (1927) navodi da je još 1134 B.C. u Kini uočeno povećanje plodnosti tla ako je ono pod vegetacijom. Isti autor spominje navod Teofrasta (372-287 B.C.) o korištenju leguminoza za povećanje plodnosti tla (cit. Hoffman and Regnier, 2005). Pokrovne kulture zajedno s reduciranom obradom tla kasnije su prepoznate kao korisne u smanjenju problema erozije (Thomas, 2002). Gusti pokrovni usjev amortizira brzinu i snagu oborine prije nego dospije na čestice tla. Gustom korjenovom mrežom fizički drži tlo na mjestu i povećava poroznost tla. Na sličan način pokrovne kulture čuvaju vlagu i sprečavaju naglo otjecanje oborina. Prema Teasdale (1993) pokrovne biljke gustim sklopom se natječu za ograničene izvore te priječe korove u završavanju životnog ciklusa. Nadzemna masa pokrovnih kultura koja nije unesena u tlo znatno smanjuje prolazak svjetlosti (nužne za klijanje korova) ili fizički priječi nicanje korova. Čak i kad sjeme korova proklije, klijanci su oslabljeni zbog sloja živog malča pokrovnih biljaka. Potiskuju korove i tijekom i nakon vegetacije (Kobayashi et al. 2003, Blackshaw et al. 2001). Osim kompeticijskog i fizičkog zaustavljanja korova u razvoju, utvrđen je i alelopatski učinak pokrovnih kultura, ovisno o vrsti pokrovne kulture (Creamer et al. 1996; Singh et al. 2003, Kruidhof and Bastiaans, 2007). I Haramoto i Gallandt (2004), zbog potencijalnog alelopatskog učinka navode važnost odabira vrsta za pokrovnost kulture. Blackshaw et al. (2001) navode da biomasa korova kod korištenja *Melilotus officinalis* L. Pall. kao pokrovne kulture može biti smanjena do razine 75-97%. Cilj istraživanja je bio utvrditi učinak pokrovne kulture na potencijal korova na šest OPG-ova.

Materijal i metode

U ljeto 2018. na šest lokacija (OPG 1 - 6) u okolici Zagreba istraživanje učinka pokrovne kulture (smjese pet biljnih vrsta) na zakorovljenost provedeno je u poljskim uvjetima. Na svakoj farmi postavljena su dva načina obrade tla. Na konvencionalnom tretmanu (bez obrade strništa) nakon žetve ozime pšenice u jesen (12. 12. 2018.) obavljeno je oranje do 25 cm dubine. Na tretmanu gdje je posijana pokrovna kultura, u ljeto 1. 8. 2018. tlo je podrivano, nakon čega je 2. 8. 2018. posijana gotova smjesa pokrovne kulture u količini 25 kg/ha. Parcela s pokrovnim kulturama nije u

jesen obrađivana. Širina parcele pojedinog tretmana iznosila je 6 m, a duljina je ovisila o duljini parcele. Udio sjemena u smjesi pokrovne kulture iznosio je: *Sinapis alba* L. (40 %), *Raphanus sativus* var. *oleiformis* (30 %), *Fagopyrum esculentum* Moench (20 %), *Camellina sativa* L. Crantz (5 %) i *Guizotia abyssinica* L.f. Cass. (5 %). Učinak pokrovne kulture u odnosu na konvencionalni tretman utvrđivan je brojem jedinki korova m^{-2} , pokrovnošću tla korovima i pokrovnom kulturom (11. 9. 2018.) te masom pokrovnih biljaka u jesen (19.11.2018.). Sva mjerenja istraživanih parametara obavljena su u četiri ponavljanja. Redukcija pokrovnosti tla korovima ovisno o tretmanu izračunata je po Abbottu (Püntener, 1981). Dobiveni podaci obrađeni su odgovarajućom analizom varijance u programu SAS (1997). Nakon signifikantnog *F*-testa ($P=0,05$) za usporedbu srednjih vrijednosti korišten je LSD test ($P=0,05$).

Rezultati i rasprava

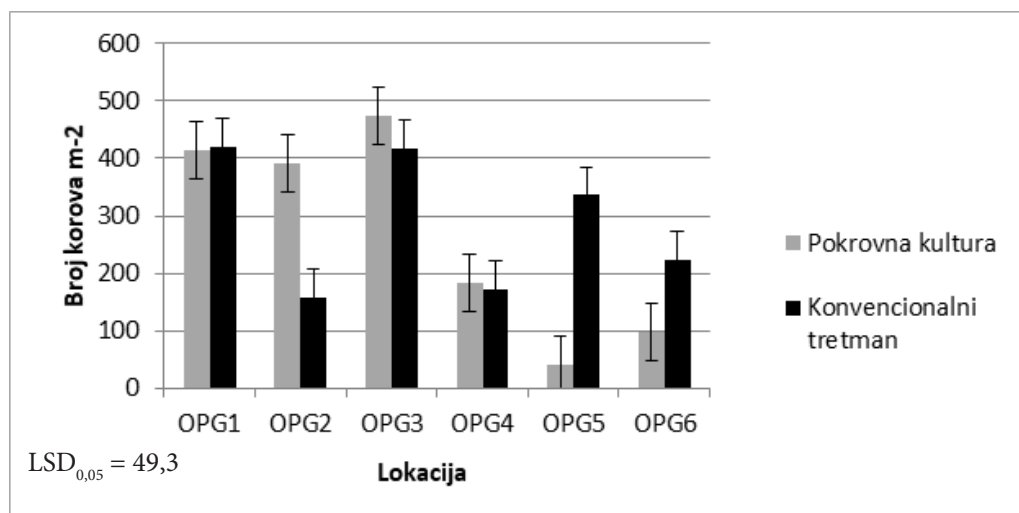
Na pokusnim parcelama su dominirale za okopavinske usjeve važne korovne vrste. Na istraživanim parcelama zakorovljenost je bila neujednačena. U tablici 1 prikazana je zastupljenost korovnih vrsta na pojedinim lokacijama (OPG-ima).

Tablica 1. Zastupljenost korovnih vrsta ovisno o lokaciji

Vrsta korova	OPG1	OPG2	OPG3	OPG4	OPG5	OPG6
<i>Abutilon theophrasti</i> Medik.	++	-	-	-	-	++
<i>Ambrosia artemisiifolia</i> L.	-	+++	-	++	++	++
<i>Cirsium arvense</i> (L.) Scop.	-	-	-	-	+	+
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	++	++	+++	++	+	-
<i>Setaria glauca</i> (L.) P. Beauv.	+++	+++	+++	+++	+++	-
<i>Sorghum halepense</i> (L.) Pers.	+	-	+++	-	-	+++

- nije bila znatnije zastupljena; + prisutna; ++ znatno prisutna; +++ naglašeno dominira

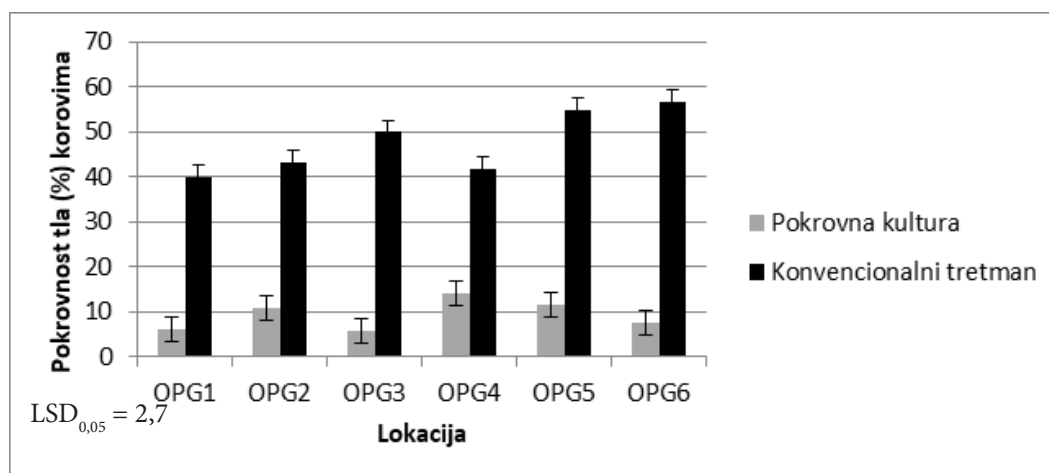
Na grafikonu 1. prikazan je broj jedinki korova po jedinici površine.



Grafikon 1. Broj korova po m^{-2} ovisno o lokaciji i tretmanu

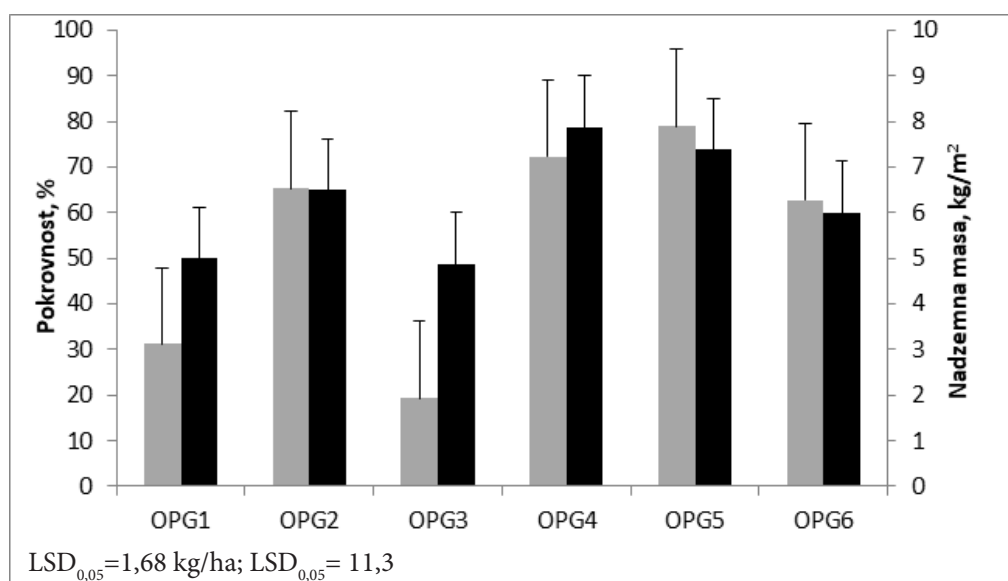
Osim na OPG5 i OPG6 broj jedinki nije bio znatnije reducirana. Naime, alternativne mjere, za razliku od herbicida, nemaju „burn down“ učinak, odnosno alternativne mjere suzbijanja korova su proces koji se razvija postupno i ne postižu se učinci u jednoj sezoni (Barić et al. 2014). Navedeno potvrđuju rezultati prikazani u grafikonu 1. Iz grafikona se vidi da je ukupan broj korova na tretmanima bio približno jednak, osim na tretmanima OPG5 i OPG6 na kojima je njihov broj bio prepolovljen. U grafikonu 1. je broj jedinki korova prikazan zbirno za sve vrste. Detaljnom

analizom rezultata o zastupljenosti korovnih vrsta po pojedinoj lokaciji utvrđeno je da na lokaciji OPG5 za razliku od ostalih, nije bila prisutna vrsta (*Sorghum halepense* (L.) Pers.) a na lokaciji OPG6 nije bila prisutna vrsta (*Setaria glauca* L.) koja je na ostalim OPG-ima bila prisutna u velikom broju (>100 jedinki po m² na konvencionalnom tretmanu). Pokrovnost površine tla nadzemnom masom korova, osim o broju jedinki, ovisna je o razvojnoj fazi korova. U grafikonu 2. prikazana je pokrovnost tla nadzemnom masom pokrovnih kultura.



Grafikon 2. Pokrovnost površine tla nadzemnom masom korova

U vrijeme ocjenjivanja (11.9.2018.) utvrđeno je da su korovi na konvencionalnom tretmanu bili u završnoj fazi životnog ciklusa (zrioba) dok su na površini s pokrovnim kulturama bili u ranim fazama razvoja (12 – 16 listova). Iz prikazanog je vidljivo da su pokrovne kulture znatno potisnule razvoj korova. Zbog prethodne obrade tla (podrivanje) korovi su nicali nešto kasnije nego na konvencionalnom tretmanu. Redukcija pokrovnosti tla korovima na tretmanu s pokrovnim biljkama iznosila je od 65,7 do 88,6 % u odnosu na konvencionalni. I Hoffman et al. (1993) su primjenom ozime grahorice (*Vicia villosa* Roth) kao pokrovne kulture postigli redukciju biomase korova za 63 do 75 %. Slično su utvrdili i Nagabhushana et al. (2001) kad navode da organski ostaci pokrovnih kultura reduciraju korove na razini 80-90 %. U grafikonu 3 prikazana je masa i pokrovnost tla nadzemnom masom pokrovnih kultura. Pokrovnost se kretala od 48,8 (OPG3) do 78,8 % (OPG4) površine tla a svježa nadzemna masa pokrovnih kultura iznosila je od 1,94 kg m² na OPG3 do 7,90 kg m² na OPG5 (grafikon 3). Blackshaw et al. (2001) a slično i Sjursen et al. (2011) navode slične rezultate gdje biomasa korova može biti potisnuta na razini 75-97%, odnosno 70 - 74 %. Na razlike u masi nadzemne mase pokrovnih kultura mogla je utjecati znatna zakorovljenost vrstom (*Sorghum halepense* L. Pers.) koja je bila naglašeno prisutna na OPG1 a pogotovo na OPG3 (176 vlati na tretmanu s pokrovnom kulturom, odnosno 124 na konvencionalnom tretmanu).



Grafikon 3. Pokrovnost tla i svježa nadzemna masa pokrovnih kultura (19.11.2018.)

Zaključak

Učinak pokrovnih kultura na redukciju broja jedinki korova nije utvrđen u prvoj godini istraživanja (2018.). Međutim, redukcija nadzemne mase korova (pokrovnost) kretala se od 48,8 do 78,8 %. Stoga bi višegodišnjom sjetvom pokrovnih kultura potencijal korova mogao biti znatno oslabljen. U istraživanju je smjesa pokrovne kulture postigle svežu nadzemnu masu od 19,4 do 79 t ha⁻². Stoga osim smanjenja potencijala korova, predstavljaju značajan potencijal za povećanje organske tvari u tlu.

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Contribution of cover crops to weed suppression

Abstract

The impact of cover crops was studied during the growing seasons of 2018 at six locations. Following cereal harvest and stubble tillage, a mixture of five cover crops was sown. The impact of cover crops was measured by botanical weed analysis in both years and compared with the standard stubble management practice. Weed cover was determined in 2018 as well as in 2019 along with weed biomass and yield components (pod number and grain weight) of soybean. Cover crops showed a positive effect on reducing weed infestation with reductions in weed cover ranging from 65.6 to 97,0% depending on location and year. Thus, cover crops showed a potential for weed suppression in integrated crop production.

Keywords: integrated weed management, number of weeds, weed density, CC density, nonchemical weed control

Influence of intercropping maize with climbing bean and fertilization with clinoptilolite on forage yield and quality

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Abstract

Maize and climbing bean intercropped in different sowing densities and pure maize crop were evaluated to determine the best intercropping system with respect to forage yield and quality. The highest dry matter yield over two years was produced by intercropping maize population 75 000 plants ha⁻¹ and 75 000 plants ha⁻¹ (MB₃) 24.2 t ha⁻¹ in fertilization with clinoptilolite, whereas the lowest yield 20.4 t ha⁻¹ for the MB₁ in control of the variant in 2018. All intercrop had higher crude protein values in dry matter 103 g kg⁻¹ for the MB₁, 113 g kg⁻¹ for the MB₂ and 125 g kg⁻¹ for the MB₃ in fertilization with clinoptilolite than the monocrop maize (78 g kg⁻¹ DM) in control of the variant in 2018. Based on forage yield and quality this study showed that among all intercropped forages the MB₃ treatment was better performing than other intercrops.

Keywords: maize, climbing bean, sowing densities, crude protein, neutral detergent fiber

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used for cows and fattening cattle. Despite its high energy content, the protein content is low (88 g kg⁻¹) compared with legumes silage (Anil et al., 2000) and needs to be supplemented with proteins for better feed quality (Stoltz et al., 2013). Intercropping maize with legumes for silage is a feasible strategy to improving the level of crude protein (Contreras-Govea et al., 2009). Although maize provides high yield in terms of dry matter, it produces low protein content in fodder. The bean (*Phaseolus vulgaris* L.) is a common legume cultivated for its edible seeds all over the world. It is slightly hairy with a well-developed root system and the stems are many branched. The bean is a fast growing, warm season legume, and, it can grow in a diverse range of environmental conditions worldwide because of its adaptability. There are many varieties of beans grown in all the regions. However, selecting high yielding (seed and herbage), disease resistant variety is most important factor for successful cultivation. In addition, the bean serves as an adequate source of protein. Furthermore, it can be planted alone or intercropped with other crops such as corn and sorghums. Javanmard et al. (2009), worked on intercropping of maize with different legumes, and pointed out that the dry matter and crude protein yields increased for all intercropping compositions compared to maize monocrop. Physiological and morphological differences between intercrop constituents influence on their ability to use resources and cereal with legume intercropping. Usually they have several advantages such as higher overall yields, better soil utilization (Dhima et al., 2007), yield stability of the cropping system (Lithourgidis et al., 2006), better use of light, water and nutrients (Javanmard et al., 2009), soil fertility through biological nitrogen fixation, increases soil conservation through greater soil coverage as compared to sole cropping and ensures better soil-susceptible crop in monoculture (Lithourgidis et al., 2006). Atmospheric nitrogen fixation using legumes plants can reduce nitrogen competition in the reciprocal intercropping system of legumes and cereals enabling the cereals to use more nitrogen in the soil (Eskandari et al., 2009). This can affect the quality of the fodder intercrop components because the protein content is directly related to the content of nitrogen in the forage plants (Putnam et al., 1985). Nutrients use efficiency can also be achieved through these

of clinoptilolite zeolite because of the unique physical and chemical properties of clinoptilolite zeolite coupled with their abundance in sedimentary deposits and in rocks derived from volcanic parent materials have made them useful in many agricultural applications (Ramesh et al., 2010). Clinoptilolite zeolite is widely used in cultivating different crops such as cereals, forage, vegetables, vine, and fruit crops due to their exceptionally high ion-exchange capacity (Butorac et al., 2002). This study was designed to determine the influence of different sowing densities of maize-climbing bean intercropping and fertilization with natural zeolite clinoptilolite on forage yield and quality.

Material and methods

A field experiment was carried out during the 2018 and 2019 growing season at experimental fields in Oborovo (45°40'54"N, 16°15'12.5"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons in 2018 and 2019

Year	Meteorological data	Month					
		April	May	June	July	August	September
2018	Mean air temperature (°C)	15.7	19.2	20.9	22.4	23.0	17.2
	Rainfall (mm)	51.6	50.5	144.4	94.4	66.1	36.4
2019	Mean air temperature (°C)	11.9	13.2	23.2	21.9	22.9	16.4
	Rainfall (mm)	76.6	185.1	79.4	61.9	40.2	66.5

The experiment was set up as a randomized complete block system with three replicates. Maize hybrid seed (KWS Kolumbaris) was obtained from Seed Company "KWS". Seed of the climbing bean cultivar "Meraviglia Di Venezia" was obtained from "Green Garden" company. The individual plot size was 50 m × 2,8 m for each treatment. The maize population 75 000 plants ha⁻¹ (SM) were spaced at 70 cm × 19 cm and climbing bean population 37 500 (MB₁), 50 000 (MB₂) and 75 000 plants ha⁻¹ (MB₃) were spaced at 70 cm × 38.1 cm, 70 cm x 28.6 cm and 70 x 19 cm, respectively, in rows alternating with maize. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha⁻¹, 100 kg P₂O₅ ha⁻¹ and 200 kg of K₂O ha⁻¹ in variant of control and additionally in the vegetation of crops (stage six maize leaves) introduced supplementation 300 kg natural zeolite clinoptilolite ha⁻¹. Clinoptilolite used in this work originated from Slovakia, and the particles size of zeolite ranged in size from 0.5 to 2.0 mm. Maize and climbing bean were sown to a depth of approximately 5 cm by maize drill on May 8, 2018 and on May 5, 2019. Herbicide Wing P (active substances 212.5 g/l dimethenamide-p and 250 g/L pendimethalin) was applied pre emergence in intercropping maize with climbing bean at a dose of 4 L ha⁻¹. The soil of the research area has a pH 4.2 (M-KCl), 3.2% humus (organic matter), and is poorly supplied with physiologically active phosphorous (4.6 mg P₂O₅/100 g soil), medium supplied with physiologically active potassium (20.0 mg K₂O/100 g soil), while the total nitrogen content averaged 0.17% in topsoil. The crops were hand harvested when the maize reached at soft dough stage and climbing bean was at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldahl (AOAC, 2000) and neutral and acid detergent fiber according to Van Soest et al. (1991). Statistical analyses: Analyses of variance were made for dry matter yield and forage quality parameters (P<0.05), and the Tukey test was used for comparing means (P<0.05). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and discussion

The differences in the yield of dry matter (Table 2) were statistically significantly (P<0.05). Dry matter yields ranged from 22.6 t ha⁻¹ (MB₃) to 19.4 t ha⁻¹ (SM) in 2018 and 20.2 t ha⁻¹ (MB₃) to 17.1 t ha⁻¹ (SM) in 2019 in control of the variant. Dry matter yield ranged from 24.2 t ha⁻¹ (MB₃) to 21.3 t ha⁻¹ (SM) in 2018 and 22.6 t ha⁻¹ (MB₃) to 18.5 t ha⁻¹ (SM) in 2019 in fertilization with clinoptilolite of the variant. Consequently, dry matter yields were higher in 2018 than in 2019. This could be due to the impact of more favorable environmental factors (effect of the year) such as

solar radiation, water and temperature during plant vegetation. According to obtained results, when climbing bean seed number increased in intercrop, dry matter yields on parcels increased. The intercropped maize with cowpea (*Vigna unguiculata* (L.) Walp.) and bean (*Phaseolus vulgaris* L.) produced higher dry matter yield than monocrop maize (Geren et al., 2008).

Table 2. Yield of dry matter of maize and maize-climbing bean intercropped

Treatments	2018			2019		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	19.4b	21.3b	20.4b	17.1c	18.5c	17.8c
MB ₁	20.4ab	22.9ab	21.7ab	17.7cb	19.7b	18.7cb
MB ₂	21.2ab	23.1ab	22.2ab	18.9b	21.3ab	20.1ab
MB ₃	22.6a	24.2a	23.4a	20.2a	22.6a	21.4a
Mean	20.9b	22.9a		18.5b	20.5a	

Different letters in the column indicate significant difference (P<0.05)

Since crude proteins are very important in cattle fodder, silage containing more crude proteins is desirable. In this study it was found that the value of crude proteins of intercropped fodder MB₁, MB₂ and MB₃ was significantly (P<0.05) higher than SM (sole crop maize) during a two year research (Table 3).

Table 3. Content of crude proteins of maize and maize-climbing bean intercropped

Treatments	2018			2019		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	78d	83d	81d	74d	79d	77c
MB ₁	94c	103c	99c	89c	97c	93b
MB ₂	102b	113b	108b	96b	105b	101b
MB ₃	112a	125a	119a	105a	116a	111a
Mean	97b	106a		91b	99a	

Different letters in the column indicate significant difference (P<0.05)

According obtained results, when climbing bean seeds number and fertilization with clinoptilolite increased in intercrops, the content of crude protein in the mixture increased. Armstrong et al. (2008) found that climbing bean intercropped with corn had the greatest potential among the climbing beans to increase crude protein concentration compared with monoculture corn. The intercropping of maize with climbing bean may serve as a way to increase crude protein and improve the overall nutritive value of silage (Grobelnik et al., 2005). In this study it was found that the yield of crude proteins of intercropped fodder MB₁, MB₂ and MB₃ was significantly (P<0.05) higher than SM (sole crop maize) during a two years research (Table 4). The results suggested that the contributions provided by legume components in the mixtures increased crude protein yields of fodder. This could be due to the greater availability of nitrogen in intercropping arrangement compared to the pure maize crop (Eskandari et al., 2009).

Table 4. Yield of crude proteins of maize and maize-climbing bean intercropped

Treatments	2018			2019		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	1.51d	1.77d	1.64c	1.27d	1.46d	1.37d
MB ₁	1.92c	2.36c	2.14b	1.58c	1.91c	1.75c
MB ₂	2.16b	2.61b	2.39b	1.81b	2.23b	2.02b
MB ₃	2.53a	3.03a	2.78a	2.12a	2.62a	2.37a
Mean	2.03b	2.44a		1.70b	2.06a	

Different letters in the column indicate significant difference (P<0.05)

Fodder produced in maize-climbing bean intercrops is important not only because of an increase in the content of crude protein, but also because of reduction in the content of neutral and acid detergent fibers. For this reason, the best option in maize-climbing bean intercropping is the use of climbing bean genotypes that provide forage with the greatest proportion of pods at harvest. In this study it was found that the neutral detergent fibers of intercropped MB₁, MB₂ and MB₃ were significantly (P<0.05) lower than SM (sole crop maize) during a two years research (Table 5).

Table 5. Content of neutral detergent fiber of maize and maize-climbing bean intercropped

Treatments	2018			2019		
	Control	Clinoptilolite	Mean	Control	Clinoptilolite	Mean
SM	373a	335a	354a	391a	363a	377a
MB ₁	357b	326b	342b	378b	351ab	365a
MB ₂	345c	317c	331c	361c	339b	350b
MB ₃	332d	308d	320d	349d	325c	337b
Mean	352a	322b		370a	345b	

Different letters in the column indicate significant difference (P<0.05)

According to the results, when climbing bean had increased seed number and fertilization with clinoptilolite in intercrop, the values of neutral detergent fibers in the mixture decreased. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006).

Conclusion

The conclusion of this study is that intercropping of maize with climbing bean at various different sowing densities was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced nutrient quality of fresh fodder. Intercropping of maize with climbing bean has increased the values of crude protein and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the MB₃ treatment (75 000 plants ha⁻¹ of maize and 75 000 plants ha⁻¹ of climbing bean) was most effective regarding the nutrient composition in fresh forage.

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Influence of intercropping maize with cowpea on forage yield and quality

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Abstract

Maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) intercropped in different sowing densities and pure maize crop were evaluated to determine the best intercropping system regard to forage yield and quality. The highest dry matter yield over two years was produced in intercrop consisting of maize population 75 000 plants ha⁻¹ and cowpea population 75 000 plants ha⁻¹ for the (MC₃) 20.6 t ha⁻¹, whereas the lowest yield 18.8 t ha⁻¹ for the MC₁. All intercrop had higher crude protein values in dry matter 91 g kg⁻¹ for the MC₁, 97 g kg⁻¹ for the MC₂ and 103 g kg⁻¹ for the MC₃, than the sole crop maize (76 g kg⁻¹). Based on forage yield and quality this study showed that among all intercropped forages the MC₃ treatment was better performing than other intercrops.

Keywords: maize, cowpea, sowing densities, crude protein, neutral detergent fiber

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used for cows and fattening cattle. Despite its high energy content, the protein content is low (88 g kg⁻¹) compared with legumes silage (Anil et al., 2000) and needs to be supplemented with proteins for better feed quality (Stoltz et al., 2013). As a cultivation system, intercropping involves planting two or more crops species in the same field (Costa et al., 2012). Intercropping maize with legumes for silage is a feasible strategy to improving the level of crude protein (Contreras-Govea et al., 2009). Appropriate spatial arrangements, planting proportions, and maturity dates of components in maize-legume intercropping enhance biological diversity and have many advantages over pure maize cropping. Although maize provides high yield in terms of dry matter, it produces low protein content in fodder. Proteins are needed for livestock growth and milk production. Javanmard et al. (2009) worked on intercropping of maize with different legumes, and pointed out that the dry matter and crude protein yields increased for all intercropping compositions compared to maize monocrop. Dahmardeh et al. (2009) concluded that intercropping of maize and cowpea resulted in more digestible dry matter and also crude protein content than maize mono-cropping. Physiological and morphological differences between intercrop constituents influence on their ability to use resources and cereal with legume intercropping. Usually they have several advantages such as higher overall yields, better soil utilization (Dhima et al., 2007), yield stability of the cropping system (Lithourgidis et al., 2006), better use of light, water and nutrients (Javanmard et al., 2009), soil fertility through biological nitrogen fixation, increases soil conservation through greater soil coverage as compared to sole cropping, and ensures better soil-susceptible crop in monoculture (Lithourgidis et al., 2006), improved soil conservation (Anil et al., 1998), and better control of pests and weeds (Banik et al., 2006; Vasilakoglou et al., 2008). Atmospheric nitrogen fixation using legumes plants can reduce nitrogen competition in the reciprocal intercropping system of legumes and cereals enabling the cereals to use more nitrogen in the soil (Eskandari et al., 2009). This can affect the quality of the fodder intercrop components because the protein content is directly related to the content of nitrogen in the forage plants (Putnam et al., 1985). This present study was designed to determine the influence of different sowing densities of maize-cowpea intercropping on forage yield and quality.

Material and methods

A field experiment was carried out during the 2018 and 2019 growing season at experimental fields in Oborovo (45°40'54"N, 16°15'12.5"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons in 2018 and 2019

Year	Meteorological data	Month					
		April	May	June	July	August	September
2018	Mean air temperature (°C)	15.7	19.2	20.9	22.4	23.0	17.2
	Rainfall (mm)	51.6	50.5	144.4	94.4	66.1	36.4
2019	Mean air temperature (°C)	11.9	13.2	23.2	21.9	22.9	16.4
	Rainfall (mm)	76.6	185.1	79.4	61.9	40.2	66.5

The experiment was set up as a randomized complete system with three replicates. Maize hybrid seed (KWS Kolumbaris) was obtained from Seed Company "KWS". Seed of the cowpea cultivar "Dolga vigna" was obtained from "Sjemenarna" company. The individual plot size was 50 m × 2,8 m for each treatment. The maize population 75 000 plants ha⁻¹ (SM) were spaced at 70 cm × 19 cm and cowpea population 37 500 (MC₁), 50 000 (MC₂) and 75 000 plants ha⁻¹ (MC₃) were spaced at 70 cm × 38.1 cm, 70 cm × 28.6 cm and 70 × 19 cm, respectively, in rows alternating with maize. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done in spring using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha⁻¹, 100 kg P₂O₅ ha⁻¹ and 200 kg of K₂O ha⁻¹. Maize and cowpea were sown to a depth of approximately 5 cm by maize drill on May 8, 2018 and on May 3, 2019, respectively. Herbicide Wing P (active substances 212.5 g/L dimethenamide-p and 250 g/L pendimethalin) was applied pre emergence in intercropping maize with cowpea at a dose of 4 L ha⁻¹. The soil of the research area has a pH 4.2 (M-KCl), 3.2% humus (organic matter), and is poorly supplied with physiologically active phosphorous (4.6 mg P₂O₅/100 g soil), medium supplied with physiologically active potassium (20.0 mg K₂O/100 g soil), while the total nitrogen content averaged 0.17% in topsoil. The fresh fodders were hand harvested when the maize reached soft dough stage and was cowpea at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldah, phosphorus was analyzed by colorimetry (AOAC, 2000) and neutral detergent fiber according to Van Soest et al. (1991). Analyses of variance were made for dry matter yield and forage quality parameters (P<0.05), and the Tukey test was used for comparing means (P<0.05). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and discussion

The differences in the yield of dry matter (Table 2) were statistically significant (P<0.05). Dry matter yield ranged from 21.0 t ha⁻¹ (MC₃) to 19.4 t ha⁻¹ (SM) in 2018. In the following growing season of 2019, the yield of dry matter yield ranged from 20.2 t ha⁻¹ (MC₃) to 17.1 t ha⁻¹ (SM). Consequently, dry matter yields were higher in 2018 than in 2019. This could be due to the impact of more favorable environmental factors (effect of the year) such as solar radiation, water and temperature during plant vegetation. According to obtained results, when cowpea had increased seed number in intercrop, dry matter yields on parcels increased. Cowpea can be intercropped with maize (Dahmardeh et al., 2009; Horvatić et al., 2018; Uher et al., 2019) and sorghum (Azraf et al., 2007) for a higher yield and quality compared with sole cropping. Legume contribution to maize in mixtures was significant and increased the total biomass yield of mixtures (Geren et al., 2008). One of the main reasons of intercropping maize and cowpea is to increase the level of crude protein in silage fodder.

Table 2. Yield of dry matter and yield of crude proteins of maize and maize-cowpea intercropped

Treatments	Yield of dry matter			Yield of crude proteins		
	in t ha ⁻¹			in t ha ⁻¹		
	2018	2019	Mean	2018	2019	Mean
SM	19.4b	17.1b	18.3b	1.51d	1.27d	1.39d
MC ₁	20.0ab	17.6ab	18.8ab	1.86c	1.57c	1.72c
MC ₂	20.6ab	19.1ab	19.9ab	2.04b	1.81b	1.93b
MC ₃	21.0a	20.2a	20.6a	2.23a	2.02a	2.13a
Mean	20.3a	18.5b		1.91a	1.67b	

Different letters in the column indicate significant difference (P<0.05)

Crude proteins are very important in cattle feed and, silage containing more crude proteins is desirable. In this study it was found that the yield of crude proteins of intercropped fodder MC₁, MC₂ and MC₃ was significantly (P<0.05) higher compared to SM (sole crop maize) during the both years of investigation (Table 2). Treatment MC₃ had the highest yield of crude protein averaging 2.23 t ha⁻¹ in 2018 and 2.02 t ha⁻¹ in 2019 in comparison to other fresh fodder mixtures (Table 2). In this study it was found that the value of crude proteins of intercropped forage MC₁, MC₂ and MC₃ was statistically significantly (P<0.05) higher compared to SM (sole crop maize) during the both years of investigation (Table 3). According to the results, when cowpea had increased seeds number in intercrops, the content of crude protein in the mixture increased.

Table 3. Content of crude protein and content of neutral detergent fiber of maize and maize-cowpea intercropped

Treatments	Crude protein			Neutral detergent fiber		
	in g kg ⁻¹ dry matter			in g kg ⁻¹ dry matter		
	2018	2019	Mean	2018	2019	Mean
SM	78d	74b	76d	367a	381a	374a
MC ₁	93c	89a	91c	355ab	368b	362b
MC ₂	99b	95a	97b	342b	356c	349c
MC ₃	106a	100a	103a	327c	338d	333d
Mean	94a	90b		348b	361a	

Different letters in the column indicate significant difference (P<0.05)

Cowpea fodder is a rich source of crude protein, giving up to 184 g kg⁻¹ (Khan et al., 2010). Results in the present study were in agreement with other studies where legumes also increased crude protein concentration in a mixture with maize (Dawo et al., 2007; Horvatić et al., 2018; Uher et al., 2019). This could be due to higher nitrogen availability for maize in intercropping compared with the sole crop (Eskandari et al., 2009). From this point of view, fodder produced in maize-cowpea intercrops is important not only to profit from the increase in the content of crude protein, but also from the reduction of the content of neutral detergent fibers. For this reason, the best option in maize-cowpea intercropping is the use of cowpea genotypes that provide forage with the greatest proportion of pods at harvest. In addition, the level of neutral detergent fibers is associated with the stage of maturity of the fodder due to the level of the cell wall components, mainly cellulose, hemicellulose and lignin (Mugweni et al., 2000). The value of a neutral detergent fiber refers to the total cell wall and consists of an acid detecting fiber fraction plus hemicellulose. In this study it was found that the neutral detergent fibers of intercropped MC₁, MC₂ and MC₃ were significantly (P<0.05) lower than SM (sole crop maize) during a two years research (Table 3). According to the results, when cowpea had increased seed number in intercrop, the values of neutral detergent fibers in the mixture decreased. Neutral detergent fiber is the measure of the total content of fiber (hemicellulose, cellulose and lignin) in silage. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006). In general, the concentration of neutral detergent fibers is

higher for grass than for legumes (Dahmardeh et al., 2009). Since smaller amounts of fiber components are used for better digestion, the cowpea intercropped plots to be superior to sole crop maize in terms of neutral detergent fiber.

Conclusion

The conclusion of this study is that intercropping of maize with cowpea at various in different sowing densities was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced the nutrient quality of fresh fodder mixture. Intercropping of maize with cowpea increased the value of crude protein and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the MC₃ treatment (75 000 plants ha⁻¹ of maize and 75 000 plants ha⁻¹ of cowpea) was most effective regarding the nutrient composition in fresh forage.

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Agronomska i gospodarska svojstva sorata pšenice Bc instituta d.d. Zagreb

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Sažetak

Cilj rada bio je utvrditi ulogu sorte i vremenskih prilika tijekom vegetacije 2018./2019. na prinos, komponente prinosa i druga svojstva ozime pšenice. U poljskom pokusu analizirano je ukupno 6 sorti pšenice Bc instituta d.d. Zagreb (Bc Anica, Bc Lorena, Bc Mandica, Bc Ljepotica, Bc Darija i Bc Opsesija) u tri ponavljanja. U usporedbi s višegodišnjim prosjekom analiziranu godinu je karakterizirala nešto manja količina oborina tijekom vegetacije uz prosječno više temperature zraka za 2°C. Analizom varijance je utvrđena statistička značajnost ($P < 0,005$) za sve ispitivane parametre osim za broj klasova po m². Općenito, određenu signifikantnost u pojedinom svojstvu su pokazale četiri sorte u istraživanju (Bc Anica, Bc Darija, Bc Lorena i Bc Mandica).

Ključne riječi: sorte pšenice, vremenske prilike, agronomska svojstva, ekonomska svojstva

Uvod

Pšenica (*Triticum aestivum* L.) je najvažnija krušarica i najznačajniji ratarski usjev širom svijeta koja se koristi ponajviše za ljudsku prehranu. Zbog širokog areala rasprostranjenosti i prilagodljivosti različitim agroekološkim uvjetima zastupljena je na oko ¼ svjetskih oranica (FAOSTAT, 2019.). Ovako velikoj zastupljenosti je zasigurno doprinijelo postojanje izuzetno velikog broja varijeteta i genotipova, odnosno sorti. S botaničkog gledišta sorta ili kultivar je naziv za skupinu biljaka koje pripadaju istoj vrsti koje se po vanjskom izgledu i proizvodnim svojstvima mogu značajno razlikovati. Navedeno su potvrdili mnogi autori temeljem ispitivanja sorti pšenice pod utjecajem različitih tretmana (Dhaka i sur., 2006.; Maras, 2010.; Jukić i sur., 2018.; Arif i sur., 2019.). Zaman i sur. (2017.) su analizirali tri sorte pšenice (Bari Gom 25, Bari Gom 26 i Sourav) te utvrdili značajne razlike između testiranih parametara. U uvjetima nedostatka vode sorta Bari Gom 26 je imala najveći broj klasića po klasu, masu 1000 zrna i žetveni indeks te najveći prinos što upućuje na važnost sorte. Značajne razlike je utvrdio i Kendal (2019.) na temelju ispitivanja 10 sorti pšenice. Autori nadalje zaključuju kako neka svojstva (broj klasova po jedinici površine, dužina klasa, broj klasića po klasu, sadržaj klorofila u listu) izravno ili neizravno utječu na prinos i kvalitetu zrna. Slično istraživanje je proveo Heđi (2019.) s 12 sorti ozime pšenice tijekom četiri godine (2015. - 2018.). Autor je utvrdio značajan utjecaj godine, sorte i njihove interakcije uz variranje prosječnih prinosa od 8,26 t ha⁻¹ do 11,90 t ha⁻¹. U Republici Hrvatskoj se na Sortnoj listi u 2019. nalazilo 177 sorti ozime pšenice različitih domaćih i stranih sjemenskih kuća (www.hapih.hr) što proizvođačima omogućuje, ali istovremeno i otežava izbor sorte. Stoga je cilj rada bio utvrditi prinos, komponente prinosa (broj klasova po m², broj zrna po klasu i masa 1000 zrna) i neka agronomska svojstva (visina biljke, masa vlati, masa klasa, dužina klasa, hektolitarska masa i žetveni indeks) šest različitih sorti pšenice Bc Instituta d.d. Zagreb u vegetacijskoj godini 2018./2019.

Materijal i metode

Poljski pokus je postavljen 24. listopada 2018. na OPG-u Šormaz u mjestu Klisa (45.48'13" SGŠ i 18.82'52" JGŠ) sjetvom šest sorti ozime pšenice sjemenske kuće Bc institut d.d. Zagreb (Bc Anica, Bc Lorena, Bc Mandica, Bc Ljepotica, Bc Darija i Bc Opsesija). Pokus je postavljen po potpuno slučajnom planu u tri ponavljanja, a veličina površine svake sorte je iznosila 420 m². Tijekom cijele vegetacije provedene su uobičajene agrotehničke mjere u

intenzivnoj proizvodnji pšenice s posebnim naglaskom na gnojidbu te zaštitu od bolesti i štetnika. Neposredno prije žetve obavljeno je mjerenje visine biljaka pomoću drvenog metra na slučajnom uzorku od 30 biljaka za svaku sortu i svako ponavljanje. Uzorci biljnog materijala uzeti su 5. srpnja 2019. godine sa 1 m² (4 x 0,25 m²) sa svake osnovne parcele uz pomoć škara, kvadrata i papirne vrećice nakon čega su utvrđeni prinosi, broj klasova po m², dužina klasa, broj zrna po klasu, masa vlata, masa klasa, hektolitarska masa i masa 1000 zrna. Iako je na određenim dijelovima pokusa bilo polegnutih biljaka za potrebe istraživanja uzorci su uzeti s mjesta gdje su biljke bile u uspravnom položaju. Broj klasova po m² je određen brojanjem svih klasova uzetih sa m² dok je vršidba uzoraka obavljena pomoću stacionirane vršilice prilagođene za manje pokuse. Masa vlata (g), dužina klasa (cm), masa klasa (g) i broj zrna po klasu određeni su na slučajnom uzorku od 30 biljaka jednostavnim mjerenjem, vaganjem i brojanjem zrna. Hektolitarska masa je određena pomoću uređaja Dicke John GAC 2100, a masa 1000 zrna ručnim brojanjem i vaganjem zrna. Žetveni indeks (ŽI) je izračunat po formuli masa zrna x 100 / ukupna biološka masa. Sva mjerenja su napravljena na Fakultetu agrobiotehničkih znanosti Osijek u Laboratoriju za analizu ratarskih usjeva. U radu su korišteni podaci srednjih mjesečnih temperatura zraka (°C) i mjesečnih količina oborina (mm) tijekom vegetacijskog razdoblja pšenice 2018./2019. godine kao i višegodišnji podatci srednjih mjesečnih temperatura zraka i mjesečnih količina oborina za razdoblje od 1961. do 1990. Svi podatci su prikupljeni na meteorološkoj postaji Osijek (aerodrom Klisa) Državnog hidrometeorološkog zavoda Republike Hrvatske. Statistička obrada podataka o istraživanim svojstvima je provedena pojedinačnom analizom varijance uz korištenje F testa. Značajnost razlika između prosječnih vrijednosti ispitivanih faktora i tretmana je ocjenjena LSD-om. Dobiveni rezultati su obrađeni u računalnim programima Excel i SAS Software 9.4. (SAS STAT, 2013.).

Rezultati i rasprava

S obzirom da vegetacija ozime pšenice traje dugo utjecaj vremenskih prilika može imati izuzetan značaj. U pogledu nedostatka vode pšenica je najosjetljivija u fazi vlatanja, zatim tijekom cvatnje i oplodnje te formiranja i nalijevanja zrna. Također, djelovanje visokih temperatura, naročito tijekom cvatnje i oplodnje, ima značajnu ulogu u opadanju prinosa (Kovačević i Rastija, 2014.). Sa stajališta potrebe pšenice prema vodi i temperaturi vegetacijska sezona 2018./2019. je bila relativno povoljna (Tablica 1.). Iako je količina oborina od listopada do travnja bila manja od prosjeka to se nije negativno odrazilo na pšenicu. S druge strane kada pšenica ima najveće potrebe za vodom količine oborina su bile veće za 39%, 102% i 21%. U istom razdoblju prosječne temperature zraka su bile više za oko 22%.

Tablica 1. Vremenski uvjeti 2018./2019. i višegodišnji prosjeci (VGP) od 1961. do 1990. za meteorološku postaju Osijek (DHMZ, 2019.)

	X.	XI.	XII.	I.	II.	III.	IV.	V.	VI.	VII.*	Ukupno/ Prosjek
2018./	Oborine (mm)										
2019.	14	32	24	42	20	11	75	119	106	45	488
VGP	41	57	52	47	40	45	54	59	88	65	548
2018./	Temperature (°C)										
2019.	14,4	7,6	1,5	0,3	4,6	9,6	13,2	14,6	23,6	22,9	11,2
VGP	11,2	5,4	0,9	-1,2	1,6	6,1	11,3	16,5	19,5	21,1	9,2
	Odstupanje (%)										
mm	-66	-44	-54	-11	-50	-76	+39	+102	+21	-30	-11
°C	+29	+41	+67	+125	+188	+57	+17	-12	+21	+9	+22

* podatci od 1.-25. srpnja 2019.

Analizom varijance je utvrđena statistička značajnost ($P < 0,005$) za sve ispitivane parametre osim za broj klasova po m^2 (Tablica 2.). Prosječno je u istraživanju utvrđeno visokih $8,18 t ha^{-1}$ zrna pšenice uz značajne razlike između sorti. Najprinosnija sorta je bila Bc Anica iako se nije značajnije razlikovala od preostale četiri sorte. Nešto slabiji prinos sorte Bc Mandica je rezultat nešto većeg napada bolesti koja je uočena prilikom uzimanja uzoraka iako su primijenjena dva prskanja protiv bolesti. Pošto klasanje kod svih sorti ne nastupa istovremeno bilo je nemoguće tretirati svaku sortu posebno što je mogući razlog napada uzročnika bolesti na Bc Mandici. Ispitivanjem četiri sorte pšenice tijekom dvije godine Fetahu i sur. (2019.) su također utvrdili signifikantne razlike i široku varijabilnost između sorti, godina i njihovih interakcija. U pogledu komponenti prinosa zabilježena je visoka signifikantnost samo kod dvije komponente. Prosječan broj zrna po klasu je bio relativno nizak (30,2) uz postignutu razliku od čak 12 zrna između sorti. Prosječna masa 1000 zrna je bila također relativno niska (39,9 g) uz variranje od 46,6 g do 33,0 g. Treba istaknuti kako je Bc Opsesija postigla najniže vrijednosti mase 1000 zrna iako je imala vrlo visok prinos zrna. Veće prosječne vrijednosti broja zrna po klasu (37,2) i mase 1000 zrna (43,4 g) utvrđene su u istraživanju Iljkić i sur. (2019.) na temelju pet sorti pšenice. U pokusu je ostvarena prosječna hektolitarska masa od $78,4 kg/ha$ što bi prema važećem Pravilniku o ugovornim odnosima pri otkupu pšenice (NN 62/2019) pšenica pripala kategoriji I. klase.

Tablica 2. Prinos, komponente prinosa i hektolitarska masa ispitivanih sorti pšenice

Sorta	Prinos t/ha	Broj klasova/ m^2	Broj zrna po klasu	Masa 1000 zrna (g)	Hektolitarska masa (kg/hl)
Bc Anica	9,64 a	633	30,2 bc	46,6 a	80,9 a
Bc Darija	8,09 ab	550	25,6 cd	45,6 a	82,0 a
Bc Ljepotica	7,91 ab	704	24,0 d	38,8 c	76,3 b
Bc Lorena	7,83 ab	595	36,1 a	42,3 b	81,3 a
Bc Mandica	7,09 b	708	33,6 ab	33,5 d	73,4 c
Bc Opsesija	8,51 ab	710	31,5 ab	33,0 d	76,3 b
Prosjeck	8,18	650	30,2	39,9	78,4
LSD 0,05	2,44	ns	5,84	3,13	1,88

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju.

Za razliku od komponenata prinosa agronomska i morfološka svojstva nemaju direktan utjecaj na prinos ali u određenoj mjeri mogu imati pozitivan ili negativan učinak. Na primjer, sorta više stabljike je manje otporna na polijeganje što može imati za posljedicu niži prinos i slabiju kvalitetu zrna. Prosječna visina biljke u istraživanju je bila 81,5 cm uz variranja od 75,6 cm (Bc Ljepotica) do 86,7 cm (Bc Mandica). Dvije najviše biljke u istraživanju su imale i najveću masu vlata što je i očekivano (Tablica 3.). Međutim, veću masu vlata je postigla i sorta prosječne visine (Bc Opsesija) što može značiti kako ima nešto deblje stijenke što je vrlo poželjno svojstvo sa stajališta toleratnosti na polijeganje. Iako dužina klasa nije direktna komponenta prinosa može imati važnu ulogu u postizanju visokih prinosa jer je dužina klasa osnova za velik broj zrna na klasu i posljedično veći prinos. Prosječna dužina klasa u istraživanju je iznosila 7,73 cm uz vrlo značajne razlike između sorti od 8,47 cm (Bc Mandica) do 6,28 cm (Bc Ljepotica). Međutim, najduži klasovi u ispitivanju nisu postigli i najveću masu klasa pri čemu je Bc Lorena imala najveću vrijednost od 1,93 g po jednom klasu. Žetveni indeks (ŽI) je pokazatelj omjera prinosa zrna i ukupnog biološkog prinosa odnosno udjela zrna u nadzemnoj masi. U provedenom istraživanju ŽI je iznosio prosječno 37,1%, ali uz izuzetno značajna variranja između sorti od 42,8% (Bc Darija) do 27,9% (Bc Mandica) uz napomenu kako je ova sorta bila najviše zaražena biljnim bolestima.

Tablica 3. Morfološka svojstva i žetveni indeks ispitivanih sorti pšenice

Sorta	Visina biljke (cm)	Masa vlati (g)	Dužina klasa (cm)	Masa klasa (g)	Žetveni indeks (%)
Bc Anica	80,5 b	1,05 bc	8,35 a	1,70 b	41,8 a
Bc Darija	81,4 b	1,00 c	7,52 c	1,65 b	42,8 a
Bc Ljepotica	75,6 c	0,97 c	6,28 d	1,29 c	36,2 b
Bc Lorena	85,5 a	1,35 a	7,65 bc	1,93 a	40,6 a
Bc Mandica	86,7 a	1,23 ab	8,47 a	1,29 c	27,9 c
Bc Opsesija	79,1 bc	1,26 ab	8,13 ab	1,64 b	33,1 b
Prosjeak	81,5	1,14	7,73	1,58	37,1
LSD 0,05	3,57	0,23	0,61	0,22	3,70

^{a,b,c} Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju.

Zaključak

Ispitivanjem šest sorti ozime pšenice Bc instituta d.d. Zagreb u istim agroekološkim uvjetima vegetacijske godine 2018./2019. utvrđena je statistička značajnost ($P < 0,005$) kod čak devet analiziranih svojstava uz izuzetak broja biljaka po m² te visoka varijabilnost kod svih svojstava. Sorta Bc Anica je postigla najviši prinos (9,64 t ha⁻¹) i masu 1000 zrna, sorta Bc Darija najveću hektolitarsku masu i žetveni indeks, a sorta Bc Lorena najveći broj zrna po klasu, masu vlati i masu klasa. Iako je imala najniži prinos vjerojatno uslijed jačeg napada biljnih bolesti Bc Mandica je ostvarila najveću dužinu klasa i najveću visinu biljke. Prikazani rezultati naglašavaju značaj i važnost izbora sorti za pojedino područje kao i preporuku sjetve više različitih visokoprinosnih sorti.

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Agronomic and economic properties of wheat varieties of Bc institute d.d. Zagreb

Abstract

The aim of this study was to determine the role of variety and weather during the 2018/2019 vegetation on yield, yield components and other characteristics of winter wheat. In the field experiment, a total of 6 wheat varieties of the Bc Institute d.d. Zagreb (Bc Anica, Bc Lorena, Bc Mandica, Bc Ljepotica, Bc Darija and Bc Opsesija) were analyzed in three repetitions. Compared to the long term mean, the 2018/2019 was characterized by slightly lower rainfall during vegetation with an average higher air temperature of 2 °C. Analysis of variance established statistical significance ($P < 0.005$) for all tested parameters except for the ear number per m^2 . In general, the four varieties in the study showed some significance in each trait (Bc Anica, Bc Darija, Bc Lorena i Bc Mandica).

Keywords: wheat varieties, weather condition, agronomic traits, economics traits

Effects of seeding rate, seed inoculation and Bio-algeen application on common vetch-wheat forage yield in Western Slavonia

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Abstract

The objective of this research was to evaluate whether the application of seed inoculants or growth bio stimulators can enable ecological management of common vetch (*Vicia sativa* L.) in mixture with wheat (*Triticum aestivum* L.) at different seeding rates. The effect of different common vetch cultivar Poppelsdorfer sowing rates (40, 60, 80, 100 grains/m²) in mixture with wheat cv. Valerius (200 grains/m²), on fodder yield was evaluated through 3 treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bv. *viciae* 1001 and Bio-algeen growth bio stimulator application). Seeding rate of 80 seeds of common vetch inoculated with *Rhizobium leguminosarum* bv. *viciae* 1001 in mixture proved to be the optimum, and there is no need to apply higher seeding rates of common vetch.

Keywords: common vetch, wheat, seeding rate, seed inoculation, growth stimulator

Introduction

The advantage of growing vetch and wheat together is that vetch has a well-developed taproot system while wheat has a hair root system (Lauk and Lauk, 2006). It provides good quality palatable forage biomass, and it is usually sown mixed with a cereal companion for animal feeding (Lithourgidis et al. 2006). Oat, barley, wheat and triticale are added to provide a climbing frame for the legumes and to increase the bulk of feed produced (Tuna and Orak, 2007). Due to its high ability to fix nitrogen and to produce quality fodder, it could serve as one of the richest, yet potentially the cheapest protein source for profitable ecological management. The price of common vetch seeds is high, so it is important to optimize the sowing rate. It is documented in previous research that mixture of legumes and small grain cereal has some advantages over pure stands, as crops within the mixtures use the environmental resources efficiently and produce more yield than their pure stands (Atis et al., 2012; Roberts et al., 1989; Štafa et al., 1998; Uher et al., 2009). Intercropping of legumes and cereals has produced higher yields than sole cereal crops primarily on soils with no N-fertiliser (Jensen, 1996; Hauggaard-Nielsen et al., 2001; Lauk and Lauk, 2006). The above-ground plant material of common vetch may contain more than 100 kg N ha⁻¹ originating from N₂-fixation (Papastylianou 1999; Mueller and Thorup-Kristensen, 2001). Cereal crops use the nitrogen fixed by the legume crops and that fact could influence not only quantity of fodder, but quality. Uher et al. 2007 reported that the highest forage pea crude protein yield was obtained in mixture with wheat compared to other cereals, and all cereals in mixtures with forage pea significantly better yielded in late boot vegetative stage compared to early head. Protein and carbohydrate rates of feed originated from mixtures are balanced and the feed from mixture has higher feeding value compared to the feed from pure sowings of mixture components (Atis et al., 2012; Caballero et al., 1995; Lithourgidis et al., 2006; Karadag and Buyukburc, 2003; Tuna and Orak, 2007; Mariotti et al., 2009; Uher, et al., 2007). Feeding vetch in mixtures with small grain cereals is reported to increase milk yields of cows and growth performance of beef cattle (Štafa et al., 2001). The choice of a legume species and compatible plant densities are very important for high forage yields and quality in intercrops with cereals (Altinok et al., 1997). The inclusion of common vetch in a crop rotation is considered a precondition to increasing the overall production of the crop rotation (Yau et al., 2003). Furthermore, it is valuable to ascertain whether the application of seed inoculants or growth bio-stimulators can enable ecological management and successful fodder production in the temperate region of Western Slavonia.

Material and methods

Research was conducted through field experiment set up on agricultural area near city of Nova Gradiška, Western Slavonia, during the vegetative seasons 2016/17 and 2017/18. The effect of different common vetch cultivar Poppelsdorfer sowing rates (40, 60, 80, 100 grains/m²) in mixture with wheat cultivar Valerius (200 grains/m²), on fodder yield was evaluated through 3 treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bv. *viciae* 1001 and Bio-algeen growth bio stimulator application). Four common vetch seeding rates (40, 60, 80, 100 grains/m²) in mixture with wheat cultivar Valerius (200 grains/m²), were grown through three treatments (control without fertilization, seed inoculation with strain *Rhizobium leguminosarum* bv. *viciae* 1001 and Bio-algeen growth bio stimulator application), in all possible combinations in a randomized, completed block experimental design with 3 replications. The dates of sowing were on 8th of October 2016 and 9th of October 2017. Common vetch and wheat seeds were mixed prior to sowing and were sown in the same row using a special plot drill. The area of the test plots was 30 square meters. The previous crop was wheat in both years of the study. A 5 m² section was harvested for green herbage in full of flowering and samples of 1 kg were dried at 60 °C for 48 h, to determine dry matter yield. The experiment was established on an anthropogenic, moderately moist soil having a slightly sandy-clayey texture. The soil characteristics were as follows: pH u nKCl of the ploughed layer was 5.25, organic matter content 2.70 %. An analysis of variance was carried out using SAS 9.3 (SAS Institute Inc. 2011). The statistical significance of the treatments was determined at the P<0.05 probability levels using the F-test and treatment means were compared by LSD.

Results and discussion

Intercropping of common vetch with wheat achieved maximum green mass yield (GMY) by increasing the seeding rate due to increased plant density, as expected. Therefore, the plots sown at higher common vetch seeding rates (80 and 100 grains/m²) covered the ground faster after emergence.

Table 1. Green mass yield of the mixtures seeding rate as affected by the treatment (t ha⁻¹).

Seeding rate / seed no. /m ²	Treatment/Year					
	Control		Growth bio stimulator		<i>Rhizobium leguminosarum</i> bv. <i>viciae</i> 1001	
	2017	2018	2017	2018	2017	2018
40 vetch + 200 wheat	32.0 ^c	30.0 ^c	32.0 ^c	29.0 ^c	35.0 ^c	33.0 ^c
60 vetch + 200 wheat	43.0 ^b	38.0 ^b	42.0 ^b	40.0 ^b	44.0 ^b	43.0 ^b
80 vetch + 200 wheat	44.0 ^b	47.0 ^a	46.0 ^a	48.0 ^a	63.0 ^a	66.0 ^a
100 vetch + 200 wheat	47.0 ^a	48.0 ^a	45.0 ^{ab}	48.0 ^a	60.0 ^a	62.0 ^a

Values with the different small letters in column are significantly different according to the LSD test at P < 0.05.

In control, the highest green mass yield (GMY) determined was achieved in both years with 100 grains/m², 47.0 t ha⁻¹ in 2017 and 48.0 t ha⁻¹ in 2018, (P<0,05), respectively. The GMY determined with growth stimulator application was significantly higher (P<0.05) with 80 grains/m², 46.0 t ha⁻¹ in 2017 and 48.0 t ha⁻¹ in 2018, compared to two lower seeding rates. In that treatment there were no significant differences between two higher seeding rates. The GMY determined with *Rhizobium leguminosarum* bv. *viciae* 1001 seed inoculation treatment in both years was not significantly different between 80 and 100 grains/m², although both those 2 higher rates had significantly higher yield (P<0.05), compared to 2 lower seeding rates (40 and 60 grains/m²). The dry matter yield (DMY) is shown on Table 2.

Table 2. Dry matter yield of the mixtures seeding rate as affected by the treatment (t ha⁻¹).

Seeding rate / seed no. /m ²	Treatment/Year					
	Control		Growth bio stimulator		<i>Rhizobium leguminosarum</i> bv. <i>viciae</i> 1001	
	2017	2018	2017	2018	2017	2018
40 vetch + 200 wheat	7.36 ^b	6.90 ^c	7.36 ^b	6.67 ^c	8.05 ^c	7.59 ^c
60 vetch + 200 wheat	9.89 ^a	8.74 ^{bc}	9.66 ^a	9.20 ^b	10.12 ^b	9.89 ^b
80 vetch + 200 wheat	10.12 ^a	10.81 ^{ab}	10.58 ^a	11.04 ^a	14.49 ^a	15.81 ^a
100 vetch + 200 wheat	10.81 ^a	11.04 ^a	10.35 ^a	11.04 ^a	13.80 ^a	14.26 ^a

Values with the different small letters in column are significantly different according to the LSD test at $P < 0.05$.

In control, the highest dry matter yield (DMY) determined was achieved in both years with 100 grains/m², 10.81 t ha⁻¹ in 2017 and 11.04 t ha⁻¹ in 2018, ($P < 0.05$), respectively. The highest DMY determined with growth stimulator application was significantly higher ($P < 0.05$) with 60, 80 and 100 grains/m² (9.66, 10.58 and 10.35 t ha⁻¹, respectively) in 2017, compared to the lowest seeding rate (40 grains/m²). The DMY determined with growth stimulator application in both years was not significantly different between 80 and 100 grains/m², although those 2 higher rates had significantly higher yield ($P < 0.05$), compared to 2 lower seeding rates (40 and 60 grains/m²). The DMY determined with *Rhizobium leguminosarum* bv *viciae* 1001 seed inoculation treatment in both years was not significantly different between 80 and 100 grains/m², although those 2 higher rates had significantly higher yield ($P < 0.05$), compared to 2 lower seeding rates (40 and 60 grains/m²). The highest DMY and the biggest potential for ecological management in both years was found by applying seed inoculation with *Rhizobium leguminosarum* bv *viciae* 1001 (14.49 t ha⁻¹ in 2017 and 15.81 t ha⁻¹ in 2018), at the seeding rate of 80 grains/m². Seed treatments and higher seeding rates of common vetch in mixture with wheat showed significant effects on forage yield.

Conclusions

This research shows that common vetch-wheat forage crop mixture could be grown under ecological management in temperate climate of Western Slavonia. Two higher seeding rates (80 and 100 grains/m²) resulted in greater green and dry matter yield compared to two lower seeding rates (40 and 60 grains/m²). Seeding rate of 80 seeds of common vetch inoculated with *Rhizobium leguminosarum* bv *viciae* 1001 in mixture with 200 seeds of wheat per 1 m² proved to be the optimum measure, and there is no need to apply higher seeding rates of common vetch.

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Influence of intercropping maize with soybean on forage yield and quality

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Abstract

Maize and soybean intercropped in various row ratios and pure maize crop were evaluated to determine the best intercropping system regard with to forage yield and quality. The highest dry matter yield over two years (19.9 t ha⁻¹) was produced by intercropping 1 row of maize with 3 rows of soybean (1M3S), whereas the lowest yield (16.3 t ha⁻¹) produced intercrop consisting of 1 row of maize with 1 row of soybean (1M1S). All intercrop had higher crude protein values in dry matter averaging 113 g kg⁻¹ for the 1M1S, 123 g kg⁻¹ for the 1M2S and 130 g kg⁻¹ for the 1M3S, than the sole crop maize (76 g kg⁻¹). Intercropping of maize with soybean reduced neutral detergent fiber content, which in turn, resulted in increased forage digestibility. Based on forage yield and quality, this study showed that among all intercropped forages the 1M3S treatment was better performing than other intercrops.

Keywords: maize sole crop, soybean, intercrop, crude protein, neutral detergent fiber

Introduction

In many regions of Europe, whole-plant maize silage is the basic feed used for cows and fattening cattle. Despite its high energy content, the protein content is low (88 g kg⁻¹) compared with legumes silage (Anil et al., 2000) and needs to be supplemented with proteins for better feed quality (Stoltz et al., 2013). As a cultivation system, intercropping involves planting two or more crops species in the same field (Costa et al., 2012). Intercropping maize with legumes for silage is a feasible strategy to improving the level of crude protein (Contreras-Govea et al., 2009). Appropriate spatial arrangements, planting proportions, and maturity dates of components in maize-legume intercropping enhance biological diversity and have many advantages over pure maize cropping. Although maize provides high yield in terms of dry matter, it produces low protein content in fodder. Proteins are needed for livestock growth and milk production. Javanmard et al. (2009) worked on intercropping of maize with different legumes, and pointed out that the dry matter and crude protein yields increased for all intercropping compositions compared to maize monocrop. The use of maize grown for silage and the seeding of soybean with maize in alternative-rows such as 1 maize + 1 soybean, 1 maize + 2 soybeans or 1 maize + 3 soybean significantly increased the quality of silage and the content of crude protein (Htet et al., 2016). Physiological and morphological differences between intercrop constituents influence on their ability to use resources and cereal with legume intercropping. Usually they have several advantages such as higher overall yields, better soil utilization (Dhima et al., 2007), yield stability of the cropping system (Lithourgidis et al., 2006), better use of light, water and nutrients (Javanmard et al., 2009), soil fertility through biological nitrogen fixation, increases soil conservation through greater soil coverage as compared to sole cropping, and ensures better soil-susceptible crop in monoculture (Lithourgidis et al., 2006), improved soil conservation (Anil et al., 1998), and better control of pests and weeds (Vasilakoglou et al., 2008). Atmospheric nitrogen fixation using legumes plants can reduce nitrogen competition in the reciprocal intercropping system of legumes and cereals enabling the cereals to use more nitrogen in the soil (Eskandari et al., 2009). This can affect the quality of the fodder intercrop components because the protein content is directly related to the content of nitrogen in the forage plants (Putnam et al., 1985). The present study was designed to determine the influence of different patterns of maize-soybean intercropping on forage yield and quality.

Material and methods

A field experiment was carried out during the 2017 and 2018 growing season at experimental fields in Oborovo (45°40'54"N, 16°15'12.5"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons in 2017 and 2018

Year	Meteorological data	Month					
		April	May	June	July	August	September
2017	Mean air temperature (°C)	11.9	17.3	22.4	23.3	22.7	14.8
	Rainfall (mm)	41.6	49.2	57.8	91.8	32.0	186.3
2018	Mean air temperature (°C)	15.7	19.2	20.9	22.4	23.0	17.2
	Rainfall (mm)	51.6	50.5	144.4	94.4	66.1	36.4

The experimental design was set up as a randomized complete block system with three replicates. Maize was seeded as sole crop (SM) and intercropped with soybean as follows: 1 maize row to 1 soybean row (1M1S), 1 maize row to 2 soybean rows (1M2S) and 1 row maize to 3 rows soybean (1M3S). Maize hybrid seed "KWS Kolumbaris" was obtained from Seed Company „KWS“. The soybean cultivar seed known as „OAC Wallace“ was obtained from „RWA Agro“ company. The individual plot size was 50 m × 2.8 m for each treatment. The maize and soybean were spaced at 70 cm × 19 cm and 70 cm × 5 cm with population of 75 189 and 285 720 plants per hectare, respectively. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done in spring using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 200 kg of N ha⁻¹, 100 kg P₂O₅ ha⁻¹ and 200 kg of K₂O ha⁻¹. Maize and soybean were sown to a depth of approximately 5 cm by maize drill on April 20, 2017 and on April 25, 2018, respectively. Herbicide Wing P (active substances 212.5 g/L dimethenamide-p and 250 g/L pendimethalin) was applied pre-emergence in intercropping maize with soybean at a dose of 4 L ha⁻¹. The soil of the research area has a pH 4.2 (M-KCl), 3.2% humus (organic matter), and is poorly supplied with physiologically active phosphorous (4.6 mg P₂O₅/100 g soil), medium supplied with physiologically active potassium (20.0 mg K₂O/100 g soil), while the total nitrogen content averaged 0.17% in topsoil. The fresh fodders were hand harvested when the maize reached soft dough stage and soybean was at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter (DM) content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein (CP) content was measured according to Kjeldahl (AOAC, 2000) and neutral detergent fiber according to Van Soest et al. (1991). Analyses of variance were made for dry matter yield and forage quality parameters (P<0.05), and the Tukey test was used for comparing means (P<0.05). Data were analyzed using SAS statistical software (SAS Institute Inc., 2013).

Results and discussion

The differences in the yield of dry matter (Table 2) were statistically significantly (P<0.05). Dry matter yields ranged from 16.0 t ha⁻¹ (1M1S) to 19.7 t ha⁻¹ (SM) in 2017. In the following growing season of 2018, the yield of dry matter yield ranged from 16.6 t ha⁻¹ (1M1S) to 20.5 t ha⁻¹ (SM). Consequently, dry matter yields were higher in 2018 than in 2017. This could be due to the impact of more favorable environmental factors (effect of the year) such as solar radiation, water and temperature during plant vegetation. The average yield of dry matter over the two years showed that 1M3S was the best intercropping production system with relatively small yield reduction compared to sole crop maize (Table 2). According to obtained results, when the number of soybean rows increased in intercrops, dry matter yields of parcels increased.

Table 2. Yield of dry matter and yield of crude proteins of maize and maize-soybean intercropped

Treatments	Yield of dry matter in t ha ⁻¹			Yield of crude proteins in t ha ⁻¹		
	2017	2018	Mean	2017	2018	Mean
SM	19.7a	20.5a	20.1a	1.46d	1.60d	1.53d
1M1S	16.0c	16.6c	16.3c	1.76c	1.93c	1.85c
1M2S	17.6b	18.4b	18.0b	2.13b	2.30b	2.22b
1M3S	19.6a	20.2a	19.9a	2.49a	2.69a	2.59a
Mean	18.2a	18.9a		1.96a	2.13a	

Different letters in the column indicate significant difference (P<0.05)

The form or system of mutually overlapping, such as the order of rows, density and genotypes used for intercropping may influence the yield of fresh fodder (Reta et al., 2010; Erdogdu et al., 2013). Herbert et al. (1984) found that yields of dry matter of maize-soybean intercrop were similar to monocrop maize. It has been reported that the maize-soybean intercrops produced a higher yield of dry matter than any of the species grown in pure crops (Geren et al., 2008; Eskandari, 2012). One of the possible explanations for higher yields for the intercrops is their ability to exploit different layers of soil without mutual competition. In addition, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetic active radiation and soil moisture during the rainy season may affect yield and potential application of the intercropping system (Anil et al., 1998; Lithourgidis et al., 2006). Geren et al. (2008) and Htet et al. (2016) indicate that the contribution of legumes in mixtures with maize was significant and increased the overall yield of the biomass. In this study it was found that the yield of crude proteins of intercropped fodder 1M1S, 1M2S and 1M3S was significantly (P<0.05) higher compared to SM (sole crop maize) during a two years research (Table 2). Treatment 1M3S had the highest yield of crude protein averaging 2.49 t ha⁻¹ in 2017 and 2.69 t ha⁻¹ in 2018 in comparison to other fresh fodder mixtures (Table 2). Reta et al. (2010) found that crude protein yields per hectare in intercrop treatments were higher (27.5 to 42.8%) than of pure maize crop. One of the main reasons of intercropping maize and soybean is to increase the level of crude protein in silage fodder. Crude proteins are very important in cattle feed and, silage containing more crude proteins is desirable. In this study it was found that the value of crude proteins of intercropped fodder 1M1S, 1M2S and 1M3S was statistically significantly (P<0.05) higher compared to SM (sole crop maize) during a two years research (Table 3). According to the results, when the number of soybean rows increased in intercrops, the content of crude proteins in the mixture increased. This could be due to the greater availability of nitrogen in intercropping arrangement compared to the pure maize crop (Eskandari, 2012). The findings in this study are consistent with other research in which legumes also increased the concentration of crude proteins when grown in mixture with maize (Baghdadi et al., 2016; Htet et al., 2016). Fodder produced in maize-soybean intercrops is important not only because of an increase in the content of crude protein, but also because of reduction in the content of neutral detergent fibers. For this reason, the best option in maize-soybean intercropping is the use of soybean genotypes that provide forage with the greatest proportion of pods at harvest. In this study it was found that the neutral detergent fibers of intercropped 1M1S, 1M2S and 1M3S were significantly (P<0.05) lower than SM (sole crop maize) during a two years research (Table 3).

Table 3. Content of crude protein and content of neutral detergent fiber of maize and maize-soybean intercropped

Treatments	Crude protein in g kg ⁻¹ dry matter			Neutral detergent fiber in g kg ⁻¹ dry matter		
	2017	2018	Mean	2017	2018	Mean
SM	74b	78c	76d	403a	423a	413a
1M1S	110a	116b	113c	370b	384b	377c
1M2S	121a	125ab	123b	378b	394b	386bc
1M3S	127a	133a	130a	385ab	403ab	394b
Mean	108a	113a		384b	401a	

Different letters in the column indicate significant difference (P<0.05)

According to the results, when soybean had increased row number in intercrop, the values of neutral detergent fibers in the mixture decreased. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh et al., 2009).

Conclusion

The conclusion of this study is that intercropping of maize with soybean at different planting patterns was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced the nutrient quality of fresh fodder mixture. Intercropping of maize with soybean has increased the value of crude protein and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the 1M3S treatment (intercropping 1 row of maize with 3 rows of soybean) was most effective regarding the nutrient composition in fresh forage.

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Influence of intercropping sweet sorghum with soybean on yield and crude protein content of fresh fodder

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Abstract

Sorghum and soybean intercropped in various row ratios and pure maize crop were to determine the best intercropping system with respect forage yield and quality. The highest dry matter yield over two years was produced by intercropping 1 row of sorghum with 3 rows of soybean and averaged 18.5 t ha⁻¹, whereas the lowest yield 16.3 t ha⁻¹ produced intercrop consisting of 1 row of sorghum with 1 row of soybean. All intercrop had higher crude protein values in dry matter averaging 109 g kg⁻¹ for the 1SB1S, 118 g kg⁻¹ for the 1SB2S and 132 g kg⁻¹ for the 1SB3S than the monocrop sorghum 76 g kg⁻¹. Intercropping of sorghum with soybean reduced neutral detergent fiber content, which in turn, resulted in increased forage digestibility. Based on forage yield and quality, this study showed that among all intercropped forages the 1SB3S treatment was better performing than other intercrops.

Keywords: sorghum sole crop, soybean, intercrop, crude protein, neutral detergent fiber

Introduction

Intercropping is the practice of cultivating two or more crops simultaneously on the same piece of land during the same time span (Guleria and Kumar, 2016). It is characterized by rotation and diversification in time and space dimensions (Biabania et al., 2008). Intercropping systems help farmers to exploit the principle of diversity (Ghosh, 2004). They help avoid reliance on a single crop and result in different products of different nature such as fodder, oil and pulses (Iqbal et al., 2018a). Another key benefit associated with intercropping is its potential to increase soil productivity per unit area and efficient utilization of farm resources (Mucheru-Muna et al., 2010). Cereals intercropping with legumes result in increased resource capture by component crops and improve soil microbial activity along with improved conversion resource efficiency that drives higher biomass production (Alvey et al., 2003). In addition, soil fertility improves when legumes are intercropped with cereal forages (Iqbal et al., 2018b). Ghosh et al. 2006, since different crops have different root lengths and so nutrients absorb from different soil horizons (Ghosh et al., 2007). Intercropping of cereals with legumes also increases productivity per unit of land area due to atmospheric biological nitrogen fixation (BNF) that takes place in the root nodules of legumes (Pal and Sheshu, 2001). Greater productivity per unit of surface area of sorghum-soybean intercropping systems resulted in 46% higher yields than their monocrop sorghum (Iqbal et al., 2017). Sorghum is an important silage crop and has an increasing popularity because of the need for relatively smaller quantities of maize water per unit dry matter production (Bean et al., 2013). Legumes contain more than twice as crude protein than forage sorghum, therefore, sorghum-legume intercropping has the potential to increase the biomass and quality of forage for per area compare to sole sorghum (Eskendari et al., 2009). Forage sorghum can be intercropped with forage legumes such as cowpea cluster bean, soybean etc. which are totally compatible with sorghum in terms of sowing time and irrigation (Iqbal et al., 2015). The present study was designed to determine the influence of different patterns of sweet sorghum-soybean intercropping on forage yield and quality of fresh fodder.

Material and methods

A field experiment was carried out during the 2015 and 2016 growing season at experimental fields in Daruvar (45°35'34"N, 17°13'25"E), Croatia. Meteorological data of the experimental site are presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons in 2015 and 2016

Year	Meteorological data	Month					
		April	May	June	July	August	September
2015	Mean air temperature (°C)	11.2	16.6	20.0	23.4	22.4	16.4
	Rainfall (mm)	34.0	173.4	34.8	50.6	71.7	95.5
2016	Mean air temperature (°C)	12.3	15.5	20.4	22.3	19.4	17.0
	Rainfall (mm)	35.3	83.7	99.6	152.6	66.2	48.5

The experimental design was set up a randomized complete block system with three replicates. Sweet sorghum was seeded as sole crop (SB) and intercropped with soybean as follows: 1 sweet sorghum row to 1 soybean row (1SB1S), 1 sweet sorghum row to 2 soybean rows (1SB2S) and 1 row sweet sorghum to 3 rows soybean (1SB3S). Sweet sorghum hybrid seed "KWS Zerberus" was obtained from Seed Company „KWS“. The soybean cultivar seed known as „OAC Wallace“ was obtained from „RWA Agro“ company. The individual plot size was 50 m × 2 m for each treatment. The sweet sorghum and soybean were spaced at 50 cm × 8 cm with population of 250 000 sweet sorghum and 250 000 plants soybean per hectare, respectively. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done in spring using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 150 kg of N ha⁻¹, 100 kg P₂O₅ ha⁻¹ and 200 kg of K₂O ha⁻¹. Sweet sorghum and soybean were sown to a depth of approximately 5 cm by drill on April 25, 2015 and on April 28, 2016. Herbicide Dual Gold 960 EC (active substances 960 g/L S-metolaklor) was applied pre-emergence and herbicide Basagran 480 (active substances 480 g/L bentazon) was applied post-emergence in intercropping sweet sorghum with soybean at a dose of 2 L ha⁻¹. The soil of the research area has a pH 5.7 (M-KCl), 2,1% humus (organic matter), and is poorly supplied with physiologically active phosphorous (14.9 mg P₂O₅/100 g soil), medium supplied with physiologically active potassium (21.5 mg K₂O/100 g soil), while the total nitrogen content averaged 0.15% in topsoil. The fresh fodders were hand harvested when the sweet sorghum reached early hard dough stage and soybean was at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldahl (AOAC, 2000) and neutral detergent fibre according to Van Soest et al. (1991). Analyses of variance were made for dry matter yield and forage quality parameters (P<0.05), and the Tukey test was used for comparing means (P<0.05). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and discussion

The differences in the yield of dry matter (Table 2) were statistically significant (P<0.05). Dry matter yields ranged from 15.9 t ha⁻¹ (1SB1S) to 18.2 t ha⁻¹ (SB) in 2015. In the following growing season of 2016, the yield of dry matter ranged from 16.7 t ha⁻¹ (1SB1S) to 19.6 t ha⁻¹ (SB). Consequently, dry matter yields were higher in 2016 than in 2015. This could be due to the impact of more favorable environmental factors (effect of the year) such as solar radiation, water and temperature during plant vegetation. The average yield of dry matter over the two years showed that 1SB3S was the best intercropping production system with relatively small yield reduction compared to sole crop sweet sorghum (Table 2). According to obtained results, when the number of soybean rows increased in intercrops, dry matter yields on parcels increased. One of the possible explanations for higher yields for the intercrops is their ability to exploit different layers of soil without mutual competition. Besides, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetic active radiation and soil moisture during the rainy season may affect yield and potential use of the intercropping system (Anil et al., 1998; Lithourgidis et al., 2006). Terzić et al., (2004) and Basaran et al., (2017) indicated that, legumes contribution to sweet sorghum in mixtures was significant and increased the dry matter yield. In this study it was found that the yield of crude proteins of intercropped fodder 1SB1S, 1SB2S and 1SB3S was significantly (P<0.05) higher than SB (sole crop sweet sorghum)

during a two year study (Table 2). Treatment 1SB3S had the highest yield of crude protein averaging 2.27 t ha⁻¹ in 2015 and 2.62 t ha⁻¹ in 2016 in comparison to other fodder mixtures (Table 2). In this study, crude protein yields per hectare (14.4% to 37.5%) were less compared to the results of other studies or research Terzić et al. (2004) and Basaran et al. (2017).

Table 2. Yield of dry matter and yield of crude proteins of sweet sorghum and sweet sorghum-soybean intercropped

Treatments	Yield of dry matter in t ha ⁻¹			Yield of crude proteins in t ha ⁻¹		
	2015	2016	Mean	2015	2016	Mean
SB	18.2a	19.6a	18.9a	1.33c	1.55d	1.44d
1SB1S	15.9b	16.7b	16.3b	1.69b	1.87c	1.78c
1SB2S	16.8a	17.7ab	17.3ab	1.92b	2.16b	2.04b
1SB3S	17.9a	19.1ab	18.5a	2.27a	2.62a	2.45a

Different letters in the column indicate significant difference (P<0.05)

One of the main reasons of intercropping sweet sorghum and soybean is the increase crude protein level in silage. Crude proteins are very important in cattle feed and, silage containing more crude proteins is desirable. In this study it was found that the value of crude proteins of intercropped fodder 1SB1S, 1SB2S and 1SB3S was significantly (P<0.05) higher compared to SB (sole crop sorghum) during a two year research (Table 3).

Table 3. Content of crude protein and content of neutral detergent fiber of sweet sorghum and sweet sorghum-soybean intercropped

Treatments	Crude protein in g kg ⁻¹ dry matter			Neutral detergent fiber in g kg ⁻¹ dry matter		
	2015	2016	Mean	2015	2016	Mean
SB	73d	79d	76d	558a	542a	550a
1SB1S	106c	112c	109c	515b	503b	509b
1SB2S	114b	122b	118b	490c	478c	484c
1SB3S	127a	137a	132a	467d	451d	459d

Different letters in the column indicate significant difference (P<0.05)

According to the results, when the number of soybean increased in intercrops, the content of crude protein in the mixture increased. The findings in this study are consistent with other research in which legumes also increased the concentration of crude proteins when grown in mixture with sweet sorghum (Terzić et al., 2004); Basaran et al., 2017). Fodder produced in maize-soybean intercrops is important not only because of an increase in the content of crude protein, but also because of reduction the content of neutral detergent fibers. For this reason, the best option in maize-soybean intercropping is the use of soybean genotypes that provide forage with the greatest proportion of pods at harvest. In this study it was found that the neutral detergent fibers of intercropped 1SB1S, 1SB2S and 1SB3S were significantly (P<0.05) lower than SB (sole crop sweet sorghum) during two years of research (Table 3). According to the results, when soybean had increased row number in intercrop, the values of neutral detergent fibers in the mixture decreased. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis et al., 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh et al., 2009).

Conclusion

The conclusion of this study is that intercropping of sweet sorghum with soybean at different planting patterns was shown to be an effective way to affect dry matter yield and crude protein yield, which in turn, enhanced the nutrient

quality of fresh fodder mixture. Intercropping of sweet sorghum with soybean has increased the value of crude protein and decreased values of neutral detergent fiber in fresh fodder mixture. Finally, the 1SB3S treatment (intercropping 1 row of sweet sorghum with 3 rows of soybean) was most effective regarding the nutrition composition in fresh forage.

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Agronomic traits of QPM maize hybrids adapted to temperate regions

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Abstract

In this paper agronomic characteristics of quality protein maize (QPM) hybrids adapted to temperate regions created in Maize Research Institut Zemun Polje are presented. Hybrid ZPQPM13 showed favorable traits for growing in these regions, and its grain yield was at the level of one of the leading ZP hybrids - ZP 606. However, grain moisture of this hybrid was 3% higher than that of ZP 606, pointing out to the tropical origin of its parental inbred lines. Namely, mother was obtained from F₁ cross between tropical and adapted lines, and father by marker assisted selection (MAS) from QPM line of tropical origin that was crossed and twice backcrossed with a commercial ZP line. In the future, in order to obtain even more adapted QPM hybrids, MAS program will continue with all the best commercial ZP inbred lines of all FAO groups.

Keywords: *Zea mays* L., *opaque2*, grain yield, grain moisture, lodging

Introduction

Maize kernel contains proportionally the highest quantity of proteins compared to the other cereals. However, its proteins are lacking two essential amino acids, lysine and tryptophan, that humans and monogastric animals cannot produce (Gernach et al., 2011). The malnutrition is the consequence of the fact that maize is a staple food in the undeveloped regions of the world. Homozygous recessive *opaque2* mutation (*o2o2*) leads to significant increase in these two amino acids, but this allele has several negative pleiotropic effects (soft kernel that crushes a lot, high incidence of lodging, susceptibility to insects and diseases) and could not be accepted commercially (Vasal, 2001). In CYMMIT (International Maize and Wheat Improvement Center) in Mexico City, by a large number of cycles of recurrent selection breeders succeeded to convert soft into hard endosperm of *o2o2* maize, and improve its agronomic traits to the level of the leading conventional hybrids (Vivek et al., 2008). Such maize was called Quality Protein Maize (QPM). Firstly developed QPM is of tropical origin, so there are constrains and difficulties to adapt it to temperate regions. Some researchers have shown positive effects in human and animal consumption with QPM (Krivanek et al., 2007; Mbuya et al., 2011). So far, a few attempts have been made to make temperate adapted QPM (Scot et al., 2009; Carena, 2013; Woral et al., 2015). In Maize Research Institute Zemun Polje we begun a QPM program in 2008, including developing new QPM lines from the crosses of tropical and commercial temperate lines, as well as marker assisted selection (MAS) for converting elite ZP inbred lines to their QPM versions (Ignjatović-Micić et al., 2013; Kostadinović et al., 2014). MAS have resulted in conversion of a line ZPL-5 of Lancaster origin, which is a father of series of the most commercial ZP hybrids (Kostadinović et al., 2016) to QPM (now called ZPL-5QPM). The rest of our commercial lines are in the last stages of conversion by MAS. Taking into consideration previously mentioned facts, the purpose of this research was an attempt to create the first ZPQPM hybrids well adapted to our climate and with agronomic traits at the level of commercial conventional hybrids.

Material and Methods

In 2015, by courtesy of M.P. Scott from Iowa State University, we have obtained a series of QPM lines (BQPM9, 10, 11, 12, 13, 15, 16 and 17) that were derived from two CYMMIT tropical lines and six lines released by Iowa State University (B91, 97, 98, 99, 100 and 113) and are adapted to the US corn belt (Worrall et al., 2015). These

lines were sown in Zemun Polje in 2016 in two replications for evaluation of agronomic traits and adaptation to our environmental conditions. Lines BQPM 9, 10 and 11 were selected for further work. On the other hand, from ZP QPM program three lines were chosen: GS-4, 5 and 6, that are derived from the same sources but in Serbian conditions. In winter nursery 2015/16 (Chile) this six lines were crossed as mothers as well as fathers with converted ZPL-5QPM line. The cross with line GS-5 as a father failed to produce seed. The hybrids were consecutively coded (Table 1).

Table 1. Codes of the experimental QPM hybrids

No.	Hybrid code	Hybrid combination
1	ZPQPM1	ZPL-5QPM × BQPM9
2	ZPQPM2	BQPM9 × ZPL-5QPM
3	ZPQPM3	BQPM10 × ZPL-5QPM
4	ZPQPM4	ZPL-5QPM × BQPM10
5	ZPQPM5	ZPL-5QPM × BQPM11
6	ZPQPM6	BQPM11 × ZPL-5QPM
7	ZPQPM9	GS-4 × ZPL-5QPM
8	ZPQPM10	ZPL-5QPM × GS-4
9	ZPQPM11	GS-5 × ZPL-5QPM
10	ZPQPM13	GS-6 × ZPL-5QPM
11	ZPQPM14	ZPL-5QPM × GS-6

In 2017 a test-trial in four locations, according to the Randomized Complete Block Design (RCBD) with three replications was conducted with obtained QPM hybrids and check hybrids: ZP 555, ZP 560, ZP 600, ZP 666 and ZP 606. The elementary plot consisted of two rows 0.75m apart with 20 hills per row 0.40m from each other. Sowing was done by hand, four plants per hill, and hills were thinned to two plants at 5-7 leaf stage. Plant density was 66,667 plants ha⁻¹. Harvesting was also done by hand. Standard agronomical practices were performed. Measured agronomic traits were plant height (PH) and ear height (EH; cm), lodged (LP) and broken plants (BP; %), grain moisture at harvest (GM; %) and grain yield (GY) adjusted to 14% grain moisture (t ha⁻¹). Two-way analysis of variance was performed in MSTAT-C program, and LSD test at 0.05 probability level between hybrid means was obtained.

Results and discussion

Replications were significant source of variation only for PH (data not shown). Differences between hybrids were significant for all traits: for PH, EH, GM and GY at $p < 0.001$, for BP at $p < 0.01$ and for LP at $p < 0.05$. Locations were significant for all the traits ($p < 0.001$). Hybrid × location interaction was significant at $p < 0.001$ for PH, EH, GM and GY, while for BP at $p < 0.05$. In Tab. 2 values of LSD test are presented. Three QPM hybrids had suitable agronomic performances, namely ZPQPM5, 6 and 13. All three hybrids had GY at the level of ZP 606, which is in fact late commercial hybrid of FAO 700, grown largely in Serbia and other countries. ZPQPM13 had higher PH than ZP 606, while other two hybrids were at the same level, while for EH ZPQPM5 and ZPQPM6 had significantly lower values than ZP 606. LP values were low for the three hybrids, but BP had very high values for QPM hybrids (over 10%), showing this trait as a possible constraint in wide production of these hybrids. GM of ZPQPM5 and ZPQPM6 was at the level of ZP 606, while for ZPQPM13 it was higher. Considering variation of three chosen QPM hybrids over locations, ZPQPM5 had PH in the range of 181.5-227 cm, EH 70.5-90.5 cm, GM 18.50-29.55% and GY 4.44-10.60 t ha⁻¹. ZPQPM6 had 185.0-237.5 and 76.0-96.0 cm PH and EH, respectively, GM 19.45-29.00% and GY 3.79-10.46 t ha⁻¹, while ZPQPM13 had these values of 178.5-242.0 cm for PH, 74.0-103.0 cm for EH, 22.20-33.55% GM and GY 3.22-11.41 t ha⁻¹. These values point out to a large difference in the environmental conditions among test-locations. One of the main impediments to the commercial use of QPM hybrids is their frequently lower grain yield than that of standard hybrids (Scott et al., 2009). However, using MAS Babu et al. (2005) and Gupta et al. (2013) obtained higher grain yields of QPM over standard hybrids, and Jompuk et al. (2011) concluded the opposite.

Agronomic traits of QPM maize hybrids adapted to temperate regions

Table 2. Average values of tested hybrids in 2017 with values of LSD test at 0.05 probability level

No.	Hybrid	PH	EH	LP	BP	GM	GY
1	ZPQPM1	219.4cdef1	80.88gh	4.804ab	5.969cde	25.15bc	4.522h
2	ZPQPM2	235.5ab	87.06ef	3.088abc	11.090abcde	26.88a	6.145def
3	ZPQPM3	223.1cd	82.64g	0.329c	8.586bcde	21.70g	5.722fg
4	ZPQPM4	219.5cdef	77.53h	1.005bc	13.260abc	21.07g	5.065gh
5	ZPQPM5	212.4g	81.00gh	1.024bc	11.180abcde	25.06bc	7.156c
6	ZPQPM6	217.4defg	87.50ef	1.266abc	16.130ab	25.16bc	7.030cd
7	ZPQPM9	223.6c	101.80a	4.774ab	8.630bcde	27.29a	5.349fgh
8	ZPQPM10	213.6fg	94.94bc	1.738abc	9.656bcde	24.35cd	5.122gh
9	ZPQPM11	218.5cdefg	97.94ab	5.025a	11.880abcd	22.24fg	5.958efg
10	ZPQPM13	221.5cde	91.88cd	0.313c	13.190abc	26.33ab	7.702bc
11	ZPQPM14	218.3cdefg	84.06fg	0.000c	18.470a	24.21cd	6.812cde
12	ZP 555	217.5cdefg	90.50de	0.000c	6.994cde	22.49efg	9.165a
13	ZP 560	238.5a	97.56b	0.000c	4.383de	21.52g	7.679bc
14	ZP 600	231.6b	94.69bc	3.125abc	5.360de	22.35fg	8.805a
15	ZP 666	221.0cde	89.94de	0.000c	6.665cde	23.39def	8.616ab
16	ZP 606	215.9efg	91.94cd	0.000c	4.135e	24.01cde	7.452c
LSD _{0.05}		6.097	4.089	3.872	7.551	2.418	0.998
Average		221.708	89.487	1.656	9.723	23.950	6.769
CV		2.75	4.57	234.09	77.73	6.49	14.76
SD		19.43	10.13	4.68	8.88	3.48	2.91

¹ - values in a column followed by all the different letters are significantly different at 0.05 probability level; CV - coefficient of variation (%); SD - standard deviation

Having the highest quantity of seed, hybrid ZPQPM5 was tested in the Pre-commission trials (from which hybrids suitable to be send into State Commission for releasing varieties are chosen), but it failed to fulfil required agronomic criteria. Since two remaining hybrids were of FAO 700-800, we have repeated the test-trial with them in 2018 at seven locations alongside with official commercial check hybrids for FAO 700: NS 6010 and AS 72 (repeated twice instead of one missing hybrid), and 800 group - ZP 735 and ZP 873, and also with ZP 606 as a check that was used in the first year of testing. The methodology of the trial was the same as in 2017, and only difference was that lodged and broken plants (LBP) were counted together before the harvest. Locations and hybrids differences for all the traits were very highly significant ($p < 0.001$), as well as interaction for all traits but LBP (data not shown). In Table 3 are presented values for LSD test of this new trial.

Table 3. Average values of tested hybrids in 2018 with values of LSD test at 0.05 probability level

No.	Hybrid	PH	EH	LBP	GM	GY
1	AS 72	256.8f1	99.30d	0.5754d	21.60d	11.43b
2	ZPQPM6	271.9d	96.05e	3.413a	26.85ab	10.02de
3	ZPQPM13	277.4c	93.27f	1.956b	26.01bc	10.59cd
4	ZP 606	267.9e	94.95ef	0.762cd	22.87d	12.55a
5	NS 6010	275.2cd	108.30c	2.022b	27.26ab	9.57e
6	AS 72	258.2f	99.90d	0.905bcd	21.69d	11.47b
7	ZP 735	299.3a	116.90a	0.933bcd	28.38a	10.10de
8	ZP 873	284.2b	114.20b	1.749bc	24.91c	11.29bc
LSD _{0.05}		3.792	2.616	1.131	1.916	0.7944
Average		273.852	102.864	1.539	24.947	10.877
CV		2.26	4.16	120.08	12.56	11.94
SD		18.74	12.43	2.30	8.28	2.78

¹ - values in a column followed by all the different letters are significantly different at 0.05 probability level; CV - coefficient of variation (%); SD - standard deviation

The most prominent difference for two QPM hybrids tested in both years was the percentage of BP in 2017 and LBP in 2018. This was most probably due to the huge difference between meteorological conditions between the two years, namely in 2017 a few storms were recorded in the test-locations, causing high LB in QPM hybrids. Since ZP 606 is one of the best and most widely grown ZP hybrid, significance of t-test for the two QPM hybrids and this check is calculated for PH, EH, GM and GY (lodged and broken stalks were omitted due to difference in measurement in two years of the research). ZPQPM13 showed better performances over ZPQPM6. Its GY was at the level of ZP 606, although its GM was 3% higher. This is most probably due to the tropical origin of the mother of this hybrid. PH was higher, but what is more important EH was at the level of ZP 606, which is favourable due to the lesser lodging. This hybrid also had the best chemical kernel characteristics of all examined QPM hybrids. Namely, ZPQPM6 had average tryptophan content of 0.075% measured in two locations over two years, while ZPQPM13 had 0.081% tryptophan, in comparison with standard hybrids (0.069%; $p < 0.01$). So ZPQPM13 was chosen and sown on 0.5 ha in mercantile production in 2019 to produce seed for feeding trials for broilers and pigs. QPM was used on a large scale for food in undeveloped countries for improving the health of populations living there (Akuamo-Boateng, 2002). QPM would be beneficial for developed countries as well, since soybean or artificial lysine should not be needed to add to the feed, thus lowering its cost (Scott et al., 2009). Due to a twofold growth and better usage of the meal made from QPM (Burgoon et al., 1992) a part of conventional maize could be used for some other purposes (bioethanol production). All of these results confirm the fact that QPM hybrids can be at the level of the best standard commercial hybrids regarding agronomic traits (Vivek et al., 2008). Also, temperate QPM inbred lines can have high combining abilities, which is in agreement with Ignjatović-Mičić et al. (2013) and Kostadinović et al. (2016).

Conclusions

Based on the results presented herein we could draw the conclusion that it is possible to create QPM hybrids suitable for temperate conditions, both in the view of good agronomic traits, as well as grain quality. This task is not easy and it is time consuming, but it is attainable and worth trying.

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Ribarstvo, lovstvo i pčelarstvo

Utjecaj vremena nasađivanja mladi na rast orade i lubina u kaveznom uzgojnom sustavu

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Sažetak

U radu su istraživane razlike u trajanju proizvodnog ciklusa, brzini rasta, konverziji hrane i preživljavanju lubina i orade s obzirom na vrijeme nasađivanja mladi u plutajuće kaveze. Mlađ lubina nasađena u travnju 2017. godine dostigla je prosječnu masu od $250 \pm 3,76$ g početkom kolovoza slijedeće godine, dok je mlađ iste veličine nasađena u kolovozu dostigla tek $117 \pm 0,83$ g do kolovoza slijedeće godine. Mlađ orade nasađena u travnju 2017. dostigla je prosječnu masu od $296 \pm 11,58$ g početkom kolovoza slijedeće godine, dok je mlađ iste veličine nasađena u srpnju dostigla $211 \pm 0,94$ g početkom kolovoza slijedeće godine. Kod obje istraživane vrste su utvrđene statistički značajne razlike u rastu s obzirom na vrijeme nasađivanja.

Ključne riječi: *Dicentrarchus labrax*, *Sparus aurata*, preživljavanje, konverzija hrane

Uvod

Razvoj mediteranske marikulture u zadnja dva desetljeća prošlog stoljeća obilježio je intenzivni razvoj kaveznog uzgoja dvije vrste bijele morske ribe, lubina, *Dicentrarchus labrax* (Linnaeus, 1758), i orade, *Sparus aurata* (Linnaeus, 1758), (Moretti i sur., 1999). Proizvodnja ove dvije vrste i danas su osnova marikulture na ovom području (FAO, 2018). Vrijeme potrebno da komarča i lubin dostignu konzumnu veličinu u ambijentalnim uzgojnim uvjetima značajno ovisi o okolišnim čimbenicima. Temperatura, kao jedan od najvažnijih abiotičkih čimbenika utječe na intenzitet metabolizma te time i na količinu konzumirane hrane i rast (Moksness i sur., 2004). Pri organiziranju proizvodnje potrebno je planirati nabavu mladi i vrijeme nasađivanja koje omogućuje iskorištenje dužeg perioda optimalne okolišne temperature. Osnovni cilj ovog rada bio je istražiti razlike u trajanju proizvodnog ciklusa, brzini rasta, visini konverzije hrane te preživljavanju lubina i orade s obzirom na vrijeme nasađivanja mladi u plutajuće kaveze.

Materijal i metode

Istraživanje je provedeno na kaveznom uzgajalištu lubina i orade tvrtke Orada Adriatic d.o.o u uvali Veli Bok na otoku Cresu, u razdoblju od 1. travnja 2017. do 31. kolovoza 2018. godine. Dva kaveza lubina (KL1, KL2) i dva kaveza orade (KO1, KO2) nasađena su u travnju 2017. godine. Druga dva kaveza orade (KO3, KO4) nasađena su u srpnju, a lubina u kolovozu (KL3, KL4) iste godine. U svaki okrugli kavez promjera 22 m i dubine 20 m nasađeno je po 130 000 jedinki. Jedinke obje vrste su do mase od 20 g hranjene komercijalnom hranom koja je, ovisno o njihovoj masi, sadržavala 51-54% proteina, 15% masti te imala energetska vrijednost 18 MJ/kg. Lubin je potom hranjen hranom koja je sadržavala, ovisno o masi jedinki, 39 – 48% proteina, 16 – 24% masti te imala energetska vrijednost 17-18 MJ/kg. Hrana orade sadržavala je 41 – 49% proteina, 16% masti te bila energetske vrijednosti 15,5 – 17,5% MJ/kg. Dnevna količina hrane za svaki kavez određivana je sukladno uputama proizvođača, u ovisnosti o prosječnoj masi jedinki u kavezu i temperaturi mora.

Svakodnevno je tijekom istraživanja mjerena temperatura mora pomoću termometra te su bilježeni podaci o utrošenoj količini hrane i broju uginulih jedinki za svaki kavez. Jednom mjesečno (početkom mjeseca) iz svakog kaveza uziman je uzorak od 30 jedinki koje su potom pojedinačno vagane baždarenom digitalnom vagom (SKALA JPL – Digitron). Prikupljeni podaci uneseni su u program Excel, te su za svaki kavez posebno izračunate slijedeće vrijednosti: broj jedinki u kavezu za svaki mjesec, prosječna mjesečna masa jedinki u svakom kavezu, ukupna masa jedinki u kavezu za svaki mjesec, ukupna utrošena mjesečna količina hrane po kavezu, prosječna dnevna količina hrane po kavezu i po jedinki (kg), prosječan mjesečni i ukupni faktor konverzije (FK) na kraju razdoblja istraživanja te postotak preživljavanja za svaki kavez.

Faktor konverzije (FK) računan je prema sljedećoj formuli:

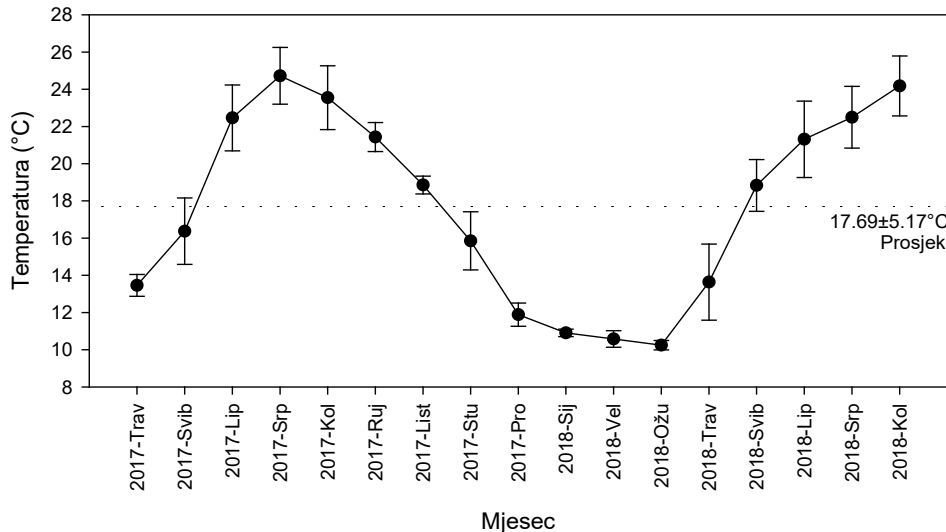
$$FK = I (g) / WD$$

gdje je I ukupna količina utrešene hrane, a WD masa ribe (Havasi i sur., 2015).

Kako bi se ispitalo da li su različite srednje mase jedinki u različitim kavezima na kraju istraživanja uvjetovane razlikama u početnoj nasadnoj masi jedinki, početne i krajnje srednje mase testirane su T i Z-testom. Za analizu sličnosti između vremenskih nizova s obzirom na različito vrijeme nasađivanja testirani su parovi vremenskih nizova Wilcoxon-Man-Whitney testom te potom rezultati potvrđeni Kruskal-Wallis testom. Za ispitivanje profila vremenskih nizova u odnosu na temperaturu i hranu kao vanjske varijable korištena je analiza unakrsnih korelacija (Cross Correlation Function) (IBM Corp., 2017).

Rezultati i rasprava

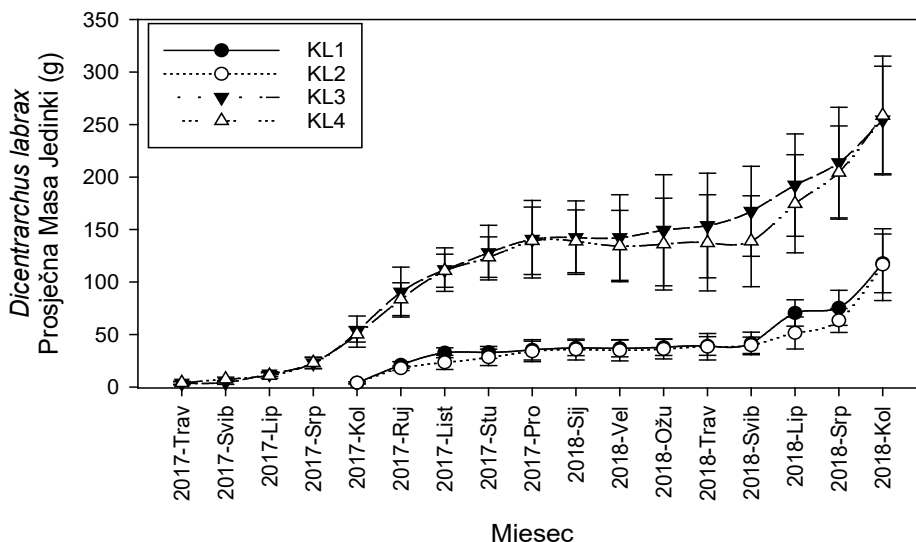
Tijekom razdoblja istraživanja dnevna temperatura mora se kretala u rasponu 10 - 27 °C. Na slici 1 prikazane su promjene srednje mjesečne temperature mora tijekom razdoblja praćenja uzgojnog ciklusa lubina i orade u uvali Veli Bok na otoku Cresu.



Slika 1. Srednje mjesečne temperature morske vode tijekom praćenja uzgojnog ciklusa lubina i orade u uvali Veli Bok na otoku Cresu

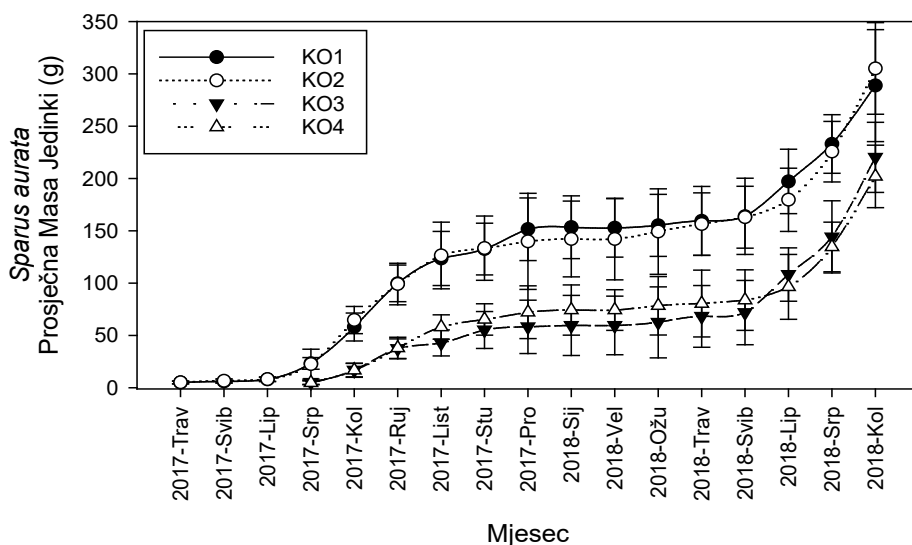
Na slici 2 prikazane su srednje mjesečne mase lubina nasađenog u travnju (KL1 i KL1) i kolovozu (KL3 i KL3) 2017. godine. Mlađ nasađena u travnju 2017. godine dostigla je prosječnu masu od $250 \pm 3,76$ g početkom kolovoza slijedeće godine, dok je mlađ iste veličine (3.9 ± 0.16 g) nasađena u kolovozu dostigla tek $117 \pm 0,83$ g do kolovoza slijedeće godine. Statistički značajne razlike između prosječnih početnih masa jedinki u oba vremena nasađivanja nisu utvrđene, dok su na kraju istraživanja utvrđene značajne razlike s obzirom na vrijeme nasađivanja (T- i Z-test). Analize sličnosti između vremenskih nizova Wilcoxon-Man-Whitney testom te potom Kruskal-Wallis testom

pokazale su dvije statistički različite grupe kaveza (KL1 i KL2) i (KL3 i KL4). Vidljivo je da su ribe prestale rasti kada je temperatura mora pala ispod 11°C te rast ponovno započinje kada temperatura prelazi 18°C. Prema podacima Düglera i sur. (2012) optimalna temperatura za mlađ lubina je oko 25°C. U ovom istraživanju je najintenzivniji rast lubina također zabilježen kada su temperature bile najviše, međutim analizom unakrsnih korelacija utvrđeno da temperatura nije imala izravan utjecaj na rast za razliku od količine hrane, koja pak ovisi o temperaturi.



Slika 2. Promjena prosječne mjesečne mase jedinki lubina nasađenih u travnju (KL1 i KL2) i kolovozu (KL3 i KL4)

Orada nasađena u travnju 2017. dostigla je prosječnu masu od 296±11,58 početkom kolovoza sljedeće godine, dok je mlađ iste veličine (5.08±0.19 g) nasađena u srpnju dostigla 211±0,94 g početkom kolovoza sljedeće godine. Statistički značajne razlike između prosječnih početnih masa jedinki u oba vremena nasađivanja nisu utvrđene (T- i Z-test). Iako su razlike u prirastu s obzirom na vrijeme nasađivanja orade manje u usporedbi s istim kod lubina, ipak su kao i kod lubina na kraju istraživanja utvrđene statistički značajne razlike s obzirom na vrijeme nasađivanja (T- i Z-test). Analize sličnosti između vremenskih nizova Wilcoxon-Man-Whitney testom te potom Kruskal-Wallis testom pokazale su dvije statistički različite grupe kaveza (KO1 i KO2) i (KO3 i KO4).



Slika 3. Promjena prosječne mjesečne mase jedinki orade nasađenih u travnju (KO1 i KO2) i u srpnju (KO3 i KO4)

Vidljivo je da orada prestaje rasti kada temperatura mora padne ispod 13°C. Ribe ponovo počinju rasti kada temperatura mora rasti iznad od 18°C. Prema podacima Katavića i sur. (2005) te Brigolinia i sur. (2008) optimalna temperatura za rast orade je od 22 do 26°C. U ovom istraživanju je najintenzivniji rast orade također zabilježen kada su temperature bile najviše i kretale se u granicama optimalnih sukladno rezultatima prethodno navedenih autora. Analiza unakrsnih korelacija pokazala je da temperatura nije imala izravan utjecaj na rast za razliku od količine hrane, koja pak ovisi o temperaturi.

Ukupni faktor konverzije kod lubina na kraju istraživanja iznosio je 1,53 za KL1, 1,35 za KL2, 1,98 za KL3 te 1,93 za KL4. Mjesečni faktor konverzije kod lubina za kaveze KL1 i KL2 kretao se u rasponu od 0.81 do 2.35, a za kaveze KL3 i KL4 od 0.36 do 2.30. Očigledno je da su razlike u visini faktora konverzije na kraju istraživanja između dvije skupine kaveza lubina uzrokovane različitom starošću jedinki te je za pretpostaviti da će kod mlađih jedinki ovaj faktor biti niži kada dostignu masu jedinki prve skupine. Slične rezultate kod lubina dobili su Jug-Dujaković i sur. (2012) te su izračunali da iskoristivost hrane lubina u uvjetima kaveznog uzgoja u Jadranskim uvjetima iznosi između 61 i 73,1%. Mjesečni faktor konverzije kod orade za kaveze KO1 i KO2 kretao se u rasponu od 0,75 do 2,07, a za kaveze KO3 i KO4 od 0,72 do 1,89. Kako promjene faktora konverzije ukazuju na fiziološko stanje ribljih populacija, to je pravilnom interpretacijom moguće okarakterizirati abiotičke i biotičke komponente okoliša u kojem jedinke obitavaju, ali i uvidjeti ekofiziološke procese promatrane vrste (Relić, 2015). Prema Moretti i sur. (1999) konzumnu masu od 400 - 450 g riba doseže u periodu od 18 - 24 mjeseca. Više temperature mora na Afričkoj obali Mediterana omogućile su da uzgojni ciklus traje kraće pa je tako za lubina potrebno 15 mjeseci da postigne navedenu konzumnu veličinu, a za oradu od 10-12 mjeseci (Moretti i sur., 1999). Prema Treer i sur. (1995), povišenjem temperature moguće je davati veće količine hrane od navedenih u tablicama te se tako postiže bolji prirast bez podizanja koeficijenta konverzije. U ovom istraživanju je najintenzivniji rast obje vrste također zabilježen kada su temperature bile najviše, a rezultati analize unakrsnih korelacija ukazuju da bi u istim ambijentalnim uvjetima na ovom uzgajalištu hranjenje većom količinom hrane od one po preporukama proizvođača omogućilo brži rast obje istraživane vrste.

Na kraju istraživanog razdoblja, preživljavanje u kavezima KL1 i KL2 bilo je 91%, dok je u kavezima KL3 i KL4 bilo 88%. Kod orade je na kraju istraživanog razdoblja preživljavanje u kavezu KO1 bilo je 89%, kavezu KO2 88%, dok je u kavezima KO3 i KO4 iznosilo 90% i 91%. S obzirom na navode Katavić i sur. (2005) da na kaveznim uzgajalištima lubina i orade očekivano preživljavanje do konzumne veličine iznosi 80-85%, moglo bi se zaključiti da je preživljavanje obje istraživane vrste u uvali Veli Bok iznad očekivanog.

Zaključak

Temperatura kao abiotički okolišni čimbenik ima veliki utjecaj na duljinu uzgojnog ciklusa te je pri organizaciji proizvodnje u kaveznom uzgoju potrebno planirati nabavu mlađi i vrijeme nasada koje omogućuje iskorištenje dužeg perioda optimalne temperature mora. Mlađ lubina potrebno je nasađivati tijekom travnja ili početkom svibnja, kako bi se maksimalno iskoristilo razdoblje intenzivnog rasta i postigla zadovoljavajuća konzumna masa prije drugog zimskog perioda u uzgojnom ciklusu. Mlađ orade nasađena u travnju dostiže minimalnu konzumnu težinu u srpnju i ostavlja više od tri mjeseca dodatnog rasta prije druge zime, pa je moguć i kasniji, svibanjski i lipanjski nasad. Pri višim temperaturama koje se nalaze unutar optimuma za lubina i oradu, hranjenje većom količinom hrane od one po preporukama proizvođača omogućilo bi brži rast obje istraživane vrste.

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Impact of stocking time on the growth of sea bream and sea bass in a cage rearing system

Abstract

The differences in duration of production cycle, growth rate, feed conversion, and survival of sea bass *Dicentrarchus labrax* (Linnaeus,1758) and gilthead sea bream *Sparus aurata* (Linnaeus,1758) were investigated in relation to the time of stocking of fingerlings in floating cages. Sea bass fingerlings stocked in April 2017, reached a weight of $250\pm 3,76$ grams at the beginning of August of the following year, while fingerlings of the same size stocked in August reached only $117\pm 0,83$ g by August of the following year. Sea bream fingerlings stocked in April 2017, reached a weight of $296\pm 11,58$ g grams at the beginning of August of the following year, while fingerlings of the same size stocked in July reached $211\pm 0,94$ g by August the following year. Statistically significant differences in growth of both investigated species with respect to the time of stockinge were found.

Keywords: *Dicentrarchus labrax*, *Sparus aurata*, survival, feed conversion

Browsing intensity of some Mediterranean plants by the European mouflon

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Abstract

Measurement of the damage to the holm oak (*Quercus ilex*), laurustinus (*Viburnum tinus*) and hawkweed oxtongue (*Picris hieracioides*) caused by the European mouflon browsing showed that there is a difference in the use of individual plant species. Most of the shoots of the holm oak and hawkweed oxtongue were browsed at the height of 42 cm, while the average height is significantly lower for laurustinus browsing: 16 cm for the main shoots and 22.6 cm for the twigs. A browse diameter is significantly larger in laurustinus (2.5 mm) than in a holm oak (1.9 mm) and hawkweed oxtongue (1.8 cm), indicating that mouflon browses more biomass from laurustinus than of other two species, which could endanger the laurustinus, and could lead to its disappearance from the natural habitat.

Keywords: bite diameter, holm oak, laurustinus, hawkweed oxtongue

Introduction

The search for food is the dominant activity of wildlife. Animals spend at least 40 to 60% daytime for food seeking (Wickstrom et al., 1984). In doing so, the herbivore selects forage at the plant level, and within each plant it selects certain palatable plant parts (Palo et al., 1992). The survival and regeneration of plants, and the location of physical and chemical defences to different parts of the plant can be influenced by several factors: the number of browses taken (i), the amount of material taken after the browsing (ii), and the part of the plant that the herbivore prefers. The browse size was not chosen at random. It is a consequence of the compromise between the architecture and plant availability (Shipley et al. 1999). Previous studies have shown that the forage value of a shoot declines with the shoot diameter (Ganga & Scogings, 2007; Wilson & Kerley, 2003; Jia et al., 1995; Danell & Bergström, 1985; Vivås & Sæther, 1987). Therefore, depending on the type of plant and the habitat on which the plant grows, the herbivore will evaluate the optimal thickness of the shoot for consumption, which will allow it to intake sufficient nutrients, and minimize the intake of secondary metabolites (chemical defence of the plant) and difficult-to-digest compounds (lignin, cellulose, hemicellulose). Therefore, herbivores tend to browse smaller diameter shoots rather than the largest diameter shoots (Vivås et al., 1991). Regardless of the criterion of ecophysiological adaptation (Hofmann, 1989; Gordon and Illius, 1988), the European mouflon (*Ovis orientalis musimon*) is considered a grazing herbivore type. However, its introduction into various parts of Europe (Tomiczek & Türcke, 2003) regardless of the climate area, has led to a shift in nutritional strategy towards a transitional ruminant type, with a tendency to increase the proportion of woody species in the diet. Since this species was introduced to the island of Rab at the end of the 20th century, in the area with complete dominance of forest vegetation, relatively rapid adaptation to the new habitat conditions was observed, that is, the browsing of woody vegetation. Therefore, the purpose of this paper is to identify ways of using individual plant species typical of the Mediterranean area, whether indigenous or allochthonous.

Material and methods

The research was conducted in a fenced part (877 ha) of the state hunting ground number VIII /6 "Kalifront" on the island of Rab. From a vegetation point of view, the hunting ground belongs to the eumediterranean vegetation zone (Trinajstić, 1986). Forests make up 95% of the hunting grounds (the forest community of holm oak and manna ash

– *Fraxino orni-Quercetum ilicis* H-ić/1956/1958). The dominant big game species game in the hunting ground is the European mouflon – *Ovis orientalis musimon* (Krapinec, 2000).

The analysis of browsing by mouflon was performed on a line, 5 m wide and 400 m long, that was cleared in the 36 year old holm oak stump, during January and February 1999 (6 months after the mouflon had been released into the hunting ground). The direction of extension of the line is NW-SE. To avoid the likelihood of recording a damage to a plant caused by a single animal, each other plant of a holm oak (*Quercus ilex*) and laurustinus (*Viburnum tinus*) was selected on the sample plot, with traces of browsing surface going from the beginning to the end of the stripe along its entire length. A browse diameter of each plant was measured as well as the height of browse of all shoots from the ground, with shoots classified into shoots and twigs. The damage to the hawkweed oxtongue (*Picris hieracioides*) was measured on a road 150 m southwest of the example surface, extending parallel to the example surface of holm oak and laurustinus damage, in the same way as in the first two species. The bite diameter was measured by a sliding calliper with an accuracy of a tenth of a millimetre, and the browse heights were measured by a steel measuring tape from the ground to the bite diameter vertically to the ground with a millimetre accuracy. The total plant height, whether damaged or not, was measured for all three plant species. Measures were taken on 10 plants of the holm oak stump, 14 plants of the laurustinus stump, and 105 hawkweed oxtongue plants.

The regression model of the dependence of the diameter and length of the shoot was made on a sample of 30 intact shoots of holm oak, laurustinus and hawkweed oxtongue. The length from the vegetative shoot apex to a certain diameter on the shoot were measured. The above analyses were made from 13 to 16 October 1999. In the area of data recording during the spring-winter period, a herd of over 30 mouflon heads was constantly inhabited, and the presence of the chital was not recorded. Testing the differences in the height of the holm oak and laurustinus was performed by a t-test. Differences in the bite diameter were tested by the Kruskal-Wallis test. The equalization of the length dependence on the shoot diameter was performed by the power function. The data obtained were analysed using Statsoft 13 software (TIBCO Software Inc., 2018).

Results and discussion

The research included two typical Mediterranean woody species and one herbaceous, which can be annual or biennial - the hawkweed oxtongue. The hawkweed oxtongue is a representative of the ruderal and weed vegetation, which colonizes the mid-season stages of post-cultivation successions of the Mediterranean area (Sans et al., 2002).

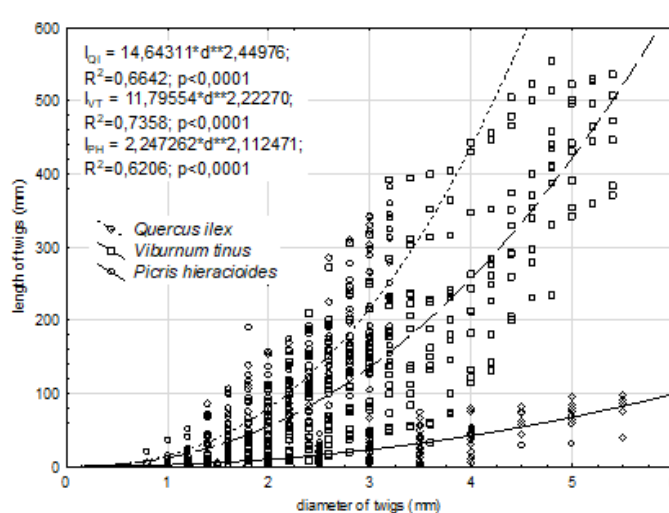
In the period from winter to autumn, the holm oak, after clear felling, has shoots from the stump 36 cm higher than the laurustinus (Table 1; $t = 5.609$; $p < 0.00001$). The height of the hawkweed oxtongue is not taken into account, because almost all the plants have been browsed and, since it is not a woody species, the stems are of generative origin. However, the number of major shoots per stump was significantly higher in laurustinus (23.5 shoots) than in holm oak (13.4; $t = -2.933$; $p < 0.01$). Except in height, a holm oak has a significantly higher number of twigs per main stem (0.87 twigs /m of shoot) compared to hawkweed oxtongue (0.4 twig /m shoots) and laurustinus (0.3 twigs/m shoots), that is, the laurustinus has significantly the lowest number of twigs per stem ($\chi^2 = 132.8715$; $p < 0.0001$), despite the fact that, unlike the previous two species, in which the leaves and twigs develop alternately, in the laurustinus they develop in the opposite sequence.

Table 1. Test results of measured parameters of the browsed plants (numbers with different letters in columns indicate significant difference)

Species	Growing Type	Absolute height (mm)	Number of shoots per stump	Number of twigs per 1 m of shoots	Sample size	High from the ground (mm)	Bite diameter (mm)
Quercus ilex	shoots	657 ^a	13,4 ^a	0,87 ^a	134	421 ^c	1,9 ^b
	twigs				537	418 ^c	1,6 ^c
Viburnum tinus	shoots	299 ^b	23,5 ^b	0,27 ^b	328	160 ^b	2,5 ^d
	twigs				168	226 ^a	2,2 ^a
Picris hieracioides	shoots			0,38 ^b	105	404 ^c	2,6 ^d
	twigs				135	436 ^c	1,8 ^{b,c}

The height of browsed shoot ranged from 115 to 1,401 mm and there is no difference in the height of shoot between the hawkweed oxtongue and the holm oak, averaging about 42 cm. However, mouflons browse laurustinus at a significantly lower height, which is 16 cm for the main shoots and 22.6 cm for the twigs ($\chi^2=638.78$; $p=0.0001$). Within each plant species, mouflons browsed twigs of a smaller bite diameter than the main shoots (Table 1). This difference is relatively small (but statistically significant) for holm oak and laurustinus, and averages only 0.3 mm. However, in the case of the hawkweed oxtongue, it is relatively large and averages 0.8 mm ($\chi^2=464.70$; $p=0.0001$). Except for the type of shoots, the difference is quite pronounced between the species. The largest bite diameter was observed in the hawkweed oxtongue shoots (2.6 mm) and the laurustinus shoots (2.5 mm), while the smallest was in the branches of the holm oak twigs (1.6 mm) and the hawkweed oxtongue twigs (1.8 mm).

Whether it is a moderate, boreal (Shipley et al. 1999) or subtropical belt (Wilson & Kerley, 2003), the bite diameter is proportional to the size of a wild ruminant. For example, in roe deer, the average bite diameter is from 1.86 mm (for grey alder – *Alnus incana*) to 3.19 mm (for a mountain ash – *Sorbus aucuparia*). According to Jia et al., (1995), 90% of bite diameters in moose are thinner than 4.8 mm, which makes the marginal value for assessing the availability of phytomass. So far, one of the highest bite diameter has been observed in black rhinoceros (*Diceros bicornis*) and in 99% of cases it reaches a value of 10 mm (Ganqa & Scogings, 2007). Difference in bite diameter may be due to the architecture or the morphology of the plant, especially if mechanical barriers (thorns and spines) are present. In prickly and thorny species, the same species of herbivores browses shoots at smaller diameters (Wilson & Kerley, 2003). If the available biomass is distributed among several shoots, then greater browsing is expected since the herbivore must move less while eating (Vivås et al., 1991). Since a holm oak grows fewer shoots per stump than the laurustinus, and the bite diameter is smaller, it can be concluded that the laurustinus is a more palatable plant species than the holm oak, or rather optimal, because it gives more shoots per stump.



The reason why mouflon feeds on hawkweed oxtongue is quite confusing. The plant is covered with strong short hairs, and also contains a bitter substance picriside A. The compound belongs to lactucin glycosides, and is a relatively common constituent of the Asteraceae plant family (Nishimura et al., 1986). It is obvious that the plant is equipped with both chemical and mechanical defence mechanisms. In the time period from logging to data recording, holm oak and laurustinus did not pass into the generative phase and mouflons browsed only the vegetative parts of the plant. However, the period of flowering of the hawkweed oxtongue is relatively long (June to October), and it might be possible that the mouflons were attracted to browsing by flowers. Summarizing the bite diameter ranges of each species, 96% of the bite diameter ranges from 0.5 to 2.4 mm in holm oak, and from 0.9 to 3.8 mm in laurustinus, while 94% of the bite diameter in hawkweed oxtongue, ranges from 0.8 to 3.7 mm. Considering the ranges of movement of the bite diameters and the regression (Fig. 1), a mouflon browses shoots of holm oak in length from 3 to 125 mm, the laurustinus in length from 9 to 229 mm and hawkweed oxtongue, from 1 to 36 mm. Based on the average bite diameters, which makes up more than 50% of the browse cases, the mouflons browse on average 71 mm of holy oak shoots, 90 mm of laurustinus shoots and 17 mm of hawkweed oxtongue shoots. In North America, regression models based on a diameter and length, or mass of shoots, began to develop as early as the 1970s (Peek et al. 1974), and serve to assess the capacity or load of habitats with wild ruminants. Depending on the part of the continent, models have been developed for a large number of woody species (MacCracken & Ballenberge, 1993; Rumble, 1985), but models made on plants of one area in another area for the same plant species are not accurate enough. This is also a disadvantage of this method, since there are no universal estimation models. However, although no regression models of mass dependence on shoot diameter were made in this paper, our models indicate that a mouflon ultimately makes more intensive use of a laurustinus in comparison with a holm oak, which, in case of a habitat overload with this species of game, could lead to the disappearance of laurustinus from the habitat.

Although a mouflon is a grazing herbivore, in a predominantly forested habitat this ruminant species has switched to a browsing type of diet, that is, it could be classified as an intermediate type. Previous research on small ruminants in the Mediterranean area (Cuartas & García-Gonzales, 1992) has shown that a European mouflon uses far less holy oak in the diet compared to Pyrenean ibex (*Capra pyrenaica*), domestic sheep (*Ovis aries*) or domestic goat (*Capra hircus*). In doing so, it completely avoids eating holm oak during May. In addition, in relation to goats, sheep generally tend to avoid the diet of holm oak (Bartolome et al. 1998), and a lower level of tannin tolerance may be the reason (Narjisse et al. 1995). Rogošić et al. (2008) in research on secondary metabolites of the macchia element, state that the following are particularly unfavourable for sheep: tannins (high content in the genera *Arbutus*, *Quercus* and *Pistacia*), terpenes (genus *Juniperus*) and iridoid glycoside (*Viburnum tinus*). Therefore, sheep prefer laurustinus to holy oak when browsing, which is also the case in this paper. In addition to holy oak and hawkweed oxtongue, laurustinus also contains secondary metabolites. These are over 10 iridoid glucosides (most commonly viburnoside A and B), including coumarin, which cause bitter taste, but most of these ingredients have a positive effect on liver function (Mohamed et al., 2005). Therefore, according to Freeland & Jansen (1974), small ruminants (including the European mouflon) in the Mediterranean area or habitat dominated by the macchia vegetation, makes their diet from a greater number of species whose forage value varies greatly with respect to secondary metabolites and nutrients. The reason for such a diet strategy is to avoid poisoning by secondary metabolites, and the mechanism of choice of mouflon meal in macchia should certainly be further investigated.

Conclusions

The study showed that in forest habitats, the European mouflon can very successfully extend the forage spectrum to woody tree species, despite chemical and mechanical strategies to defend the plant species. In doing so, it shows a tendency for more intensive use of laurustinus compared to the holy oak, which could endanger the native Mediterranean vegetation.

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Razina brštenja pojedinih sredozemnih biljnih vrsta od strane europskog muflona

Sažetak

Izmjera oštećenosti hrasta crnike, lemprike i runjikastog jagušca brštenjem od strane europskog muflona je pokazala kako postoji razlika u načinu korištenja pojedinih biljnih vrsta. Glavnina odgrizanja izbojaka hrasta crnike i runjikastog jagušca odvija se na visinama od 42 cm, dok je prosječna visina brštenja lemprike bila signifikantno niža i iznosila je 16 cm za glavne izbojke i 22,6 cm za ogranke. Promjer pregrizne plohe signifikantno je veći kod lemprike (2,5 mm) nego kod hrasta crnike (1,9 mm) i runjikastog jagušca (1,8 cm) što ukazuje da muflon s lemprike brsti veću biomasu nego s ostale dvije vrste, a što bi moglo dovesti do nestanka lemprike iz staništa.

Ključne riječi: europski muflon, hrast crnika, lemprika, runjikasti jagušac

Procjena brojnosti populacije jarebice kamenjarke (*Alectoris graeca*) na području značajnog krajobraza vodenog toka i kanjona rijeke Čikole

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Sažetak

Procjena brojnosti jarebice kamenjarke (*Alectoris graeca*) provedena je osluškivanjem spontanog pjevanja i primjenom metode vokalnog podraživanja na području značajnog krajobraza „Vodeni tok i kanjon rijeke Čikole“. Istraživanje je provedeno tijekom svibnja 2018. godine. Primjenjena metoda je invazivna, a rezultati ovise o razdoblju godine, razdoblju dana te meteorološkim prilikama. Istraživano područje je podjeljeno na 43 kvadranta. Na istraživanom području utvrđena je gustoća populacije od 1,1 parova na 100 ha, odnosno procijenjena je brojnost od 12 parova.

Ključne riječi: *Alectoris graeca*, značajni krajobraz, brojnost, vokalno podraživanje

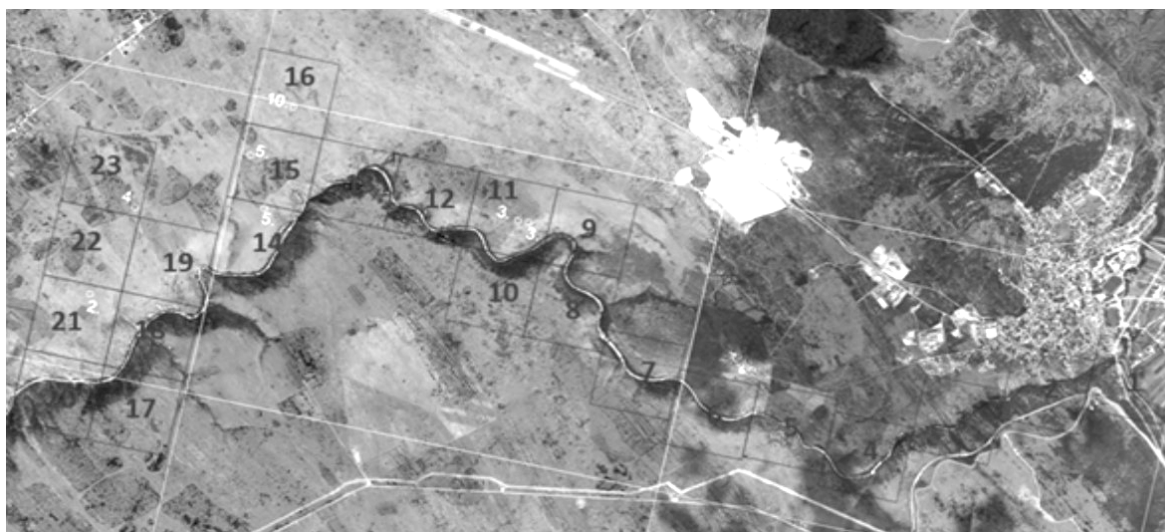
Uvod

Jarebica kamenjarka-grivna (*Alectoris graeca* M.) jedini je autohtoni pripadnik roda *Alectoris* na području Hrvatske (Randi, 2006). Osim ove vrste u Hrvatskoj obitava i alohtona vrsta jarebica čukar (*Alectoris chucar* L.), koja je iz umjetnog uzgoja naseljavana za potrebe komercijalnog lova na otoke sjevernog Jadrana (Janicki i sur., 2007). Jarebica kamenjarka girvna rasprostranjena je u Hrvatskoj po čitavom priobalju, uključujući otoke ali i planinske predjele južnih i zapadnih obronaka gorja primorskog i dalmatinskog zaleđa i istarskog priobalja (Grubešić i sur., 2011). Dugogodišnji trend brojnosti ove vrste u Hrvatskoj je negativan, a kao najznačajniji uzrok navode se promjene u staništu radi depopulacije prostora, napuštanja poljoprivrede i stočarstva, sukcesije vegetacije, promjene u načinu gospodarenja šumom i dr. (Grubešić i sur., 2011). Randi (2006) također navodi da radi značajnih promjena u staništu u mnogim prirodnim staništima, a posebice na Apeninima i u Grčkoj brojnost ove vrste se smanjuje, pri čemu napominje i problem hibridizacije između jarebice kamenjarke-grivne i jarebice čukar.

Cilj ovoga istraživanja je procjena brojnosti, odnosno gustoće populacije jarebice kamenjarke (*Alectoris graeca*) na području značajnog krajobraza „Vodeni tok i kanjon rijeke Čikole“ metodom vokalnog podraživanja mužjaka u vrijeme reprodukcije („playback“ metoda), ali i testiranje primjenjivosti ove metode u našim geografskim uvjetima. Naime, ova metode se često koristi za monitoring populacije jarebice kamenjarke u Francuskoj i Italiji (Bernard-Laurent i Laurent, 1984., Bernard-Laurent i Boev, 1997., Maurino i sur., 2013., Amici i sur., 2013.), dok nema podataka da je primjenjivana u Hrvatskoj. Pravilnik o sadržaju, načinu izrade i postupku donošenja, odnosno odobravanja LGO, programa uzgoja divljači i programa zaštite divljači (NN 40/06, 92/08, 39/11, 41/13) predviđa primjenu metode osluškivanja, međutim u slučaju vokalnog podraživanja, radi upotrebe uređaja za reprodukciju glasanja mužjaka, potrebno je ishoditi dozvolu nadležnog ministarstva. Osluškivanje, kao metoda procjene brojnosti kamenjarke naročito je primjenjiva u strmim, teško pristupačnim područjima, kakva uobičajeno nastanjuje jarebica kamenjarka. Osluškivati možemo cijelu godinu, bilo obiteljska jata ili pjevajuće mužjake, dok metodom vokalnog podraživanja možemo samo brojati parove u vrijeme reprodukcije, odnosno od početka ožujka do kraja svibnja (Pintur, 2010). Temeljem glasanja mužjaka moguće je razlikovati vrste unutar roda *Alectoris*, kao što su npr. jarebica kamenjarka i jarebica čukar (Bernard-Laurent i Laurent, 1984).

Materijal i metode

Istraživanje je provedeno na području značajnog krajobraza „Vodeni tok i kanjon rijeke Čikole“ tijekom svibnja 2018. godine. Veličina istraživanog područja je 1075 ha. Kanjon je dug 13 km, a na mjestima dubok 130 m. Kanjon se proteže od grada Drniša do utoka u rijeku Krku kod jezera Torak, gdje dijeli granicu s NP Krka. Cijelo područje je područje Natura 2000, odnosno POP - područje značajno za očuvanje ptica. Klima je na ovom području mediteranska, ljeta su duga i vruća, zime kratke i tople (Anonymous, 2006). Nadmorska visina platoa kod kanjona Čikole je 273 m pa je lokalitet dodatno zanimljiv u smislu broja kamenjarki koje se gnijezde na područjima nižih nadmorskih visina. Dio kanjona je još uvijek minski sumnjivo područje. Procjena brojnosti kamenjarke grivne provedena je metodom vokalnog podraživanja, odnosno primjenom playback tehnike pri čemu su brojani „pjevujući“ mužjaci. Prije početka vokalnog podraživanja evidentirana su spontana pjevanja mužjaka. Metoda je provedena u transekt točkama (Bernard-Laurent i Laurent, 1984., Amici i sur., 2013., Lo Valvo i sur., 2013., Sorace i sur., 2013.), a istraživano područje je podijeljeno mrežom kvadranta (500x500 m) (43 kvadranta) pri čemu je u sredini svakog kvadranta bila točka s koje je puštena snimka pjevanja mužjaka i slušan odaziv. Unutar područja od 100 ha nalazile su se 4 točke transekta. Nakon osluškivanja spontanijeh pjevanja (30 minuta nakon dolaska na transekt točku), reproducirana je audio snimka pjeva mužjaka 20 sekundi u smjeru sjevera (N), nakon čega se 20 sekundi slušalo, a zatim istim protokolom u smjeru istoka (E), pa juga (S) i na kraju zapada (W). Početak „pjevanja“ se izvodio u vremenskom intervalu od četiri sata ujutro tj. pola sata prije svitanja pa do tri i pol sata nakon svitanja te uvečer tri sata prije mraka (Bernard-Laurent i Laurent, 1984.). Tijekom podraživanja evidentirani su meteorološki uvjeti na terenu, a posebno intenzitet vjetra. U slučaju pjevanja mužjaka, kao odgovor na reprodukciju snimke ili u slučaju spontanog pjeva mužjaka, radi izračuna gustoće populacije u obzir je uzeta pretpostavka da je u tom razdoblju godine (reprodukcije) mužjak u paru s ženkom (Sorace i sur., 2013). Gustoća populacije je izračunata na način da je omjer broja izbrojanih mužjaka, odnosno parova i površine pomnožen sa 100. Uz pomoć tog podatka je procijenjena i veličina populacije koja se nalazi na području značajnog krajobraza „Vodeni tok i kanjon rijeke Čikole“.



Slika 1. Podjela istraživanog područja prema kvadrantima

Rezultati i rasprava

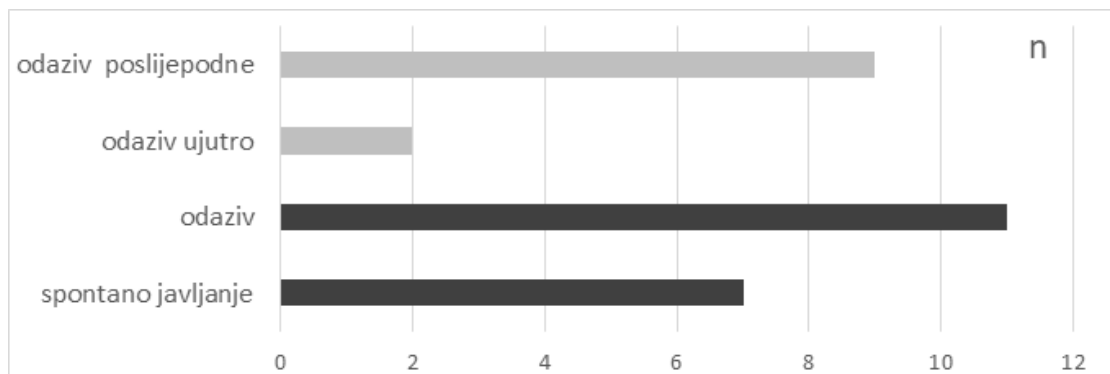
Rezultati praćenja spontanijeh i potaknutijeh javljanja mužjaka kamenjarke grivne, prikazani su prema kvadrantima u tablici br. 1. Audio snimka mužjaka, kojom su vokalno podraživani mužjaci u staništu reproducirana je na 43 točke (središnje mjesto 43 kvadranta). Tijekom osluškivanja zabilježeno je 7 spontanijeh javljanja, odnosno 11 javljanja nakon vokalnog podraživanja (grafikon 1). Na navedenom području smo utvrdili 12 parova jarebica kamenjarki, odnosno 1,11 parova na 100 ha lovno produktivne površine. Prisutnost kamenjarke čukar nije utvrđena.

Tablica 1. Broj utvrđenih parova prema kvadrantima

R.br. kvad.	Centar kvadranta (x/y)	Datum i vrijeme	Metorološke prilike	Spontano javljanje ili odaziv	Broj parova
11.	468718.1215 4857065.2280	12.05.2018 19.00-20.00	oblačno, bez vjetra	spontano	1 par
		18.05.2018. 07.00-8.00	oblačno, bez vjetra	odaziv	
15.	467218.1215 4858065.2280	18.05.2018. 19.00-20.00	sunčano, bez vjetra	odaziv	1 par
		18.05.2018. 19.00-20.00	sunčano, bez vjetra	odaziv	
21.	466218.1215 4856315.2280	12.05.2018. 16.00-17.00	oblačno, bez vjetra	spontano	1 par
		13.05.2018. 12.00-13.00	oblačno, vjetar 5km/h	spontano	
26.	465218.1215 4855565.2280	14.05.2018. 06.00-07.00	vedro, vjetar 10km/h	spontano	1 par
		14.05.2018. 06.00-07.00	vedro, vjetar 10km/h	spontano	
27.	465218.1215 4856065.2280	17.05.2018. 19.00-20.00	sunčano, bez vjetra	odaziv	1 par
		14.05.2018. 07.00-08.00	vedro, vjetar 10km/h	spontano	
28.	464718.1215 4856065.2280	17.05.2018. 19.00-20.00	sunčano, bez vjetra	odaziv	2 para
		19.05.2018. 19.00-20.00	sunčano, bez vjetra	odaziv	
29.	464718.1215 4855565.2280	17.05.2018. 18.00-20.00	sunčano, bez vjetra	odaziv	2 para
		12.05.2018.	sunčano, bez vjetra	spontano	
38.	462218.1215 4854315.2280	10:25	vjetra	spontano	1 par
		19.05.2018. 08:16	oblačno, vjetar 5km/h	odaziv	

Lovnogospodarska osnova za ovo područje predviđa gustoću populacije od 1.40 parova na 100 ha lovneproduktivne površine (Anonymous, 2006). Iako je utvrđena gustoća populacije na ovom području manja od one predviđene lovogospodarskom osnovom, istu možemo smatrati zadovoljavajućom. Naime, riječ je o području na kojem se kamenjarka lovi, a u vrijeme brojanja bilo je dosta promijenljivo vrijeme, što može utjecati na preciznost metode (Sorace i sur., 2013). Istraživanja koja su proveli Sorace i sur. (2013). u nekoliko talijanskih regija pokazala su slične ili niže rezultate u područjima na kojima se lovi (0.12-2.02 para), dok je na zaštićenim područjima, na kojima nema lova, utvrđena veća gustoća populacije (NP Maiella 4.2 parova na 100 ha). U istom istraživanju je utvrđeno da se u regijama Calabria, Campania i Basilicata kamenjarka uglavnom očuvala na višim područjima, što ukazuje na preferabilnost i kvalitetu staništa (Sorace i sur., 2013.). Amici i sur. (2013.) su objavili rezultate šestogodišnjeg monitoringa u Lazio regiji i srednjim Apeninima. Njihovi rezultati pokazuju da su gustoće populacije u zaštićenim područjima 0.12 do 0.62 parova na 100 ha, dok su na lovnim područjima od 0.12 do 0.45 parova na 100 ha.

Procjena brojnosti populacije jarebice kamenjarke (*Alectoris graeca*) na području značajnog krajobraza vodenog toka i kanjona rijeke Čikole



Grafikon 1. Odnos spontanih i potaknutih javljanja (odaziv ujutro/poslijepodne)

U Hrvatskoj se procjena brojnosti najčešće utvrđuje brojanjem na pokusnim plohama i cijelogodišnjim osmatranjem, dok objavljenih podataka o brojnosti kamenjarke primjenom metode vokalnog podraživanja nema. Tijekom provedbe ovog istraživanja mužjaci su se najviše oglašavali, odnosno najbolji odaziv je bio u popodnevnom satima, iako Bernard-Laurent i Laurent (1984) navode da je najbolje ovu metodu provoditi u jutarnjim satima. Uzroke boljeg popodnevnog odaziva možemo tražiti u boljim vremenskim uvjetima tijekom popodnevni sati, budući je cijeli svibanj bio neuobičajeno kišovito na ovom području. Metodom vokalnog podraživanja utvrđeno je više mužjaka, nego metodom osluškivanja spontanog javljanja. Od 7 mužjaka koji su se javljali spontano i jednog kojeg smo utvrdili bez pjevanja, dobili smo odaziv 6 mužjaka nakon vokalnog podraživanja, ili 75 %. Sara (1989) navodi da je na Siciliji utvrđen prosječan odaziv od 59 %, dok prema Bernard-Laurent i Laurent (1984), u optimalnim uvjetima, pogreška brojanja ovom metodom ne prelazi 10%.

Zaključak

Utvrđena brojnost kamenjarki na istraživanom području je nešto manja od one predviđene lovnogospodarskim osnovom. Najznačajnije prednosti ove metode, u odnosu na metode koje iziskuju podizanje kamenjarki pomoću pasa, su jednostavnost izvedbe te njena primjenjivost na teško pristupačnim terenima. Brojanje reproduktivnih parova tijekom predinkubacijskog razdoblja, obzirom na migracijske sklonosti ove vrste, pokazalo se dobrim, budući su tada kamenjarke vezane za područje gniježđenja. Osim toga, primjenom ove metode ne dolazi do brojanja drugih vrsta jarebica na područjima gdje dijele teritorij s jarebicom kamenjarkom, kao što je npr. jarebica čukara. Nedostaci ove metode su ovisnost o meteorološkim uvjetima, invazivnost, relativno kratko vremensko razdoblje primjenjivosti tijekom godine te mogućnost odaziva mužjaka koji nema svoju ženku. Radi kvalitetnije procjene proletne brojnosti te prirasta, uz ovu metodu poželjno je primjeniti i druge metode poput cjelogodišnjeg praćenja i brojanja, posebice uz pomoć pasa.

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Estimation population size of the rock partridge (*Alectoris graeca*) in the area of protected landscape of the water course and the canyon of the Čikola River

Abstract

The estimation of the rock partridge (*Alectoris graeca*) abundance was carried out by listening to spontaneous singing and applying the playback call count technique in the area of protected landscape of the water course and the canyon of the Čikola River. This survey was conducted in May 2018. The used playback call count technique is an invasive method and results depend on the season, time of day and meteorological conditions. The study area was divided into 43 quadrants. The population density of 1.1 pairs per 100 ha was determined in the studied area, ie the number of 12 pairs was estimated.

Keywords: rock partridge, protected landscape, spring density, playback call count

Kraniometrijska obilježja šljuke bene (*Scolopax rusticola* L.) s područja Dalmatinske zagore

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Sažetak

Šljuka bena (*Scolopax rusticola* L.) tradicionalna je i iznimno atraktivna lovna vrsta u Republici Hrvatskoj. Cilj ovog istraživanja bio je obraditi njena kraniometrijska obilježja te statistički utvrditi eventualne razlike između mužjaka i ženki. U radu je analizirano 50 uzoraka prikupljenih tijekom 2 lovne godine u zajedničkom otvorenom lovištu br. XVII/135 "Vrgoračko jezero" koje se nalazi na području Dalmatinske zagore. Svakoj šljuki je određena dob te spol, a potom je na obrađenim lubanjama izmjereno 8 kraniometrijskih parametara. Temeljem statističke obrade dobivenih podataka zaključeno je kako nije moguće pouzdano utvrditi dob i spol jedinki šljuke bene temeljem kraniometrijskih obilježja.

Ključne riječi: Šljuka bena, *Scolopax rusticola* L., kraniometrija, Dalmatinska zagora

Uvod

Šljuka bena (*Scolopax rusticola* L.) je još i danas slabo istražena ptica (Piersma i sur., 1996.). U Hrvatskoj su do sad provedena malobrojna istraživanja nad ovom vrstom. Šprem i sur. (2010.) te Pervan i sur. (2018.) su obrađivali morfometriju na manjem broju uzoraka, dok ostali autori uglavnom iznose literaturne navode stranih znanstvenika. Šljuka bena ili euroazijska šljuka nastanjuje područje umjerenog i hladnog pojasa Europe i Azije. Migratorna je vrsta, a samo su ptice koje nastanjuje Britansko otočje i Francusku stanarice. Tradicionalna je i iznimno atraktivna lovna vrsta te ju se prema Zakonu o lovstvu svrstava u sitnu pernatu divljač (Anonymous, 2018., 2019a.). Lovostajom je zaštićena od 01. ožujka do 30. rujna (Anonymous, 2019b.). Dozvoljen je odstrijel do 3 kljuna/lovac/dan, odnosno da ukupni odstrijel ne prelazi 4 kljuna/100 ha površine lovišta obrasle drvenastom vegetacijom (Anonymous, 2006a.). U Hrvatskoj se putem komercijalnog lova na šljuku ostvaruju značajni prihodi, a odstrijel se na području Europe procjenjuje na oko tri do četiri milijuna jedinki (Ferrand i Gossmann, 2001.) s time da samo francuski lovci odstrjele cca 30-40% ove kvote (Ferrand i Gossmann, 2000.). Uvrštena je u Crvenu knjigu ugroženih ptica Hrvatske (Čiković i Radović, 2013.), te je potrebno utvrditi brojnost, proučiti ekologiju vrste i osigurati stabilnu populaciju koja će ostati atraktivna lovna vrsta, bez ugrožavanja ekološke stabilnosti. Zakonom o lovstvu (Anonymous, 2018., 2019a.) divljač je dobro od interesa za Republiku Hrvatsku i ima njezinu osobitu zaštitu, a kako je šljuka bena još i danas slabo istražena ptica, potrebno je posvetiti pozornost u istraživanjima ove vrste kako bi se omogućio uvid u stanje njene populacije. Poznavanje njenih bioloških i morfoloških obilježja u konačnici može pridonijeti i strateškom planiranju gospodarenja ovom vrstom i nastavak dosadašnje tradicije. (Piersma i sur., 1996.).

Materijal i metode

Za potrebe istraživanja prikupljeno je 50 uzoraka šljuke bene (*Scolopax rusticola* L.), unutar nekoliko lovnih dana (05. siječanj – 15. siječanj) tijekom dvije lovne godine (2014/2015 i 2015/2016). Uzorci su prikupljeni u zajedničkom otvorenom lovištu broj XVII/135 „Vrgoračko jezero“ na području Splitsko – dalmatinske županije kojim gospodari „LU Split“. Lubanje ptica su iskuhane, te izbijeljene pomoću 30 % vodikovog – peroksida. Mjerenje je napravljeno uz pomoć digitalne pomične mjerke marke MIB, sa preciznošću od dvije decimale. Deskriptivna statistika i T-test podataka napravljene su u programu IBM SPSS Statistics, Version 22. Na svakom uzorku izmjereno je 8 kraniometrijskih mjera prema Driesch (1976): ukupna dužina lubanje (GL), kondilobazalna dužina lubanje (CBL),

najveća širina lubanje (GB), najveća širina lubanje kod čeonog izdanka (GBP), najmanja širina između očne šupljine (SBO), najveća visina u središnjoj ravnini (GH), duljina od medijalno trokutastog izbočenja do najviše aboralne točke čeonog izdanka (LP) i najveća duljina od vrha kljuna do čeonog izdanka (LI). Sve šljuke odstrijeljene su u skladu sa tada važećim Zakonom o lovstvu (Anonymous, 2005., 2009., 2014.) i lovnogospodarskom osnovom za zajedničko otvoreno lovište broj XVII/135 -"Vrgoračko Jezero" (Anonymous, 2008.). Odstrjel šljuka izvršen je različitim tehnikama pojedinačnog lova, buširanjem s psom i večernjim preletom temeljem Pravilnika o uvjetima i načinu lova, nošenju lovačkog oružja, obrascu i načinu izdavanja lovačke iskaznice, dopuštenju za lov i evidenciji o obavljenom lovu (Anonymous, 2010a.). Tijekom lova isključivo je korišteno lovačko oružje i naboji propisani Pravilnikom o načinu uporabe lovačkog oružja i naboja (Anonymous, 2006b., 2010b.). Svako šljuki je neposredno nakon odstrijela određena dob prema obrascu obojanosti perja (Blasco-Zumeta i Heinze, 2006.) dok je spol određen razudbom i pregledom unutarnjih spolnih organa.

Rezultati i rasprava

Od 50 analiziranih uzoraka 13 je pripadalo muškim, a 14 ženskim juvenilnim jedinkama te 15 muškim i 8 ženskim adultnim jedinkama. Rezultati T-testa pod pretpostavkom da varijance nisu jednake kod juvenilnih i adultnih jedinki jedinki su prema oznakama navedenih mjera prikazani u tablici 1.

Tablica 1. Prikaz ukupnih rezultata T-testa kod juvenilnih i adultnih jedinki šljuke bene (*Scolopax rusticola* L.)

Oznaka mjere (dob)	t	df	p - vrijednost
GL (juvenile)	-0.64613	24.992	0.5241
GL (adult)	-2.0362	10.639	0.06741
CBL (juvenile)	0.69701	24.992	0.4922
CBL (adult)	-1.4785	12.008	0.165
GB (juvenile)	1.0717	21.459	0.2958
GB (adult)	0.82332	10.643	0.4284
GBP (juvenile)	1.1653	24.814	0.255
GBP (adult)	0.91323	13.092	0.3776
SBO (juvenile)	1.5027	23.95	0.146
SBO (adult)	0.79458	14.711	0.4395
GH (juvenile)	-0.90043	20.448	0.3784
GH (adult)	0.40576	20.897	0.689
LP (juvenile)	0.57296	24.949	0.5718
LP (adult)	-0.71937	20.95	0.4799
LI (juvenile)	0.78289	24.987	0.441
LI (adult)	-1.8288	10.092	0.0971

Navedeni rezultati kazuju da nema statistički značajne razlike koja bi mogla poslužiti za razlikovanje juvenilnih i adultnih jedinki oba spola. Pregledom dostupne literature ustanovljeno je da kranimetrija šljuke bene (*Scolopax rusticola* L.) u Republici Hrvatskoj do sada nije podrobije istraživana te su ovdje iskazani podaci rezultat prvog preliminarnog istraživanja na ovom prostoru. Određivanje dobi šljuke bene moguće je utvrditi na temelju boje i istrošenosti letnog perja, dok je spol sa sigurnošću moguće utvrditi razudbom i vizualnim pregledom unutarnjih reproduktivnih organa. Dužina kljuna u nekim slučajevima može ukazivati na spol no statistički nije pouzdan parametar (Pervan i sur., 2018.). U morfometrijskom istraživanju na neobrađenim glavama (52 uzoraka u svježem stanju) isti autori navode kako je kljun kod ženskih jedinki češće duži nego kod muških jedinki, tj da kod ženskih adultnih jedinki prosječna dužina iznosi 7,62 cm, a kod juvenilnih jedinki 7,55 cm dok kod adultnih mužjaka dužina iznosi 7,30 cm, a kod juvenilnih mužjaka dužina iznosi 7,20 cm. Arradis i sur. (2005.) navode kako je spol moguće

utvrditi mjerenjem veličine srednjeg nožnog prsta te DNA analizom. U ovom istraživanju rezultati izmjere dužine od vrha kljuna do čeonog izdanka (LI) kazuju da srednja vrijednost kod adultnih ženki iznosi 8,70 cm, kod juvenilnih ženki 8,60 cm, dok kod adultnih mužjaka iznosi 8,35 cm, a kod juvenilnih mužjaka iznosi 7,99 cm. Izmjerene vrijednosti su veće u ovom istraživanju nego istraživanju Pervana i sur. iz 2018. godine no treba uzeti u obzir da se ovdje radi o otkuhanim i očišćenim lubanjama te krajnjoj točki izmjere (čeonni izdanak) što u morfometrijskom istraživanju na neobrađenim lubanjama nije bilo moguće izmjeriti. Tablica 2 prikazuje sumarni prikaz kraniometrijskih obilježja šljuke bene svih 50 uzoraka po kraniometrijskim mjerama, minimalnim i maksimalnim vrijednostima, medijanu, srednjoj vrijednosti i standardnoj devijaciji dok Tablica 3 prikazuje usporedne rezultate ovog istraživanja i istraživanja Schafera i Schmitza (2016.) na šest istih izmjera.

Tablica 2. Sumarni prikaz kraniometrijskih obilježja šljuke bene (*Scolopax rusticola* L.) s područja Vrgoračkog jezera (N = 50). Puni nazivi kratica oznaka mjera su navedeni u tekstu.

Oznaka mjere	Min (mm)	Max (mm)	Median	Srednja vrijednost (mm)	SD
GL	104,30	124,0	111,30	111,7	3,75
CBL	86,15	101,61	93,00	92,91	3,47
GB	21,16	24,82	23,50	23,50	0,79
GBP	16,10	20,44	18,95	18,87	0,88
SBO	8,34	13,78	11,14	11,21	1,19
GH	20,49	27,08	24,93	24,78	1,00
LP	22,91	28,16	26,48	26,44	0,98
LI	78,97	97,71	84,72	85,21	3,79

U Njemačkoj su Schafer i Schmitz (2016.) proveli istraživanje kraniometrijskih obilježja šljuke bene (*Scolopax rusticola* L.) na uzorku od 10 lubanja iz kolekcija Prirodoslovnog muzeja u Stuttgartu. Pri odabiru uzoraka pazili su na omjer spolova (1:1), a izmjeru su napravili na temelju mjera koje opisuju King i McLelland (1978.) koji su kraniometrijske mjere imenovali drugačijim nazivljem od autora po kojem su obrađeni uzorci u ovom radu. Rezultati Schafera i Schmitza (2016.) prikazuju niže vrijednosti od rezultata dobivenih u ovom istraživanju. Svrha njihovog rada bila je izraditi determinacijski ključ za određivanje vrsta, a korištenjem 6 istih kraniometrijskih mjera bez obzira na njihov različit naziv moguće je usporediti rezultate.

Tablica 3. Usporedba šest izmjera kranimetrijskih obilježja šljuke bene iz dva istraživanja

Oznaka mjere	Autori, godina	N	Srednja vrijednost (mm)	Min	Max	SD
GL	Schafer i Schmitz, 2016.	10	112,9	104,5	119,6	17,9
	Pervan i sur., 2019.	50	111,7	104,30	124,0	3,75
GB	Schafer i Schmitz, 2016.	10	23,0	22,2	23,9	0,3
	Pervan i sur., 2019.	50	23,50	21,16	24,82	0,79
SBO	Schafer i Schmitz, 2016.	10	10,6	9,3	12,5	1,0
	Pervan i sur., 2019.	50	11,21	8,34	13,78	1,19
GH	Schafer i Schmitz, 2016.	10	23,3	22,0	24,3	0,5
	Pervan i sur., 2019.	50	24,78	20,49	27,08	1,00
LP	Schafer i Schmitz, 2016.	10	28,9	27,7	30,6	0,8
	Pervan i sur., 2019.	50	26,44	22,91	28,16	0,98
LI	Schafer i Schmitz, 2016.	10	84,0	76,0	89,0	16,0
	Pervan i sur., 2019.	50	85,21	78,97	97,71	3,79

Sve kranimetrijske mjere dobivene u ovom istraživanju pokazuju veće srednje vrijednosti od njemačkog istraživanja, osim vrijednosti duljine od medijalno trokutastog izbočenja do najviše aboralne točke čeonog izdanka (LP) no ovdje napominjemo kako je u njihovom istraživanju korišten značajno manji broj uzoraka (10) čija dob nije utvrđivana.

Zaključak

Ovo istraživanje dalo je uvid u opća kranimetrijska obilježja šljuke bene po prvi puta u Hrvatskoj. Temeljem dobivenih rezultata zaključujemo kako ne postoji statistički značajna razlika između kranimetrijskih obilježja juvenilnih i adultnih jedinki oba spola šljuke bene. Samo cjelovit, interdisciplinarni i sveobuhvatan pristup može polučiti zadovoljavajuće rezultate u poznavanju biologije šljuke bene te time ukazati na eventualne nužne promjene u lovnom gospodarstvu, a obzirom da je šljuka bena u Hrvatskoj predstavlja tradicionalno vrlo atraktivnu lovnu divljač i značajan je resurs u lovno-gospodarskom smislu, njeno očuvanje i daljnje istraživanje se i samo nameće.

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Craniometrical features of Eurasian woodcock (*Scolopax rusticola* L) in the area of Dalmatian Hinterland

Woodcock (*Scolopax rusticola* L.) is a traditional and very attractive game species in Croatia. The aim of this study was to evaluate its craniometric features and to statistically determine possible differences between males and females. In this research we analyzed 50 specimens collected during 2 hunting years in the common open hunting ground no. XVII / 135 “Vrgoračko Jezero” located in the area of the Dalmatian Hinterland. Each woodcock was determined by age and sex, and then 8 craniometric factors were measured on the treated skulls. Based on the statistical analysis of the obtained data it was concluded that it is not possible to reliably determine the age and sex of individual woodcock specimens by craniometric features.

Keywords: woodcock, *Scolopax rusticola* L., craniometry, Dalmatian Hinterland

Fish assemblage of the artificial flood protection channel Kupa-Kupa

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Abstract

The objective of this paper is to determine the qualitative and quantitative composition of the fish community, and distribution of native and non-native fishes in the channel Kupa-Kupa. Fish community of the channel Kupa-Kupa was analyzed from September 2017 to September 2019. At eight different locations, 23 species from eight families were found. The dominant species was European bitterling *Rhodeus amarus* which takes up 32.83% of total number of fish caught, while the least represented is Asp *Leuciscus aspius* with one individual. During this research, one endemic fish species was found, as well as two invasive alien species (IAS). Non-native species hold a significant share in the fish community of the channel Kupa-Kupa.

Keywords: fish community, channel Kupa-Kupa, endemic species, non-native species

Introduction

Fishes are appropriate indicators of trends in aquatic biodiversity because their enormous variety reflects a wide range of environmental conditions (Moyle et al., 1992). Fresh waters are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems (Dudgeon et al., 2006). Anthropogenic impacts such as flow regulation, channelization, and bank stabilization, eliminate upstream-downstream linkages and isolate river channels from riparian/floodplain systems and contiguous groundwater aquifers (Ward, 1998). Floods lead to increased resource availability (e.g., food and shelter), with positive effects on the development of the initial stages of fish and on the maintenance of high levels of species diversity (Agostinho et al., 2004a, 2007), allows species dispersal across several plain environments (Thomaz et al., 2007). The life cycle of a fish species, especially its reproduction, depends on the hydrological regime (Vasconcelos et al., 2014). Annual variation in the hydrograph affects species with distinct life history strategies differently, and influences the composition and structure of fish assemblages. Large floods were associated with higher species richness (Agostinho et al., 2004b). Human activities usually shift the balance among fish species, causing the extirpation of many indigenous species and the dominance of a reduced set of often introduced fish species (Balirwa et al., 2003). Croatian freshwater ichthyofauna has been previously researched (Čaleta et al., 2019), as well as the ichthyofauna of one location on the lower course of the Kupa River (Delić et al., 2014). Previous papers on the composition of ichthyofauna in the channel Kupa-Kupa are unknown. The objective of this paper is to determine the qualitative and quantitative composition of the fish community, and distribution of native and non-native fishes in the channel Kupa-Kupa.

Materials and methods

Study area

The beginning of the drainage section of the channel Kupa-Kupa is located on the near vicinity of Karlovac, it was built in 1984 (Mujić, 2016) to protect the area from floods and it is 21.8 km long (Hrvatske vode, 2014). It connects the middle and the lower course of the Kupa River from Mahično to Donja Kupčina. Fishponds Kupa, Crna mlaka

and lake Šumbar are located along the flood channel, and fishpond Pisarovina downstream of the channel. The bottom of the channel varies in substrate. In the very beginning near Mahično the bottom is made of concrete without sediment residue, while in its central part it is muddy. The final third of the channel has a rocky bottom with smaller size rocks. The channel margins are without vegetation and are regularly mowed. Riparian vegetation was found along most of the channel during the two years of research. The vegetation of the bed channel includes common reed, sedge, water lilies and coontails. The regime of floods in the channel Kupa-Kupa is most intense in spring and autumn. The channel is also used for sport fishing purposes.

Fishing samplings

Ichthyofauna samples were collected 11 times, from August 2017 to August 2019, from eight different locations in the channel Kupa-Kupa. The samples from three locations were collected twice (Mahično, mouth of the Bukovac creek and the blue bridge in Rečica), while the samples from other locations were collected once. In result analysis, the results from the locations sampled twice were not summed, but calculated individually for each electrofishing. The sampling locations were marked according to UTM coordinates (WGS84) and were named after the nearest place according to the map: No.1) 45°32'45"N, 15°31'55"E-Mahično, No.2) 45°32'33"N, 15°34'11"E-Topolovka, No.3) 45°32'37"N, 15°35'27"E-Bugarski vrt, No.4) 45°32'44"N, 15°38'23"E-mouth of the Bukovac creek, No.5) 45°32'39"N, 15°39'43"E-blue bridge in Rečica, No.6) 45°32'31"N, 15°42'07"E -Čadinjak, No.7) 45°32'25"N, 15°43'53"E-Dubrave, No.8) 45°31'52"N, 15°47'21"E-mouth of the Kupčina River (Figure 1).

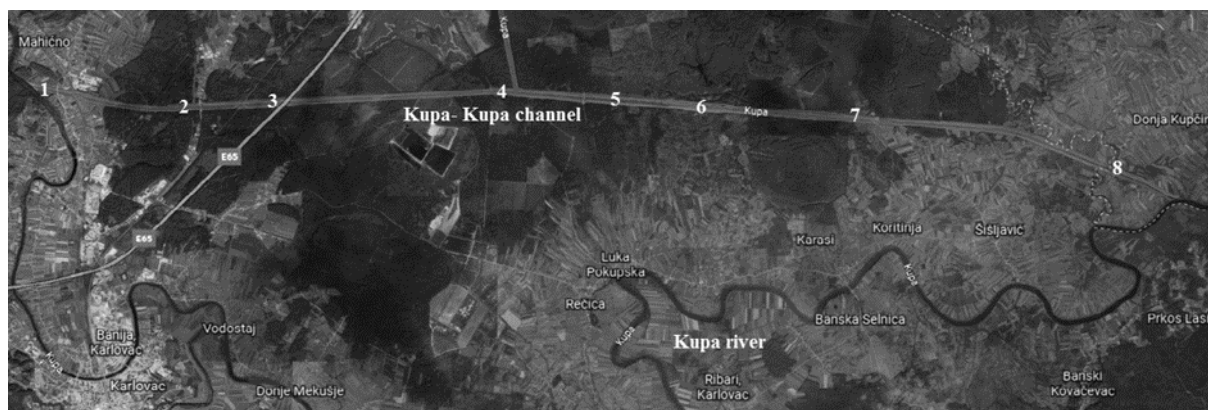


Figure 1. Map of the channel Kupa-Kupa with sampling locations

The samples were collected with a Hans-Grassl IG600 electrofishing device, with a maximum power of 650W of direct current and 1200W of pulsating electric current. The length of the transect of each location was 100 m. The width of the water column channel at sampling sites varied. The maximum width of the sampling channel was at locations No.1) and No.5), where the sampling width was 25 m. In the narrowest part of the channel sampling, the width was 3m, at location No.2). Samplings are done throughout the channel width at all sampling locations. After collecting the samples, the species were determined according to Kottelat and Freyhof (2007) determination key. The determined individuals were classified according to family and origin. The composition of fish community was calculated as a percentage of each species in the total number of fish caught in all fish species. All individuals were placed in an aerated tank with a capacity of 1 hL and transported to JU Aquatika -freshwater aquarium Karlovac for the purpose of the exhibition. MS EXCEL 2016 was used for data analysis.

Results and discussion

In total, 3 567 individuals were collected at eight locations on the channel Kupa-Kupa, from Mahično to Donja Kupčina. Fish from the Cyprinidae family are most numerous in the ichthyofauna of the channel Kupa-Kupa, with 14 species and relative abundance of 79.22% (Figure 2a). From eight families 23 species were identified (Table 1.). The most numerous species were European bitterling *Rhodeus amarus* with 1 171 individuals, followed by Bleak *Alburnus alburnus* with 696 individuals, and the least numerous is Asp *Leuciscus aspius* with one individual.

Table 1. Qualitative and quantitative composition of the fish community of the channel Kupa-Kupa

Taxon	%	Biogeographical status
Cyprinidae		
Bleak <i>Alburnus alburnus</i> (Linnaeus, 1758)	19.51	Native
Asp <i>Leuciscus aspius</i> (Linnaeus, 1758)	0.03	Native
White bream <i>Blicca bjoerkna</i> (Linnaeus, 1758)	0.45	Native
Prussian carp <i>Carassius gibelio</i> (Bloch, 1782)	9.08	Non-native
Common nase <i>Chondrostoma nasus</i> (Linnaeus, 1758)	0.14	Native
Carp <i>Cyprinus carpio</i> Linnaeus, 1758	0.28	Native
Danube gudgeon <i>Gobio obtusirostris</i> Valenciennes, 1842	0.90	Native
Pseudorasbora <i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)	7.71	IAS (EU)
European bitterling <i>Rhodeus amarus</i> (Bloch, 1782)	32.83	Native
Roach <i>Rutilus rutilus</i> (Linnaeus, 1758)	1.99	Native
Rudd <i>Scardinius erythrophthalmus</i> (Linnaeus, 1758)	0.28	Native
Chub <i>Squalius cephalus</i> (Bonaparte, 1837)	5.21	Native
Tench <i>Tinca tinca</i> (Linnaeus, 1758)	0.59	Native
Vimba bream <i>Vimba vimba</i> (Linnaeus, 1758)	0.20	Native
Cobitidae		
Balkan spined loach <i>Cobitis elongata</i> Heckel & Kner, 1858	0.31	Endemic
Danubian spined loach <i>Cobitis elongatoides</i> Băcescu & Mayer, 1969	8.19	Native
Balkan golden loach <i>Sabanejewia balcanica</i> (Karaman, 1922)	0.11	Native
Centrarchidae		
Pumpkinseed <i>Lepomis gibbosus</i> (Linnaeus, 1758)	6.06	IAS (EU)
Esocidae		
Pike <i>Esox lucius</i> Linnaeus, 1758	1.96	Native
Percidae		
Perch <i>Perca fluviatilis</i> Linnaeus, 1758	1.46	Native
Gobiidae		
Pontian monkey goby <i>Neogobius fluviatilis</i> (Pallas, 1814)	1.43	Non-native
Ictaluridae		
Black bullhead <i>Ameiurus melas</i> (Rafinesque, 1820)	0.93	Non-native
Siluridae		
Catfish <i>Silurus glanis</i> Linnaeus, 1758	0.34	Native

When these results are compared to previous research of the lower course of the Kupa River (Delić et al., 2014), in the channel Kupa-Kupa was recorded six fish species less. Except for Black bullhead *Ameiurus melas*, all other species recorded in the channel Kupa-Kupa were also found in the lower course of the Kupa River (Delić et al., 2014). The Cobitidae family is represented by three species from two genera, including Balkan spined loach *Cobitis elongata* which is an endemic species of the Danube River Basin (Čaleta et al., 2015). This species was found at four locations marked by numbers 1), 5), 6), and 7). Danubian spined loach *Cobitis elongatoides* is the most represented species from this family, and these results are in accordance with previous research of the lower course of the Kupa River

(Delić et al., 2009). At the location No. 5) *C. elongatoides* is the dominant species, and in two samplings conducted at the same location on February 20, 2019 and March 15, 2019 this species took up a relative density of 50% and 66% of the entire fish community. The least represented species from this family is Balkan golden loach *Sabanejewia balcanica* found only at the location No. 5), with only four individuals. All the species from this family were also found in previous research of the lower course of the Kupa River (Delić et al., 2014). It was observed that in colder periods of the year individuals from this species prefer the central part of the channel, while in warmer periods of the year from March to late September most of the population of this family tends to reside in the zone of the riparian vegetation. This type of behaviour could be possible connected with undetermined fractional spawning of this species (Lodi and Malacarne, 1990), and the males from the *Cobitis* genus follow the females into the thick vegetation during spawning season (Bohlen, 2008). Other fish families were represented by one species.

In this research out of the 23 species of fish, 18 species are native, while according to the latest Croatian freshwater fish checklist (Čaleta et al., 2019), Prussian carp *Carassius gibelio*, Pseudorasbora *Pseudorasbora parva*, Pumpkinseed *Lepomis gibbosus*, Pontian monkey goby *Neogobius fluviatilis* and *A. melas* are non-native species. The total number of non-native species is 25,2% of the total number of all species caught in the channel Kupa-Kupa. Species *P. parva* and *L. gibbosus* are on the EU invasive species list (EU 2016/1141, EU 2019/1262) and according to the results of this research, they take up more than 50% of the population of non-native species and 13.8% of the entire fish community of the channel Kupa-Kupa (Figure 2b).

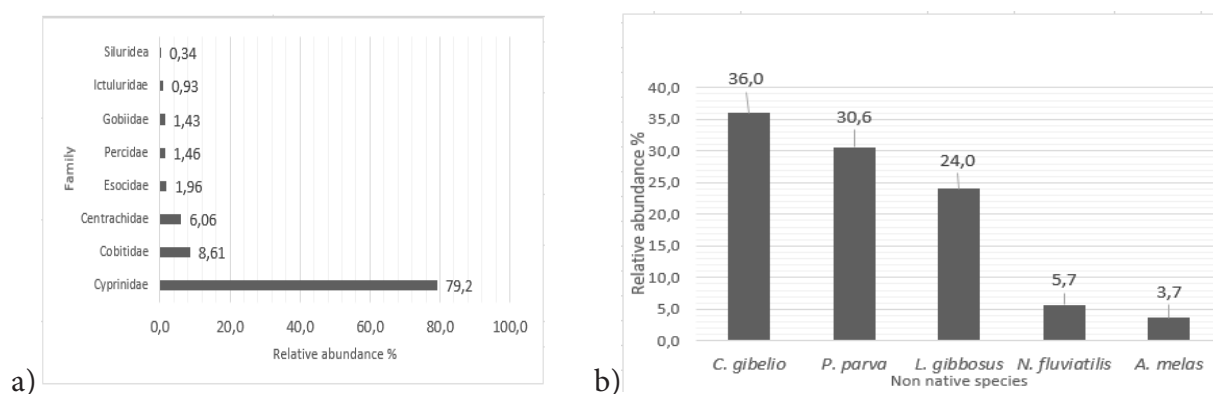


Figure 2. Relative abundance of species with respect to family a), and relative abundance of each non-native species out of the entire population of non-native fish in the channel Kupa-Kupa b)

Among the non-native species, the most represented one is *C. gibelio*, found in all locations, while *N. fluviatilis* inhabits the channel from the location No. 4) to the end of the channel in all locations explored. According to the Jakovlić et al. (2015), the westernmost area of *N. fluviatilis* is in the Korana River in Karlovac and the Kupčina River, approximately two km to the west of location No. 4). The spreading of *N. fluviatilis* to the Kupčina River from the Kupa River was most likely made possible by the channel Kupa-Kupa, because the Kupčina River does not have direct contact with the Kupa River or the Sava River where *N. fluviatilis* was found (Jakovlić et al, 2015). The results of this research confirm the distribution and habitat of *N. fluviatilis* from the location No. 8), to the location No. 4), or less than 9 km from the place where the middle course of the Kupa River flows into the beginning of the channel near Mahično. *A. melas* has the smallest population out of the non-native species, it was recorded only at locations No. 1) and 8). Periodic oscillations were found at the same location in the diversity of species, but also in the abundance of individuals.

Conclusion

Even though it connects the middle and the lower course of the Kupa River, according to its species of ichthyofauna the channel Kupa-Kupa belongs to the lower course of the Kupa River. The ichthyofauna of the channel Kupa-Kupa is richest in species from the Cyprinidae family, and it is also habitat for the endemic species *C. elongata*. Non-native species hold a significant share in the fish community of the channel Kupa-Kupa.

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Sastav riblje zajednice rasteretnog poplavnog kanala Kupa-Kupa

Sažetak

Cilj ovoga rada je utvrditi kvalitativni i kvantitativni sastav ihtiocenoze, te rasprostranjenost autohtonih i alohtonih vrsta riba u kanalu Kupa-Kupa. Analiziran je sastav riblje zajednice od rujna 2017. do rujna 2019., na osam različitih lokacija utvrđeno je 23 vrste riba iz osam porodica. Gavčica *Rhodeus amarus* s 32,83 % od ukupnog broja svih ulovljenih jedinki čini dominantnu vrstu, dok je najmanje zastupljena vrsta bolen *Leuciscus aspius* s jednom ulovljenom jedinkom. Tijekom ovoga istraživanja zabilježena je i jedna endemska vrsta, kao i dvije invazivne strane vrste (IAS). Rezultati ovoga rada pokazuju značajan udio alohtonih vrsta riba u ihtiocenozi kanala Kupa-Kupa.

Ključne riječi: ihtiocenoza, kanala Kupa-Kupa, endemska vrsta, alohtone vrste

Kompeticija u prehrani između unesene kalifornijske pastrve (*Oncorhynchus mykiss*) i ugrožene Europske jegulje (*Anguilla anguilla*) iz rijeke Žrnovnice

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Sažetak

Kompeticijski odnos za hranu od strane unesenih vrsta može utjecati na brojnost autohtone ihtiofaune. Cilj rada bio je utvrditi sastav prehrane kalifornijske pastrve i europske jegulje iz rijeke Žrnovnice, analizirati konzumirane svojte plijena i utvrditi postoji li kompeticija u prehrani između ove dvije vrste. Ulovljenim primjercima izdvojeno je probavilo te su determinirane i analizirane svojte plijena iz sadržaja probavila. Europska jegulja i kalifornijska pastrva hrane se sličnim svojtima, najčešća hrana su im rakušci iz roda *Gammarus* sp. Indeksom preklapanja prehrambenih navika je utvrđena sličnost njihove prehrane što upućuje na zaključak da je kalifornijska pastrva jedna od prijetnji za već vrlo ugroženu europsku jegulju u kraškim vodama Jadranskog sliva.

Ključne riječi: Kalifornijska pastrva, Europska jegulja, *Gammarus* sp., prehrana, kompeticija

Uvod

Alohtone i potencijalno invazivne vrste predstavljaju veliku prijetnju biološkoj raznolikosti (Britton i sur., 2011). Vrste s negativnim ekološkim učincima mogu biti prijetnja autohtonim vrstama i zajednicama na nekom staništu jer kroz biološke mehanizme kompeticije za hranu i prostor često uzrokuju smanjenje i/ili nestanak autohtonih vrsta (Baiser i Lockwood 2011). Kada se dvije vrste preklapaju u vremenu, prostoru i prehrambenim resursima javlja se kompeticija (Amundsen, 2004). Zanimljivo je istražiti kompeticiju u prehrani između kalifornijske pastrve (*Oncorhynchus mykiss*) koja je alohtona vrsta i ugrožene autohtone vrste Europske jegulje (*Anguilla anguilla*). Kalifornijska pastrva najraširenija je unesena riblja vrsta u svijetu (Stanković i sur., 2015). Potkraj devetnaestog stoljeća iz sjevernoameričkih pacifičkih pritoka prenesena je u europske vode, tako i u Hrvatsku (Treer i sur., 1995). Mlad kalifornijske pastrve se hrani planktonom i beskralježnjacima, a odrasle jedinke hrane se makrozoobentosom i manjim ribama (Povž i Sket, 1990). Suprotno tome jegulja je katadromna riba zmijolikog tijela, koja živi u kopnenim vodama cijele Europe (Treer i sur., 1995). Brojnost europske jegulje danas je u velikom padu na cijelom europskom kontinentu (Piria i sur., 2014). Prema IUCN kriterijima za procjenu ugroženosti vrsta, europska jegulja procijenjena je kao kritično ugrožena vrsta (CR), odnosno suočena je sa vrlo visokim rizikom od izumiranja (Jacoby i Gollock, 2014). Ona ima oportunistički tip hranjenja, odnosno konzumira plijen koje je u određenom trenutku najpristupačniji (Bouchereau i sur., 2006). Podataka o prirodnoj prehrani navedenih vrsta u Hrvatskoj još uvijek nema. Dosadašnja istraživanja govore o prehrani europske jegulje u zavičajnom staništu (Bouchereau i sur., 2009) i kalifornijske pastrve u unesenom staništu (Metcalf i sur., 1997), međutim u dostupnoj literaturi nema podataka o prehrani ova dva obligatorna predatora kada nastanjuju isto stanište. Stoga, cilj ovog istraživanja bio je: i) utvrditi sastav prehrane kalifornijske pastrve i europske jegulje iz rijeke Žrnovnice; ii) analizirati konzumirane svojte plijena iii) utvrditi postoji li kompeticija u prehrani između kalifornijske pastrve i europske jegulje.

Materijali i metode

Istraživanje je provedeno u gornjem toku rijeke Žrnovnice 2. listopada 2016. godine. Žrnovnica izvire u podnožju Mosora, a ulijeva se u more u stobrečkoj uvali (nekoliko kilometara od Splita u smjeru Omiša), (Klanjec i Donevski, 2010). Uzorkovanje ihtiofaune obavljeno je u vremenskom intervalu od 30 minuta, pomoću uređaja za elektroribolov. Primjerci su sortirani prema vrstama i pohranjeni na -10 °C u hladnjak sve do trenutka analize. Uzorkovanim jedinkama izmjerena je totalna dužina tijela (TL) pomoću ihtimetra na točnost od 0,5 centimetara, dok je masa jedinki izvagana s vagom, preciznošću od $\pm 0,001$ g temeljem čega je izračunat faktor kondicije (CF), važno je istaknuti da se faktor kondicije može uspoređivati samo za jedinke iste starosti, istog spola koje su uzorkovane u istoj sezoni (Treer i Piria, 2019). U laboratoriju je izdvojeno probavilo nakon rezanja kod jednjaka i analnog otvora. Izmjerena je dužina probavila, izvagana je masa punog i praznog probavila te je izdvojen sadržaj. Za determinaciju svojiti plijena korištena je Promea Tech lupa. Svojte su determinirane do najnižih taksonomskih kategorija (vrsta), ako je to bilo moguće. Za utvrđivanje dinamike hranjenja izračunat je koeficijent punoće probavila (Jr%) i koeficijent praznog probavila (V%) (Treer i Piria, 2019). Pri obradi svojiti plijena korišten je postotak učestalosti pojavljivanja (F %), postotak brojnosti (N %) i postotak mase (W %), a za utvrđivanje preklapanja prehrane korišten je indeks preklapanja prehranbenih navika, Schoener indeks(α) (Treer i Piria, 2019). Vrijednosti Schoener indeksa preko 0,60 označava biološki signifikantnu vrijednost i dokazuju međusobnu kompeticiju (Lorenzoni i sur., 2002).

Rezultati

Ukupno je analizirano 17 jedinki europske jegulje duljine od 33,5 do 53,0 centimetara i 7 kalifornijskih pastrva duljine od 13,5 do 28,0 centimetara. Tijekom analize ispunjenosti probavila (Tablica 1) niti jedno probavilo u potpunosti nije bilo prazno. Uočeno je da veće jedinke imaju veći koeficijent punoće probavila. Naime, kod manjih jedinki europske jegulje prosječna punoća probavila (Jr%=0,73) bila je manja od većih jedinki (Jr%=0,92). Kod kalifornijske pastrva koeficijent punoće probavila nešto je veći u odnosu na jegulju. Manje jedinke imaju manju prosječnu punoću probavila (Jr%=1,01) od većih jedinki (Jr%=1,43).

Tablica 1. Koeficijent punoće (Jr%) i koeficijent praznih probavila (V%) europske jegulje (AA) i kalifornijske pastrve (OM), ukupan broj jedinki (UK) za dva dužinska razreda(cm).

Parametar	AA		UK		OM		UK
Broj jedinki	12	5	17	4	3	7	
Dužina(cm)	33 - 39,5	41,5 - 53	33 - 53	13,5 - 18,5	20 - 28	13,5 - 28	
Jr%	0,73	0,92	0,79	1,01	1,43	1,19	
V%	0	0	0	0	0	0	

U prehrani europske jegulje (Tablica 2) dominiraju rakušci *Gammarus* sp. Zatim, u nešto manje broju, prisutni su kukci redova Trichoptera (ličinaka kućica, bez kućice), Diptera (Chironomidae), Plecoptera i mekušci razreda Gastropoda (por. Lymnaeidae, Neritidae). Prehrana kalifornijske pastrve (Tablica 2) slična je prehrani europske jegulje. Rakušci *Gammarus* sp. dominiraju u prehrani pastrve, dok su ostale vrste prisutne u nešto većem broju nego kod jegulje: kukci redova Trichoptera (ličinaka kućica, bez kućice), Diptera (Chironomidae), Plecoptera, Ephemeroptera i mekušci razreda Gastropoda (*Theodoxus fluviatilis*, Neritidae). Dominantna vrsta u prehrani obje vrste su rakušci *Gammarus* sp.

Tablica 2. Frekvencije učestalosti pojavljivanja (F%), postotak brojnosti (N%), postotak mase (W%), faktor kondicije (CF) europske jegulje (AA) i kalifornijske pastrve (OM) (n - broj analiziranih jedinki, n.d. - nije determinirano).

Svojta	AA			OM		
	CF=0.1819, n=17			CF=1.0971, n=7		
	N	F	W	N	F	W
<i>Gammarus</i> sp.	76,15	21,57	20,68	47,67	17,95	13,67
Trichoptera (ličinačka kućica)	4,62	9,80	0,52	4,65	12,82	9,43
Trichoptera (bez kućice)	3,85	3,92	0,57	13,95	7,69	2,17
Chironomidae	3,08	3,92	0,02	2,33	2,56	0,06
Plecoptera	1,54	5,88	0,08	6,98	10,26	2,90
Gastropoda (Lymnaeidae)	2,31	1,96	0,02			
Gastropoda (<i>Theodoxus fluviatilis</i>)				4,65	7,69	8,04
Gastropoda (Neritidae)	3,85	7,84	0,54	6,98	5,13	6,24
Simuliidae	0,77	1,96	0,02	1,16	2,56	0,15
Pisces n.d.	0,77	1,96	2,22			
Sjemenke n.d.	0,77	1,96	0,02			
Pulmonata (kopneni puževi)	0,77	1,96	8,04			
Coleoptera	0,77	1,96	0,28	1,16	2,56	0,04
Kopneni insekt (n.d. osica)				1,16	2,56	0,60
Ephemeroptera	0,77	1,96	0,07	9,30	10,26	1,41
Detritus		33,33	66,93		17,95	55,29

Uzorkovane jedinke jegulje podijeljene su na dva dužinska razreda (Tablica 3). Iz tablice se može uočiti divergencija u preferiranim svojstama (Piria i sur., 2007). Kod jedinki prvog razreda dužine 33,5-39,5 centimetara, uzorak je imao širok spektar svojti plijena gdje uočavamo brojčanu dominaciju *Gammarus* sp. (N%=79,21). Jedinke drugog razreda dužine 41,5-53,0 centimetara karakterizira nešto viši kondicijski faktor i veća izbirljivost kod izbora hrane.

Tablica 3. Frekvencija učestalosti pojavljivanja (F%), postotak brojnosti (N%), postotak mase (W%), faktor kondicije (CF) europske jegulje (AA) (n - broj analiziranih jedinki, n.d. - nije determinirano).

Duljina jedinke	33,5-39,5 cm			41,5-53 cm		
	n=12			n=5		
Veličina uzorka	CF=0,1772±0,0126			CF=0,1932±0,013		
Faktor kondicije	N%	F%	W%	N%	F%	W%
<i>Gammarus</i> sp.	79,21	22,86	23,97	65,52	18,75	17,51
Trichoptera (ličinačka kućica)	3,96	8,57	0,62	6,9	12,5	0,43
Trichoptera (bez kućica)	2,97	2,86	0,32	6,9	6,25	0,81
Chironomidae	0,99	2,86	0,01	10,34	12,5	0,04
Plecoptera	0,99	2,86	0,06	3,45	6,25	0,09
Gastropoda (Lymnaeidae)	2,97	2,86	0,05			
Gastropoda (Neritidae)	3,94	8,57	1,1	3,45	6,25	0
Simuliidae	0,99	2,86	0,4			
Pisces n.d.	0,99	2,86	4,52			
Sjemenke n.d.	0,99	2,86	0,04			

Kompeticija u prehrani između unesene kalifornijske pastrve (*Oncorhynchus mykiss*) i ugrožene Europske jegulje (*Anguilla anguilla*) iz rijeke Žrnovnice

Pulmonata (kopneni)	0,99	2,86	16,4			
Coleoptera				3,45	6,25	0,54
Ephemeroptera	0,99	2,86	0,14			
Detritus		34,29	52,73		31,25	80,57

Uzorkovane jedinke kalifornijske pastrve podijeljene su na dva dužinska razreda (Tablica 4), u ovom slučaju na jedinke prvog razreda od 13,5 do 18,5 centimetara i jedinke drugog razreda od 20,0 do 28,0 centimetara dužine (Tablica 4). U sastavu prehrane prvog razreda dominira *Gammarus* sp. (N%=63,41), dok kod jedinki drugog razreda može se vidjeti manji pad u brojnosti *Gammarus* sp. (N%=33,33). Preklapanje prehrane između kalifornijske pastrve i europske jegulje u rijeci Žrnovnici potvrđeno je Schoenerovim indeksom (> 0,60).

Tablica 4. Frekvencije učestalosti pojavljivanja (F%), postotak brojnosti (N%), postotak mase (W%), faktor kondicije (CF) kalifornijske pastrve (OM) (n - broj analiziranih jedinki, n.d. - nije determinirano).

Duljina jedinke	13,5-18,5 cm			20-28 cm		
	n=4			n=3		
Veličina uzorka	CF=1,1648±0,0968			CF=1,0067±0,0346		
Faktor kondicije	N%	F%	W%	N%	F%	W%
<i>Gammarus</i> sp.	63,41	21,05	32,12	33,33	15	8,17
Trichoptera (ličinačka kućica)	7,32	15,79	0,77	2,22	10	12,02
Trichoptera (bez kućice)	4,88	5,26	0,51	22,22	10	2,66
Chironomidae	4,88	5,26	0,25			
Plecoptera	7,32	10,53	7,82	6,67	10	1,43
Gastropoda (<i>Theodoxus fluviatilis</i>)	2,44	5,26	0,94	6,67	10	10,16
Gastropoda (Neritidae)				13,33	10	8,1
Simuliidae				2,22	5	0,19
Coleoptera	2,44	5,26	0,19			
Kopneni insekt (n.d. osica)				2,22	5	0,77
Ephemeroptera	7,32	10,53	2,18	11,11	10	1,18
Detritus		21,05	55,23		15	55,31

Rasprava

Veliki problem, ne samo za jegulje već i za cijelu ihtiofanu, predstavlja kanaliziranje rijeke Žrnovnice (Piria i sur., 2016). Utvrđen je pad faktora kondicije za kalifornijsku pastrvu povećanjem totalne dužine tijela, dok je kod jegulje utvrđen porast faktora kondicije s porastom totalne dužine tijela. Dobiveni rezultati kondicijskog faktora za jedinke europske jegulje analizirane ovim istraživanjem su suprotni prethodnim istraživanjem jedinki istog vodotoka gdje je za veće jedinke utvrđen pad kondicije (Piria i sur., 2016). To upućuje na mogućnost poboljšanja uvjeta staništa za jegulju. Također, sukladno novim zakonskim propisima kalifornijska pastrva se više ne približava u ovaj vodotok pa je moguće da je njihova populacija opala i tako manje konkurira za stanište i hranu. Prema dostupnoj literaturi prehrana obje istraživane vrste je vrlo različita ovisno o raspoloživoj hrani, međutim kod kalifornijske pastrve obično u prehrani dominiraju ličinke kukaca (Metcalf i sur., 1997), a kod jegulje prevladavaju rakušci (Bouchereau i sur., 2009). Prehrana obje vrste se u rijeci Žrnovnici bazira na rakušcima, odnosno hrane se sličnim svojstama što upućuje na činjenicu da je rijeka Žrnovnica znatno bogatija skupinom Crustacea nego vodenim kukcima (Rađa i Puljas, 2010). Prema tome, kalifornijska pastrva predstavlja značajnog kompetitora za jegulju prema raspoloživoj hrani u rijeci Žrnovnici, a moguće i u ostalim kraškim vodama Jadranskog sliva gdje su prisutne obje vrste.

Zaključak

Dobivenim rezultatima istraživanja potvrđena je kompeticija u prehrani kalifornijske pastrve i europske jegulje u rijeci Žrnovnici. Preporučuje se provoditi redovito praćenje stanja, kako bih se utvrdila daljnja Europske jegulje kao ugrožene vrste.

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Competition for food between introduced rainbow trout (*Oncorhynchus mykiss*) and endangered European eel (*Anguilla anguilla*) from the Žrnovnica River

Abstract

The competitive attitude to food by the species introduced can affect the abundance of native ichthyofauna. The aim of the study was to determine the composition of the diet of Rainbow trout and European eel from the Žrnovnica River, to analyze the prey species consumed and to determine if there was a dietary competition between the two species. The captured specimens were digested individually and prey species were determined and analyzed from the digestive contents. European eel and Rainbow trout feed on similar taxa, their most common food being shellfish of the genus *Gammarus* sp. The index of overlap in eating habits establishes the similarity of their diet, which suggests that trout are one of the threats to the already endangered European eel in the karst waters of the Adriatic basin.

Keywords: Rainbow trout, European eel, *Gammarus* sp., diet, competition

Analiza ulova ribe mrežom poponicom u Medulinskom zaljevu

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Sažetak

Ulov ribe mrežom poponicom istraživan je od svibnja do rujna 2018. godine u Medulinskom zaljevu. Analizirani su ukupna brojčana zastupljenost svake pojedinačne vrste u ukupnom ulovu, maseni udio svake gospodarski važne vrste u ukupnom ulovu ribe te njihov prosječni mjesečni maseni udio. Zabilježeno je 27 vrsta riba, od čega 19 gospodarski važnih. Najveću brojčanu zastupljenost među gospodarski važnim vrstama imala je vrsta škarpun, *Scorpaena porcus* (27,3 %), a od gospodarski nevažnih lumbrak, *Symphodus tinca* (13,64 %). Prosječna mjesečna masena zastupljenost pojedinačnih vrsta ovisila je o sezoni uzorkovanja. S obzirom na utvrđene mjesečne razlike kao i za potpunu inventarizaciju vrsta potrebno je provesti dopunska istraživanja tijekom cijele godine.

Ključne riječi: gospodarski ribolov, mreža poponica, Medulinski zaljev

Uvod

Mali obalni ribolov po količini ostvarenog ulova u Republici Hrvatskoj zauzima drugo mjesto, odmah iza pelagičkog ribolova. U njegovoj provedbi poglavito se koriste jednostruke i trostruke mreže stajačice (Matić-Skoko i sur., 2010). Trostruke mreže stajačice koriste se zbog učinkovite lovnosti i širokog raspona veličinskih frakcija lovljenih organizama (Stergiou i sur., 2006). Također, koriste se još za prikupljanje i obradu podataka iz gospodarskog ulova u cilju izvještavanja o rezultatima iz zakonom propisanog monitoringa kojim se procjenjuje utjecaj ribolova na morski ekosustav (Matić-Skoko i sur., 2010). Međutim, nerijetko je upitna vjerodostojnost tako sakupljenih podataka jer očividnici o ostvarenim lovinama ne odražavaju stanje realno ostvarenog ulova (Vrgoč, 2008). Najčešće korišteni tip mreže stajačice u gospodarskom ribolovu na Jadranu je mreža poponica. Poznato je da je to neselektivni ribolovni alat koji spada u kategoriju trostrukih mreža stajačica (Jardas, 1979). Međutim, podaci o broju ribljih vrsta koje se love mrežom poponicom nisu sustavno prikazani za cijeli Jadran. Postoji više istraživanja kojima su izneseni podaci o ulovu mrežom poponicom (Matić-Skoko i sur., 2008; Dulčić i sur., 2013), ali najveći broj istraživanja odnosi se samo na srednji i južni Jadran. Mreže poponice se također široko koriste u gospodarskom ribolovu uz istočnu obalu sjevernog Jadrana, ali podaci o ulovu još uvijek nisu sustavno obrađeni, a posebice kad se radi o specifičnim akvatorijima kao što je Medulinski zaljev. Naime, na sjevernom Jadranu, zbog klimatskih specifičnosti, obitavaju vrste riba koje podnose veća kolebanja temperaturnih promjena (Treer i sur., 1995), pa se prema tome ribolovni ulov istim ribolovnim alatom može razlikovati od onog iz toplijeg dijela Jadranskog mora. Analizom ulova s područja zapadne obale Istre u toplijem razdoblju godine uočen je najmanji broj ribljih vrsta (Draščić, 2018). Medulinski zaljev predstavlja važno ribolovno područje u južnoj Istri, registrirano je 76 profesionalnih ribara, a samo područje se odlikuje značajnim biološkim vrijednostima podmorja i sastavni je dio ekološke mreže Natura 2000 (LAGUR Istarska batana, 2017).

Stoga je cilj ovog rada prikazati podatke ulova riba mrežom poponicom u Medulinskom zaljevu, u toplijem razdoblju godine kada je očekivan najmanji broj ribljih vrsta, kako bi se utvrdila lovnost gospodarski važnih vrsta riba u odnosu na one koje nisu važne za gospodarsku djelatnost. Osim toga, zbog neselektivnosti ovog alata biti će moguće analizirati sastav ihtiofaune Medulinskog zaljeva, čiji podaci još uvijek nisu u potpunosti poznati.

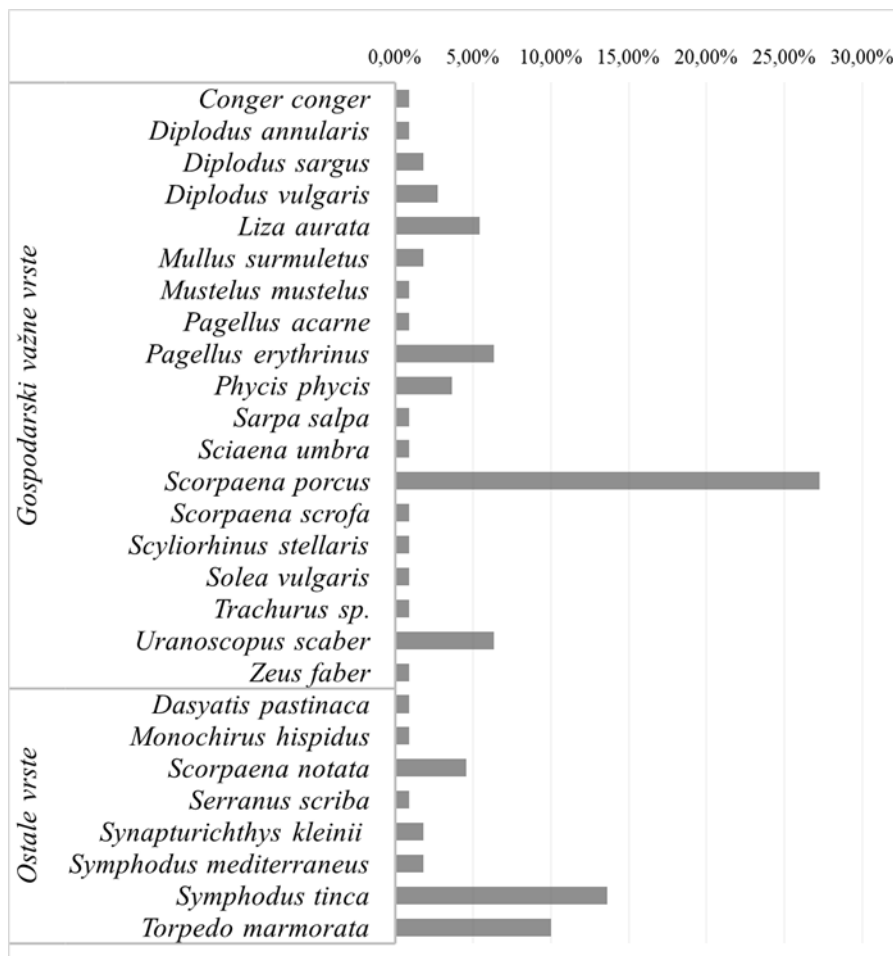
Materijal i metode

Za potrebe ovog istraživanja ishođena je dozvola Ministarstva poljoprivrede, Rješenje Uprave ribarstva (KLASA UP/I-324-01/18-01/69; URBROJ: 525-13/1766-18-2) za polaganje mreža poponica za razdoblje od svibnja do rujna 2018. godine. Korištene su standardne mreže poponice; visine 1.8 m, duljine 50 m, veličine oka mahe 40 mm, popona 150 mm i s trofilnom debljinom konca mrežnog tega. Mreže su pri polaganju bile povezane u niz od 5 pojedinačnih mreža, ukupne duljine od 250 m. Polaganje je obavljeno 2 puta mjesečno u Medulinskom zaljevu (44°47'34.6"N; 13°56'31.0"E) u periodu od svibnja do rujna 2018. godine u večernjim satima, a podizane su tijekom sljedećeg jutra. Raspon dubina na kojima su mreže polagane varirao je između 15 i 30 m. Položeno je ukupno 3000 m poponica, a ostvareno je i analizirano 12 lovina. Ulovljene ribe raspoređene su u skupine vodeći se odredbama Pravilnika o obliku, sadržaju i načinu vođenja i dostave podataka o ulovu u gospodarskom ribolovu na moru (NN 38/2018). Određene su vrste, izbrojane su jedinke i izmjerena masa ulovljenih jedinki. Utvrđen je ukupan broj jedinki svake pojedinačne vrste, a potom su izračunati ukupna brojčana zastupljenost pojedine vrste u istraživanom razdoblju, maseni udio svake pojedinačne gospodarski značajne vrste (vrste koja se po Pravilniku pojedinačno evidentira) u ukupnom ulovu ribe te prosječan mjesečni maseni udio gospodarski značajnih vrsta u mjesečnom ulovu. Za obradu podataka korišten je program Microsoft Excel (2016).

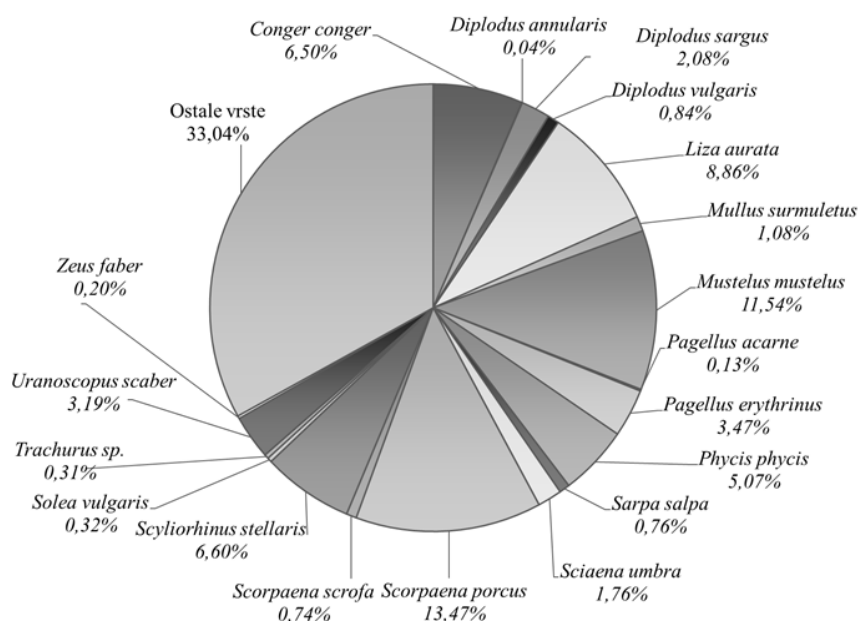
Rezultati i rasprava

Postotak brojčane zastupljenosti vrsta u ukupnom ulovu riba u razdoblju svibanj - rujan 2018. prikazan je na slici 1, a njihov maseni udio u ukupnom ulovu, izražen u postotcima, na slici 2. Zabilježeno je ukupno 27 vrsta riba, od čega je 19 (70%) gospodarski značajnih dok osam (30%) pripada skupini ostalih, odnosno gospodarski nevažnih vrsta. Najveću brojčanu zastupljenost među gospodarski važnim vrstama imala je vrsta škarpun, *Scorpaena porcus* (27,3 %), a od gospodarski nevažnih lumbrak, *Symphodus tinca* (13,64 %). Ukupna masena zastupljenost gospodarski nevažnih vrsta iznosila je 33,04 %. U periodu istraživanja utvrđene su razlike u prosječnoj mjesečnoj zastupljenosti pojedinih gospodarski važnih vrsta riba. U svibnju je najveću prosječnu masenu zastupljenost imala mačka mrkulja, *Scylliorhinus stellaris* (18,46 %), u lipnju ugor, *Conger conger* (33,26 %), u srpnju i kolovozu *S. porcus* (18,35 i 61,82 %) a u rujnu *M. mustelus* (38,1 %). Varirala je i prosječna mjesečna masena zastupljenost gospodarski nevažnih vrsta pri čemu su najveće vrijednosti utvrđene u srpnju (80,68 %), a najmanje u kolovozu (Slika 3).

U priobalju Istre od 2011. do 2017. godine analizirane su lovine primjenom mreža salpare, listarice, prostice i rakovice oka mahe 40 mm te analizom ribarskih očevidnika. Utvrđeno je ukupno 20 gospodarski važnih vrsta, pri čemu je njihova najmanja brojčana zastupljenost ustanovljena tijekom ljetnih mjeseci (Draščić, 2018). Diskutabilan je relativno mali broj gospodarski značajnih vrsta riba sakupljenih u periodu od sedam godina u odnosu na ovo petomjesečno istraživanje, moguće iz razloga što se analiza lovina obavljala dijelom i na temelju ribarskih očevidnika uz upitnu točnost determinacije lovine. Dulčić i sur. (2013) su tijekom 2010. godine u srednjem i južnom Jadranu proveli istraživanje mrežom poponicom oko mahe 40 mm. Pri tom je položeno 6.680 m mreža poponica na različitim dubinama (5 - 103 m) te je ostvareno 18 lovina u kojima su zabilježene 24 vrste riba.

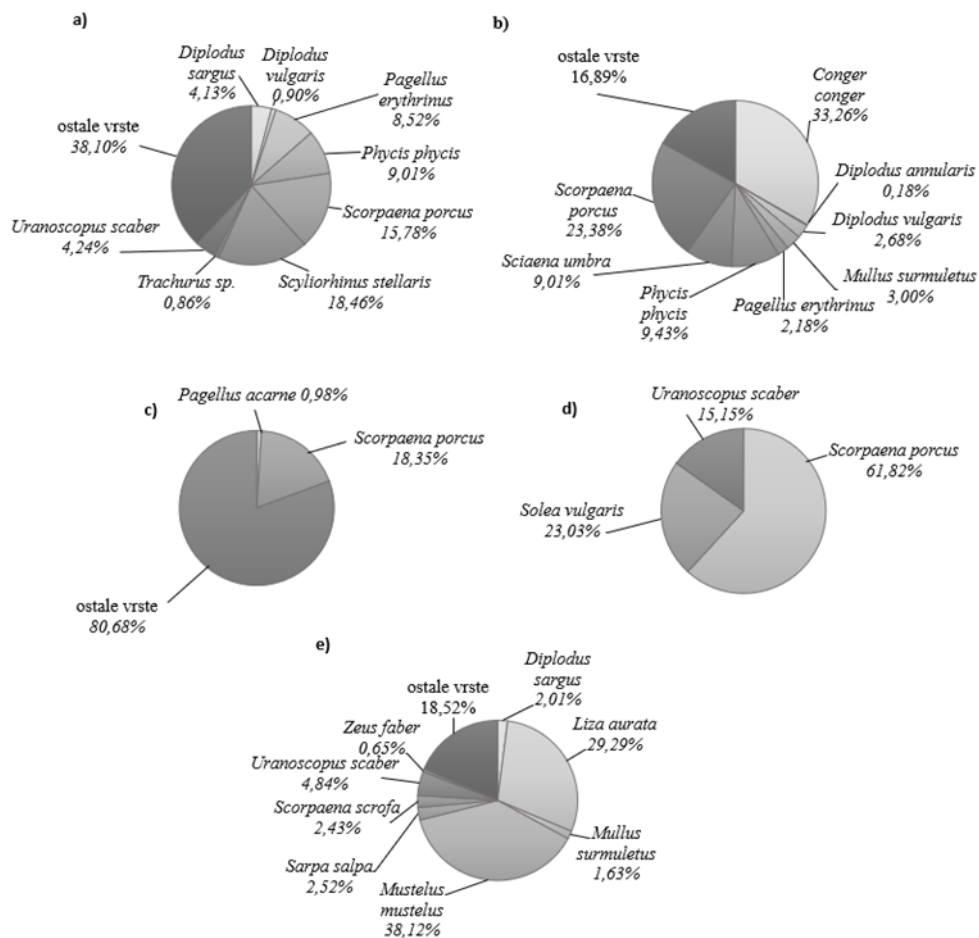


Slika 1. Zastupljenost vrsta (%) u ukupnom ulovu riba u razdoblju svibanj - rujan 2018.



Slika 2. Maseni udio (%) gospodarski važnih vrsta u ukupnom ulovu ribe

Analiza ulova ribe mrežom poponicom u Medulinskom zaljevu



Slika 3. Prosječna masena zastupljenost (%) pojedinačnih vrsta u ukupnom ulovu ribe u razdoblju svibanj - rujan 2018. (a-svibanj, b-lipanj, c-srpanj, d-kolovoz, e-rujan)

Od gospodarski važnih vrsta riba najveća ukupna masena zastupljenost u cjelokupnom periodu istraživanja odnosila se na vrste škarpina, *Scorpaena scrofa* (10,4 %), tabinja mrkulja, *Phycis phycis* (6,9 %) i kovač, *Zeus faber* (5,3 %). Iste vrste zabilježene su i u Medulinskom zaljevu, međutim njihova je ukupna masena zastupljenost bila manja. U Medulinskom zaljevu istraživanje je provedeno s dvostruko manjim rasponom mreža poponica koje su polagane na manjim dubinama, pri čemu je utvrđen veći broj ribljih vrsta (27), što bi moglo ukazivati na veliku bioraznolikost u akvatoriju Medulinskog zaljeva. Tijekom 2006. godine je na sedam postaja srednjeg i južnog Jadrana (Pag, Zadar, Šibenik, Vis, Split, Ploče i Dubrovnik) u cilju procjene stanja priobalnih ribljih zajednica provedeno istraživanje poponicom srednjeg oka mahe 28 i 32 mm (Matić-Skoko i sur., 2008). Ostvarene su 54 lovine, a duljina pojedine mreže varirala je od 340 do 400 m. Determinirane su ukupno 82 vrste riba, pri čemu je od gospodarski važnih vrsta najveća brojčana i masena zastupljenost na većini postaja utvrđena za vrste *S. porcus* i trlju kamenjarku, *Mullus surmuletus*, te za gospodarski nevažnu vrstu *S. tinca*. Podaci u pogledu brojčane i masene zastupljenosti pojedinih vrsta u ukupnom ulovu slični su rezultatima ovog istraživanja. Iako je u Medulinskom zaljevu, u odnosu na istraživanja Matić – Skoko i sur. (2008), uočen manji broj ulovljenih vrsta ipak činjenica da je tijekom ljetnih mjeseci vjerojatna manja brojnost ribljih vrsta (Draščić, 2018) mogla je utjecati na rezultate ovih istraživanja. Izneseni rezultati upućuju na činjenicu da se brojčano razlikuju najzastupljenije riblje vrste Medulinskog zaljeva od onih lovljenih na srednjem i južnom Jadranu. Međutim, za sustavan popis ribljih vrsta i još detaljniju sliku o ihtiocenozi Medulinskog zaljeva, potrebno je provesti dopunska istraživanja koja će obuhvatiti cijelu godinu.

Zaključak

Lovnost gospodarski važnih vrsta je različita tijekom ranog odnosno kasnog ljeta, pa je tako u svibnju najzastupljenija bila *S. stellaris*, u lipnju *C. conger*, u srpnju i kolovozu *S. porcus* a u rujnu *M. mustelus*. Također razlikovala se i mjesečna zastupljenost ostalih vrsta. S obzirom na ove mjesečne razlike kao i za potpunu inventarizaciju vrsta potrebno je provesti dopunska istraživanja tijekom cijele godine.

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Fish catch analysis of the “poponica” net in Bay of Medulin

Abstract

The catch of the “poponica” trammel net in the Bay of Medulin, from May to September 2018, was analyzed. A total of 27 fish species were identified, 19 of which are commercially important. The total numerical representation of each species, the weight fraction of each commercially important species in the total fish catch, and their average weight fraction for each month was analyzed. The largest number among the economically important species was the Black scorpionfish, *Scorpaena porcus* (27.3%), and of the economically insignificant species was the East Atlantic peacock wrasse, *Symphodus tinca* (13.64%). Analysis indicates that seasonal changes in the average weight representation of individual species are present in the total monthly catch. Given the established monthly differences, and for the completion of species inventory, additional surveys should be carried out throughout the whole year period.

Keywords: commercial fisheries, trammel net “poponica”, Bay of Medulin

Results of the first generation of Carniolan honey bees (*Apis mellifera carnica*) selection to *Varroa destructor* resistant traits

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Abstract

Ectoparasitic mite *Varroa destructor* is a major threat for beekeeping worldwide. As the drugs to fight Varroa mites are losing on its importance in beekeeping, the possible sustainable solution is recognised through the breeding of mite-resistant honey bees. Here we present the results from the first generation of Carniolan bee selection for the recapping behaviour (REC) and suppressed mite reproduction (SMR). In 2016, 51 colonies (initial population) were examined and colony with highest REC and SMR was used for grafting of the first generation. In 2018, 27 daughter queens were evaluated for the same traits. The first generation queens had higher REC for 28%, REC of infested brood cells for 33% and SMR for 4%. The high variability of recapping potentially gives the possibility to include this trait in selection strategies. Future research should focus on heritability estimations and reasons how recapping of brood cells reduces the success of varroa reproduction.

Keywords: *Apis mellifera carnica*, *Varroa destructor*, suppressed mite reproduction, recapping, selection

Introduction

Despite the many problems that beekeeping faces today, *Varroa destructor* is the most significant cause of honey bee (*Apis mellifera* L.) colony losses in the North hemisphere (Rosenkranz et al., 2010; Neumann and Carreck, 2010). The loss of honey bee colonies is strongly influenced by the synergistic effects of *V. destructor* mites, viruses, *Nosema ceranae*, pesticides and climate change (Le Conte et al., 2010). Climate change causes the early onset of brood development in the spring and the longer brood development in the fall, allowing mites to have more reproductive cycles throughout the year (Le Conte and Navajas, 2008). The location and density of bee colonies and the density of hives in a given area have a significant impact on the spread of bee disease (Seeley and Smith, 2017). The hygienic behaviour of bees is the ability of bees to recognize, open and remove a sick, injured or dead brood (Rothenbuhler, 1964.). Hygienic behaviour is the basic mechanism of resistance of bees to American foulbrood (causative agent *Paenibacillus larvae*) and chalkbrood (causative agent *Ascosphaera apis*) (Gilliam et al., 1983). To some extent, it reduces the rate of reproduction and spread of *V. destructor* and viruses (Spivak and Reuter, 1998). Certain bees in the colony are able to recognize the presence of mites in the brood through the wax capping and may remove such brood. This form of hygienic behaviour, specifically directed at the brood with mites, is called varroa sensitive hygiene - VSH (Harbo and Harris, 2005). This defence mechanism does not kill the mites, but does affect the success of a single mite reproductive cycle. As a consequence, a large proportion of mites found in colonies with a highly pronounced VSH trait will not be able to reproduce. This failure in *V. destructor* reproduction is called suppressed mite reproduction - SMR (Harbo and Harris, 1999.). In contrast to the VSH trait (where hygienic removal of the brood also has an economically negative effect on the bee colony), simple opening and closing the brood capping - recapping (REC) can reduce the success of reproduction of *V. destructor* (Oddie et al., 2018). The aim of this research was to examine the success of selection of honey bees on varroa resistant traits, i.e. recapping behaviour and suppressed mite reproduction.

Materials and methods

The research was conducted in Baranja, north-east Croatia on Carniolan honey bee (*Apis mellifera carnica*). In 2016 brood samples were taken from the 51 colonies that were never selected for SMR and REC (initial population of a sister queens). The queen from the colony with highest SMR and REC results (32% of infertile mites and 41% of total recapped brood cells) was used to rear the daughter queens in 2017 (first generation after selection, 27 colonies with sister queens) from which in 2018 brood samples were taken. The brood samples were taken in July of both years, and stored at -18 °C until analysis. The evaluation of the SMR trait was based on an estimate of the proportion of *V. destructor* that fail to reproduce. For this analysis, stereomicroscope of 5x to 10x magnification were used. Each sample was checked until 35 single infested brood cells with mite were found. To determine the infertility of mites, a brood in development stage of purple eyes (7 days after the capping) to the pre-hatching brood (12 days after the capping) was analysed. After opening of each brood cell, the cell contents were removed and the presence of *V. destructor* was determined. If mite was found, the brood age, number of adult mites, age of eldest daughter, and presence of males were determined. Foundress *V. destructor* without offspring (infertility), with younger offspring than expected (delayed development) or without the presence of a male, was considered as infertile mite (Martin, 1994). When analysing the infertility of mites, each brood cell was carefully opened to determine if the capping was open and closed again (recapped). This was determined by examination of the inner side of capping - the untouched capping is shiny on the inside because it contains a cocoon, while the recapped cell lacks the shiny cocoon (Harris et al., 2012). The statistical program SPSS v20 (SPSS Inc., 2011) was used for statistical data processing. The Mann-Whitney U test was used to determine differences between the generations. The phenotypic mean values with standard deviations and 95% confidence intervals are presented in results.

Results and discussion

Suppressed mite reproduction was higher in first generation (Table 1) but did not differ significantly to the initial population ($U = 516,5$; $p = 0,070$). Recapping of all brood cells was significantly higher in the first generation (Table 1, $U = 286$; $p < 0,001$). Also, recapping of infested brood cells ($U = 272,5$; $p < 0,001$) and recapping of non-infested brood cells ($U = 288$; $p < 0,001$) was significantly higher in the first generation (Table 2). As reported in several studies, bees uncap and recap again the brood cells infested with *V. destructor* (Boecking and Spivak, 1999, Arathi et al., 2006). Villa et al. (2009) indicated 38% of totally recapped brood cells, while Kirrane et al. (2015) reported high rates of recapping of infested brood cells for Italian bees (64.36%) and Russian bees (77.81%), which is in line with our data from 2018. Recapping of brood cells plays a major role in survival of naturally selected bees and Oddie et al. (2018) reported that four separate bee populations, which independently survived for more than 17 years without treatment against varroa, had a significantly higher number of recapped brood cells compared to local, control colonies. However, at the moment, it is still unclear how recapping affects the mite reproduction. As brood cells infested with mites are warmer (Bauer et al., 2018), the change of temperature in the cell may affect the mite reproduction.

Table 1. The mean average values with standard deviation and 95% confidence interval for SMR and REC

Year	N	SMR				Recapping of all brood cells			
		Mean	SD	95% CI		Mean	SD	95% CI	
				Lower bound	Upper bound			Lower bound	Upper bound
2016	51	27,86	14,57	23,99	31,74	13,31	17,13	10,42	22,21
2018	27	32,11	12,52	26,78	37,44	41,22	27,23	33,12	49,32

Table 2. The mean average values with standard deviation and 95% confidence interval for recapping of infested brood cells and recapping of non-infested brood cells.

Year	N	Recapping of infested brood cells				Recapping of non-infested brood cells			
		Mean	SD	95% CI		Mean	SD	95% CI	
				Lower bound	Upper bound			Lower bound	Upper bound
2016	51	46,35	31,44	38,18	54,53	14,65	16,38	8,96	20,34
2018	27	79,59	24,69	68,36	90,83	38,37	26,49	30,55	46,19

The change of CO₂ level, change of humidity, air flow or reduction of chemical compounds (*V. destructor* sex pheromones) may also have an impact on the success of mating of mites (Ziegelmann et al., 2013). One additional benefit of brood recapping may happen when mites enter such temporary opened cell where it will fail to reproduce (Büchler and Kovačić, 2016). Frey et al. (2013) artificially infested the brood of different stages of development and showed that the mites that were inserted into the brood at the older stage had a significantly higher proportion of infertile mites. Sulimanović et al. (1982) reported 21% of infertile mites, while Kulinčević et al. (1988) state 9%, which is less than found in this study. De Guzman et al. (2008) found a relatively high proportion of infertile mites (39.84%) in *A.m. ligustica* and even higher (51.06%) in Russian bees, known for better resistance than European honey bees. Also, Locke and Fries (2011) found 52% of infertile mites in the survivor bee population on the Gotland Island, while they recorded 22% of infertile mites at the control susceptible colonies. The results of SMR in this research are in the line with the results of cited authors, however lower in comparison to different resistant populations. Results presented here are just the beginning of selection for SMR and REC, and further research are necessary to make the estimations of heritability.

Conclusions

The results of this study indicate the possibilities of selection of SMR and REC, traits associated with *V. destructor* resistance. The differences between generations, as well as the high variability of SMR and REC indicate a good opportunity to initiate breeding towards *V. destructor* resistance. However, the high standard deviations of the mean values obtained in this study imply that the more comprehensive research should be carried out in order to obtain reliable estimation of investigated parameters.

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Rezultati selekcije prve generacije sive pčele (*Apis mellifera carnica*) na svojstva otpornosti na grinju *Varroa destructor*

Sažetak

Grinja *Varroa destructor* glavna je prijetnja pčelarstvu u cijelom svijetu. Kako lijekovi za zaštitu pčela gube značaj u pčelarstvu, jedino održivo rješenje je uzgoj pčela s povećanom otpornosti. Ovdje su prikazani rezultati prve generacije odabira sive pčele na svojstva otklapanja legla (REC) i potisnutog razmnožavanja grinje (SMR). Tijekom 2016. godine ispitana je 51 zajednica (početna populacija) te je zajednica s najviše izraženim svojstvom REC i SMR korištena za uzgoj matica kćerki (prva generacija). U 2018 godini, 27 zajednica s maticama kćerkama ocjenjeno je na ista svojstva. Prva generacija imala je više otklopljenih stanica legla za 28%, otklapanje zaraženih stanica legla za 33% i SMR za 4%. Velika varijabilnost svojstva otklapanja legla potencijalno daje mogućnost selekcije na ovo svojstvo. Buduća istraživanja trebaju se usredotočiti na izračun heritabiliteta svojstva te na koji način otklapanje legla smanjuje uspješnost razmnožavanja grinje.

Ključne riječi: *Apis mellifera carnica*, *Varroa destructor*, potisnuto razmnožavanje varoe, otklapanje, selekcija

Povijest i pregled istraživanja potočne pastrve crnomorskog sliva u Hrvatskoj

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Sažetak

Potočna pastrva (*Salmo trutta*) je jedna od najpoznatijih slatkovodnih ribljih vrsta, cijenjena je za konzumaciju, ali i vrlo atraktivna za ribolov. Upravo zbog toga, kroz povijest je bilo mnogo pokušaja očuvanja kao i širenja areala rasprostranjenja ove vrste. Tako se zbog potreba poribljavanja dopremala oplodena ikra i mlađ potočne pastrve iz susjednih zemalja te unosila u vodotoke crnomorskog sliva u Hrvatskoj. Vrlo je malo istraživanja divljih populacija potočne pastrve. Uglavnom se baziraju na prehrani i morfologiji, a novija istraživanja na njihovoj genskoj raznolikosti. Iz ovog pregleda vidljivo je da nedostaju istraživanja crnomorske pastrve iz otvorenih voda u Hrvatskoj te da nema pouzdanog podatka o njihovim izvornim populacijama u mnogim vodotocima.

Ključne riječi: pastrva, poribljavanje, prehrana, morfologija, genetika

Uvod

Potočna pastrva (*Salmo trutta*) oduvijek je bila vrlo atraktivna riblja vrsta za uzgoj, poribljavanje, ribolov i nezaobilazna za ljudsku prehranu. Sukladno tome tijekom povijesti bila je predmetom manipulacije od strane ljudi, pa je tako bilo mnogo pokušaja njena uzgoja prvenstveno za prehranu, a potom za reintrodukciju u otvorene vode. Iako je to jedna od najpoznatijih vrsta slatkovodnih riba, potočna pastrva Balkanskog poluotoka je do danas ostala jedna velika nepoznanica i predmet mnogih kontraverzi (Simonović i sur., 2017). Tome napose pridonosi činjenica da je tijekom 20. stoljeća potočna pastrva bila prenošena iz jednog vodotoka u drugi, iz jednog sliva u drugi, unesena u jezera i potoke gdje prethodno nije bila prisutna. Matični stok za potrebe umjetnog mriještenja uvezen je iz različitih dijelova Europe te su nakon mriješta mlade ličinke puštane u otvorene vode diljem Balkana (Taler, 1951). Isti scenarij nije zaobišao niti Hrvatsku što je znatno pridonijelo činjenici da još uvijek u jadranskom kao ni u crnomorskom slivu, sistematika Salmonida nije potpuno razriješena (Čaleta i sur., 2019). Osim toga, prirodna rasprostranjenost dunavske linije potočne pastrve u Hrvatskoj još uvijek nije potpuno jasna s obzirom da je vrsta naseljena u pojedine vodotoke gdje prethodno nije zabilježena (Anonymus, 1927). Vrlo je interesantno da su istraživanja biologije i ekologije na divljim populacijama potočne pastrve iz crnomorskog sliva, posebice ona koja se odnose na Hrvatsku, vrlo oskudna. Postoje tek nekoliko navoda literature u kojima su dati podaci o morfologiji, prehrani, staništu i genskoj raznolikosti (Štefanac i Bunjevčić, 1982; Trožić-Borovac, 2002; Knežević, 2007; Jadan i sur., 2007; Simonović i sur., 2017; Škraba i sur., 2017).

Stoga je ovaj rad usmjeren na pregled dostupne literature o povijesnim poribljavanjima potočne pastrve te na pregled istraživanja biologije i ekologije koja su provedena na potočnoj pastrvi upravo na području Hrvatskog dijela crnomorskog sliva.

Poribljavanja pastrvom u vodotocima crnomorskog sliva od 1880 do 1948

Tijekom 19. i početkom 20. stoljeća za vrijeme Austro-Ugarske monarhije, događala su se vrlo intenzivna poribljavanja s alohtonim jedinkama potočne pastrve u Europi (Kohout i sur., 2012), pa tako i na Balkanskom poluotoku (Simonović i sur., 2017). Nakon drugog svjetskog rata poribljavanja i prijenosi jedinki različitog genetskog materijala su se također vrlo intenzivno nastavili (Taler, 1951) čemu je danas vrlo teško ući u trag. Problem unosa i prijenosa potočne pastrve, posebno na području Balkanskog poluotoka, vrlo je kompleksan, a posebice stoga što ne postoje referentni podaci o izvornim populacijama koje su nastanjivale pojedine vodotoke. Molekularnim analizama je potvrđeno da u neke naše vodotoke je unesena nezavičajna pastrva atlantske linije koja predstavlja prijetnju za izvornu gensku raznolikost pastrva zavičajne dunavske linije (Simonović i sur., 2014). Ipak, zbog intenzivnog poribljavanja sa različitim genskim materijalom u nekim našim vodotocima je dokazano da postoje hibridne jedinke (Jadan i sur., 2007; Škraba i sur., 2017), a za mnoge vodotoke još uvijek nije poznata izvorna genska kombinacija (Simonović i sur., 2017). Uz današnje molekularne analize korisno je pronaći stare zapise o poribljavanjima kako bi bilo moguće bolje rekonstruirati porijeklo (Tablica 1). Prema prvim dostupnim zapisima još iz kraja 19. i početka 20. stoljeća potočna pastrva je u crnomorskom slivu u Hrvatskoj bila više puta poribljavana u vodama gdje prethodno nije bila zabilježena, kao što su primjerice potok Bliznec i Kraljevec koji se nalaze na obroncima Medvednice (Anonymus, 1927). Naime, u to vrijeme lokalno stanovništvo nije poznavalo tu vrstu, pa su potoci u više navrata ostajali bez ribe zbog devastacije ribljeg stoka (Anonymus, 1927). U dostupnoj literaturi nije navedeno odakle je unesena oplodena ikra potočne pastrve u ribogojilište „Kraljičin Zdenac“ kada je ono počelo s radom 1894. godine, međutim postoji podatak da je ondje potočna pastrva godine 1928. unesena iz Austrije (Kajtner, 1928) te su naredna poribljavanja vršena potomcima uzgojenim na istom ribogojilištu. Osim potočnom pastrvom, poribljavanja su vršena i s kalifornijskom pastrvom (Anonymus, 1932), koja se također uzgajala na ribogojilištima „Čabranka“ i „Kraljičin Zdenac“ još od 1894. (Bojčić, 1982). Osim poribljavanja na Medvednici i Samoborskom gorju, pronađeni su i podaci o poribljavanjima rijeke Gacke i jezera Kozjak na Plitvičkim jezerima (Tablica 1).

Tablica 1. Povijesni podaci o poribljavanju pastrvom različitih vodotoka crnomorskog sliva u Hrvatskoj (ZRD-Zagrebačko ribolovno društvo)

Izvor poribljavanja	Godina	Vodotok	Porijeklo	Napomena	Izvor
Ribogojilište Čabranka	1882-1897	Čabranka	Nije poznato	Uzgoj za poribljavanje	Bojčić 1982
ZRD iz Ribogojilišta Kraljičin Zdenac	1895	Bliznec	Nije poznato	pastrva prethodno nije bila zabilježena	Anonymus, 1927
ZRD iz Ribogojilišta Kraljičin Zdenac	1895	Kraljevec	Nije poznato	pastrva prethodno nije bila zabilježena	Anonymus, 1927
ZRD iz Ribogojilišta kraljičin Zdenac	1903	Različiti potoci na Medvednici	Nije poznato		Anonymus 1903
ZRD iz Ribogojilišta Kraljičin Zdenac	1928	Bliznec, Bidrovec, Trnava, Sv. Šimun, Medveščak	Austrija	Ponovljeno poribljavanje	Kajtner 1928
ZRD iz Ribogojilišta Kraljičin Zdenac	1932	Bregana, Lipovačka gradna, Slapnica, Kupčina, Markuševac, Štefanovac, Bistrica potok, Sunko, Dretulja	Nije poznato	Ponovljeno poribljavanje	Anonymus, 1932
Ribogojilište Turković, Ogulin	1935	Rijeka Gacka	Nije poznato	Prijenos potočne i kalifornijske pastrve	Taler, 1951
Ribogojilište Turković, Ogulin	1935	Plitvička jezera, jezero Kozjak	Dunavska	Prijenos potočne i kalifornijske pastrve	Taler, 1951
Rijeka Vitunjčica	1948	Rijeka Gacka	Dunavska		Štefanac i Bunjevčić, 1982

Istraživanja biologije i ekologije potočne pastrve

Morfološka istraživanja prirodnih populacija potočne pastrve dunavskog sliva naročito su oskudna. Pronađen je članak gdje su izneseni samo podaci za pastrve iz rijeke Gacke o broju ljusaka u lateralnoj liniji (min=113; max=120), ispod (min=24; max=28) i iznad (min=25; max=28) nje, te o broju piloričkih nastavaka (Ekotip 1 min=36 max=44; Ekotip 2 min=45, max=50) na jedinkama uzorkovanim 1974. g. (Štefanac i Bunjevčić, 1982). Međutim autori su uočili je postojanje dvije linije pastrva (Ekotip 1 i 2) u rijeci Gackoj koje su se razlikovale u broju piloričkih nastavaka i vanjskoj morfologiji. Navedeno je da ekotip 1 predstavlja Gacku pastrvu, a ekotip 2 pastrvu iz rijeke Vitunjčice. Navedeno je da ekotip 1 posjeduje izražajne crvene pjege nepravilno razasute po čitavom tijelu, a središte svake crvene pjege je tamnije obojeno. Između crvenih točaka razasute su crne mrlje u obliku nepravilnog slova X, a na svakom škržnom poklopcu 3-5 crnih mrlja. Masna peraja nosi od 1-3 crvene točkice. Također, glava joj je zdepastija s manje izražajnih zuba od ekotipa 2. Ekotip 2 nema pjega na škržnom poklopcu, ima izduženiju glavu i vitkije tijelo (Štefanac i Bunjevčić, 1982). Da postoje dvije linije pastrva u rijeci Gackoj potvrđeno je i molekularnom analizom gdje je utvrđeno da rijeku Gacku nastanjuju nezavičajna atlantska linija i zavičajna dunavska linija pastrve (Jadan i sur., 2007), što se podudara s prethodno navedenim opisom. Međutim, za rijeku Gacku je još uvijek vrlo diskutabilno porijeklo navedene dunavske linije pastrve, pa neki autori impliciraju da je pastrva ondje nezavičajna vrsta (Jelić i sur., 2016; Simonović i sur., 2017).

Podaci o prirodnoj prehrani potočne pastrve iz Dunavskog sliva pronađeni su za jedinke koje obitavaju u rijekama Uni (Trožić-Borovac, 2002) i Gackoj (Knežević, 2007). Rijeka Una je uzorkovana na 15 lokacija u gornjem toku 2001. godine (od graničnog pojasa sa Hrvatskom do Bihaća), te su uzorkovane i analizirane 103 jedinke. Prehranu potočne pastrve, iz rijeke Une, sačinjavali su akvatični beskralješnjaci iz šest redova kukaca (Plecoptera, Ephemeroptera, Trichoptera, Diptera, Coleoptera i Hymenoptera). U najvećoj brojnosti u probavilima potočne pastrve pronađeni su tulari (Trichoptera), (37,20 %), a znatno su bile zastupljene ličinke dvokrilaca (Diptera), trzalaca (Chironomidae), rakušci (Amphipoda), a u manjem broj jedinki pronađen je peš *Cottus gobio* kao plijen (Trožić-Borovac, 2002), (Tablica 2). Istraživanje prehrane potočne pastrve iz rijeke Gacke provedeno je na 172 jedinke, a lovljene su na 10 različitih postaja 1998. i 1999. godine (Knežević, 2007). Najveći postotak brojnosti u prehrani potočne pastrve imale su svoje oblici (Nematoda) (34,42 %), jednakonošci (Isopoda) (32,23 %) i rakušci (Amphipoda) (18,82 %). Najmanji postotak brojnosti ima imago dvokrilaca (Diptera), (0,01 %) i vretenca (Odonata) (0,02 %) (Tablica 2).

Tablica 2. Sastav prehrane potočnih pastrva iz rijeke Une i Gacke (prema Trožić-Borovac, 2002; Knežević, 2007)

Svojte	Rijeka Una, N=103		Rijeka Gacka, N=172	
	Ukupan broj nađenih svojti	% od ukupnog broja svojti	Ukupan broj nađenih svojti	% od ukupnog broja svojti
Nematodes			5437	34,42
Plecoptera	12	0,36	51	0,32
Imago n. det.	10	0,30		
<i>Perla</i> sp.	46	1,39		
<i>Leuctra</i> sp.	21	0,64		
Ephemeroptera			205	1,30
Baethidae	24	0,73		
<i>Baëthis</i> sp.	268	8,10		
Trichoptera	1160	35,08	699	4,43
Hydropsychidae	70	2,12		
Coleoptera			5	0,03
<i>Elmis</i> sp. (imago)	5	0,15		
<i>Dytiscus</i> sp.	2	0,06		
<i>Oulimnius</i> sp.	4	0,12		
Elminthidae	3	0,09		

Hymenoptera	5	0,15		
Formicidae	3	0,09		
Odonata			3	0,02
Diptera				
Imago n.det.	121	3,66	1	0,01
Simuliidae	93	2,81	76	0,48
<i>Simulium</i> sp.	75	2,27		
Chironomidae	248	7,50	869	5,50
Larve	175	5,29		
Lutke	195	5,90		
Athericidae	100	3,02		
Oligochaeta	14	0,42	162	1,03
Arachnidae				
Hydrachnidae	14	0,42		
Crustacea				
Amphipoda	50	1,51		
<i>Gammarus</i> sp.	113	3,42	2973	18,82
<i>Gammarus balcanicus</i>	230	6,95		
Isopoda				
<i>Asellus</i> sp.	70	2,12	5090	32,23
Gastropoda	5	0,15	224	1,42
<i>Viviparus</i> sp.	12	0,36		
Hirudinea	150	4,54		
Pisces				
<i>Cottus gobio</i>	7	0,21		
UKUPNO	3307	100	15795	100

Zaključak

Prikazani podaci ukazuju da je potočna pastrva tijekom povijesti bila uzgajana i poribljavana u različite vodotoke. Nasadni materijal za uzgoj i poribljavanje bio je iz različitih izvora. Morfološke različitosti dvije linije potočne pastrve uočene u rijeci Gackoj potvrđene su i molekularnim analizama. Prehrana pastrva iz rijeke Une i Gacke sastoji se pretežno od ličinki trzalaca (Trichoptera), kao i od različitih svojti terestričkih kukaca.

Zahvala

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History and review of research of brown trout from Black Sea Basin in Croatia

Abstract

The brown trout (*Salmo trutta*) is one of the most famous freshwater fish species, appreciated for consumption but also very attractive for fishing. For this reason, there have been many attempts throughout history to preserve and expand the range of distribution of this fish species. Thus, due to the stocking purposes, fertilized eggs and fingerlings of brown trout were brought from neighboring countries and released into the waters of the Black Sea basin in Croatia. There is very little research on wild trout populations, mainly it is based on diet, morphology, and more recent on their genetics. This review shows that basic research on brown trout populations from inland waters of Black Sea Basin in Croatia is missing and that there is no reliable data on their native populations in many watercourses.

Keywords: trout, stocking, diet, morphology, genetics

Učinkovitost različitih metoda ekstrakcije mikroplastike iz mediteranske dagnje *Mytilus galloprovincialis* (Lamarck, 1819)

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Sažetak

Čestice plastike manje od 5 mm nazivaju se mikroplastikom, a mogu se pronaći u okolišu i u organizama na koje potencijalno mogu utjecati. Razvijene su brojne metode za ekstrakciju mikroplastike iz različitih organizama pa tako i školjkaša. U sklopu ovog istraživanja uspoređene su tri različite metode ekstrakcije mikroplastike iz mediteranske dagnje *Mytilus galloprovincialis* (Lamarck, 1819): ekstrakcija pomoću kalijeveg hidroksida (KOH), dušične kiseline (HNO₃) te vodikovog peroksida (H₂O₂) pri temperaturama od 24°C i 60°C. Sve su metode bile učinkovite, a pronađen je prosječno isti broj čestica mikroplastike na sobnoj (11,67±1,72) i na povišenoj temperaturi (11,67±6,13).

Ključne riječi: školjkaši, mikroplastika, razgradnja tkiva

Uvod

Plastične čestice manje od 5 mm nazivaju se mikroplastikom, a mogu se pronaći u različitim okolišima kao što su atmosfera, tlo, slatkovodni i morski okoliš. U vodenom okolišu mikroplastika se pronalazi na plažama, kontinentskoj podini, dubokomorskom sedimentu te u površinskim i podzemnim vodama. Također, zabilježen je unos hranom u mnogobrojne morske organizme među kojima su komercijalno važne vrste riba i beskralježnjaka (Lusher i sur., 2017). Zagađenje mikroplastikom izaziva pozornost znanstvenika i istraživača zbog velike količine u okolišu i mogućih bioloških učinaka na morske organizme (Santana, 2015).

Dagnje mogu biti model vrsta za indikaciju onečišćenja mikroplastikom u različitim područjima jer je rod *Mytilus* široko rasprostranjen, jedinke filtriraju velike količine vode, relativno su nepokretne i lako dostupne tijekom cijele godine te su važna komponenta prehrane različitih predatora, a njima se hrane i ljudi (Browne i sur., 2008).

Cilj ovog istraživanja je odrediti učinkovitost razgradnje tkiva dagnje *Mytilus galloprovincialis* (Lamarck, 1819) vodikovim peroksidom, dušičnom kiselinom i kalijevim hidroksidom na sobnoj i povišenoj temperaturi radi ekstrakcije mikroplastike i utvrđivanja broja i oblika čestica mikroplastike koji se pri tom pronađu.

Materijal i metode

Tijekom istraživanja pratila se učinkovitost razgradnje tkiva školjkaša 30%-tnim vodikovim peroksidom (H₂O₂), 55%-tnom dušičnom kiselinom (HNO₃) i 10%-tnim kalijevim hidroksidom (KOH) na dvjema različitim temperaturama (24°C i 60°C), pri čemu je svaki pokus izveden u triplicatu. U pokusima je ukupno korišteno 18 jedinki dagnje uzgojenih u Malostonskom zaljevu. Sveže jedinke su u metalnim spremnicima prenešene u laboratorij nakon čega je slijedila morfometrijska obrada. Pomoćnim mjerilom preciznosti 0,1 mm izmjerene su duljina – udaljenost od anteriornog do posteriornog ruba, visina – udaljenost od dorzalnog do ventralnog ruba i širina – najveća udaljenost lijeve od desne ljuštare. Radi mjerenja mase analitičkom vagom preciznosti 0,001 g (Mettler Toledo) svakoj je dagnji odvojeno meko tkivo od ljuštare. Prije početka pokusa uključena je vodena kupelj kako bi se zagrijala na temperaturu od 60°C. Tkivo svake dagnje stavljeno je u 50 ml odgovarajuće kemikalije u staklenoj čaši a potom je svaka čaša stavljena na sobnu temperaturu (24°C) ili u vodenu kupelj (60°C), te su bilježene promjene pri razgradnji tkiva. Nakon završetka razgradnje sadržaj svake čaše profiltriran je kroz filter od staklenih mikrovlakana veličine pora od

2,7 μm (Munktell MGD) uz pomoć ručne vakuumske pumpe (Nalgene). Nakon filtracije površina filtera pažljivo je pregledana pod lupom (Olympus SZX9) s povećanjem 10x.

Rezultati i rasprava

Ukupno je analizirano 18 dagnji čija je duljina bila u rasponu od 50 do 80 mm, u prosjeku $67,37 \pm 9,72$ mm. Širina dagnji iznosila je od 30 do 55 mm, u prosjeku $40 \pm 7,63$ mm. Visina dagnji iznosila je od 15 do 40 mm, u prosjeku $25,4 \pm 6,59$ mm. Raspon mase mekog tkiva bio je od 2 do 8 g, prosječno $4,6 \pm 1,55$ g, a za svaki tretman birane su jedinke približnih veličina. Pri razgradnji tkiva primijećene su promjene poput pojave pjene, promjene boje i izbjeljivanja uzorka, a razgradnja se smatrala završenom kad je u čaši ostala prozirna tekućina bez tkiva (Tablica 1).

Tablica 1. Tijek razgradnje tkiva

Vrijeme (min)	H ₂ O ₂ (30%) 24°C	H ₂ O ₂ (30%) 60°C	HNO ₃ (55%) 24°C	HNO ₃ (55%) 60°C	KOH (10%) 24°C	KOH (10%) 60°C
15				promjena boje u žutu, pojava pjene		promjena boje u smeđu
30	pojava guste pjene		promjena boje u žutu	završetak razgradnje	zamućenje, promjena boje u smeđu	završetak razgradnje
45	zamućenje					
60		pojava pjene				
75		izbjeljivanje uzorka				
120			završetak razgradnje			
1150					završetak razgradnje	
1200		završetak razgradnje				
6300	završetak razgradnje					

U istraživanju su razgrađivane dagnje *M. galloprovincialis* u različitim kemikalijama: vodikovu peroksidu (30%), dušičnoj kiselini (55%) i kalijevom hidroksidu (10%) pri temperaturama od 24°C i 60°C. Svaka metoda bila uspješna u razgradnji tkiva u određenom vremenu, a kako kemikalije imaju različitu brzinu djelovanja na tkivo, eksperiment je trajao pet dana. Ukupno je razgrađeno 18 jedinki dagnje i u svim uzorcima pronađena je isključivo nitasta mikroplastika, prosječno $11,67 \pm 3,93$ čestica po jedinki, što je iznosilo 2,54 čestica po gramu mokrog tkiva dagnje (Tablica 2).

Tablica 2. Ukupan broj čestica nitaste mikroplastike pronađene na filter-papiru nakon svakog tretmana

Tretman	H ₂ O ₂ (30%) 24°C	H ₂ O ₂ (30%) 60°C	HNO ₃ (55%) 24°C	HNO ₃ (55%) 60°C	KOH (10%) 24°C	KOH (10%) 60°C
Ukupan broj čestica	32	20	35	27	38	58

Budući da se dagnje hrane filtriranjem pri čemu mogu filtrirati oko dvije litre morske vode na sat (Durve, 1963) ne iznenađuje da se u uzorcima pronalaze čestice koje su prisutne u njihovu okolišu pa se u literaturi navodi kako je mikroplastika pronađena i u drugim područjima i to u dagnjama i iz prirodnih kao i iz uzgojenih populacija (De Witte i sur., 2014.; Mathalon i Hill, 2014.; Van Cauwenbergh i sur., 2014, 2015). Tako su u uzorcima dagnji *M. edulis* iz prirodnih populacija duž francuske, belgijske i nizozemske obale Sjevernog mora zabilježene prosječno $0,2 \pm 0,3$ čestice mikroplastike po gramu mokrog tkiva (Van Cauwenberghe i sur., 2015), dok je u uzorcima dagnji *M. edulis* uzgojenih u Njemčkoj po gramu mokrog tkiva zabilježeno prosječno $0,36 \pm 0,07$ čestica (Van Cauwenberghe i Janssen 2014). Zatim, u belgijskim dagnjama *M. edulis* iz prirodnih populacija primijećeno je prosječno između 0,26 i 0,51 vlakana po gramu mokrog tkiva, dok je u uzgajanim populacijama primijećeno prosječno 0,35 vlakana po gramu mokrog tkiva (De Witte i sur., 2014). U odnosu na navedena istraživanja u našem je eksperimentu pronađen prosječno veći broj čestica po gramu mokrog tkiva dagnje *M. galloprovincialis* što se može objasniti primjenom filtra manjih pora koji podrazumijeva veće zadržavanje čestica. Tako je najveći broj čestica mikroplastike zabilježen u istraživanju kanadskih dagnji *M. edulis* u kojem je korišten filter najmanjih pora; u prirodnih populacija pronađene su prosječno oko 34 čestice po dagnji, dok je u uzgojenim dagnjama zabilježeno oko 75 čestica (Mathalon i Hill, 2014). Uz to, u ovom istraživanju autori navode kontaminaciju mikroplastikom iz zraka koja se ne može isključiti niti iz našeg istraživanja s dagnjom *M. galloprovincialis*. Nadalje, u našem istraživanju korištene su uzgojene dagnje kod kojih je dokazano kako sadrže više mikroplastike od onih iz prirodnih populacija s istog područja, a što se pripisuje neprestanom kontaktu s plastičnim materijalima koji se upotrebljavaju pri uzgoju (Mathalon i Hill, 2014). Osim toga, niti u jednom istraživanju nije se mjerila veličina ni volumen čestica mikroplastike tako da broj čestica ne govori o ukupnoj količini prisutne mikroplastike.

Potrebno je istaknuti i kako je usporedba ovakvih istraživanja vrlo nezahvalan i težak posao jer osim eksperimentalnih organizama, niti primijenjene metode ekstrakcije nisu identične. Postupci ekstrakcije uglavnom su se zasnivali na istom slijedu, od sakupljanja jedinki preko morfometrijskih mjerenja, izolacije tkiva, do razgradnje i filtracije te konačne analize materijala na filter-papirima (Tablica 3). Kako su u literaturi navedeni i nedostaci nekih metoda poput razgradnje nekih vrsta mikroplastike u 69%-tnoj dušičnoj kiselini (Claessens i sur., 2013) u našem je istraživanju korištena 55%-tna dušična kiselina. Zatim, iako smo modificirali i opisanu primjenu vodikva peroksida (Mathalon i Hill, 2014), primijetili smo pojavu guste pjene koja zaostaje na stijenkama uzrokujući gubitak materijala, što su uočili i drugi autori (Claessens i sur., 2013). U skladu s novijim studijama (Kühn i sur., 2017) u ovom istraživanju smo kao alkalni medij za razgradnju tkiva upotrijebili kalijevu lužinu. U provedenom su istraživanju sve kemikalije na različitim temperaturama bile učinkovite u razgradnji, ali razgradnja nije jednako dugo trajala, a izolacija mikroplastike iz svih jedinki ukazala na uspješnost odabranih metoda ekstrakcije.

Tablica 3. Usporedba istraživanja mikroplastike u školjkašima

Organizam	Reagens	Broj uzoraka / Volumen reagensa	Procedura	Izvor
<i>M. edulis</i>	HNO ₃ , 69%	3 kom, 20 ml	24 h, kuhanje 2 sata, filtracija 5 µm, sušenje filtra 40°C/24 h	Van Cauwenberghe i sur. (2014, 2015)
<i>M. edulis</i>	H ₂ O ₂ , 30%	5 kom, 150-200 ml otopine	55-65°C do isparavanja, flotacija pomoću koncentrirane fiziološke otopine, filtracija 0,8 µm	Mathalon i Hill (2014)
<i>M. edulis</i>	mješavina HNO ₃ , 69% i HClO ₄ , 68% omjer 4:1 (v:v)	5 kom, 500 ml otopine na 100 g tkiva	24 h, kuhanje 10 min, razrjeđivanje toplom vodom, drugo kuhanje, filtracija 10-20 µm	De Witte i sur. (2014)

Pronađen je prosječno jednak broj čestica nitaste mikroplastike na sobnoj i na povišenoj temperaturi. Uz to, najmanji prosječan broj čestica je pronađen u tretmanu s H₂O₂, što se može pripisati gubitku materijala radi pjenjena, a najveći s KOH iz čega se može zaključiti da je i u ovom istraživanju došlo do razgradnje nekih vrsta mikroplastike u jakoj kiselini. S obzirom na navedeno, potrebna su daljnja istraživanja različitih kemikalija i/ili modifikacije opisanih metoda te mogućnosti primjene enzimskih metoda.

Zaključak

Dagnje su sesilni organizmi dostupni tijekom cijele godine koji se hrane filtriranjem pa su vrlo pogodni modelni organizmi za ispitivanje izloženosti mikroplastici. Svaka metoda bila je uspješna u razgradnji tkiva u određenom vremenu, a kako kemikalije imaju različitu brzinu djelovanja na tkivo, eksperiment je trajao pet dana. S obzirom na primijećene nedostatke kemijskih metoda i radi pronalaska optimalne metode koja će dati pouzdane rezultate u prihvatljivom vremenskom periodu potrebna su daljnja istraživanja i/ili modifikacije opisanih metoda te ispitivanje mogućnosti primjene enzimskih metoda.

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Efficiency of different methods for microplastics extraction from Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819)

Abstract

Plastic particles smaller than 5 mm are called microplastics and can be present in the environment and in numerous organisms where they can have an effect. Many methods have been developed for extracting microplastics from the tissues of various organisms, including shellfish. In this study different methods of extraction of microplastics from mussels *Mytilus galloprovincialis* (Lamarck, 1819) were compared: extraction using potassium hydroxide (KOH), nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) at temperatures of 24°C and 60°C. All methods were effective; in average, an equal number of microplastics particles were found at room (11,67±1,72) and elevated temperatures (11,67±6,13).

Keywords: bivalves, microplastics, tissue digestion

Indeks kondicije bežmeka *Uranoscopus scaber* Linnaeus, 1758 u južnom Jadranu

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Sažetak

Na ukupnom uzorku od 1279 jedinki bežmeka *Uranoscopus scaber* Linnaeus, 1758. prikupljenih na području južnog Jadrana u razdoblju od listopada 2011. do rujna 2012. godine određen je indeks kondicije. Vrijednosti za ukupni uzorak su bile u rasponu od 0,649 do 2,359 sa srednjom vrijednosti 1,541. Indeks kondicije analiziran je i s obzirom na spol, a njegova srednja vrijednost je iznosila 1,589 za ženke (N = 699) i 1,490 za mužjake (N = 580). Uspoređujući dobivene indekse kondicije s drugim istraživanjima može se zaključiti da su vrijednosti iz našeg uzorkovanja bliže najvišim dosad objavljenim vrijednostima za indeks kondicije bežmeka.

Ključne riječi: *Uranoscopus scaber*, indeks kondicije, Jadransko more

Uvod

Bežmek *Uranoscopus scaber* Linnaeus, 1758. je bentoska predatorska vrsta koja je rasprostranjena u čitavom Sredozemnom moru te istočnom Atlantiku (od Biskajskog zaljeva do Senegala). Trom je i aktivan noću, a lovi se najčešće koćom, poponicama i obalnim mrežama potegačama (Jardas, 1996). Iako bežmek nije gospodarski značajna vrsta u ribarstvu, važna je komponenta u prehrambenom lancu (Demirhan i sur., 2007; Çoker i sur., 2008.). Indeks kondicije opisuje fizičko stanje ribe te njegovo kolebanje može ukazivati na promjene koje se pojavljuju u njenim različitim životnim fazama. Također, indeks kondicije svoju primjenu ima i kod uspoređivanja različitih populacija istih vrsta riba. Cilj ovog rada je odrediti indeks kondicije bežmeka u južnom Jadranu te usporediti južnojadransku populaciju bežmeka s ostalim populacijama bežmeka na području Sredozemnog mora.

Materijal i metode

Uzorci bežmeka analizirani u ovom istraživanju prikupljeni su mjesečno, u razdoblju od listopada 2011. do rujna 2012. godine na području južnog Jadrana (N = 1279). Jedinke su lovljene na dubinama od 100 do 140 metara pridnenom mrežom potegačom – koćom. Nakon ulova uzorci su pohranjeni na led i ručnim hladnjakom preneseni u laboratorij. Ukupna duljina tijela jedinki mjerena je ihtimetrom s točnošću od 0,1 mm. Masa tijela je mjerena tehničkom vagom s točnošću od 0,01 g. Svim jedinkama određen je spol. Indeks kondicije izračunat je uz pomoć kubičnog ili Fultonovog koeficijenta (Ricker, 1975): $IK = 100 W L t^{-3}$, gdje su W -masa ribe, L -duljina ribe.

Rezultati i rasprava

Vrijednosti indeksa bile su u rasponu od 0,649 do 2,359 sa srednjom vrijednosti 1,541. Osim za ukupni uzorak indeks kondicije analiziran je i s obzirom na spol, a njegova srednja vrijednost iznosila je 1,589 za ženke i 1,490 za mužjake (Tablica 1). Statistički značajna razlika između indeksa za mužjake i ženke nije zabilježena ($t = 1,086$, $P = 0,283$).

Tablica 1. Promjene indeksa kondicije (IK) u odnosu na ukupnu duljinu i masu tijela ženki (N = 699), mužjaka (N = 580) i ukupnog uzorka (N = 1279) bežmeka u južnom Jadranu

Duljinski razredi (cm)	Ženke		Mužjaci		Ukupni uzorak	
	\bar{x} W(g)	IK	\bar{x} W(g)	IK	\bar{x} W(g)	IK
8	-	-	11,52	1,909	11,52	1,909
9	-	-	12,72	1,384	12,74	1,384
10	17,24	1,525	18,05	1,493	17,86	1,501
11	24,05	1,639	21,78	1,448	21,97	1,464
12	31,59	1,568	28,78	1,485	29,45	1,505
13	39,29	1,588	36,79	1,481	37,60	1,516
14	48,82	1,603	46,31	1,512	47,08	1,540
15	59,89	1,604	54,91	1,499	57,54	1,554
16	70,79	1,609	66,65	1,498	68,80	1,556
17	86,38	1,633	78,16	1,466	83,35	1,572
18	98,80	1,572	95,15	1,515	97,43	1,551
19	115,06	1,575	114,80	1,547	114,97	1,565
20	133,48	1,564	128,24	1,486	131,60	1,536
21	157,81	1,605	143,23	1,483	153,34	1,568
22	173,86	1,542	157,37	1,396	167,02	1,481
23	193,23	1,501	189,75	1,500	192,02	1,501
24	230,11	1,592	221,01	1,503	227,08	1,562
25	276,78	1,686	262,45	1,528	274,99	1,666
26	291,58	1,563	114,09	0,649	261,99	1,411
27	273,70	1,390	367,45	1,815	336,2	1,673
28	-	-	-	-	-	-
29	-	-	-	-	-	-
30	486,64	1,698	-	-	486,64	1,698
31	-	-	-	-	-	-
32	618,7	1,870	-	-	618,7	1,870
33	-	-	-	-	-	-
34	-	-	-	-	-	-
35	-	-	-	-	-	-
36	337,85	0,724	-	-	337,85	0,724
Srednja vrijednost	117,38	1,589	84,01	1,490	102,25	1,541

Statističkom metodom višestruke regresije srednje vrijednosti indeksa kondicije dobivene su za ženke ($IK = 1,546$), mužjake ($IK = 1,483$) i ukupni uzorak ($IK = 1,525$). Vrijednosti dobivene statističkom metodom višestruke regresije (dobivene procjenom) su nešto niže od vrijednosti dobivenih empirijski tj. utvrđene su relativno slične vrijednosti.

Za južnojadransku populaciju bežmeka srednja vrijednost indeksa kondicije iznosila je 1,541. U dostupnoj literaturi višu srednju vrijednost iznose Sađlam & Sađlam (2013) 1,67 za područje Crnog mora i Sanz (1985) za područje Balearskog mora 1,603 (Tablica 2). Iako je srednja vrijednost indeksa za ženke (1,5363) za područje Balearskog mora bila neznatno niža nego u ovom istraživanju (1,589), vrijednost za mužjake je bila znatno viša. Naime u Balearskom moru srednja vrijednost indeksa kondicije za mužjake iznosila je 1,6567 (Sanz, 1985), dok je u ovom istraživanju iznosila 1,490. Najniže srednje vrijednosti indeksa kondicije u dostupnoj literaturi registriraju Ak i sur. (2011) koji za područje Crnog mora iznose srednju vrijednost indeksa 0,991 za ženke te 0,956 za mužjake. Zabilježene razlike u indeksima kondicije mogu biti posljedica starosti, spola, zrelosti gonada, punoće probavila riba (Barnham & Baxter 1998). Također moguće je da na više vrijednosti indeksa kondicije utječu i povoljniji abiotski čimbenici u određenim područjima uzorkovanja.

Tablica 2. Vrijednosti indeksa kondicije bežmeka u različitim istraživanjima ove vrste u Jadranskom, Sredozemnom i Crnom moru

Područje	Autori	Indeks kondicije		
		Ukupni uzorak	Ženke	Mužjaci
Balearsko more	Sanz (1985)	1,603	1,5363	1,6567
Sredozemno more	Rizkalla & Backhoum (2009)	-	1,400	1,439
Crno more	Ak i sur. (2011)	-	0,991	0,956
Crno more	Sağlam & Sağlam (2013)	1,67	-	-
Jadransko more	ovo istraživanje	1,541	1,589	1,490

Osim navedenih autora vrijednosti indeksa kondicije iznose Rizkalla & Backhoum (2009) te navode da u Sredozemnom moru (obala Egipta) ne postoji značajna razlika između indeksa za mužjake i ženke. Također navode i promjene indeksa u odnosu na ukupnu duljinu tijela. Najviša vrijednost indeksa za mužjake zabilježena je pri duljinskom razredu od 12 cm, dok je u ovom istraživanju najviša vrijednost zabilježena pri duljinskom razredu od 8 cm što potvrđuje da su visoke vrijednosti indeksa karakteristične za ribe najmanjih ukupnih duljina.

Zaključak

Na ukupnom uzorku od 1279 jedinki bežmeka prikupljenih na području južnog Jadrana određen je indeks kondicije. Vrijednosti su bile u rasponu od 0,649 do 2,359 sa srednjom vrijednosti 1,541. Srednja vrijednost indeksa za žene iznosila je 1,589, a za mužjake 1,490. Vrijednosti indeksa kondicije dobivene u ovom istraživanju su u skladu s najvišim dosad objavljenim vrijednostima za indeks kondicije bežmeka.

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The condition index of the stargazer *Uranoscopus scaber* Linnaeus, 1758. from the southern Adriatic

Abstract

The condition index was determined using a sample of 1279 specimens of the stargazer, *Uranoscopus scaber*, collected from the southern Adriatic in the period from October 2011 to September 2012. The values for the total sample ranged from 0.649 to 2.359 with a mean of 1.541. Mean condition indices for females and males were 1.589 and 1.490, respectively. It can be concluded that the values for the condition index from our research were close to the highest published values for the condition index for the stargazer.

Keywords: *Uranoscopus scaber*, condition index, Adriatic Sea

Utjecaj snage pčelinje zajednice na skupljanje pčelinjeg otrova metodom elektrostimulacije

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Sažetak

Cilj je istraživanja bio utvrditi utjecaj snage pčelinje zajednice na količinu pčelinjeg otrova elektrostimulacijom pri skupljanju na ulazu u košnicu i unutar košnice, kao i utvrditi postoje li razlike u količini pčelinjeg otrova skupljenog u jutarnjim, popodnevnim i predvečernjim satima. Utvrđene su statistički značajne razlike u masi pčelinjeg otrova između skupine jakih (0,193 g) i srednje jakih (0,164 g) te između srednje jakih i slabih (0,111 g) pčelinjih zajednica. Utvrđena je prosječno veća masa pčelinjeg otrova kod skupljača postavljenih na satonošama (0,165 g) u odnosu na leto košnice (0,148 g). Statistički značajna razlika je bila utvrđena u količini skupljenog otrova između jutarnjih (0,173 g) i popodnevnih sati (0,131 g), kao i u količini između predvečernih (0,166 g) i popodnevnih sati, dok između jutarnjih i predvečernih sati nije bilo statistički značajne razlike.

Ključne riječi: pčelinja zajednica, pčelinji otrov, metoda elektrostimulacije

Uvod

Proizvodnja meda u posljednje vrijeme postaje sve upitnija zbog češćih ekstremnih klimatskih promjena (suše, dugotrajna kišna razdoblja). Zbog navedenog se razloga nameće potreba razvijanja proizvodnje i drugih pčelinjih proizvoda. Jedan od pčelinjih proizvoda koji zaslužuje pozornost svakako je pčelinji otrov. Naime, proizvodnja pčelinjeg otrova nema dugu tradiciju, a u posljednje vrijeme potražnja za potrebe farmaceutske i kozmetičke industrije sve više raste, kako kod nas, tako i u Europi. Sve do 60-ih godina prošlog stoljeća proizvodnja je pčelinjeg otrova bila ograničena nedostatkom prirodnih tehnika za njegovo skupljanje. Do tada se otrov dobivao iz ekstrakata žalčanih aparata ili pažljivim odstranjivanjem otrovnog mjehura. Pčelinji se otrov dobivao i omamljivanjem pčela pomoću etera ili kloroforma. Navedene metode skupljanja otrova su bile spore i izvedive samo u laboratorijskim uvjetima, a posljedica je toga bila iznimna skupoća. Sredinom 50-ih godina prošlog stoljeća započela je primjena skupljanja otrova elektrošok metodom, tj. iritiranjem pčela slabo izmjeničnom strujom (Kezić i sur., 2014). Stoga je potrebno upotpuniti saznanja o mogućnostima proizvodnje pčelinjeg otrova suvremenom metodom elektrostimulacije radilica te je cilj istraživanja bio utvrditi utjecaj snage pčelinje zajednice na količinu pčelinjeg otrova elektrostimulacijom pri skupljanju na ulazu u košnicu i unutar košnice, kao i utvrditi postoje li razlike u količini pčelinjeg otrova skupljenog u jutarnjim, popodnevnim i predvečernjim satima.

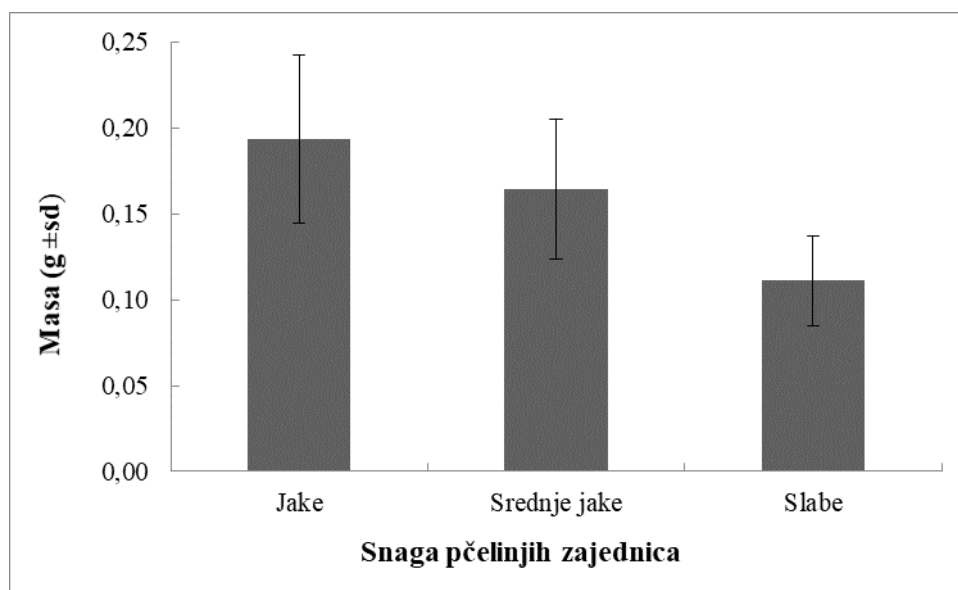
Materijal i metode

Istraživanje je bilo provedeno tijekom lipnja i srpnja 2017. godine na OPG-u Matijević u Daruvarskom Brestovcu. „Liebefeld“ je metodom (Imdorf i Gerig, 2001) utvrđena snaga zajednica te je izdvojeno 18 pčelinjih zajednica koje su bile podijeljene u dvije skupine od po devet zajednica. Svaku su skupinu činile tri slabe, zatim tri srednje jake te tri jake pčelinje zajednice. U skupinu slabih zajednica uvrštene su bile zajednice kod kojih se broj utvrđenih pčela kretao od 20 270 do 32 480. Kod skupine srednje jakih zajednica izabrane su bile zajednice kod kojih se broj utvrđenih pčela kretao od 32 810 do 40 790, a u skupinu jakih zajednica utvrđeni se raspon broja pčela kretao od 46 960 do 61 530. U istraživanju je bio korišten elektrostimulacijski skupljač otrova s istosmjernom strujom prema modelu pčelara Ivana Curiša. Postupak skupljanja pčelinjeg otrova provodio se tijekom tri uzastopna dana i to u 9:00, 13:00 i 17:00 na tri pčelinje zajednice različite snage sa skupljačima postavljenim na ulazu u košnicu (leto) i tijekom 10:00, 14:00

i 18:00 na skupljačima postavljenim unutar košnice (satonoše). Ukupno su bila izvršena tri ponavljanja s razmakom od tjedan dana.

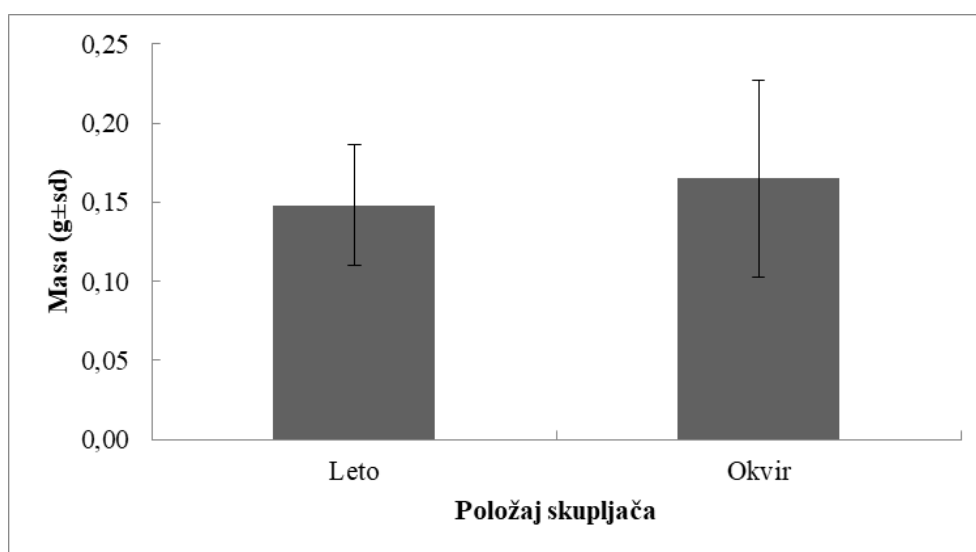
Rezultati i rasprava

Tijekom istraživanja ukupno su bila prikupljena 162 uzorka pčelinjeg otrova, od čega su po svakoj pokusnoj skupini pčelinjih zajednica skupljena njih 54. Uspoređujući prosječne mase skupljenog pčelinjeg otrova između ispitivanih skupina pčelinjih zajednica (grafikon 1) utvrđena je statistički značajna razlika s obzirom na njihovu snagu ($F=59,79$; $p<0,001$).



Grafikon 1. Prosječna masa pčelinjeg otrova između skupina pčelinjih zajednica različite snage

Statistički značajna razlika utvrđena je kod skupine jakih (0,193 g) u odnosu na skupinu srednje jakih (0,164 g) i slabih (0,111 g), kao i između skupine srednje jakih i slabih pčelinjih zajednica. Nešto niže prosječne vrijednosti zabilježene su u istraživanju Rybak (2008) koji navodi prosječnu masu pčelinjeg otrova od 0,112 g s rasponom od 0,063 g kod slabih do 0,159 g kod jakih pčelinjih zajednica.

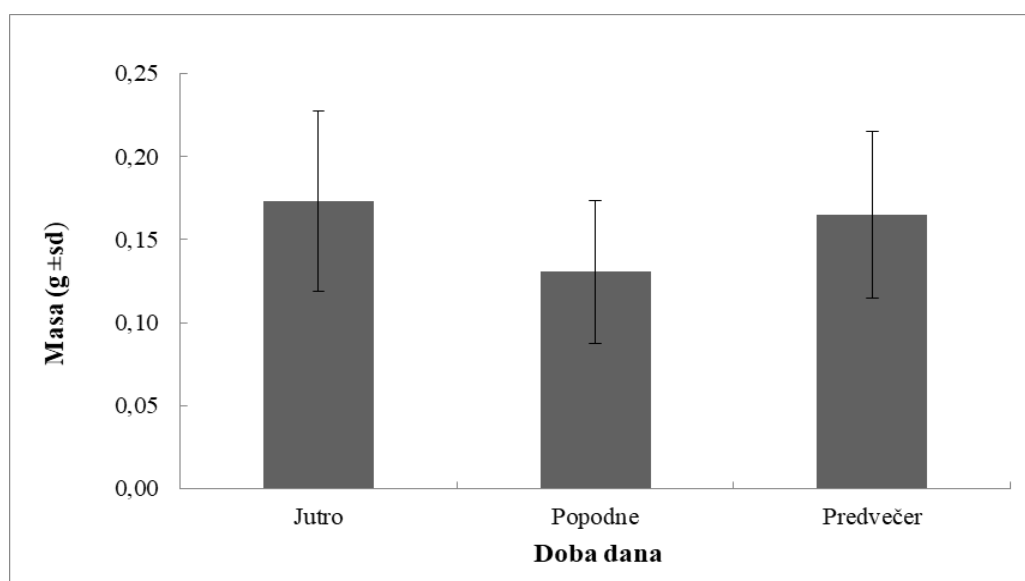


Grafikon 2. Prosječna masa pčelinjeg otrova s obzirom na položaj skupljača pčelinjeg otrova

Uspoređujući prosječnu masu pčelinjeg otrova skupljenog na satonošama okvira i na letu košnice utvrđena je statistički značajna razlika ($F=4,56$; $p<0,03$). Iz grafikona 2. je razvidno da je utvrđena prosječno veća masa pčelinjeg otrova kod skupljača postavljenih na satonošama okvira (0,165 g) u odnosu na one koji su bili na letu košnice (0,148 g).

U istraživanju koje je provedeno u Poljskoj, Nenchev i Stoichev (prema Sanad i sur., 2013) su utvrdili da su radilice ispuštale od 60,85 do 65,57% otrova na staklo skupljača postavljen na leto. Također navode kako povećano trajanje elektrostimulacije od 60 na 90 minuta nije rezultiralo većom količinom prikupljenog pčelinjeg otrova košnice.

Promatrajući masu skupljenog pčelinjeg otrova tijekom dana utvrđena je statistički značajna razlika ($F=11,14$; $p<0,001$). Prema utvrđenim prosječnim vrijednostima u jutarnjim satima (0,173 g) masa se pčelinjeg otrova statistički značajno razlikovala od mase skupljene u popodnevnim satima (0,131 g). Također, statistički značajna razlika u masi pčelinjeg otrova utvrđena je i između predvečernjih (0,166 g) i popodnevni sati, dok između jutarnjih i predvečernjih nije bilo statistički značajne razlike (grafikon 3).



Grafikon 3. Prosječna masa pčelinjeg otrova skupljenog u različito doba dana

U istraživanju Sanad i Mohanny (2013) najviša je prosječna masa otrova ustanovljena u razdoblju od 16:00 do 18:00 i iznosila je 0,166 g, dok je najniža prosječna masa (0,080 g) bila ustanovljena u razdoblju od 13:00 do 15:00. Nowar (2016) također navodi najveću prosječnu masu otrova skupljenu u popodnevnim, odnosno večernjim satima između 19:00 i 21:00 u količini od 0,158 g, a najnižu masu (0,110 g) u vremenu između 15:00 i 17:00 sati. Uspoređujući količini skupljenog otrova u odnosu na godišnje doba, Sanad i Mohanny (2013) navode da je najbolje razdoblje za skupljanje pčelinjeg otrova u ljeti (kolovoz) od 16:00 do 18:00, kada je i zabilježena najveća prosječna masa pčelinjeg otrova od 0,185 g/danu. Također su utvrdili da je najsigurnije vrijeme za skupljanje pčelinjeg otrova između 13:00 i 15:00 sati, kada je i utvrđen najmanji broj uginulih radilica (26,74/dnevno), kao nuspojava skupljanja pčelinjeg otrova.

Različite su metode skupljanja pčelinjeg otrova uspoređivane s obzirom na količinu skupljenog otrova, zatim njihov utjecaj na pčelinju zajednicu i njezino zimovanje, kao i na njezinu opću produktivnost (med, pelud, vosak). Utvrđeno je da skupljanje pčelinjeg otrova nema štetnih učinaka na snagu zajednice, leglo i produktivnost. Međutim, prikupljanje otrova može nepovoljno utjecati na prezimljavanje zajednice (Skubida i sur., 1995).

Zaključci

Na osnovu snage prosječno najveću je količinu pčelinjeg otrova skupila skupina jakih pčelinjih zajednica. S obzirom na položaj skupljača pčelinjeg otrova prosječno je veća masa utvrđena kod onih postavljenih na satonošama. Uspoređujući vrijeme skupljanja utvrđena je statistički značajna razlika između jutarnjih i popodnevni sati te između predvečernjih i popodnevni sati, dok između jutarnjih i predvečernjih sati nije bilo statistički značajne razlike.

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Influence of the honey bee colony strength on collecting bee venom by electro stimulation method

Abstract

The aim of the study was to determine the effect of honey bee colonies strength on the amount of bee venom by electrostimulation during collection at the entrance of the hive and inside the hive, as well as to determine whether there are differences in the amount of bee venom collected in the morning, afternoon and evening. The study showed statistically significant differences in bee venom weight between strong (0.193 g), medium strong (0.164 g) and between medium strong and weak (0.111 g) honey bee colonies. Considering the position of the collector, a statistically significant difference in the weight of bee venom was found with the collectors on frames (0.165 g) compared to collector on entrance of the hive (0.148 g). A statistically significant difference was also found in the amount of collected bee venom in the morning (0.173 g) compared to the amount collected in the afternoon (0.131 g), as well as the amount in the early evening (0.166 g) and afternoon hours. There were no statistically significant differences between the morning and the early evening hours in the amount of collected bee venom.

Keywords: honey bee colony, bee venom, electro stimulation method

Financijski učinci lovnog turizma u istočnoj Hrvatskoj

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Sažetak

Primarna zadaća lova, osiguravanje čovjekove egzistencije, danas je gotovo zanemariva, a sve više jača gospodarski značaj te rekreacijski boravak u prirodi. Lovci kao nositelji lovnih aktivnosti brinu za očuvanje biljnog i životinjskog eko sustava, a istovremeno kroz lovni turizam i trofejni odstrjel nastoje ostvariti dodatne prihode i ekonomsku korist, kako za lovačku, tako i za širu društvenu zajednicu. Cilj rada je pokazati postojanje resursa za razvoj lovnog turizma u istočnoj Hrvatskoj. Primjenom matematičkog modela eksponencijalne funkcije na podatke o broju stranih lovaca turista u Osječko-baranjskoj županiji i analizom financijskog izvješća Hrvatskih šuma, Uprave šuma Osijek u proteklom razdoblju uočava se mogućnost razvoja lovstva u istočnoj Hrvatskoj.

Ključne riječi: financijski učinci, istočna Hrvatska, lovni turizam, lovstvo

Uvod

Lovstvo i lov kao njegova sastavnica i danas, početkom 21. stoljeća, su i dalje prisutna i nezamjenjiva ljudska djelatnost. Primaran motiv lova, osiguranje hrane i egzistencije, postaje gotovo zanemariv u odnosu na nove motive bavljenja lovom, a to su sportsko i rekreacijsko uživanje u prirodi. Lovci kao nositelji lovnih aktivnosti brinu za očuvanje biljnog i životinjskog eko sustava, a istovremeno kroz lovni turizam i trofejni odstrjel nastoje ostvariti dodatne prihode i ekonomsku korist, kako za lovačku, tako i za širu društvenu zajednicu. Lovni turizam se može definirati kao „kretanje i aktivan boravak turista-lovca u specifičnom okruženju–lovištu, kao dijelu zdrave prirodne sredine, radi lova (odstrjela, hvatanja, promatranja, odnosno snimanja) divljači, čime njegovi sudionici (lovci–turisti) zadovoljavaju snažan motiv (primarnu hobi aktivnost koja je nekima sklonost, a nekima strast). Pri tom isti plaćaju naknadu za odstrijeljenu divljač (trofeji, meso, koža i drugo), prateće usluge u lovu (usluge stručnih pratitelja, transport i dr.), smještaj i ishranu u odgovarajućim ugostiteljskim objektima, kao i druge ugovorene usluge, po u to vrijeme važećim cjenicima“ (Prentović, 2014:24). Isti autor (Prentović, 2014) navodi kako lovni turizam kao gospodarska djelatnost istovremeno ima rekreativnu, ekološku, ekonomsku, edukativnu i kulturnu funkciju.

Neophodni resursi za ostvarivanje lovnog turizma su divlje životinje i njihova staništa, odnosno poljoprivredne površine i površine pod šumama koje su istovremeno dijelovi pojedinih lovišta. To su uglavnom područja ruralnog prostora koji je najzastupljeniji na području istočne Hrvatske. Upravo zbog te prostorne povezanosti i prirodnog okruženja lovni turizam je komplementaran s ruralnim ili seoskim i ekoturizmom. Ova dva oblika turističke ponude doprinose razvoju tzv. zelenog turizma te se može reći da to čini i lovni turizam. To postaje vidljivo kroz primjenu zelenog turizma što u praksi znači „manji ekološki otisak, doprinos ciljevima zaštite prirode, jačanje konkurentnosti i tržišne otpornosti, stvaranje novih poduzetničkih prilika, široku primjenu uz pomoć EU fondova te povećanje dobiti i koristi za lokalnu ekonomiju“ (Carić, 2018:79).

Financijski učinci lovnog turizma mogu se pratiti analiziranjem različitih čimbenika kao što su: izdane dozvole za lov stranim državljanima, broj odstrijeljene divljači i naplaćeni trofeji, broj dolazaka i noćenja stranih i domaćih turista lovaca u lovačkim i drugim smještajnim objektima te odnosom prihoda i rashoda nastalih uz sam lov i lovnju djelatnost pojedinog poslovnog subjekta koji se bavi lovnim turizmom. Analiziranjem nekih od navedenih čimbenika koji su svojevrsni pokazatelji ostvarenih učinaka u lovnom turizmu u prošlom razdoblju može se doći

do informacija koje bi mogle pomoći u daljnjem razvoju lovstva i lovnog turizma, posebice na području istočne Hrvatske.

Materijal i metode

Prikupljeni su, analizirani i prikazani sekundarni podatci iz baze podataka Lovačkog saveza Osječko-baranjske županije kao jedne od uspješnijih županija u ostvarivanju lovne djelatnosti na području istočne Hrvatske te podatci Hrvatskih šuma d.o.o., Uprave šuma Osijek koja se također intenzivno bavi lovnim turizmom. Financijski učinci lovnog turizma praćeni su temeljem broja izdanih dozvola strancima u razdoblju od 2008. do 2018. godine, a obrađeni su metodom analize ekspanzije trenda. Analiza prihoda, rashoda i ostvareni financijski rezultat lovnog turizma Hrvatskih šuma, Uprave šuma Osijek u razdoblju od 2014. do 2018. godine također su jedan od analiziranih pokazatelja financijske učinkovitosti lovnog turizma.

Rezultati i rasprava

Temeljni preduvjet za odvijanje lova stranog lovca turista je izdana dozvola za lov koju mora zatražiti lovoovlaštenik lovišta u čijem lovištu će se odvijati lov, od Hrvatskog lovačkog saveza. Na temelju podataka o broju izdanih dozvola stranim državljanima u periodu od 2008.g do 2018. godine u Osječko-baranjskoj županiji (Tablica 1) uočava se njihov rast.

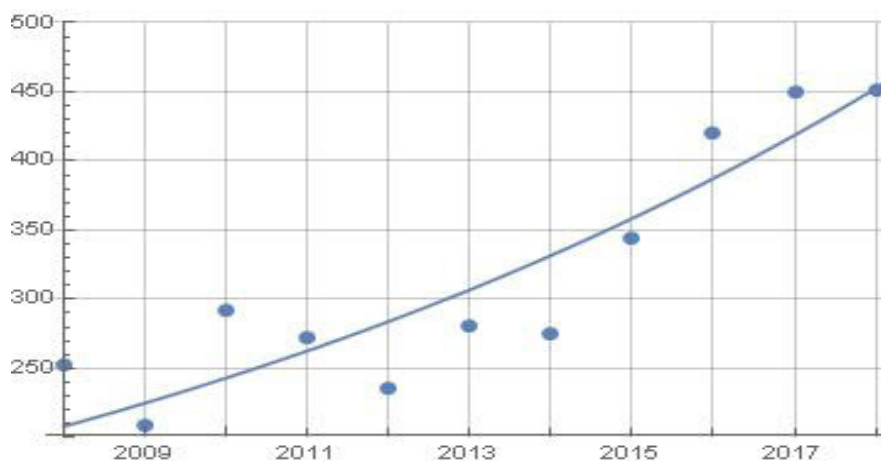
Tablica 1. Izdane dozvole za lov stranim državljanima u OBŽ-u

Godina	Državna lovišta	Zajednička lovišta	Ukupno
2008.	175	78	253
2009.	94	115	209
2010.	173	119	292
2011.	211	62	273
2012.	162	74	236
2013.	156	125	281
2014.	172	103	275
2015.	254	90	344
2016.	294	126	420
2017.	315	135	450
2018.	297	155	452

Izvor: Lovački savez Osječko-baranjske županije

Uočava se kako je, osim u 2009. godini, izdano znatno više dozvola stranim državljanima za državna lovišta u odnosu na zajednička lovišta. Jedan od razloga takvoj situaciji svakako leži u činjenici da zajedničkim lovištima gospodare lovačka društva koja ustrojena kao udruge ne smiju ostvarivati značajnije prihode, a time i moguću dobit. Analizirajući podatke o broju izdanih dozvola za lov strancima (Tablica 1) koji su izravni korisnici lovnog turizma u razdoblju 2008. do 2018. godina te primjenjujući ekspanziju trenda model-funkciju moguće je pratiti kretanje, odnosno promjene (rast ili pad) broja izdanih dozvola strancima (Grafikon 1).

Ekspanzija trenda model funkcija je, odnosno procjenjuje se kako je prosječna godišnja stopa rasta ukupno izdanih dozvola za strance 7,8%.



Grafikon 1. Ukupno izdane dozvole za strance u OBŽ-u od 2008. - 2018. godine

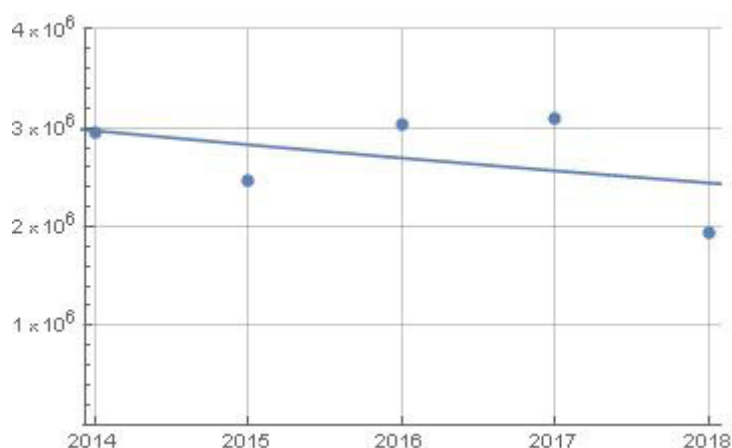
Na temelju navedenih i prikazanih podataka može se očekivati kako će se trend povećanja nastaviti i u sljedećem razdoblju. Promatra li se na isti način prosječna godišnja stopa rasta izdanih dozvola strancima u Hrvatskim šumama d.o.o., Uprava šuma Osijek, kao najvećeg koncesionara državnih lovišta ona je 15,4%, (. Analiziraju li se podatci lovne djelatnosti Hrvatskih šuma, Uprava šuma Osijek kroz prihode i rashode, odnosno ostvaren financijski rezultat u periodu od 2014. do 2018. godine (Tablica 2) jasno je vidljivo ostvarivanje pozitivnog financijskog učinka.

Tablica 2. Financijski rezultat Hrvatskih šuma d.o.o., Uprava šuma Osijek (2014.-2018.) (u HRK)

Godina	2014.	2015.	2016.	2017.	2018.
Prihodi	8.123.214	7.655.534	8.237.238	8.263.315	7.280.914
Rashodi	5.167.774	5.178.514	5.193.679	5.140.594	5.340.855
Dobit/gubitak	2.955.440	2.477.020	3.043.555	3.095.721	1.940.055

Izvor: Hrvatske šume d.o.o., Uprava šuma Osijek

Navedeni podatci iz prethodne tablice jasno pokazuju ostvarivanje pozitivnog financijskog rezultata, ali s trendom pada. Analizira li se promjena dobiti (Grafikon 2) eksponencijalnom model funkcijom stopa promjene financijskog rezultata (dobiti) je -4,9% , odnosno (odnosno ($f(t)=3.12282 \times 10^6 e^{(-0.04895 t)}$)).



Grafikon 2. Financijski rezultat – dobit

Prosječna stopa promjene ostvarene dobiti je negativnog predznaka, odnosno ima trend opadanja, ali Hrvatske šume

d.o.o., Uprava šuma Osijek i dalje ostvaruju dobit u djelatnosti lovstva. Pokazatelji rasta rashoda i smanjenja prihoda trebaju biti indikatori koji upozoravaju na nužnost kontrole rashoda kako bi se održao pozitivan poslovni rezultat u budućem razdoblju.

Zaključak

Uz doprinos održavanju prirodnog okruženja lovačke aktivnosti moguće je usmjeriti i za stvaranje doprinosa široj zajednici kroz ostvarivanje financijske koristi od trofejnog odstrjela i lovnog turizma. Uz Hrvatske šume i druge pravne osobe koje su koncesionari državnih lovišta te lovačka društva Osječko-baranjske županije i istočne Hrvatske, ovisno o bonitetu lovišta i lovnogospodarske osnove, imaju potencijala za bavljenje lovnim turizmom. Lovni turizam, kao poseban oblik turističke ponude, daje mogućnosti za izravno stvaranje financijske dobiti te izravno i neizravno može pridonijeti gospodarskom razvoju istočne Hrvatske. Navedeni podatci o rastu interesa stranih lovaca turista, odnosno povećanje broja izdanih lovačkih iskaznica strancima u Osječko-baranjskoj županiji od 7,8% kroz prošlo desetogodišnje razdoblje kao i ostvarena dobit Hrvatskih šuma, Uprave šuma Osijek od lovne turističke djelatnosti dokaz su mogućeg daljnjeg razvoja lovstva u smislu lovnog turizma i ostvarivanje pozitivnih financijskih efekata. Potrebno je analizirati ostale čimbenike financijskog učinka lovnog turizma, kako bi se utvrdilo kako oni djeluju na negativnu stopu promjene financijskog rezultata od 4,9% te odrediti načine rada koji bi utjecali na njezino ponovno povećanje.

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Financial effects of hunting tourism in eastern Croatia

Abstract

The original purpose of hunting has become virtually negligible: the exchange value and the use value of hunting have gradually declined, while the significance of economic and recreation gradually increases. As hunting industry stakeholders, the hunters work to conserve plant and animal ecosystems while trying to earn an extra income and obtain an economic benefit for the hunting community as well as the broader social community through hunting tourism and trophy hunting. The aim of this study is to show the existence of resources for development of hunting tourism in eastern Croatia. Applying the mathematical model of the exponential function to the number of foreign tourist hunters in the Osijek-Baranja County data and analysing the financial report of the Hrvatske šume (Croatian Forests), the Croatian Forests Administration of Osijek, in the past period reveals the possibility of hunting development in eastern Croatia.

Keywords: financial effect, eastern Croatia, hunting tourism, hunting

Length-weight relationship of grass goby *Zosterisessor ophiocephalus* from Novigrad Sea - eastern Adriatic Sea

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Abstract

This study describes length-weight relationship of grass goby in the Adriatic Sea. The total lengths (TL) of 1229 specimens were sampled from September 2010 – August 2011 in eastern Adriatic (Novigrad Sea) with average value 12.3 ± 2.7 . Length - weight relationship was described by the equation: $W = a L^b$. The values of the regression coefficient 'b' obtained for the length-weight relationship for the species of grass goby was 2.92 with respective r^2 value of 0.969.

Keywords: morphometric, interpopulation, intrapopulation

Introduction

Grass goby, *Zosterisessor ophiocephalus* (Pallas, 1814) (Gobiidae) is an inshore species that usually occurs in brackish waters of estuaries and lagoons of the Mediterranean and Black Sea including Adriatic Sea and Sea of Azov (Jardas, 1996). It is widespread in the Adriatic Sea, especially in Novigrad Sea and Karin Sea. Seagrass meadows and associated soft substrates provide an essential habitat for life and reproduction of this species (Gandolfi et al., 1991; Malavasi et al., 2003). Length-weight relationship depends mostly on ecological conditions and changes in growth allometry point to changes in body shape, physiological changes and increase or decrease in growth rate (Frost, 1945). Furthermore, allometry changes in dependence with body length, age, sex and sampling location. Length-weight relationship can be used in research of fish populations in brackish waters under illegal fisheries pressure or under conservation efforts (Gurkan et al., 2010). Length-weight relationship of Gobids has been investigated widely from Mumbai coast, India (Jaiswar et al., 2018), to the Mediterranean Sea (Maci et al., 2009, Koutrakis and Tsikliras, 2003; Gurkan et al., 2010; Özaydin and Taskavak, 2006). The length-weight relationship (Wt/Lt) has been widely used in fish biology for several purposes (e.g. to estimate the mean weight of the fish, based on the known length, (Beyer 1987, Borges et al., 2003; Mendes et al., 2004). Conversion of the length equations to weight for equivalent of growth in weight can be used for morphometric interspecies, interpopulational and intrapopulational comparisons as well as to assess the index of well-being of the fish populations (Bolger and Connolly, 1989). The length-weight equation is also a quantitative expression of the development at corporal level of an organism.

The objective of the study was to determine the length-weight relationship of grass goby populations in the Adriatic Sea.

Materials and methods

Samples of grass goby were collected from Novigrad Sea (Figure 1) (eastern Adriatic Sea) on monthly basis (September 2010 – August 2011). The Novigrad Sea, Croatia (44°12'N, 15°30'E) is an estuarine embayment of 29 km² on the northeastern Adriatic coast receiving freshwater from the Zrmanja River and a few nearby seasonal creeks. It is connected to the Velebit channel to the north by the narrow Maslenica channel, and to the Karin Sea to the south by the Karin channel. Salinity in the study site ranges from 10 to 30 PSU (Schultz et al. 2011), and water surface temperature of the Zrmanja estuary ranges from 6.7 to 26.6°C (Viličić 2011). Sea bottom types include rocky

bottom covered with algae, muddy and sandy ground partially covered with sea grass and gravel ground (Mokos, 2017). In total 1229 samples were analyzed. Laboratory work included measure of total and standard length down to the nearest 0.1 mm using Vernier caliper, body weight using digital scale down to the nearest 0.1g. Specimens were also sexed using binocular microscope. Analysis of length-weight relationship of grass goby was performed on entire sample with functional regression (Ricker, 1975) of Log transformed values:

$$\log W = \log a + b \log Lt \text{ and exponential equation: } W = a * Lt^b$$

Where: Lt = total length, W = body weight, a and b are constants.

Variability statistical methods in data analysis used following measures: group mean (\bar{X}), standard deviation ($\pm SD$) and variability coefficient (V), which was calculated using equation: $V = 100 * SD / \bar{X}$

To assess the occurrence of sexual dimorphism both in females and in males, t -test ($p < 0.05$) was used. The data was analyzed in 7.0 software (Statsoft, USA)

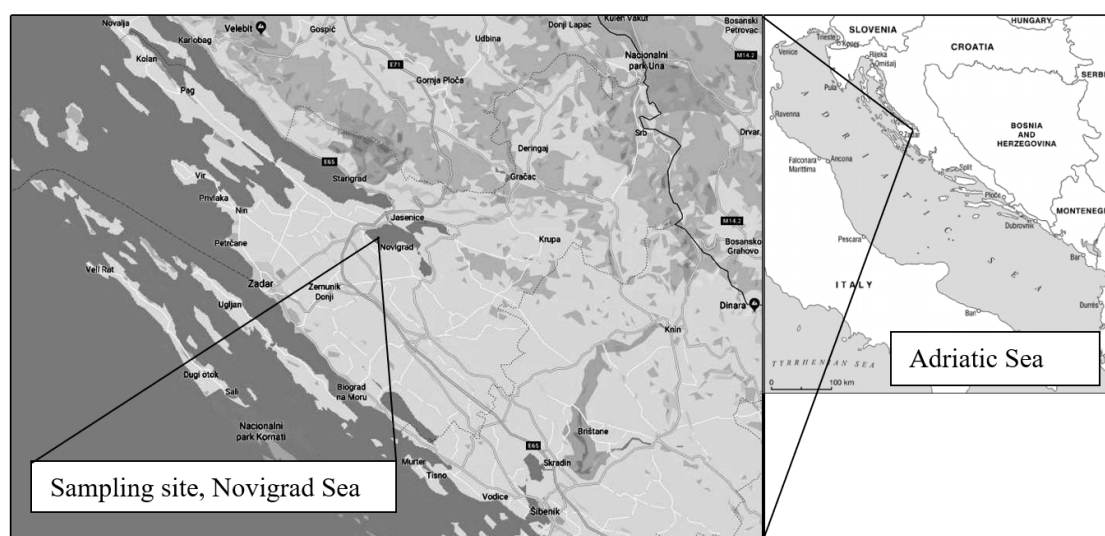


Figure 1. Sampling site, Novigrad Sea, Adriatic Sea

Results and discussion

Average values of grass goby total length were 12.30 ± 2.70 cm (in range 4.3-20.0 cm). In the present study calculated constant b was 2.920 for total sample, 2.890 for females and 2.895 for males (Figure 2). The results indicate negative allometric growth for grass goby. Difference in exponent b was significantly different between males and females (t -test, $p < 0.05$). Most abundant individuals of both sexes fall into length groups 11.0 and 14.0 cm, with less abundance in smaller and larger length groups. Weight of fish increasingly fluctuates with the increase of total length or growth. Individuals with highest body weight fall into largest size group, which was recorded for males and females, as well for total sample. Higher mid body weight values were noted for female size groups of 7.0-12.0 cm and for males in size groups of 14.0-20.0 cm. Comparing values of b for *Z. ophiocephalus* from this study with previous research from northeastern Aegean Sea (Turkey) and northern Aegean estuaries (Greece) significant difference is indicated (Table 1). In research conducted in Turkey a positive allometry was recorded, while in research conducted in Greece (Porto Lagos) correspond closely with this research values. Therefore, mentioned differences can be attributed to different ecological and physical factors in investigated areas. Negative allometry for grass goby was reported by Hajji et al. (2013) for mid Mediterranean Sea, also. According to Dulčić and Kraljević (1997) parameters of length-weight relationship can fluctuate with season, different biological and ecological factors in certain area and sex and maturity of particular species.

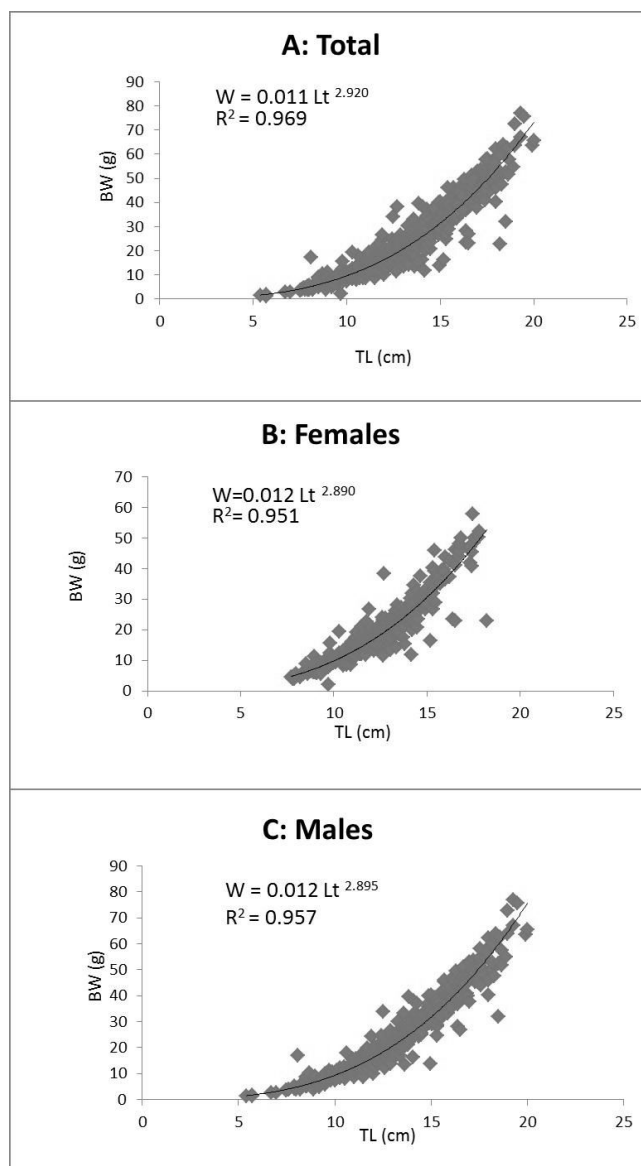


Figure 2. Length-weight relationship of grass goby, *Zosterisessor ophiocephalus* from Novigrad Sea. 1A) total sample (N=1229), 1B) females (N=500), 1C) males (N=565).

Table 1. The values of regression parameters *a*, *b* and total length range (Lt [cm]) for grass goby (*Zosterisessor ophiocephalus*) in different research areas from Aegean, Mediterranean and Adriatic Sea.

Area	Author	Lt range (cm)	<i>a</i>	<i>b</i>
Aegean estuary (Porto- Lagos, Greese)	Koutrakis & Tsikliras (2003)	10.20-16.50	0.011	2.998
Eastern Aegean Sea (Turkey)	Özaydin & Taskavak (2006)	9.30-20.50	0.004	3.306
Northern Aegean Sea (Turkey)	Gurkan et al. (2010)	6.50-7.00	0.004	3.339
Mid Mediterranean Sea	Hajii et al. (2013)	10.50-20.20	0.020	2.811
Aegean Sea (Bay of Izmir)	Akyol (2003)	8.0-23.3	0.008	3.060
Eastern Adriatic Sea (Novigrad Sea)	this paper	4.3-20.0	0.011	2.920

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Dužinsko - maseni odnos glavoča travaša *Zosterisessor ophiocephalus* u Novigradskom moru – istočno Jadransko more

Sažetak

U ovom radu prikazan je dužinsko - maseni odnos glavoča travaša u Jadranskom moru. Jedinke (N=1229) su uzorkovane od rujna 2010 do kolovoza 2011 u istočnom Jadranu (Novigradsko more) a prosječna vrijednosti totalne dužine (TL) iznosila je $12,3 \pm 2,7$. Dužinsko - maseni odnos je opisan formulom $W = a L^b$. Vrijednost regresijskog koeficijenta "b" iz dužinskog - masenog odnosa za glavoča travaša iznosi 2,92 ($r^2=0,969$).

Ključne riječi: morfometrija, inter-populacija, intra-populacija

Analiza prisutnosti čestica mikroplastike u želucima triju komercijalnih vrsta riba na području južnog Jadrana

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Sažetak

Analiza prisutnosti mikroplastike u želucima odrađena je na trlji od blata, *Mullus barbatus* Linnaeus, 1758, bukvi, *Boops boops* (Linnaeus, 1758) i molu, *Merluccius merluccius* (Linnaeus, 1758). Ukupno je analizirano 150 jedinki. Ukupna duljina analiziranih jedinka trlje od blata bila je u rasponu od 14,10 do 22,30 cm ($17,07 \pm 1,46$ cm), bukve od 14,60 do 19,90 cm ($16,79 \pm 1,40$ cm), a mola u rasponu od 19,60 do 30,40 cm ($24,22 \pm 3,41$ cm). Mikroplastika je pronađena u želucima bukve ($n=5$) i mola ($n=7$) dok kod trlje od blata nije zabilježena. Pronađena mikroplastika bila je u obliku čestica i niti.

Ključne riječi: mikroplastika, *M. barbatus*, *B. boops*, *M. merluccius*, južni Jadran

Uvod

U svega nekoliko desetljeća, od početka komercijalne upotrebe sintetičkih polimera, došlo je do njihove akumulacije u okoliš. Zbog upotrebe plastike u svakodnevnom životu, raznih industrijskih aktivnosti, lošeg upravljanja otpadom, ali i činjenice da biološki nije razgradiva, njena prisutnost u morskom okolišu postala je stalna (Andrady, 1994; Barnes i sur., 2009; Jambeck i sur., 2015; Kühn i sur., 2016). Prema istraživanju Eriksen i sur. (2014) oko 270 000 tona plastike pluta oceanima i ugrožava gotovo 700 različitih vrsta morskih organizama. Mikroplastika predstavlja zagađenje u obliku sitnih čestica veličine manje od 5 mm na čijoj se površini mogu nakupljati toksične tvari. Riba mikroplastiku mogu progutati tijekom hranjenja, te na taj način toksične tvari ulaze u prehrambeni lanac (Foekema i sur., 2013). Progutana mikroplastika može uzrokovati smetnje tijekom probave i ovisno o svojoj veličini i obliku može se zadržati u probavnom sustavu sve dok jedinka ne ugine (Caron i sur., 2016). Također je moguć negativni utjecaj na endokrini sustav što može dovesti do smanjenja reproduktivnog kapaciteta organizma (Talsness i sur., 2009; Lithner i sur., 2011). Cilj ovog istraživanja bio je dobiti podatke o prisutnosti mikroplastike u želucima trlje od blata *Mullus barbatus* Linnaeus, 1758, bukve, *Boops boops* (Linnaeus, 1758) i mola, *Merluccius merluccius* (Linnaeus, 1758). Odabrane vrste imaju različita staništa i različite strategije hranjenja, a predstavljaju jedne od komercijalno najdostupnijih vrsta na tržištu.

Materijali i metode

Uzorci su prikupljeni povlačnom mrežom - kočom i vršama uz pomoć lokalnih ribara tijekom 2017. na području Koločepskog kanala, južni Jadran. Nakon ulova, jedinke su transportirane u laboratorij Sveučilišta u Dubrovniku na daljnju obradu.

Rad je izvod iz diplomskog rada Irene Glavor, mag. ing. maricult. naslova „Mikroplastika u probavilima trlje od blata, *Mullus barbatus* (Linnaeus, 1758), bukve, *Boops boops* (Linnaeus, 1758) i mola, *Merluccius merluccius* (Linnaeus, 1758) na području južnog Jadrana”

Uzorcima je mjerena ukupna duljina tijela (Lt) ihtiomrom s točnošću od 0,1 mm i ukupna masa tijela (W) tehničkom vagom s točnošću od 0,01 g. Spol jedinka određivan je temeljem vanjskog izgleda gonada. Za potreba određivanja prisutnosti mikroplastike, ribama su odstranjivani želuci i pohranjivani u petrijeve zdjelice. Uzorci su uranjeni u otopinu kalijeva hidroksida (oko 45 ml) u koncentraciji od 1M, kako bi se razgradio organski materijal. Sadržaj je nakon 24 sata pregledavan pomoću invertnog mikroskopa s integriranom kamerom (Olympus Inverted Microscope

IX17), te je fotografiran svaki uzorak. Metoda pohranjivanja sadržaja želuca u otopinu kalijeva hidroksida preuzeta iz Kühn i sur. (2016).

Rezultati i rasprava

Tijekom istraživanja ukupno je analizirano 150 jedinka. Podaci o ukupnoj duljini i masi tijela jedinki svih vrsta dati su u Tablici 1. Zbog načina rukovanja nakon ribolova na 10 (20%) prikupljenih jedinka mola nije bila moguća analiza. Ukupni uzorak trlje od blata sastojao se od 39 (78%) ženka i 11 (22%) mužjaka, a bukve 8 (16%) jedinka neodređenog spola, te 29 (58%) ženke i 13 (26%) mužjaka. Od ukupno 40 analiziranih jedinka mola 16 (32%) su bile ženke, a 24 (48%) mužjaci.

Tablica 1. Vrijednosti ukupnih duljina i masa analiziranih jedinki trlje od blata (*Mullus barbatus*), bukve (*Boops boops*) i mola (*Merluccius merluccius*) na području južnog Jadrana

	n	ukupna duljina (cm)				ukupna masa (g)			
		min	max	mean	SD	min	max	mean	SD
M. barbatus	50	14,1	22,30	17,07	1,46	33,14	113,74	51,14	15,17
B. boops	50	14,6	19,90	16,79	1,40	28,55	87,19	48,63	12,08
M. merluccius	40	19,6	30,40	24,22	3,41	45,02	185,73	95,53	43,76

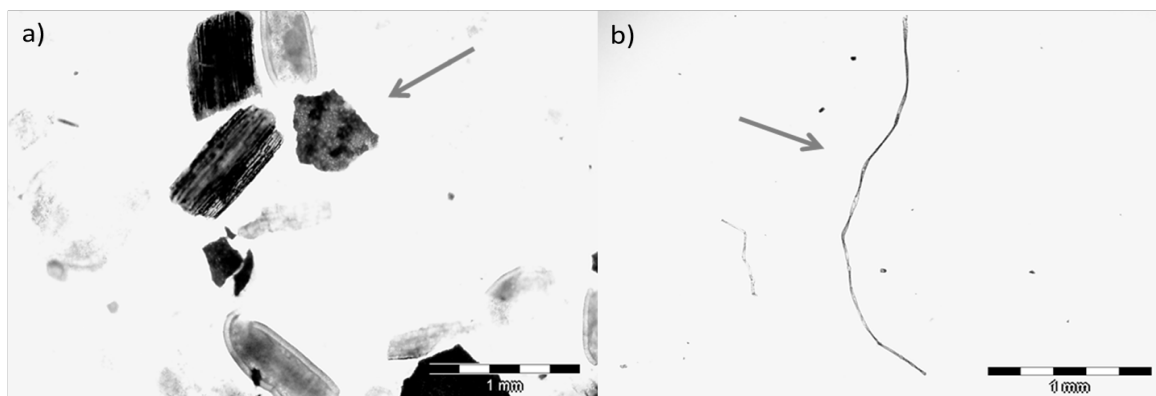
n=broj uzorka, *min*= najmanja ukupna duljina/masa, *max*=najveća ukupna duljina/masa, *mean*=srednja vrijednost ukupne duljine/mase, *SD*=standardna devijacija

Mikroplastika je pronađena u želucima bukve (*n*=5) i mola (*n*=7). U Tablici 2 prikazane su vrijednosti ukupne duljine (cm), mase (g) i spol jedinki bukve u kojima je pronađena mikroplastika.

Tablica 2. Broj uzorka, ukupna duljina (cm), ukupna masa (g) i spol jedinka bukve (*Boops boops*) kod kojih je pronađena mikroplastika

Broj uzorka	ukupna duljina (cm)	ukupna masa (g)	spol
1b	15,3	39,2	ž
2b	15,6	42,85	no
3b	17,1	49,87	ž
4b	17,1	46,79	m
5b	19,9	76,07	ž

Mikroplastika u obliku sitnih čestica promjera 545 μm pronađena je u uzorku 1b (Slika 1a), u ostalim uzorcima pronađena je u obliku niti (Slika 1b). U istraživanju na području Španjolske također je potvrđena prisutnost kod bukve, gdje je 68% uzoraka (*n*=337) sadržavalo neki oblik mikroplastike (Nadal, 2016). Razlike u rezultatima mogu biti zbog nejednakog broja uzorkovanih jedinki, ali i opterećenja ekosustava mikroplastikom na istraživanim područjima.



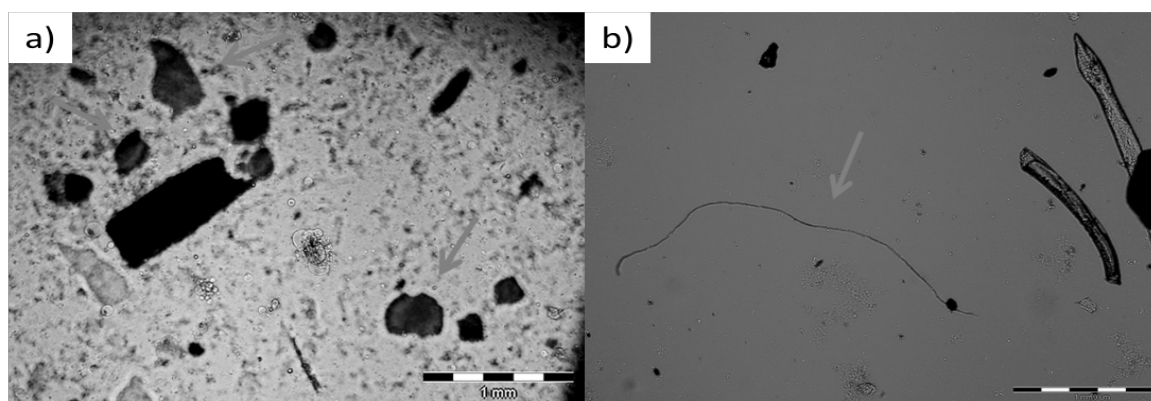
Slika 1. Mikroplastika u obliku čestica (a) i niti (b) pronađena u želucima bukve, *Boops boops* (Linnaeus, 1758) na području južnog Jadrana (scale bar 1 mm)

U Tablici 3 prikazane su vrijednosti ukupne duljine (cm), ukupne mase (g) i spol jedinki mola kod kojih su pronađene čestice mikroplastike.

Tablica 3. Broj uzorka, ukupna duljina (cm), ukupna masa (g) i spol jedinka mola (*Merluccius merluccius*) kod kojih je pronađena mikroplastika

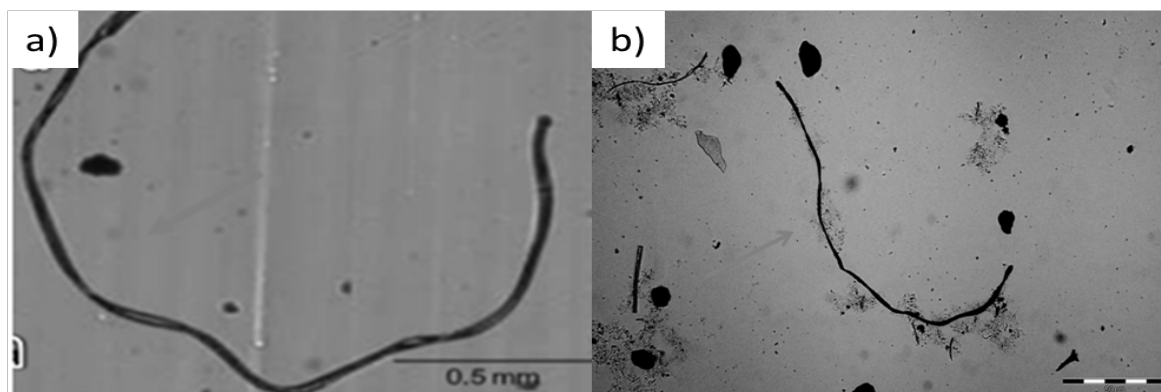
Broj uzorka	ukupna duljina (cm)	ukupna masa (g)	spol
1m	26,8	133,14	ž
2m	22,1	66,42	m
3m	19,6	45,02	ž
4m	23,2	68,30	m
5m	20,5	52,07	ž
6m	21,3	63,54	m
7m	23,4	80,80	m

Mikroplastika u obliku čestica promjera od 113,90 do 256,12 μm pronađena je u uzorku 1m, 5m i 6m (Slika 2a), a u ostalim uzorcima pronađena je u obliku niti (Slika 2b).



Slika 2. Mikroplastika u obliku čestica (a) i niti (b) pronađena u želucima mola (*Merluccius merluccius*) na području južnog Jadrana (scale bar 1 mm)

Na Slici 3a. vidljiva je nit pronađena u želucu mola, ulovljenog na području Španjolske u usporedbi s niti pronađenoj tijekom ovog istraživanja (Slika 3b). Postotak riba koje su sadržavale mikroplastiku iznosio je 16,7% (n=12) (Bellás i sur., 2016).



Slika 3. Usporedba pronađene mikroplastike u želucu mola, *Merluccius merluccius* (a) (Bellás, 2016) (scale bar 0,5 mm) i u ovom istraživanju (b) (scale bar 50 μm)

Kod jedinki trlje od blata nije zabilježena prisutnost mikroplastike. Za razliku od ovog istraživanja na području Španjolske, gdje je istraživano na većem uzorku, čestice su pronađene u 18,8 % (n=128) jedinka (Bellás i sur., 2016).

Zaključak

Analizama probavila trlje od blata, bukve i mola utvrđeno je prisustvo mikroplastike u želucima bukve (n=5) i mola (n=7). Pronađena mikroplastika bila je u obliku sitnih čestica i niti. Za određivanje sastava i porijekla mikroplastike potrebne su daljnje laboratorijske analize.

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Analysis and the presence of microplastic particles in the stomachs of three commercial fish species in the southern Adriatic

Abstract

The analysis of the presence of microplastic in the stomach of the red mullet, *Mullus barbatus* Linnaeus, 1758, bogue, *Boops boops* (Linnaeus, 1758) and European hake, *Merluccius merluccius* (Linnaeus, 1758) were performed. A total of 150 individuals were analysed. The total body length of red mullet ranged from 14.10 to 22.30 cm (17.07 ± 1.46 cm), bogue from 14.60 to 19.90 cm (16.79 ± 1.40 cm), and European hake from 19.60 to 30.40 cm (24.22 ± 3.41 cm). Microplastic was found in the stomachs of bogue (n=5) and European hake (n=7), while in the red mullet it was not recorded. The microplastics found were in the form of particles and fibres.

Keywords: microplastic, *M. barbatus*, *B. boops*, *M. merluccius*, southern Adriatic

Morphometric characteristics of the snail *Phorcus turbinatus* (Born, 1778) (Mollusca: Gastropoda) from the marine lake Mrtvo more (Lokrum Island, South Adriatic)

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Abstract

The aim of this research was to contribute to the knowledge of morphometric characteristics of mediolittoral sea snail *Phorcus turbinatus* (Born, 1778). Sampling was carried out eight times, comprehending all seasonal changes. The mean shell height of the sampled population ($n = 668$) was $Sh = 13.57$ mm, while the mean shell width was $Sw = 14.51$ mm. The smallest specimen measured 3.80 mm shell height and 4.30 mm shell width. The largest measured specimen had 26.85 mm shell height and 25.35 mm shell width. Positive allometric growth was determined, with specimens shell growing more in width until they reach sexual maturity, which is at approximately 11 mm, and more in height afterward.

Keywords: *Phorcus turbinatus*, morphometric characteristics, South Adriatic,

Introduction

Gastropod species of the genus *Phorcus* Risso, 1826 are herbivorous marine snails inhabiting rocky shores from the Mediterranean Sea to the North-eastern Atlantic Ocean including the Macaronesia Archipelagos of Madeira, Canaries, Azores and Cape Verde (Crothers, 2001). Together with limpets and periwinkles, they are the most important grazers present in the intertidal zone and as such have an important role in regulating algae populations (Templado and Rolan, 2012). Furthermore, they are used as biomonitors of heavy metals contamination in the coastal systems (Boucetta et al., 2016, Duysak and Ersoy 2014). Intertidal communities are under increasing environmental stress and anthropogenic pressures which are affecting the functioning of biological systems at different levels of organization (Sousa et al., 2017). It is of great importance to protect them and in order to do so, knowing the biological and ecological traits of the species inhabiting this area is necessary. Research on genus *Phorcus* is mainly addressed to the species *Phorcus lineatus* inhabiting shores from Morocco to the Ireland and Great Britain (Sousa et al., 2017). Although they share some common characteristics, the ecology of each species differs due to different environmental conditions; therefore, further research is needed in order to dispose of more information about this important mediolittoral species. The aim of this research was to contribute to the knowledge of the morphometric characteristics of the species *Phorcus turbinatus* (Born, 1778), a common sea snail in a mediolittoral zone of the rocky habitats in the Adriatic Sea.

Materials and methods

Samples were randomly picked by hand from marine lake Mrtvo more on Lokrum island. Measurements were carried out eight times, *in situ*, from July 2017 to May 2018, comprehending all seasonal changes. Whenever it was possible, a sample measured at minimum of 100 individuals, although it was not attainable for every fieldwork due to the withdrawal of individuals under the rocks during low tide and the population mortality which appeared in the summer period. In total 668 individuals were measured for shell height (Sh) and shell width (Sw) with a digital calliper to the nearest 0.05 mm. Recorded data were put into tables and analysed with statistical package Minitab.

Results and discussion

The mean shell height values in each sample differed and ranged from 12.10 ± 0.29 to 14.98 ± 0.34 mm, while the mean shell width ranged from 12.02 ± 0.31 to 16.18 ± 0.28 mm (Table 1). Poppe and Goto (1991) indicated that the average width of the grown-up individuals varies between 15.00 and 38.00 mm. In Mrtvo more 46.7% of sampled individuals ($n= 312$) were in that range, but the highest shell width value in overall sampled population measured only $Sw_{max} = 25.35$ mm (Table 2). The maximum size of the specimens varies in different habitats because it depends on the food availability and ecological conditions (Sousa et al., 2018).

Table 1. *Phorcus turbinatus* shell height (*Sh*) and width (*Sw*) (n – number of individuals in a sample, \bar{x} – sample mean, $SE \bar{x}$ – standard error of the sample mean, *st. dev.* – standard deviation, *min* – minimum value, *max* – maximum value)

Sampling	n (ind.)		\bar{x} (mm)	$SE \bar{x}$ (mm)	<i>st.dev</i> (mm)	<i>min</i> (mm)	<i>max</i> (mm)
14 th July 2017	25	<i>Sh</i>	12.10	0.29	9.65	9.65	14.15
		<i>Sw</i>	12.02	0.31	1.54	10.01	15.40
6 th September 2017	22	<i>Sh</i>	12.29	0.89	4.19	3.80	21.00
		<i>Sw</i>	12.86	0.78	3.65	4.30	18.00
16 th October 2017	15	<i>Sh</i>	14.83	1.23	4.76	10.55	25.50
		<i>Sw</i>	14.16	1.06	4.12	10.50	25.35
25 th October 2017	131	<i>Sh</i>	11.32	0.16	1.82	7.20	18.00
		<i>Sw</i>	12.34	0.15	1.73	8.10	18.30
5 th December 2017	105	<i>Sh</i>	14.33	0.31	3.18	6.35	26.85
		<i>Sw</i>	15.13	0.28	2.86	6.80	25.30
29 th March 2018	112	<i>Sh</i>	13.53	0.43	4.56	5.30	22.90
		<i>Sw</i>	14.47	0.36	3.77	6.30	22.05
27 th April 2018	143	<i>Sh</i>	14.31	0.36	4.30	4.35	21.85
		<i>Sw</i>	15.41	0.29	3.58	5.15	21.35
17 th May 2018	115	<i>Sh</i>	14.98	0.34	0.34	3.80	21.80
		<i>Sw</i>	16.18	0.28	0.28	7.05	22.70

Mean shell height (*Sh*) of the overall sampled population ($n = 668$) was 13.57 mm, while the mean shell width (*Sw*) was 14.51 mm (Table 1).

Table 2. *Phorcus turbinatus* shell height (*Sh*) and width (*Sw*) values of the sampled population (n – number of individuals in a sample, \bar{x} – sample mean, $SE \bar{x}$ – standard error of the sample mean, *st. dev.* – standard deviation, *min* – minimum value, *max* – maximum value)

	n (ind.)	\bar{x} (mm)	$SE \bar{x}$ (mm)	<i>st.dev</i> (mm)	<i>max</i> (mm)	<i>min</i> (mm)
<i>Sh</i>	668	13.57	0.15	3.84	26.85	3.80
<i>Sw</i>	668	14.52	0.13	3.33	25.35	4.30

Species belonging to the genus *Phorcus* have a high growth rate during the first year after settlement and it decreases thereafter due to reaching sexual maturity (Crothers 2001). In most researched species of this genus, *Phorcus lineatus* da Costa 1778, it was observed that in the first six months post settlement specimens can grow up to 8.00 mm in shell width and 11.00 - 15.00 mm by the end of the first year when they reach sexual maturity (Fretter et al., 1976). The highest abundance of juvenile specimens ($Sw < 11$ mm) in our research was observed during autumn (September, October) and in spring (March, April), which corresponds with Schifano (1983) findings that spawning

events occur during autumn and spring. The smallest specimen was measured during sampling in September ($Sh = 3.80$, $Sw = 4.30$ mm) (Table 1). There is statistically significant difference between values of shell height and width (T-test; $p < 0.05$, T- value = - 4.82). *P. turbinatus* grows more in width until it reaches 11 mm what is evident from the regression equation for measured specimens that had shell width values less than 11 mm: $Sh = -0.644 + 0.9339$. After they reach this stage there is more expressive growth in height with continuing shell width growth. Pearson's correlation coefficient is $r = 0.938$ ($p = 0.000$) which indicates a strong positive correlation between the height and width. Regression analysis confirmed a positive allometric growth; for every mm of shell width growth there is 1.089 increase in shell height (Figure 1).

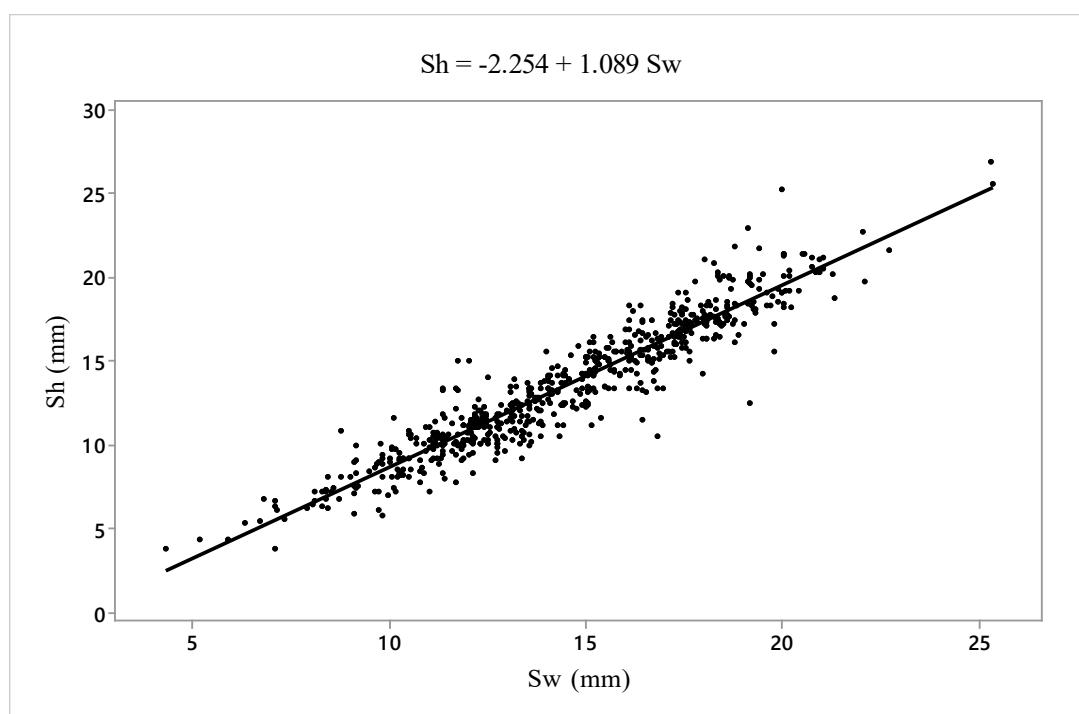


Figure 1. Shell height (Sh) and shell width (Sw) linear model of the total sampled population of *Phorcus turbinatus*, Mrtvo more

Conclusions

Sea snail *P. turbinatus* is among most common species inhabiting Mrtvo more. By analysing its morphometric characteristics it was confirmed that this species has positive allometric growth. Growth in shell width is more rapid than in shell height until individuals reach 11 mm shell width. Afterwards, there is more expressive growth in shell height with slower, but continuous growth in shell width. The smallest specimens were most abundant during the autumn and spring, which also indicated spawning during this period. Further research is needed in order to determine the growth rate and other traits of this species.

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Morfometrijske značajke puža *Phorcus turbinatus* (Born 1778) (Mollusca: Gastropoda) iz Mrtvog mora (otok Lokrum, južni Jadran)

Sažetak

Cilj ovog istraživanja bio je doprinijeti saznanjima o morfometrijskim značajkama mediolitoralnog puža *Phorcus turbinatus* (Born, 1778) Srednja vrijednost visine kućice istraživane populacije ($n = 668$) iznosila je $Sh=13,57$ mm, dok je srednja vrijednost širine kućice iznosila $Sw= 14,51$ mm. Najmanja izmjerena jedinka imala je visinu kućice 3,80 mm, a širinu 4,30 mm. Najveća izmjerena jedinka imala je visinu kućice 26,85 mm, a širinu 25,35 mm. Utvrđen je pozitivni alometrijski rast. Kućice rastu više u širinu sve do dostizanja prve spolne zrelosti pri otprilike 11 mm širine kućice, a zatim više u visinu uz manju, ali i dalje kontinuiranu stopu rasta u širinu.

Ključne riječi: *Phorcus turbinatus*, morfometrijske značajke, južni Jadran

Uzroci obojenja mišićnog tkiva svježeg brancina (*Dicentrarchus labrax*) žučnim bojama

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Sažetak

Cilj istraživanja bio je utvrditi utjecaj temperature mora, dana gladovanja i odležavanja ribe na ledu na kvalitetu mesa brancina, odnosno na pojavu obojanosti mesa žučnim bojama. Utvrđeno je kako dulje gladovanje utječe na obojanost mišićnog tkiva i na povećanje mase žučnog mjehura istraživane vrste. Temperatura mora nije bila povezana s obojanošću fileta, ali se njezin utjecaj značajno odražavao na indeks kondicije, hepatosomatski indeks i omjer mase žučnog mjehura i mase žučnog mjehura s jetrom. Visceralna mast u utrobi ribe i oko žučnog mjehura reducirala je obojenje mišićnog tkiva žučnim bojama.

Ključne riječi: *Dicentrarchus labrax*, žučni mjehur, obojenje, žučne boje, gladovanje

Uvod

Brancin (*Dicentrarchus labrax*) je jedna od glavnih morskih vrsta u akvakulturnoj proizvodnji u mediteranskim zemljama (Čakli i sur., 2006). Na tržištu se nudi kao cijeli proizvod, očišćena riba ili u obliku fileta. Morska riba iz uzgoja, a napose karnivori, zahtjeva hranidbu s kvalitetnim životinjskim proteinima, odnosno najčešće ribljim brašnom. Postoje mnogi pokušaji zamjene ribljeg brašna s jeftinijom hranom biljnog podrijetla, a kao jedna od posljedica javlja se pojava „zelene jetre“ (Goto i sur., 2001; Takagi i sur., 2005). Obojanost mišićnog tkiva žučnim bojama nastaje kao normalna postmortalna promjena kada stjenka žučnog mjehura uslijed smrti stanica postaje propusna za žučne boje koje se preslikavaju na okolna tkiva. Ako je žučni mjehur naslonjen na mišićno tkivo preslikavanje će se odraziti i na njemu. Obojani dio mišićnog tkiva je okusom gorak i treba ga odstraniti prije konzumacije (Jones, 1969).

Cilj istraživanja bio je utvrditi utjecaj potencijalnih čimbenika: temperature mora, dana gladovanja i odležavanja ribe na ledu na kvalitetu mesa brancina, odnosno na pojavu obojanosti mesa žučnim bojama. Također, uz indeks kondicije ispitivanih jedinki, željelo se utvrditi i utjecaj mase žučnog mjehura i mase jetre na pojavu povećane koncentracije žuči.

Materijali i metode

Istraživanje je provedeno na uzgajalištu tvrtke Cromaris d. d. na lokaciji otoka Bisage tijekom 2015. i 2016. godine. Pokus se izvodio u dva kaveza sa po 6000 jedinki i to: u kontrolnom kavezu u kojem se riba svakodnevno hranila i pokusnom u kojem je riba gladovala. Dan prije svakog uzorkovanja, ribe su hranjene *ad libitum* komercijalnom ribljom hranom za brancina u obliku ekstrudiranih peleta. Tijekom istraživanja izvršena su 4 uzorkovanja, a svaki ciklus uzorkovanja trajao je 10 dana. Uz svako testiranje mjerena je i temperatura mora. Prvi ciklus uzorkovanja počeo je 14.6.2015. drugi 20.7.2015., treći 7.12.2015. i četvrti ciklus 2.3.2016. Po dvadeset riba iz kontrolnog kaveza uzorkovano je 3., 5. i 10. dana svakog ciklusa istraživanja, dok je deset riba iz kontrolnog kaveza stavljeno na led te uzorkovano nakon 24 h. Riba u pokusnom kavezu su gladovale 10 dana i uzorkovanje se vršilo 6, 12, 24 h nakon zadnjeg hranjenja. Od drugog dana uzorkovanje se vršilo jednom dnevno. U oba kaveza se svaki uzorak sastojao od 20 riba koje su se odmah analizirale i uzorak od 10 riba koje su se analizirale nakon 24 h ležanja na ledu. Odmah po

izlovu riba se eviscirala, mjerila i detektirala obojanost mišićnog tkiva. Mjerena je ukupna duljina tijela (TL) (cm), masa tijela (g), masa trbušnih organa (g), masa jetre i žučnog mjehura (g), masa jetre (g), duljina žučnog mjehura van jetre (mm) i procjenjivao se intezitet obojenja mišićnog tkiva (0 nije obojeno, + slabo obojeno, ++ srednje obojeno i +++ jako obojeno). Nakon toga, riba bi se stavila na led 24 h kako bi se imitirali uvjeti skladištenja. Na temelju izmjerenih podataka, izračunao se indeks kondicije (IK), hepatosomatski indeks (HSI) i omjer mase žučnog mjehura i mase žučnog mjehura s jetrom (HI). Indeks kondicije (IK) prikazan je Fultonovim ili kubičnim indeksom kondicije:

$$IK = W L^{-3} \cdot 100$$

gdje je *W* – masa u gramima, *L* – ukupna dužina tijela u centimetrima (Ricker, 1975).

Hepatosomatski indeks (HSI) prikazan je kao omjer mase jetre i ukupne mase tijela ribe (Wootton i sur., 1978):

$$HSI = \frac{\text{masa jetre (g)}}{\text{ukupna masa tijela (g)}} \cdot 100$$

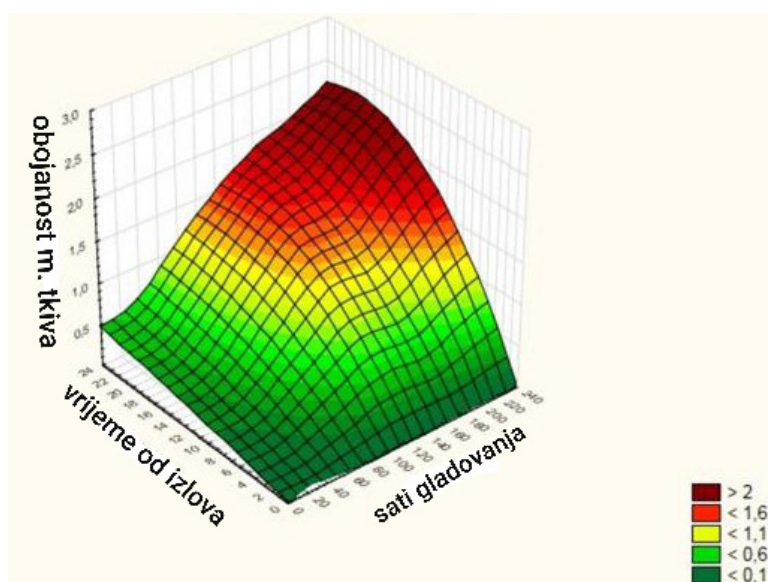
Omjer mase žučnog mjehura i mase žučnog mjehura s jetrom (HI) izračunat je prema Banaee i suradnicima, 2013.

$$HI = \frac{\text{masa žučnog mjehura (g)}}{\text{masa žučnog mjehura (g) + masa jetre (g)}} \cdot 100$$

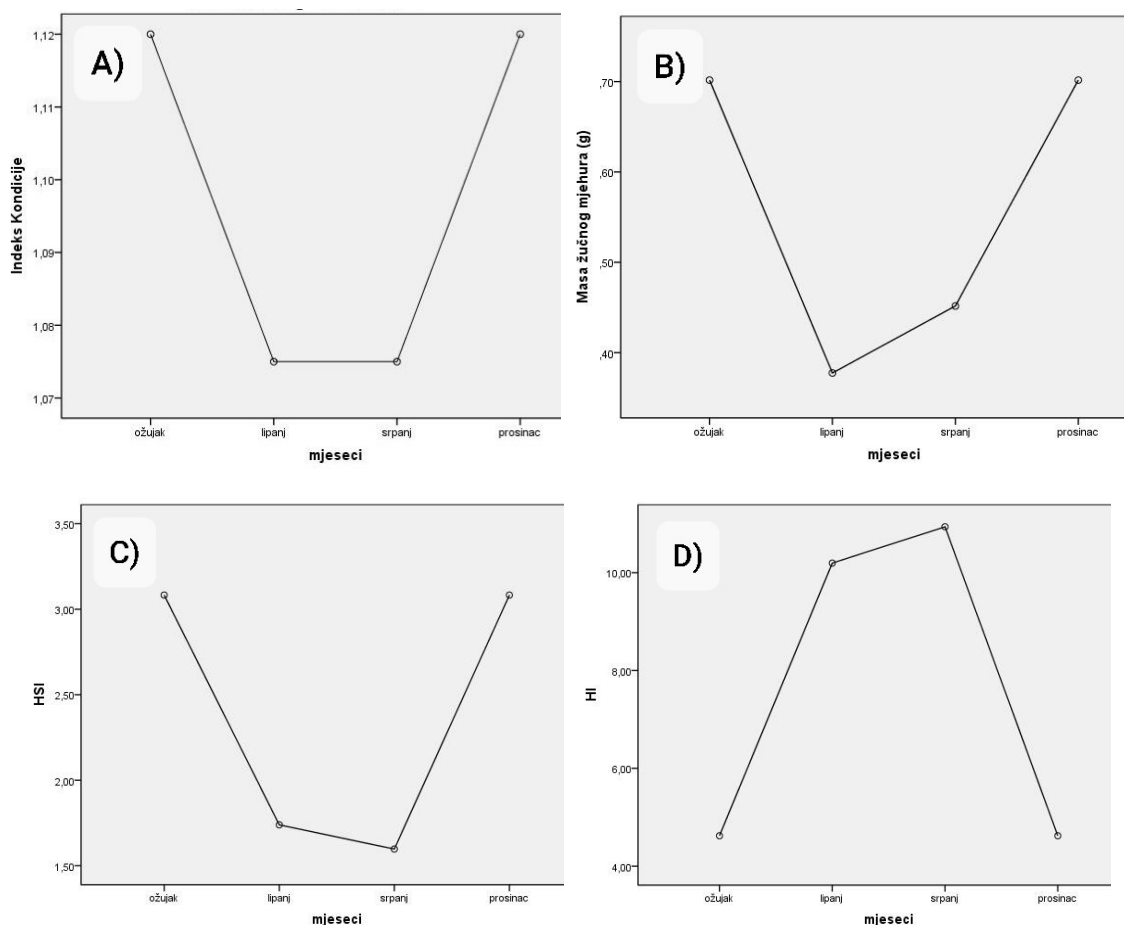
Usporedba navedenih čimbenika sa sezonom (mjesecima) u istraživanju izvršena je regresijskom analizom a rezultati su testirani LSD testom s razinom značajnosti ($p < 0,05$) uz pomoć računalnog programa SPSS ver. 19.0 (IBM, StatSoft., 2010).

Rezultati

Dulje gladovanje prije izlova te dulje vrijeme proteklo od izlova do evisceracije, povezano je sa češćom i izraženijom pojavom obojenosti mišićnog tkiva i obratno, što su te dvije varijable vremenski kraće to je manje riba imalo izraženo obojenje (Slika 1; Tablice 1, 2, 3, 4 i 5 u Dodatku).



Slika 1. Prikaz utjecaja dviju varijabli (sati gladovanja i vremena proteklog od izlova do obrade ribe) na obojenje mišićnog tkiva



Slika 2. Grafički prikaz rezultata A) indeksa kondicije (IK); B) mase žučnog mjehura, C) hepatosomatskog indeksa (HSI) i D) omjera mase žučnog mjehura i mase žučnog mjehura s jetrom (HI) kroz sezone (mjeseci) istraživanja

Temperatura mora prilikom istraživanja je bila: u prvom ciklusu 22.31 °C, drugom 25.38 °C, trećem 16.59 °C i četvrtom 13.35 °C. U 'hladnom periodu godine' (tijekom mjeseca ožujka i prosinca) indeks kondicije je bio veći (1,1-1,2 ±0,58), dok je u toplom periodu (lipanj, srpanj) bio niži (1,07-1,08 ±0,79). Hemosomatski indeks (HSI) u hladnom periodu je bio također veći (2,58-3,08±0,53) u odnosu na topli period (1,6-1,74±0,95). Omjer mase žučnog mjehura i mase žučnog mjehura s jetrom (HI) u toplom periodu je bio veći (10,2-10,94 ±0,96), dok je u hladnom periodu bio niži (4,62-6,43 ±0,96). Indicirana je statistički značajna razlika između mjeseci toplije sezone (lipanj, srpanj) za varijable: indeks kondicije ($p=0,019$), masa žučnog mjehura ($p<0,001$), hepatosomatski indeks ($p<0,001$) te omjer mase žučnog mjehura i mase žučnog mjehura s jetrom (HI) ($p<0,001$).

Rasprava

Pojavnost obojenja je bila prisutna kod svih izmjerenih temperaturnih vrijednosti, odnosno nije utvrđena razlika u obojanosti fileta s obzirom na temperaturu mora. Međutim, temperatura mora je utjecala na povezane varijable koje su utjecale na obojenje mišićnog tkiva, a to su indeks kondicije, hepatosomatski indeks i omjer mase žučnog mjehura i mase žučnog mjehura s jetrom. U hladnom periodu godine indeks kondicije i hepatosomatski indeks su bili veći. Dok je omjer mase žučnog mjehura i mase žučnog mjehura s jetrom u toplom periodu pokazivao višu vrijednost. Zbog bržeg metabolizma u toplom periodu brže nastupaju razlike u masi između jetre i žučnog mjehura, jetra brže gubi na masi, a žučni mjehur se brže puni. Zbog toga je i omjer između njihovih masa veći. Zbog sporijeg metabolizma i smanjene hranidbe žuč se neće previše trošiti tako da je masa žučnog mjehura veća u hladnom periodu (Pointet i Milliet, 2000).

Indeks kondicije pokazuje opće stanje riba, kao i promjene koje se događaju zavisno od lokacije i fizioloških ciklusa u životu riba. On se smanjuje što je duži period gladovanja (Treer, 2008), a kod smanjenog indeksa kondicije smanjuje se i količina visceralnog masnog tkiva koje onemogućuje kontakt žučnog mjehura s mišićnim tkivom. U lipnju i srpnju indeks kondicije bio je najniži nakon perioda niskih temperatura i sniženog metabolizma ribe. Indeks kondicije se povećavao kroz ljetne mjesece kad je ribi dostupna veća količina hrane i kad je temperatura mora pogodna za rast. Indeks kondicije se tako povećavao sve do rujna i zatim postepeno padao prema zimskim mjesecima. Na početku hladnog perioda riba je bila u najboljoj kondiciji.

Žučni mjehur se puni i postaje teži kod riba koje gladuju (Love, 1958), a masa jetre se smanjuje (Bogut i Bavčević, 2016). Prema tome, žučni mjehur više prominira izvan rubova jetre i u većem je kontaktu sa okolinom, te se hepatosomatski indeks smanjuje s duljim gladovanjem, a omjer mase žučnog mjehura i mase žučnog mjehura s jetrom se povećava (Collins i Anderson 1995). U ovom istraživanju u pokusnom kavezu u kojem je riba gladovala, dulji sati gladovanja su utjecali na veću i jaču pojavu obojenja, shodno tome, masa žučnog mjehura se povećavala. Žučni mjehur više prominira izvan rubova jetre i u većem je kontaktu sa okolinom. Suprotno tome, u kontrolnom kavezu gdje se riba hranila svakodnevno, pojavnost obojenja je bilo jako niska. Količina visceralne masti u ribi koja je direktno povezana s indeksom kondicije. Viscelarna mast umanjuje obojenje fileta bez obzira koliko dugo riba leži na ledu. Naime, što je veća količina masti u utrobi ribe veće su šanse da ta mast obuhvati žučni mjehur i tako fizički spriječi njegovo preslikavanje na mišićno tkivo.

Zaključak

U istraživanju je utvrđeno kako gladovanje bitno utječe na obojenje mišićnog tkiva. Što je riba dulje gladovala, veće je obojenje mišićnog tkiva i masa žučnog mjehura. Nadalje, što je dulje trajanje gladovanja i dulje vrijeme proteklo od izlova do obrade ribe, to je bila češća i intenzivnija pojava obojanosti mišićnog tkiva žučnim bojama. Temperatura mora nije bila značajno povezana s obojanošću fileta, ali se njezin utjecaj odražavao na indeks kondicije, hepatosomatski indeks i omjer mase žučnog mjehura i mase žučnog mjehura s jetrom.

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Causes of coloration of muscular tissue at fresh sea bass (*Dicentrarchus labrax*) with bile pigments

Abstract

In the present study, longer fasting was found to affect the occurrence of coloration of muscle tissue and the increase of gallbladder mass of the sea bass. Sea temperature was not related to the coloration of muscular tissue, but it has a influence on the condition index, the hepatosomatic index and the ratio of the gall bladder weight and the weight of the gallbladder with the liver. Visceral fat in the intestines of the fish and around the gallbladder has reduced the coloration of the muscular tissue with bile colors.

Keywords: *Dicentrarchus labrax*, gall bladder, coloration, bile pigments, starvation

Dodatak (Appendix)

Tablica 1. Prikaz postotka obojenosti kontrolnog uzorka između uzorkovanja nakon izlova i nakon držanja na ledu 24 sata

Broj ciklusa	Redni dan uzorkovanja	Uzorkovanje nakon izlova Postotak obojenosti u uzorku				Uzorkovanje nakon 24h na ledu Postotak obojenosti u uzorku			
		nije obojeno	slabo obojeno	srednje obojeno	jako obojeno	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno
1	3	95	5						
	5	100							
	10	65							
2	3	100				60	30	10	
	5	100				70	30		
	10	100	35			60	40		
3	3	100				40	60		
	5	90	10				90	10	
	10	90	10			50	50		
4	3	100				60	20	10	
	5	100				50	30	20	10
	10	100				80	10	10	

Tablica 2. Prikaz postotka obojenosti pokusnog uzorka u prvom ciklusu uzorkovanja

Sati gladovanja	Uzorkovanje nakon izlova			
	Postotak obojenosti u uzorku			
	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno
6	95	5		
12	100			
24	100			
48	85	15		
72	30	65	5	
96	100			
120	90	10		
144	65	30	5	
168	90	10		
192	100			
216	45	55		
240		55	40	5

Tablica 3. Prikaz postotka obojenosti pokusnog uzorka između uzorkovanja nakon izlova i nakon držanja na ledu 24 sata u drugom ciklusu uzorkovanja

Sati gladovanja	Uzorkovanje nakon izlova				Uzorkovanje nakon 24h na ledu			
	Postotak obojenosti u uzorku				Postotak obojenosti u uzorku			
	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno
6	100							
12	100							
24	100				70	20	10	
48	100				20	60	20	
72	100					20	60	30
96	100					20	40	40
120	100					50	30	20
144	100						40	60
168	95	5				10	30	60
192	100					10	60	30
216	100					20	40	40
240	100					20	40	60

Tablica 4. Prikaz postotka obojenosti pokusnog uzorka između uzorkovanja nakon izlova i nakon držanja na ledu 24 sata u trećem ciklusu uzorkovanja

Sati gladovanja	Uzorkovanje nakon izlova Postotak obojenosti u uzorku				Uzorkovanje nakon 24h na ledu Postotak obojenosti u uzorku			
	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno
6	100				100			
12	75	25			100			
24	100				100			
48	100				100			
72	100				30	70		
96	100				20	70	10	
120	100					80	20	
144	100					50	50	
168	100					50	40	10
192	100					10	90	
216	100				10	20	40	30
240	65	35				10	40	50

Tablica 5. Prikaz postotka obojenosti pokusnog uzorka između uzorkovanja nakon izlova i nakon držanja na ledu 24 sata u četvrtom ciklusu uzorkovanja

Sati gladovanja	Uzorkovanje nakon izlova Postotak obojenosti u uzorku				Uzorkovanje nakon 24h na ledu Postotak obojenosti u uzorku			
	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno	nije obojeno	slabo obojeno	srednje obojeno	jako obojeno
6	100				100			
12	80	15		5	100			
24	100				80	10	10	
48	100				50	50		
72	100				60	20	20	
96	100				20	70	10	
120	100				20	40	30	10
144	100				10	30	60	
168	100				10	20	30	30
192	100					50	50	
216	100						90	10
240	100						30	70

Effects of silicate concentrations on the growth of the diatom *Chaetoceros cf. neogracilis* in aquaculture conditions

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Abstract

This study presents the results of cultivation experiments of the diatom *Chaetocheros cf. neogracilis* at varying concentrations of silicates. The growth rate of *C. cf. neogracilis* and the length of each stage of cultivation at different concentrations of silicates were observed. Four experimental groups were grown as starter cultures and then upscaled to experimental stage using standard cultivation protocols and Guillard's F/2 medium differing in levels of added silicate: „S+/+“ and „S+/++“ groups had standard amounts of silicates (1 mg L⁻¹) during the start phase and standard (1 mg L⁻¹) or double (2 mg L⁻¹) during the experimental phase, respectively; „S-/+“ and „S-/++“ groups had no silicates added (0 mg L⁻¹) during the start phase and standard (1 mg L⁻¹) or double (2 mg L⁻¹) during the experimental phase, respectively. All groups showed similar maximum cell concentrations and growth in the lag phase, but an observable trend of higher maximum concentrations in those that had access to silicates within the start cultures (S+/+ and S+/++) was noticed, although differences were not statistically significant. Higher silicate levels in the experimental phase significantly contributed to greater stability of the culture by prolonging the stationary growth phase in groups S+/++ and S-/++ and not causing a sudden decrease in cell concentrations after the culture reached its peak levels as was the case with groups where standard levels of silicates were added (S+/+ and S-/+).

Keywords: phytoplankton, microalgae, stationary phase, concentration peak, culture crash

Introduction

Phytoplankton is intensively used in aquaculture as food for zooplankton, larval stages of most meroplanktonic invertebrates and all life stages of bivalves (FAO, 1996). Diatoms (Bacillariophyceae), especially, are important for the growth and development of bivalves due to their nutritional composition (Gosling, 2003; Arapov et al., 2010; Perneta et al., 2012). They are likely the most diverse of all phytoplankton classes with an estimated 100.000 or more species (Mann et al., 2017). Diatoms differ from other phytoplankton taxa by having cell walls made of silica. This makes silicates a key nutrient for successful proliferation both in nature and culture conditions (Paasche, 1973; Crawford et al., 2001). In a culture environment, there are several stages of microalgae growth (FAO, 1996). The first phase is the lag or induction phase characterized by a slow increase in cell density, likely due to physiological adaptation to the high availability of nutrients. This is followed by an exponential growth phase ending in a decline of relative growth as certain chemical or physical factors start limiting further growth. This marks the onset of the stationary phase which differs in length in different species under different conditions and ends in a death phase that is marked by a rapid decline of cell density leading to a culture crash.

The goal of this study was to examine the potential of a diatom culture deprived of silicates to re-establish itself under conditions of normal and increased silicate levels. Furthermore, the effects of different concentrations of silicates had on the dynamics of microalgal growth was closely observed. As the model organism for this study, the small bilateral centric diatom *Chaetoceros cf. neogracilis* was chosen. They are one of the most commonly used diatoms in aquaculture; hardy species, easy to cultivate that even in nature readily outcompete other species of microalgae when nutrients are plentiful (Simon, 1978).

Materials and methods

The microalgae used in the experiment were obtained from the experimental bivalve hatchery in Institute of Agrifood Research and Technology (IRTA), Sant Carles de la Rapita, Spain. All microalgal stages were cultured using borehole seawater, salinity 35, filtered through 0,45 μm filter. The ambient air temperature was maintained at 19°C and 24 h fluorescent lighting was provided at the intensity of 1000-1500 lux for starter cultures and 2500 lux during the experimental phase. Guillard's *f/2* culture medium was prepared according to Creswell (2010) and added to the water at 1 mg L⁻¹ (Guillard, 1975), with silicates being added in the form of Na₂SiO₃ at concentrations according to the experimental design. Culture vessels were closed off with cotton caps wrapped in sterile gauze. Erlenmeyer flasks of 250 mL were used for start cultures, while aerated 5 L Erlenmeyer flasks were used to culture the microalgae during the experimental phase. Setup and handling of the cultures were performed according to standard procedures of microalgae culture (FAO, 1996), with special care being taken to ensure sterile conditions during the experiment. Starter cultures were inoculated with 30 mL of an existing *C. cf. neogracilis* culture, grown for 7 days and used to inoculate the 5 L experimental cultures at 70 cells μL^{-1} . The experimental cultures were grown for 24 days (until crashing). Half of the starter cultures were given normal levels of silicates (1 mg L⁻¹) and the other half was completely void of silicates. The experimental cultures were given normal levels of silicates (1 mg L⁻¹) or double levels of silicates (2 mg L⁻¹) to create 4 experimental groups, each in duplicate (n=2) (Table 1). Experimental group „S+/+“ (Table 1), which had standard levels of silicates (1 mg L⁻¹) in both the start and experimental phase acted as the control for this experiment.

Table 1. Concentrations of silicates in the four experimental groups during each culture phase.

	Experimental group			
	S+/+	S+/++	S-/+	S-/++
Start culture (250 mL)	1 mg L ⁻¹	1 mg L ⁻¹	0 mg L ⁻¹	0 mg L ⁻¹
Experimental culture (4 L)	1 mg L ⁻¹	2 mg L ⁻¹	1 mg L ⁻¹	2 mg L ⁻¹

Cell concentrations were counted using a Neubauer counting chamber under an Olympus IX71 inverted microscope at the end of the start phase and daily during the experimental phase, taking care to sample at the same time each day. Sampling was performed by taking a subsample from each flask and using it to load the counting chamber, where cells were counted in triplicate. The average values from these counts were used for further statistical analyses.

A one-way ANOVA test was used to determine differences in concentrations between cultures without and with silicates during the start phase (n=8) and between concentration peaks in the experimental phase using the statistical software SPSS v.20 (SPSS Inc., Chicago, IL, USA).

Table 2. Culture dynamics of *C. cf. neogracilis* in each experimental group during the experimental culture phase. Concentration values are presented as MEAN \pm SD (n=2).

	Experimental group			
	S+/+	S+/++	S-/+	S-/++
End of exponential phase (day)	15	15	16	14
Concentration peak (cells μL^{-1})	1220,0 \pm 53,4	1211,1 \pm 251,4	1152,2 \pm 328,4	911,1 \pm 157,1
Duration of stationary phase (days)	0	6	0	6
Concentration during stationary phase (Mean \pm SD; cells μL^{-1})	-	1088,9 \pm 91,3	-	880,9 \pm 29,0
Start of culture crash (day)	16	21	17	20

Results and discussion

Cell concentrations of *C. cf. neogracilis* at the end of the start phase were significantly higher ($p < 0.05$) in flasks containing 1 mg L^{-1} of silicates ($371 \pm 46 \text{ cells } \mu\text{L}^{-1}$) than in those without silicates ($168 \pm 51 \text{ cells } \mu\text{L}^{-1}$) which was to be expected as silicates are key to the proliferation of diatoms (Crawford et al., 2001). Nevertheless, each culture was given an equal start in the experimental phase by adjusting for differences in concentration, with the addition of standard or double levels of silicates. However, peak concentrations in the experimental phase were still observed to be higher in treatments that had access to silicates during the start phase, although not statistically different, while those cultured in mediums deprived of silicates did not reach these levels at any point during the experiment (Table 2) regardless of newly availability of silicates. This could be attributed to the health and physiological readiness of the diatoms to grow and utilize abundant nutrients in the culture medium (FAO, 1996).

On the other hand, regardless of previous silicate availability, it was observed that in experimental treatments with double concentrations of silicates (S+/++ and S-/++) cells had a fast exponential growth phase, quickly reaching their concentration peaks and the phase of declining relative growth which was followed by a long stationary phase that lasted around 6 days (Figure 1; Table 2). In contrast, treatments where standard amounts of silicates were provided during the experimental phase (S-/++ and S+/++), a somewhat longer exponential phase ending in a single concentration peak and no stationary phase were observed (Figure 1; Table 2). These results indicated that increased silicate levels in the culture medium induced longer stationary phases regardless of previous availability of silicates, without the addition of other key nutrients. This is likely because other nutrients that have previously been accumulated into cells can be shared onto daughter cells at cell division, while silicates cannot; new silicates are deposited at each cell division and if there are no available silicates in the culture medium, cell division stalls and a rapid culture crash can follow (Flynn, 2019). Thus, we can speculate that cultures S+/++ and S-/++, which had prolonged stationary phases, were able to maintain relatively stable cell concentrations for 6 days due to higher levels of silicates in the medium, but could not grow further in abundance due to limitations of using cell-incorporated nutrients.

Although nutritional quality and reproductive potential of microalgae is known to decrease during the stationary phase (FAO, 1996), we cannot disregard the benefits of maintaining such a culture, even at somewhat lower nutritional values, before using them as live feed in situations, especially in commercial conditions when harvesting a culture must be postponed.

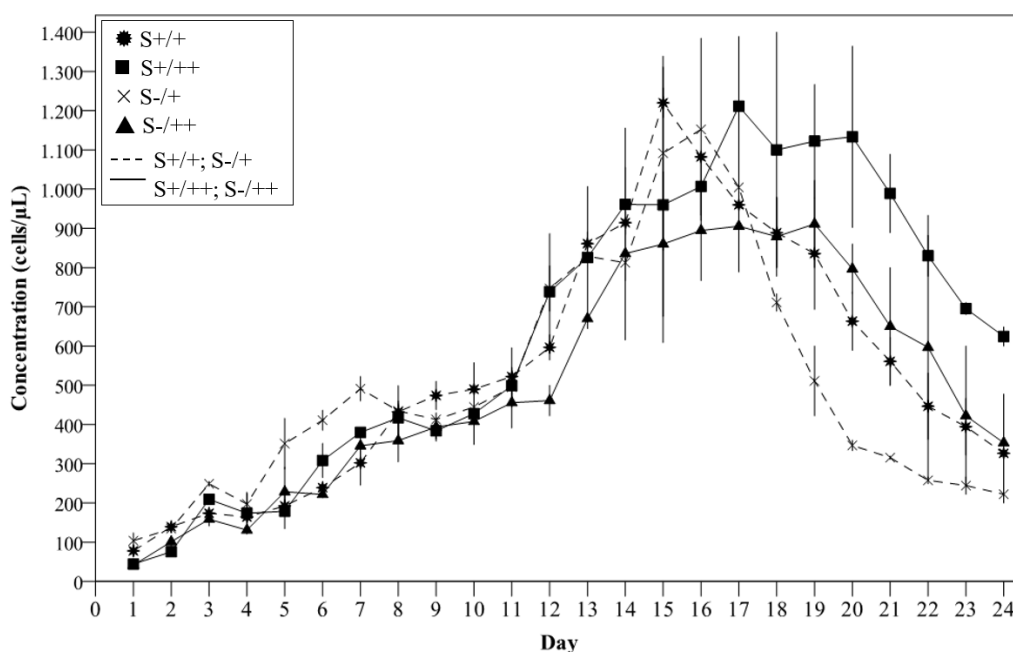


Figure 1. The growth curves of *C. cf. neogracilis* from the 4 experimental treatments. Values are presented as $MEAN \pm SE$ ($n=2$).

Conclusions

These results may contribute to better management of diatom phytoplankton cultures in a commercial aquaculture setting if cultures cannot be harvested at the moment of their peak concentration. By adding double amounts of silicates to that proposed by standard procedures, culture life of *C. cf. neogracilis* can be extended by six days, as observed under conditions used in this study. We can also speculate that adding additional silicates several days after stocking algae, but before the peak of the exponential phase would also result in extending the life of a culture, so these measures could be used as a last resort and not standard practise.

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Utjecaj različitih koncentracija silikata na abundanciju dijatomeje *Chaetoceros cf. neogracilis* (Schutt, 1985) u uzgojnim uvjetima

Sažetak

Ovaj rad predstavlja rezultate uzgoja dijatomeje *Chaetoceros cf. neogracilis* pri različitim koncentracijama silikata. Opisani su rast i duljina svake faze uzgoja pri različitim i promjenjivim razinama silikata. Korištene su 4 eksperimentalne grupe koje su prvotno uzgajane u početnim kulturama i kasnije prebačene u veće eksperimentalne kulture pri standardnim uzgojnim uvjetima koristeći Guillardov F/2 hranjivi medij, ali s različitim koncentracijama silikata: grupe „S+/+“ i „S+/++“ imale su standardne koncentracije silikata tijekom početnog dijela uzgoja (1 mg L^{-1}) i standardne (1 mg L^{-1}), odnosno duple (2 mg L^{-1}) koncentracije silikata tijekom eksperimentalnog dijela; grupe „S-/+“ i „S-/++“ nisu imale silikata u hranjivom mediju tijekom početnog dijela uzgoja (0 mg L^{-1}), ali su im dodane standardne (1 mg L^{-1}), odnosno duple (2 mg L^{-1}) koncentracije silikata tijekom eksperimentalnog dijela. Sve grupe su pokazale sličan rast tijekom „lag“ faze rasta, no grupe koje su imale pristup silikatima u početnim kulturama imale su nešto više najveće koncentracije (S+/+ i S+/++), iako rezultati nisu bili statistički značajni. U eksperimentalnim kulturama, povećana koncentracija silikata doprinjela je povećanoj stabilnosti kulture produljenjem stacionarne faze rasta u grupama S+/++ i S-/++, za razliku od kolapsa kulture zabilježene neposredno nakon postizanja najveće koncentracije alga što je zabilježeno u grupama sa standardnom koncentracijom silikata (S+/+ i S-/+).

Ključne riječi: fitoplankton, mikroalge, stacionarna faza, peak koncentracije, pad kulture

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Stočarstvo

Čimbenici plodnosti krmača banijske šare svinje

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Sažetak

Cilj rada je bio utvrditi i analizirati čimbenike plodnosti banijske šare svinje. Istraživanje je provedeno na 298 zapisa o prasnju krmača banijske šare svinje prikupljenih na 136 životinja u razdoblju od 2014. do lipnja 2019. godine. Statističkom analizom podataka utvrđen je značajan utjecaj sezone prasnja, gospodarstva te rednog broja prasnja, dok utjecaj nerasta nije bio statistički značajan. Utvrđen je postupni rast broja živooprasene prasadi nakon svakog sljedećeg prasnja sve do petog prasnja. Utvrđene razlike u broju živooprasene prasadi između gospodarstava kreću od 4 do 6 prasadi u leglu, što upućuje na razlike u tehnologiji i uvjetima držanja svinja. Poboljšanjem plodnosti banijske šare, selekcijom i tehnološkim postupcima može se utjecati na ekonomičnost uzgoja ove pasmine.

Ključne riječi: svinje, banijska šara svinja, veličina legla

Uvod

Banijska šara svinja hrvatska je autohtona pasmina koja je 2018. godine uvrštena na Popis pasmina, sojeva i hibrida domaćih životinja koje se uzgajaju u Republici Hrvatskoj i Popis izvornih i zaštićenih pasmina domaćih životinja (Škorput i sur., 2019). Nastanak banijske šare svinje, koji seže u 19. stoljeće, vezan je uz nastojanja da se unaprijeđe tadašnje domaće populacije svinja uvođenjem stranih pasmina boljih proizvodnih svojstava, poput njemačke oplemenjene svinje ili berkšira. Stoga su u vrijeme nastanka krmače ove pasmine imale visoki potencijal za plodnost, što potvrđuju Šram i sur. (1950), navodeći kako krmače banijske šare svinje po plodnosti odstupaju od drugih domaćih pasmina. Unatoč dobrim proizvodnim i reproduktivnim svojstvima, uvozom plemenitih, visoko selekcioniranih pasmina smanjuje se broj životinja ove pasmine, te je početkom stoljeća preostalo vrlo malo primjeraka. Kroz proces revitalizacije uzgoja banijske šare svinje koji je započeo 2014. godine povećan je broj jedinki (Salajpal i sur., 2017), te se posljednjih godina broj rasplodnih životinja u populaciji banijske šare ustalio. U 2018. godini populaciju je sačinjavalo 96 krmača i 28 nerastova (Ministarstvo poljoprivrede, 2019).

Plodnost je ekonomski važno svojstvo u svinjogojskoj proizvodnji, jer izravno utječe na ekonomičnost same proizvodnje. Stoga je upravljanje reprodukcijom u stadu od velike važnosti za postizanje ekonomične proizvodnje. Tijekom razdoblja revitalizacije banijske šare svinje uspostavljena je baza podataka za plodnost, s ciljem dobivanja informacija potrebnih za upravljanje pasminom i selekciju. S obzirom na navedeno, cilj rada je bio analizirati plodnost banijske šare svinje, te analizirati čimbenike koji utječu na veličinu legla.

Materijal i metode

Podaci

Podatke za analizu ustupilo je Ministarstvo poljoprivrede. Niz podataka sadržavao je 298 zapisa o plodnosti krmača banijske šare svinje prikupljenih na 136 životinja u razdoblju od 2014. do lipnja 2019. godine. Niz podataka sadržavao je sljedeće varijable: uzgojnu organizaciju životinje, prema podacima iz područnih ureda Hrvatske agencije za poljoprivredu i hranu; identifikacijski broj životinje; identifikacijski broj stada; redni broj prasnja krmače; datum prasnja; identifikacijski broj nerasta – oca legla; datum pripusta; broj živooprasene prasadi. Zbog malog broja životinja koje su imale više od 5 prasnja, peto prasnje spojeno je sa podacima iz sljedećih prasnja.

Statistička analiza

Statistička analiza provedena je pomoću programskog paketa SAS 9.4. (SAS Inc, 2013). Osnovna statistika dobivena je korištenjem procedure MEANS, dok je utjecaj pojedinih čimbenika na plodnost banijske šare svinje ispitan korištenjem GLM procedure. Za analizu utjecaja pojedinih čimbenika na veličinu legla korišten je sljedeći statistički model:

$$y_{ijkl} = \mu + S_i + H_j + P_k + B_l + e_{ijkl}$$

gdje je:

y_{ijkl} - vektor opažanja za veličinu legla;

S_i - fiksni utjecaj sezone prasnjenja, definirane kao interakcija mjeseca i godine pripusta;

H_j - fiksni utjecaj gospodarstva

P_k - fiksni utjecaj rednog broja prasnjenja

B_l - fiksni utjecaj nerasta – oca legla.

Izbor sistemskih utjecaja u modelu temelji se na značajnosti utjecaja, koeficijentu determinacije i stupnjevima slobode. Rezultati su prikazani kao srednje vrijednosti dobivene metodom najmanjih kvadrata (LSMEAN) ili kao odstupanja LSMEAN vrijednosti od prosjeka populacije u obliku grafikona.

Rezultati i rasprava

Osnovna statistika za broj **živooprasene** prasadi u populaciji banijske šare svinje prikazana je u Tablici 1. Uočena vrijednost upućuje na nisku prosječnu plodnost banijske šare svinje. Međutim, uočen je visoki koeficijent varijabilnosti za analizirano svojstvo, što je posljedica velikih razlika u veličini legla različitih krmača i legala.

Tablica 1. Osnovna statistika za broj živorođene prasadi u populaciji banijske šare svinje

Broj	Srednja vrijednost	Koeficijent varijabilnosti	Standardna devijacija	Minimum	Maksimum
298	7,06	33,59%	2,37	0	13

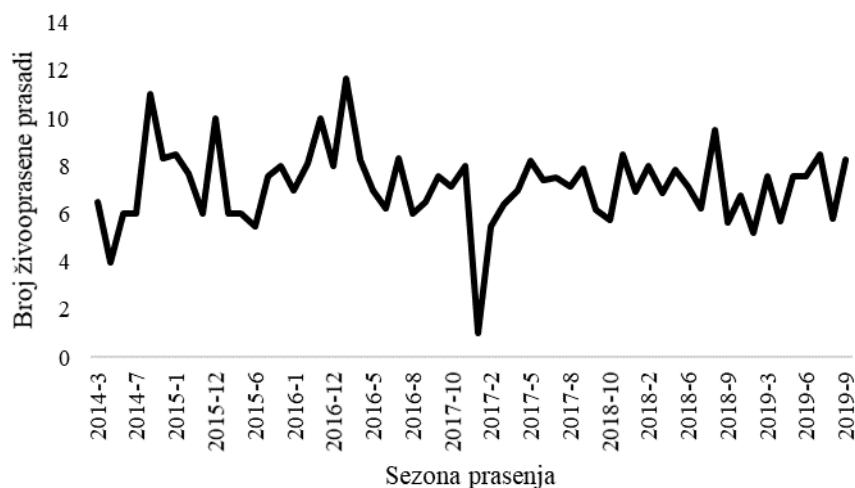
Prosječna plodnost banijske šare svinje veća je nego u crne slavonske i turopoljske svinje, koja u prosjeku iznosi 6,12 za crnu slavonsku svinju (Škorput i sur., 2014) i 4,47 za turopoljsku svinju (Karolyi i sur., 2018).

Utjecaj sezone prasnjenja na veličinu legla bio je statistički značaj (Tablica 2). Također, statistički značajan utjecaj na broj živooprasene prasadi imali su i redni broj prasnjenja te gospodarstvo. Utjecaj nerasta – oca legla – nije bio statistički značajan.

Tablica 2. Analiza značajnosti utjecaja u modelu za broj živooprasene prasadi banijske šare svinje

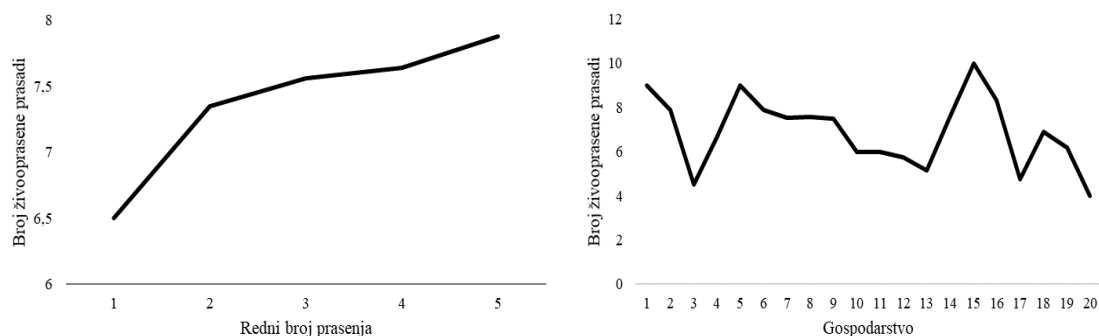
Utjecaj	Stupnjevi slobode	Srednja suma kvadrata	F vrijednost	Pr>F
Sezona	57	7,5717438	2,04	0,0009
Redni broj prasnjenja	4	12,913260	3,53	0,0100
Gospodarstvo	19	9,7083864	2,61	0,0013
Nerast	28	4,4869439	1,21	0,6419

Utjecaj sezone prasnja na broj živooprasene prasadi u krmača i nazimica banijske šare svinje prikazan je u Grafikonu 1. Uočena je visoka varijabilnost u broju živooprasene prasadi između sezona. Sezonske razlike u plodnosti svinja posljedice su kratkoročnih klimatskih promjena tijekom godine, kao i dugoročnih promjena uvjetovanih promjenama tehnološkog procesa tijekom vremena (Clark i Lehman, 1986; Quesnel i sur., 2005). Posebno oštar pad vidljiv je početkom 2017. godine, što se može pripisati ekstremnim klimatskim uvjetima, te jako izraženoj pojavi sezonske neplodnosti. Dobiveni rezultati su u skladu s rezultatima Malovrh i sur. (1996), koji su utvrdili značajan utjecaj sezone na veličinu legla. Pri tome promjene u veličini legla nisu bile periodičke, što upućuje da uzroci tih promjena leže i u drugim okolišnim uvjetima, kao što su hranidba i upravljanje uzgojem. Menčík i sur. (2015) utvrdili su značajan utjecaj godišnjeg doba prasnja na broj živooprasene prasadi crne slavonske svinje, koja rezultira manjim leglima u zimskom periodu, pripisujući te razlike sezonskoj neplodnosti svinja.



Grafikon 1. Utjecaj sezone na broj živooprasene prasadi u krmača i nazimica banijske šare svinje

Najveći broj prasadi u leglima banijske šare svinje uočen je u petom prasnju, te nema očekivanog pada nakon četvrtog prasnja (Grafikon 2a). Ovaj se zaključak temelji na manjem broju podataka iz legala s višim rednim brojem prasnja. Uz to, djelomično se takav rezultat može pripisati i osrednjoj plodnosti banijske šare svinje, zbog čega nakon postizanja maksimalne plodnosti u trećem ili četvrtom leglu nema izraženog pada u veličini legla, kao što je slučaj u plemenitim pasmina (Vincek i sur., 2005; Freyer, 2018).



Grafikon 2a i 2b. Utjecaj rednog broja prasnja i gospodarstva na broj živooprasene prasadi u krmača i nazimica banijske šare svinje

Uočena je iznimno velika varijabilnost između gospodarstava u broju živooprasene prasadi u analiziranoj populaciji (Grafikon 2b). Visoka varijabilnost između gospodarstava ukazuje na visok genetski potencijal za veličinu legla,

međutim njegovo ostvarivanje iziskuje određene smještajne i mikroklimatske uvjete. Također, različite prakse pri otkrivanju estrusa te upravljanje laktacijom i odbićem značajno mogu utjecati na broj živooprasene prasadi u leglu (Luković i sur., 2006). Gospodarstva na kojima se drže krmače i nazimice banijske šare svinje pripadaju različitim sustavima, te uvjeti variraju od ekstenzivnog načina držanja na otvorenom, do intenzivnog načina držanja svinja. Menčik i sur. (2015) navode da je utjecaj sezone prasnja na veličinu legla posebno izražen u otvorenim sustavima držanja.

Zaključak

Sezona pripusta, redni broj prasnja, te gospodarstvo imali su statistički značajan utjecaj na veličinu legla banijske šare svinje, dok utjecaj nerasta – oca legla - nije utvrđen. Banijska šara svinja dostiže maksimalnu plodnost do petog legla, te nema veći pad veličine legla nakon četvrtog prasnja, što se može pripisati i osrednjoj plodnosti te manjem broju podataka kod višeg rednog broja prasnja. Značajne razlike u veličini legla uočene su između gospodarstava zbog korištenja različitih tehnologija i načina uzgoja. S obzirom na navedeno, postoji prostor za povećanje prosječne veličine legla banijske šare svinje, prije svega unaprjeđenjem tehnoloških postupaka u uzgoju, čime se može utjecati na ekonomičnost uzgoja banijske šare svinje.

Napomena

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Factors influencing litter size sows in Banija spotted pig

Abstract

The aim of the study was to determine the factors that influence litter size in Banija spotted pig population. The study was conducted on 298 farrowing records from 136 Banija spotted sows collected between 2014 and June 2019. Statistical analysis revealed statistically significant effect of the farrowing season, herd, and parity, while the impact of boar was not statistically significant. A gradual increase in the number of live piglets was observed after each subsequent farrowing until the fifth parity. Significant differences in the number of liveborn piglets between herds were found, indicating differences in technology and production conditions. The differences range from 4 to 6 piglets per litter. Improving litter size in Banija spotted pig may positively affect the breeding efficiency of this breed.

Keywords: Pigs, Banija spotted pig, number of piglets born alive

Fenotipske odlike različitih dobnih kategorija hrvatske bijele koze

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Sažetak

Cilj istraživanja bio je utvrditi fenotipske odlike različitih dobnih kategorija hrvatske bijele koze te ih usporediti s našim izvornim pasminama koza. Istraživanje je provedeno na 42 hrvatske bijele koze podijeljene u dobne skupine (<2, 3 - 5 i > 5 godina), a obuhvaćalo je uzimanje tjelesnih mjera i vaganje koza. Utvrđen je značajan utjecaj dobi na tjelesnu masu (39,14 kg - 46,05 kg), dužinu trupa (62,43 cm - 66,32 cm) i opseg prsa (77,71 cm - 84,77 cm) te indekse anamorfoznosti, tjelesnih proporcija i mišićavosti. Ostale fenotipske odlike hrvatske bijele koze povećavale su se sukladno dobi, ali razlike nisu bile značajne. Usporedbom hrvatske bijele koze s našim izvornim pasminama koza uočeno je da su one manjeg tjelesnog okvira.

Ključne riječi: koze, dob, tjelesne mjere, indeksi tjelesne razvijenosti

Uvod

Prepoznavanje, očuvanje i poticanje uzgoja specifičnih pasmina domaćih životinja za određeno uzgojno područje i zemlju mjere su koje je obvezno provoditi iz zootehničkih, etičkih, kulturno-znanstvenih i drugih razloga, a sve u cilju očuvanja vlastitog identiteta (Antunović i sur., 2007). Prema podacima FAO (2015) u Svijetu se ukupno uzgaja 576 lokalnih pasmina koza, dok je taj broj u Europi s Kavkazom 218. Prema podacima Eurostat-a u 2018. godini se u Republici Hrvatskoj uzgajalo 80.000 koza, a analizom brojnog stanja koza u zadnjih 5 godina vidljiv je značajan porast (za 31,7%). U Hrvatskoj se uzgaja šest pasmina koza, od kojih su tri izvorne pasmine gdje ubrajamo i hrvatsku bijelu kožu. Prema podacima Ministarstva poljoprivrede procijenjena populacija hrvatske bijele koze je 5000 grla, a uzgojno valjana populacija je relativno mala i iznosi 227 grla kod 8 uzgajivača (198 koza, 14 jarica i 15 jarčeva). Prosječna veličina stada hrvatske bijele koze je 28 grla. U ukupnoj uzgojno valjanoj populaciji koza u Hrvatskoj udio uzgojno valjanih hrvatskih bijelih koza je svega 3,07% (Ministarstvo poljoprivrede, 2019). Stoga je obvezno povećanje broja hrvatske bijele koze pod selekcijskim obuhvatom, uz kvalitetniju organizaciju uzgoja i praćenje proizvodnih svojstava, a što bi dodatno pomoglo u samoodrživosti uzgoja ove pasmine (Antunović i sur., 2018). Iako se hrvatska bijela koza ubraja u kombinirane pasmine (meso-mlijeko) još uvijek je njen glavni proizvod jareće meso. Koze imaju bijelu boju dlake koja je nešto duža u odnosu na europske bijele pasmine koza. Pretpostavlja se da je nastala u procesu popravljanju mliječnosti kroz oplemenjivanje domaćih autohtonih koza s uvezenim im inozemnim jarčevima, najčešće sanske pasmine. Hrvatska bijela koza je otporna, prilagodljiva, vrlo pokretna i živahna koza. Prosječna tjelesna masa hrvatske bijele koze je od 35 do 45 kg s prosječnom plodnosti 150%. U laktaciji od 250 do 280 dana daje 250 – 350 l mlijeka (Mioč i Pavić, 2002). Prema podacima Ministarstva poljoprivrede (2019.) u populaciji uzgojno valjanih hrvatskih bijelih koza indeks jarenja je 1,0 a veličina legla 1,14. U performance testu odabrane muške jaradi u „field“ uvjetima utvrđena je prosječna porodna masa od 2,15 kg, prosječni dnevni prirast 209 g te završna tjelesna masa 25 kg.

Cilj ovoga rada bio je utvrditi fenotipske odlike različitih dobnih kategorija hrvatske bijele koze te ih usporediti s rezultatima dosadašnjih istraživanja provedenih na hrvatskim izvornim pasminama koza.

Materijal i metode

Istraživanje je provedeno na 42 hrvatske bijele koze na obiteljskom gospodarstvu iz Šibensko-kninske županije, koje

se dugi niz godina bavi uzgojem ove pasmine koza. Koze su bile podijeljene na osnovi dobi u tri dobne skupine i to: do dobi 2 godine, od 3 do 5 godina i starije od 5 godina. Koze su boravile na ispaši, a po povratku u staju dobivale su sijeno i 0,25 kg smjese krmiva (60% kukuruz, 30% ječam i 10% pšenične posije) te su na raspolaganju imale stočnu sol i vodu. Utvrđivanje tjelesnih mjera koza provedeno je uz pomoć Lydtinova štapa i mjerne vrpce, a određivanje tjelesne mase izvršeno je pomoću stočne vage. Izmjerama su bile obuhvaćene sljedeće fenotipske odlike: visina grebena, dužina trupa, širina prsa, opseg prsa, dubina prsa i opseg cjevanice. Nakon toga su izračunati indeksi anamorfoznosti i tjelesnih proporcija prema Chiofalo i sur. (2004) te indeks tjelesne kompaktnosti, mišićavosti, prsa i širine prsa prema Činkulov i sur. (2003):

Indeks anamorfoznosti: $(\text{opseg prsa, cm})^2 / \text{visina grebena, cm}$;

Indeks tjelesnih proporcija: $(\text{tjelesna masa, kg} / \text{visina grebena, cm}) \times 100$;

Indeks tjelesne kompaktnosti: $(\text{opseg prsa, cm} / \text{dužina trupa, cm}) \times 100$;

Indeks mišićavosti: $(\text{opseg prsa, cm} / \text{visina grebena, cm}) \times 100$.

Indeks prsa: $(\text{širina prsa, cm} / \text{dubina prsa, cm}) \times 100$

Indeks širine prsa: $(\text{širina prsa, cm} / \text{visina grebena, cm}) \times 100$.

Dobiveni rezultati su statistički obrađeni pomoću računalnog statističkog programa SAS/STAT 9.3 (2013) te prikazani kao srednja vrijednost, standardna devijacija i ukupna standardna greška. Podaci su analizirani GLM procedurom, a razlika između srednjih vrijednosti je utvrđena Tukey testom na razini značajnosti $P < 0,05$.

Rezultati i rasprava

U tablici 1 prikazani su rezultati opisnih statističkih pokazatelja analiziranih fenotipskih odlika hrvatske bijele koze.

Tablica 1. Rezultati opisnih statističkih pokazatelja analiziranih fenotipskih odlika hrvatske bijele koze

Odlika	Srednja vrijednost	SD	Min	Max	CV(%)
Tjelesna masa, kg	44,87	4,81	29,00	52,00	10,72
Visina grebena, cm	64,29	2,56	59,00	69,00	3,98
Dužina trupa, cm	65,24	2,57	60,00	69,00	3,93
Širina prsa, cm	16,24	2,30	12,00	20,00	14,16
Dubina prsa, cm	27,63	1,89	24,00	32,00	6,85
Opseg prsa, cm	83,34	5,60	64,00	94,00	6,72
Opseg cjevanice, cm	8,66	0,49	8,00	9,00	5,83
Indeks anamorfoznosti	108,32	11,97	69,42	129,94	11,05
Indeks tjelesnih proporcija	69,75	6,40	46,03	79,03	9,18
Indeks prsa	58,71	6,64	44,44	68,97	11,32
Indeks širine prsa	25,24	3,20	19,05	30,77	12,66
Indeks tjelesne kompaktnosti	127,71	6,38	100,00	136,23	5,00
Indeks mišićavosti	129,61	6,81	108,47	141,94	5,26

SD-standardna devijacija; Min i Max- minimalna i maksimalna vrijednost; CV-koeficijent varijacije

Uspoređujući ove rezultate sa standardima fenotipskih odlika hrvatske bijele koze navedene u Programu uzgoja koza (Mioč i sur., 2012), prema kojem je prosječna tjelesna masa hrvatske bijele koze 35-45 kg, visina grebena 58 cm, dužina trupa 66,7 cm, širina prsa 19,1 cm, dubina prsa 30,2 cm, opseg prsa 83,9 cm a opseg cjevanice 8,2 cm vidljivo je da su one bile relativno podjednake.

U tablici 2. prikazane su fenotipske odlike različitih dobnih kategorija hrvatske bijele koze.

Tablica 2. Fenotipske odlike različitih dobnih kategorija hrvatske bijele koze

Odlika	Dobne kategorije, godine			SEM	P-vrijednost	Promjena < 2 - > 5
	< 2	3 - 5	> 5			
	Srednja vrijednost					
Tjelesna masa, kg	39,14 ^b	46,08 ^a	46,05 ^a	0,75	0,001	+17,65
Visina grebena, cm	62,86	65,08	64,32	0,40	0,190	+2,32
Dužina trupa, cm	62,43 ^b	64,92 ^{ab}	66,32 ^a	0,40	<0,001	+6,23
Opseg prsa, cm	77,71 ^b	84,00 ^a	84,77 ^a	0,87	0,010	+13,12
Dubina prsa, cm	26,57	26,92	28,36	0,30	0,050	+6,74
Širina prsa, cm	14,71	16,42	16,64	0,36	0,150	+9,16
Opseg cjevanice, cm	8,14	8,25	8,50	0,08	0,150	+4,42

SEM = srednja standardna greška; ^{a,b} P<0,05

Analizirajući tablicu 2 vidljivo je da je dob imala značajan utjecaj na većinu tjelesnih mjera i tjelesnu masu hrvatske bijele koze. Utvrđena je značajno veća tjelesna masa u odraslih koza (> 5 godina) u odnosu na prvojarice (< 2 godine) kao i dužina trupa i opseg prsa. Utvrđena visina grebena te širina i dubina prsa kao i opseg cjevanice također su bili veći u odraslih koza ali razlike nisu bile značajne ovisno o njihovoj dobi. Prvojarice ove pasmine su ostvarile 82,35% tjelesne mase, 97,68% visine grebena, 93,77% dužine trupa te od 86,88% opsega prsa odraslih koza (>5 godina). U istraživanju Antunovića i sur. (2019) provedenim na istarskim kozama utvrđeno je da su mlade koze (<2 godine) postigle 87,45% tjelesne mase, 92,68% visine grebena te 94,28% dužine trupa odraslih koza (>5 godina). Mlade hrvatske šarene koze prosječne dobi 13 mjeseci (od 9 do 18 mjeseci) ostvarile su 64,2% tjelesne mase, 89,1% visine grebena te 88,1% dužine trupa u odnosu na odrasle koze (> 3,5 godina; Mioč i sur., 2008).

Analizom navedenih fenotipskih odlika hrvatske bijele koze vidljivo je da je tjelesna masa varijabilna te ovisi o puno čimbenika poput genetskih obilježja, hranidbe i dr. Ostale tjelesne mjere koze povećavale su se sukladno dobi. Navedeno ukazuje na kasnozrelost hrvatske bijele koze.

Usporedbom rezultata predmetnih istraživanja s fenotipskim odlikama hrvatske šarene koze (Mioč i sur., 2008), vidljivo je da su odrasle hrvatske bijele koze teže za 2,04 kg i višlje u grebenu za 3 cm, ali su imale kraći trup za 2,74 cm, uža i plića prsa za 1,25 cm i 2,28 cm te sličan opseg prsa i cjevanice. Usporedimo li fenotipske odlike hrvatske bijele koze s odlikama istarske koze (Antunović i sur., 2019) vidljivo je da je ona značajno manjih tjelesnih okvira. Naime, u navedenom istraživanju utvrđena je prosječna tjelesna masa istarske koze 65,67 kg, visina grebena 69,63 cm a dužina trupa 79,73 cm.

U tablici 3 prikazane su indeksi tjelesne razvijenosti hrvatske bijele koze. Odrasle koze (> 5 godina) imale su značajno veće indekse anamorfoznosti, tjelesnih proporcija i mišićavosti za razliku od prvojarica (< 2 godine). Međutim, iako su utvrđeni i veći indeksi prsa, širina prsa i tjelesne kompaktnosti u odraslih koza u odnosu na prvojarice (< 2 godine) nije bilo statističkih razlika.

Uspoređujući hrvatsku bijelu kozu s ostalim našim izvornim pasminama koza vidljivo je također da je ona manjeg tjelesnog okvira od istarske koze i hrvatske šarene koze. Indeksi tjelesne razvijenosti hrvatske bijele koze bili su znatno lošiji u usporedbi s indeksima istarske koze, osim indeksa kompaktnosti, koji su prikazani u istraživanju Antunovića i sur. (2019). Navedene fenotipske odlike hrvatske bijele koze u usporedbi s našim izvornim pasminama koza ukazuju na manje tjelesne okvire i nešto kompaktniju građu tijela.

Tablica 3. Indeksi tjelesne razvijenosti različitih dobnih kategorija hrvatske bijele koze

Pokazatelj	Dobne kategorije, godine			SEM	P -vrijednost
	< 2.	3.- 5.	> 5.		
	Srednja vrijednost				
Indeks anamorfoznosti	96,25 ^b	108,64 ^{ab}	111,99 ^a	0,87	0,007
Indeks tjelesnih proporcija	62,44 ^b	70,75 ^a	71,52 ^a	1,00	0,002
Indeks tjelesne kompaktnosti	124,45	129,44	127,80	1,00	0,264
Indeks mišićavosti	123,72 ^b	129,16 ^{ab}	131,73 ^a	1,06	0,020
Indeks prsa	55,42	60,95	58,53	1,04	0,216
Indeks širine prsa	23,40	25,27	25,80	0,50	0,226

Mean = srednja vrijednost; SEM = srednja standardna greška; ^{a,b} P<0,05

Zaključak

Na temelju dobivenih rezultata može se zaključiti da utvrđene fenotipske odlike hrvatske bijele koze ukazuju na podudarnost s onima navedenim u Programu za uzgoj koza. Utvrđeno je da su prosječna tjelesna masa, dužina trupa i opseg prsa hrvatskih bijelih koza starijih od pet godina značajno veći, kao i indeksi anamorfoznosti, tjelesnih proporcija i mišićavosti u odnosu na koze u dobi do dvije godine. Ostale fenotipske odlike koza nisu se značajno razlikovale s povećanjem dobi. Usporedimo li je s našim izvornim pasminama (hrvatskom šarenom i istarskom kozom) može se zaključiti da je hrvatska bijela koza manjih tjelesnih okvira, ali nešto kompaktnije građe.

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Phenotypic traits of Croatian white goats in various ages

The aim of this research was to determine the phenotypic characteristics of Croatian white goats in various ages and to compare them with local breeds of goats. The study was conducted with 42 does of Croatian white goat divided according to the age (<2, 3 - 5 and >5 years). Body measures were carried out as well as body weight of does. The significant effect of age on body weight (39.14-46.05 kg), body length (62.43-66.32 cm), chest circumference (77.71-84.77 cm), anamorphosis index, body proportions and muscularity indices were determined. Other phenotypic characteristics of Croatian white goat have increased with the age, although differences were not significant. Comparing Croatian white goat with Croatian local breeds, it is evident that it has smaller body frame.

Keywords: goats, age, body measures, indices of body development

Pokazatelji proizvodnje mesa križevačke kukmaste kokoši

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Sažetak

Istraživanje je provedeno na 203 pilića izvorne hrvatske pasmine križevačka kukmasta kokoš s ciljem utvrđivanja pokazatelja proizvodnje mesa te morfometrijskih svojstava jedinki starosti 5 mjeseci. Prosječna živa masa pijetlova na kraju istraživanja bila je 2738, a kokoši 1856 grama. Prosječna konverzija krmne smjese u prvih 8 tjedana iznosila je 3,56, a za razdoblje od 8. do 20. tjedna starosti 7,15 dok je mortalitet po spomenutim fazama iznosio 3% odnosno 11%. Prosječan randman klanja iznosio je 78,53%, udio prsa 20,18%, filea 11,52%, bataka 14,27% te zabataka 14,59%. Prosječne vrijednosti morfometrijskih svojstava bile su redom: duljina trupa 20,60; prsne kosti 14,49; batka 15,86; piska 11,92; glave 8,11 i kljuna 2,08 potom širina trupa 7,36; glave 3,26 i piska 1,45 te dubina prsiju 12,95 centimetara.

Ključne riječi: izvorne pasmine peradi, križevačka kukmasta kokoš, morfometrijska svojstva, pokazatelji proizvodnje mesa

Uvod

Križevačka kukmasta kokoš nastala je osamdesetih godina prošlog stoljeća selekcijskim radom na domaćim kokošima uzgajanim u Kalničkom prigorju. Spomenute domaće kokoši karakterizirala je osrednja kukmica te srednje teško tijelo, a planski su križane sa pijetlovima orpington pasmine. Prema Pintiće i sur. (2008) uzgoj su započeli supružnici Josip i Katica Vojta iz Križevaca 1948. godine. Već 1993. godine pasmina je promovirana i prvi put prikazana na izložbi malih životinja u Križevcima. Prema tadašnjem opisu pasminu karakterizira bujno perje žute boje, crno obrubljeno. Tijelo je kvadratično, skladno, a pijetlovi su posebno ponosnog držanja. Glava je srednje veličine. Na njoj se nalazi kukmica narančasto-žute boje kod pijetlova, a crno-smeđa kod kokoši. Krijesta je jednostavna, mesnata i nazubljena, djelomično ili potpuno viseća. Podušnjaci su crveni, veliki i izduženi kod pijetlova dok su kod kokoši mali, gotovo neugledni. Podbradnjaci su kod pijetlova također veliki i crveni, a kod kokoši mali i okruglasti. Oči su narančasto-žute, a kljun je jak, kratak i malo povijen, boje slonove kosti. Noge su kratke, kod pijetlova svijetle boje, a kod kokoši nešto tamnije nijanse. Vrat je kod pijetlova srednje dug, dobro obrastao perjem pa djeluje poput grive narančasto-žute boje. Kod kokoši je kratak, a perje crno-smeđe boje. Leđa su sedlasta i kod kokoši kratka. Pokrovno perje je žuto, obrubljeno tamnom, gotovo crnom bojom. Rep je srpastog izgleda s mnogo srpastog perja narančaste boje, kod nekih primjeraka pomiješan s bijelom bojom. Kod kokoši je rep malen i tamno-smeđe boje. Prsa su široka, dobro pokrivena tamnosmeđim do crnim perjem. Pokrovno perje je žuto obrubljeno tamnom bojom, a kod kokoši izgleda kao krljušt. Bataci su mesnati, obrasli perjem crno-smeđe boje. Krila su srednje veličine, dobro priljubljena uz tijelo. Ima čvrstu konstituciju i mirnog je temperamenta. Meštrović i sur. (2015) uz opis pasmine navode i tjelesne mase kokoši i pijetlova starosti 12 mjeseci koje su u njihovom istraživanju iznosile 2,95 odnosno 2,71 kg.

Istraživanje za ovaj rad je provedeno u sklopu projekta praćenja proizvodnosti križevačke kukmaste kokoši i utvrđivanja vrijednosti tjelesnih izmjera kokoši i pijetlova sa svrhom izrade pasminskog standarda potrebnog za njezino priznavanje kao izvorne hrvatske pasmine.

Materijal i metode

Istraživanje je provedeno na 203 pilića križevačke kukmaste kokoši iz 5 rasplodnih jata sa 5 gospodarstava na području grada Križevaca. Inkubiranje rasplodnih jaja provedeno je u višeslojnom inkubatoru Kalimero K-720 na Visokom gospodarskom učilištu u Križevcima. U prvih 18 dana inkubiranje je provedeno u uvjetima temperature od 37,5°C uz relativnu vlagu 70% i osigurano automatsko okretanje jaja. U posljednja tri dana inkubacije temperatura je bila 37,2°C, a relativna vlažnost 80%. U prvih 8 tjedana pilići su držani u zatvorenim objektima s potpunom kontrolom mikroklimatskih uvjeta, a nakon toga premješteni su u objekte uz koje se nalazio zatravljeni ispušni kanal. Prilikom premještanja pilića izvršeno je određivanje spola te su pilići u daljnjem uzgoju držani odvojeno po spolovima. Sa starošću od 8 tjedana zbog malog broja pilića na gospodarstvima 4 i 5, pilići su spojeni u jednu grupu te su u rezultatima prikazivani kao gospodarstvo 4. Hranidba je čitavo vrijeme bila *ad libitum*. Za sve grupe je korištena ista kompletna krmna smjesa koja je s nutritivnog stajališta prilagođena dobi pilića (Tablica 1). Prva četiri tjedna korištena je početna PPT 1 (starter) kompletna krmna smjesa, a od 4. do 16. tjedana PPT 2 potpuna krmna smjesa za piliće u rastu. Istu smjesu pijetlovi su dobivali do kraja istraživanja, a pilenke su od 16. tjedna dobivale potpunu krmnu smjesu za nesilice (PN-NES).

Tablica 1. Nutritivna vrijednost krmnih smjesa korištenih u istraživanju

Krmna smjesa ¹		PPT 1	PPT 2	PN-NES
Sirove bjelančevine	%	21	18	16
Sirova mast	%	4	2,5	3,5
Sirova vlakna	%	4	4,5	4
Sirovi pepeo	%	6	6	13,5
Metionin	%	0,48	0,35	0,28
Lizin	%	1,2	0,9	0,82
Na	%	0,19	0,19	0,15
P	%	0,65	0,65	0,6
Ca	%	1	1	4

¹PPT 1 potpuna krmna smjesa od 1. do 4. tjedna; PPT 2 potpuna krmna smjesa od 4. do 16. tjedna; PN-NES potpuna krmna smjesa za nesilice (PN-NES)

Tijekom uzgoja praćeni su osnovni proizvodni rezultati: prirast tjelesne mase i potrošnja krmne smjese odnosno konverzija kao i mortalitet. Vaganje pilića provedeno je jednom mjesečno, a nakon završnog vaganja (sa starošću od 5 mjeseci) na 20 muških i 20 ženskih jedinki izvršeno je određivanje morfometrijskih svojstava te klanje i klaonička obrada. U svrhu određivanja morfometrijskih svojstava izvršeno je mjerenje duljine trupa, prsne kosti, batka, piska, glave, kljuna potom širina trupa, glave i piska te dubina prsiju. Mjerenje je izvršeno pomoću šestara i pomične mjerke kako opisuju Bedeković i sur. (2019). Prilikom klaoničke obrade određen je randman te udjeli pojedinih dijelova u trupu.

Rezultati istraživanja obrađeni su statističkim programom Statistica 13.4.0.14 (TIBO Software, Inc 1984 – 2018) korištenjem GLM postupka. U analizi značajnosti razlika između pojedinih istraživanih svojstava upotrijebljena je ANOVA multivarijantni linearni model, a značajnost razlika utvrđena je primjenom Tukey HSD testa.

Rezultati i rasprava

Istraživanje je započelo inkubiranjem jaja križevačke kukmaste kokoši prikupljenih sa 5 obiteljskih gospodarstava, a rezultati inkubiranja prikazani su u Tablici 2. Prosječna ukupna valivost je bila niska, iznosila je svega 52% s razlikama po gospodarstvima od najmanje 16% do najviše 72%. Bolje rezultate valivosti (82%) u ranijim istraživanjima na istoj pasmini navode Pintiće i sur. (2010.). Razlog niske valivosti u našem istraživanju je, kao što se vidi iz tablice, niska oplodnost. Ona je vjerojatno posljedica bujnosti perja koje ometa kopulaciju tako da bi kod rasplodnih jedinki bilo uputno podrezivati perje u području kloake.

Pokazatelji proizvodnje mesa križevačke kukmaste kokoši

Tablica 2. Rezultati inkubiranja

gospodarstvo	uloženo jaja	prosječna masa (g)	oplođeno (n)	oplođeno (%)	izvaljeno pilića	valivost (%)
1	100	57,19	75	75	72	72
2	91	54,90	69	76	62	68
3	88	54,86	44	50	33	37,5
4	59	54,58	29	51	28	47
5	50	61,42	8	16	8	16
ukupno	388	56,59	225	66	203	52

Tablica 3. Mase pijetlova i pilenki tijekom uzgoja po gospodarstvima

dob pilića (tjedana)	gospodarstvo	spol	
		pijetlovi	pilenke
8	1	827,15 ^{aA}	673,20 ^{bA}
	2	827,24 ^{aA}	715,81 ^{bA}
	3	660,81 ^B	555,50 ^B
	4	836,45 ^A	736,87 ^A
	prosjeck	798,43 ^a	682,97 ^b
11	1	1281,00 ^a	1040,43 ^b
	2	1404,23 ^a	1131,73 ^b
	3	1327,75 ^a	1021,29 ^b
	4	1380,75 ^a	1132,83 ^b
	prosjeck	1345,11 ^a	1083,60 ^b
16	1	2083,29 ^a	1606,03 ^b
	2	2164,44 ^a	1661,67 ^b
	3	2178,94 ^a	1597,85 ^b
	4	2132,75 ^a	1696,61 ^b
	prosjeck	2135,28 ^a	1642,65 ^b
20	1	2733,89 ^a	1848,07 ^b
	2	2747,37 ^a	1778,74 ^b
	3	2825,37 ^a	1876,50 ^b
	4	2611,58 ^a	1944,13 ^b
	prosjeck	2737,94 ^a	1855,89 ^b

^{a,b} vrijednosti u istom redu tablice označene različitim slovima značajno se razlikuju između spolova ($P < 0,05$)

^{A,B} vrijednosti u istom stupcu tablice označene različitim slovima značajno se razlikuju između gospodarstava ($P < 0,05$)

U Tablici 3 prikazana je masa pilića tijekom uzgoja s obzirom na spol i gospodarstvo. Pjetlići su imali statistički značajno veće mase od pilenki ($p < 0,05$) gotovo pri svim vaganjima. Razlike u prosječnim masama pilića po gospodarstvima bile su statistički značajne ($P < 0,05$) sa starošću od 8 tjedana. Najmanje tjelesne mase imali su pilići na gospodarstvu 3, dok su sa starošću od 11 tjedana najmanje mase imali pilići na gospodarstvu 1, a najveće na gospodarstvu 2. Prosječne žive mase jedinki sa 16 i 20 tjedana nisu bile statistički značajno različite između gospodarstava. Na završnom vaganju najveću prosječnu živu masu imali su pjetlovi na gospodarstvu 3 (2825,37 g), a pilenke na gospodarstvu 4 (1944,13 g). Značajno manje tjelesne mase pijetlova križevačke kukmaste kokoši u dobi od 6 mjeseci od 2,33 kg navode Meštrović i sur. (2018) dok su mase pilenki od 1,91 kg slične tjelesnim masama utvrđenim u našem istraživanju.

U Tablici 4 prikazana je konverzija krmne smjese i mortalitet jedinki s obzirom na gospodarstvo i spol. U prvih 8 tjedana uzgoja pilići su držani u skupinama mješovitog spola tako da se prikazane vrijednosti odnose na gospodarstva. U navedenom razdoblju prosječna konverzija iznosila je 3,56 s time da je ona najveća i gotovo dvostruko veća kod gospodarstva 3 u odnosu na gospodarstvo 4. Smatramo da je razlog tako velike konverzije kod gospodarstva 3 rastep hrane. Za razdoblje od 9. do 20. tjedna starosti konverzija je ujednačena s obzirom na spol i gospodarstvo te iznosi prosječnih 7,15. Prosječan mortalitet je iznosio u prvoj fazi 3%, a u drugoj 11% s time da se može primijetiti veći mortalitet u drugoj fazi na gospodarstvu 1 na kojem je iznosio 14,5%.

Tablica 4. Konverzija krmne smjese i mortalitet s obzirom na gospodarstvo i spol

gospodarstvo		1		2		3		4		prosjeck
1-8	konverzija	3,15		3,68		5,25		2,72		3,56
tjedna	mortalitet, %	3		2		3		0		3
		m ¹	ž ²	m ¹	ž ²	m ¹	ž ²	m ¹	ž ²	
9-20	konverzija	6,67	7,07	6,89	6,15	7,19	7,67	8,08	7,53	7,15
tjedna	mortalitet, %	15	14	17	0	0	7	0	0	11

¹jedinke muškog spola

²jedinke ženskog spola

Relativno velika konverzija krmne smjese ostvarena u našem istraživanju bila je i za očekivati s obzirom da se radi o izvornoj pasmini. Tako Kalić (2012) navodi konverziju krmne smjese pilića kokoši hrvaticice od 5,40, a Bedeković i sur. (2019) navode gotovo jednaku konverziju od 7,12 kao u ovom istraživanju. No, pojedini autori poput Wanga (2009) te Mikulskog i sur. (2011) veću konverziju te manje priraste pripisuju načinu držanja odnosno omogućenom kretanju peradi na ispuštima.

Tablica 5. Randman i udjeli pojedinih dijelova trupa

parametar	masa (g)	%		spol		spol	
		m ¹	ž ²	m ¹	ž ²	m ¹	ž ²
		masa (g)	masa (g)	%	%		
randman (%)		78,53				80,23	77,78
dijelovi trupa	prsa s kostima	375,72	20,18	427,65	323,8	19,42	21,30
	file	214,40	11,52	254,4	174,4	11,55	11,47
	bataci	265,55	14,27	325,85	205,25	14,79	13,50
	zabataci	271,62	14,69	325,35	217,9	14,77	14,33

¹jedinke muškog spola

²jedinke ženskog spola

U Tablici 5 prikazan je randman te udjeli pojedinih dijelova u trupu s obzirom na spol. Kod pijetlova se može primijetiti veći randman te udio bataka i zabataka u trupu dok je kod kokoši primjetan veći udio prsa s kostima. Znatno niže randmane izvornih pasmina navode Bedeković i sur. (2019) kod kokoši hrvaticice od 71,43% te Raphulu i sur. (2015) kod Venda pasmine od 63,08%.

U Tablici 6 prikazana su morfometrijska svojstva križevačke kukmaste kokoši s obzirom na spol. Za sve izmjere utvrđena je statistički značajna razlika između spolova.

Tablica 6. Morfometrijska svojstva križevačke kukmaste kokoši kod starosti 5 mjeseci

mjera	spol		
	pijetlovi	pilenke	prosjeak
duljina trupa (cm)	21,81 ^a	19,40 ^b	20,06
duljina prsne kosti (cm)	15,25 ^a	13,73 ^b	14,49
duljina batka (cm)	17,40 ^a	14,33 ^b	15,86
duljina piska (cm)	13,19 ^a	10,65 ^b	11,92
širina trupa (cm)	8,06 ^a	6,67 ^b	7,36
dubina prsa (cm)	13,75 ^a	12,15 ^b	12,95
duljina glave (cm)	8,38 ^a	7,85 ^b	8,11
duljina kljuna (cm)	2,26 ^a	1,91 ^b	2,08
širina glave (cm)	3,49 ^a	3,03 ^b	3,26
širina piska (cm)	1,57 ^a	1,32 ^b	1,45

^{a,b} vrijednosti u istom redu tablice označene različitim slovima značajno se razlikuju ($P < 0,05$)

Zaključak

U provedenom istraživanju utvrđeni su proizvodni pokazatelji proizvodnje mesa križevačke kukmaste kokoši (prosječna tjelesna masa pijetlova i kokoši kod starosti od 5 mjeseci, prosječna konverzija, mortalitet, randman klanja te udjeli pojedinih dijelova u trupu) kao i prosječne vrijednosti morfometrijskih svojstava. Daljnja istraživanja trebala bi ići u smjeru utvrđivanja proizvodnih pokazatelja u proizvodnji jaja, utvrđivanja kakvoće mesa i jaja te genotipa križevačke kukmaste kokoši kako bi se sa kompletnim podacima o fenotipskim i genetskim svojstvima pasmine išlo ka dobivanju statusa izvornosti pri Ministarstvu poljoprivrede te Zaštićene oznake izvornosti mesa i jaja.

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Meat production indicators of Križevci crested hen

Abstract

The study was conducted on 203 chickens of the native Croatian breed Križevci crested hen with the aim of determining the indicators of meat production and morphometric characteristics of 5 months old individuals. The average live weight of the roosters at the end of the study (20 weeks) was 2738 and the hens was 1856 g. The average conversion rate in the first 8 weeks was 3.56, and in the period from 8 to 20 weeks was 7.15, while the mortality rate by the mentioned phases was 3% and 11%, respectively. The average carcass percentage was 78.53%, shares of breast 20.18%, fillet 11.52%, drumsticks 14.27% and thighs 14.59%. The average values of the morphometric properties were as follows: length of body 20.60, breastbone 14.49, drumstick 15.86, shank 11.92, heads 8.11 and beak 2.08 then width of body 7.36, head 3.26 and shank 1.45 and breast depth 12.95 centimetres.

Keywords: native breed of poultry, Križevci crested hen, morphometric traits, meat production indicators

The concentration of zinc, iron and manganese in the sheep's milk and yogurt

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Abstract

The aim of this study was to determine the concentration of zinc, iron and manganese in the raw sheep's milk and the effect of milk heat treatment (95°C/5 min) on Zn, Fe and Mn concentration in set sheep's yogurt on the 1st day of storage and after 21 days of storage. In the bulk sheep's milk samples (n=8) from the East Friesian breed and accordingly in yogurt samples (n=8), concentrations of zinc, iron and manganese were measured by inductively coupled plasma emission spectroscopy (ICP-AES). The concentrations of zinc (402±19 µg/100g), iron (53±4 µg/100g) and manganese (8±1 µg/100g) in yogurt compared to their concentrations in raw milk were not significantly changed.

Keywords: heat treatment, storage time, micro minerals, sheep's milk yogurt

Introduction

Sheep's milk yogurt is relatively little present on the market due to the seasonal character of production of sheep's milk. Significant production of sheep's milk yogurt is traditionally present in Bulgaria, Turkey, Greece and the Middle East. Also, in some Asian and European countries sheep's milk in the raw material for production of yogurt-like products, such as frozen yogurt (like ice cream) and dried or condensed yogurt with a consistency similar to soft cheese (Bonczar and Regula, 2003). Flavored variants of sheep's milk yogurt with added honey, fruits, nuts, grains and chocolate are also present on the market. Compared with yogurt from cow's milk, sheep's milk yogurt has higher nutritional and physiological significance for human diet (Samaržija, 2015). Yogurt contains a relatively high concentration of trace elements that are essential for the human organism, irrespective of the type of milk used (Cashman, 2006). Most of the minerals present in yogurt are in ionic form. Therefore, in comparison with milk where minerals are mainly part of inorganic ions or organic molecules, bioavailability of minerals in yogurt is more pronounced for human organism (Zamberlin et al., 2012; Samaržija, 2015). The concentrations of essential trace elements (zinc, iron and manganese) in cow's milk are lower than in sheep's milk. The average concentration of zinc in sheep's milk is 415, iron 62-100 and manganese 5.3 µg/100g. For the comparison, concentration of zinc in cow's milk is 74-145, iron 12.6-70 and manganese 1.3-4 µg/100g (Jenness, 1980; Park and Chukwu, 1988; Coni et al., 1999; Park, 2006; Deutchen Forschungsanstalt für Lebensmittelchemie, 2016). Consequently, sheep's milk yogurt naturally contains essential trace elements in relatively high concentrations. However, there are relatively little data about the mineral composition of sheep's milk and yogurt.

In addition, standard technological processes and the usual storage time of 21 to 28 days at 4°C, among other effects, change physical and chemical properties of yogurt. The aim of this study was to determine the effect of heat treatment of sheep's milk (95°C/5 min) and storage time of yogurt on 21st day (4°C) on the change of concentrations of zinc, iron and manganese naturally contained in raw milk.

Material and Methods

The selection of milk for yogurt production was based on the physical, chemical and microbiological analysis of bulk sheep's milk milked from the East Friesian breed (n=8) and according to the Croatian regulation for quality of fresh raw milk (Narodne novine, 2000). The acidity of milk was established by determining the pH value by potentiometric method and titratable acidity (°SH) by Soxhlet Henkel method (Sabadoš, 1996). Freezing point of milk was determined by thermistor method (Croatian Standards Institute, 2010). Milk fat, protein, lactose, dry

matter and non-fat dry matter content was determined by infrared spectrometry (International Organisation for Standardisation, 2013). The total number of aerobic mesophilic bacteria was determined by flow cytometry on the instrument BactoScan FC (Foss Electric, Danska) (Croatian Standards Institute, 2008) and the total number of somatic cells by the fluoro-opto-electronic method (Croatian Standards Institute, 2007). Sheep's milk selected for the production of yogurt was collected four times (Milk 1, Milk 2, Milk 3 and Milk 4). The samples for analysis and milk for experimental production of set sheep's milk yogurt were taken on a family farm which holds 150 sheep's of Eastern Friesian breed, located in Varaždin County. Prior to yogurt production, sheep's milk was homogenized at a temperature of 55°C/MPa and then heat-treated at a temperature of 95°C/5 min, without previous standardization of milk fat and non-fat dry matter. Cooled to a temperature of 43°C, milk was inoculated with yogurt culture LBB BY (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; (LB Bulgaricum, Bulgaria) in a concentration of 2%. The fermentation of yogurt in cups of 150 mL volume was carried out in controlled conditions of thermo-chamber at a temperature of 42°C to the pH value 4.6, and then terminated by quick cooling (4°C). Yogurt samples were stored in refrigeration glass case at a temperature of 4°C until analysis. To analyse the concentrations of zinc, manganese and iron in milk (n=8) and yogurt (n=8), their adherent samples weighing $2.5 \text{ g} \pm 0,1 \text{ mg}$ were burned at 550°C in a microwave oven to constant mass of ash. Obtained mass of ash was then dissolved in concentrated HNO₃ and quantitatively transferred to a volumetric flask of 50 mL and then made up to a volume of 50 mL with redistilled water. The concentrations of zinc, iron and manganese were determined by optical emission spectrometry inductively coupled plasma method (ICP-AES) on the instrument Vista-MPX ICP-OES (International Organisation for Standardisation, 2008).

Statistical analysis was conducted with univariate analysis of variance (ANOVA) using Dunnett's multiple comparison test where the control group was the data of mineral composition in milk. Tukey's test was used to make multiple comparisons.

Results and Discussion

Physico-chemical and hygienic quality of milk that was chosen for the test production of set sheep's milk yogurt is shown in Table 1. According to the overall quality, selected milk (Milk 1, Milk 2, Milk 3 and Milk 4) did not differ significantly ($p > 0.05$) except for the hygienic parameters.

Table 1. Physico-chemical and hygiene parameters of the quality of sheep's milk used to produce set sheep's milk yogurt

Physico-chemical and hygienic parameters	Milk 1*	Milk 2*	Milk 3*	Milk 4*
pH	6.61±0.03	6.61±0.03	6.65±0.04	6.63±0.03
Titrate acidity (°SH)	9.57±0.10	9.60±0.10	9.54±0.08	9.33±0.06
Freezing point (°C)	-0.588±0.004	-0.582±0.003	-0.586±0.004	-0.578±0.003
Milk fat (g/100g)	6.41±0.06	5.19±0.05	5.70±0.05	6.47±0.06
Protein (g/100g)	4.53±0.01	4.44±0.01	4.45±0.01	4.38±0.01
Lactose (g/100g)	4.71±0.05	4.74±0.05	4.67±0.04	4.50±0.02
Dry matter (g/100g)	16.43±0.05	15.18±0.03	15.67±0.04	16.20±0.04

The concentration of zinc, iron and manganese in the sheep's milk and yogurt

Non-fat dry matter (g/100g)	10.02±0.06	9.99±0.05	9.67±0.05	9.72±0.06
Somatic cell count (cell/mL)	133200±16500 ^a	114000±14000 ^b	565000±70000 ^c	782000±97000 ^d
TBN** (cfu/mL)	4000±500 ^e	27400±3200 ^f	35000±4100 ^g	65000±8000 ^h

* average result of five parallel measurements ± SE; ** TBN – total number of aerobic mesophilic bacteria

^{a-h} different letters in the same row indicate significant differences within specific property ($p < 0.05$)

The concentrations of manganese and zinc in the analyzed samples of milk (Milk 1, Milk 2, Milk 3 and Milk 4) were higher, and concentration of iron was about the same (Table 2) compared to the values for the same minerals obtained from other authors (Mn 5.3, Zn 74 - 145, Fe 12.6 - 70 µg/100g) (Jenness, 1980; Park and Chukwu, 1988; Coni et al., 1999; Park, 2006; Deutschen Forschungsanstalt für Lebensmittelchemie, 2016). Higher concentrations of zinc (692 µg/100g) and iron (76.6 µg/100g) and equal concentration of manganese (7.9 µg/100g) in sheep's milk was determined by Martín-Hernández et al. (1992). The difference in data for the concentrations of these elements in sheep's milk is primarily due to their concentration in the soil and feed with significant differences between and within different geographical areas (Malbe et al., 2010).

Table 2. The concentration of trace elements zinc, iron and manganese in sheep milk

Trace element (µg/100g)	Milk 1*	Milk 2*	Milk 3*	Milk 4*
Zinc	400±20	420±10	380±10	400±10
Iron	49±2	52±9	48±2	51±3
Manganese	8.0±1.0	7.8±1.0	8.0±1.0	8.0±1.0

* average result of three parallel measurements ± SE

Selection of milk (Milk 1, Milk 2, Milk 3, Milk 4) did not affect the concentration of zinc, iron and manganese in yogurt. Table 3 shows the results for concentrations of the studied trace elements for all analyzed yogurts from 1st to 21th day of storage. Results are not in accordance with the research conducted by Zurer-Cosano et al. (1994) who found that the concentration of iron in yogurt decreases by the heat treatment of milk. On the contrary, Malbe et al. (2010) found that the concentration of iron in yogurt is not changed by the heat treatment of milk, as it is with zinc and manganese confirmed in this study.

Table 3. The concentrations of zinc, iron and manganese in sheep's yogurt on the 1st and 21th day of storage

Trace element (µg/100g)	Storage (day)	
	1st	21th
Zinc	398±30	402±19
Iron	52±4	53±4
Manganese	8±1	8±1

* average result of three measurements ± SE

Zinc is an important element for the growth, sexual development, normal functioning of the immune system and other physiological processes in the body. It is a component of the hormone insulin and helps in the functioning of many other hormones important to reproduction and synthesis of DNA, RNA and proteins. Zinc is also an important co-factor for the operations of many cellular enzymes involved in most metabolic processes (Roohani et al., 2013). Iron is an essential trace element that participates in catalytic reactions of metabolic functions of the organism. It is the component of hemoglobin, myoglobin, cytochromes and plays an important role in the transport, storage and use of oxygen. Also, the iron in the body acts as a co-factor for the activity of many cellular enzymes (Abbaspour et al., 2014). For the nutritional value of yogurt, manganese is a co-factor of the enzymes involved in the synthesis of mucopolysaccharides, and non-specific co-factor of many other cellular enzymes, such as arginase, glutamine synthase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (Navarro-Alarcón et al., 2005). Unchanged concentrations of zinc, iron and manganese in yogurt during the period of storage as compared to their natural concentration in sheep's milk also points to the fact that there is no contamination of products with these trace elements during production.

Conclusion

The concentrations of zinc, iron and manganese in sheep's milk yogurt are not changing by using the standard temperature of heat-treatment of milk (95°C/5 min) and during the storage time of 21 days. Different concentrations of these trace elements in sheep's milk yogurt are primarily related to their concentrations within different geographical areas. For this reason, to determine the nutritional value of sheep's milk yogurt related to the trace elements, it seems justified to determine them separately for each area of dairy production.

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Utjecaj boje polietilenske folije na kvalitetu biljne mase silirane u velike bale

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Sažetak

Cilj istraživanja bio je utvrditi utjecaj boje polietilenske (PE) folije na hranjivu vrijednost biljne mase djetelinsko travne smjese (DTS) klupčaste oštrice (*Dactylis glomerata* L.) i lucerne (*Medicago sativa* L.) silirane u velike valjkaste bale. Biljna masa je bila pokošena u fazi cvatnje klupčaste oštrice i balirana u valjkaste bale promjera 1,2 m. Tretmani pokusa uključivali su primjenu PE folije crne, zelene i bijele boje. Nakon fermentacije u natkrivenom prostoru, bale su otvorene 10 mjeseci nakon baliranja, te su uzeti uzorci fermentirane biljne mase. Uzorci su analizirani na sadržaj suhe tvari (ST), sirovih proteina (SP), neutralnih detergent vlakana (NDV), kiselih detergent vlakana (KDV), metaboličke energije (ME) i probavljivost organske tvari (POT). Boja folije utjecala je samo na sadržaj ST ($P < 0,05$), dok se sadržaj SP, NDV, KDV, ME kao i POT nije razlikovala ovisno o boji PE folije ($P > 0,05$). Može se zaključiti da u uvjetima dužeg perioda skladištenja, boja folije ne utječe na pokazatelje hranjivosti silaže DTS-a silirane u velikim valjkastim balama.

Ključne riječi: siliranje, polietilenska folija, boja, kvaliteta, silaža

Uvod

Poželjan tijek mliječno-kiselinske fermentacije biljnog materijala unutar bale ovijene PE folijom te dobro i stabilno čuvanje silaže ovise između ostaloga o nepropusnosti PE folije za plinove odnosno njejoj sposobnosti da spriječi prodor zraka i osigura anaerobne uvijete unutar bale (Paillat i Gaillard, 2001). Prodor zraka u silažu pospješuje razvoj aerobnih bakterija, gljivica i plijesni i glavni je razlog aerobnog kvarenja što za posljedicu ima gubitak ST i hranjivih tvari te nakupljanje patogena i mikotoksina (Scudamore i Livesey, 1998). PE folije koje se koriste za ovijanje bala imaju malu gustoću te su propusne za kisik i druge plinove (McNally i sur., 2005). Nepropusnost PE folije za plinove ovisi o debljini (Snell i sur., 2003) i površinskoj temperaturi folije (Daponte, 1994; Nentwig, 1994; Borreani i Tabacco, 2008). Visoke površinske temperature PE folije rezultiraju većom propusnosti folije za plinove, a k tome kod visokih temperatura folije postaju podložnije mehaničkim oštećenjima i perforacijama (Snell i sur., 2003). Osim debljine folije (Snell i sur., 2003) na površinsku temperaturu PE folije može utjecati i boja folije (Snell i sur., 2003). Na izravan utjecaj boje folije (crna, prozirna, zelena, svijetlozelena i bijela) na sastav plinova unutar silirane mase ukazuju rezultati istraživanja McEniry i sur. (2011) prema kojima su tijekom perioda skladištenja bale ovijene crnom folijom imale su najnižu koncentraciju CO₂. S obzirom na utjecaj boje PE folije na površinsku temperaturu i propusnost PE folije Lingvall (1995) zaključuje da u područjima sa većom sunčevom radijacijom korištenje svijetlih ili bijelih folija smanje temperaturu folije i prodor O₂ u silažnu masu. Slično tome, rezultati istraživanja (McEniry i sur., 2011) ukazuju da će kod tamnih folija koje brže apsorbiraju solarnu radijaciju doći do većeg rasta temperature te će tako tamne PE folije postati propusnije za plinove u usporedbi sa svijetlim folijama.

Osim o kvaliteti biljnog materijala prije siliranja kvaliteta silaže ovisi i o tijeku fermentacije u bali-silosu. Budući da boja folije utječe na površinsku temperaturu PE folije a time i na propusnost PE folije na plinove, za pretpostaviti je da će korištenje PE folija različitih boja rezultirati promjenama u kvaliteti silirane biljne mase. Cilj istraživanja bio je utvrditi utjecaj boje PE folije (zelena, crna i bijela) na pokazatelje hranidbene vrijednosti biljne mase silirane u velike valjkaste bale.

Materijal i metode

Za ovo istraživanje korištena je djetelinsko-travna smjesa (DTS) sastavljena od lucerne (*Medicago sativa* L.) i klupčaste oštrice (*Dactylis glomerata* L.). Košnja je obavljena u fazi cvatnje klupčaste oštrice. Nakon provenjavanja u trajanju od 24 sata, biljna je masa balirana valjkaste bale promjera 0,12 m korištenjem balirke „John Deer“, tip 575. Bale su ovijene mrežom širine 1,23 metara.

Sve su bale ovijene sa 6 slojeva PE folije širine 750 mm i debljine 25 µm. Pokus je uključivao tri tretmana – korištenje PE folije crne (i), zelene (ii) i bijele (iii) boje. Za potrebe pokusa odvojeno je po 5 bala od svakoga tretmana. Bale su ostavljene fermentirati u natkrivenom prostoru.

Za potrebe uzorkovanja bale su otvorene 10 mjeseci nakon baliranja. Botanički sastav smjese utvrđen je razdvajanjem i vaganjem botaničkih sastavnica (trava, lucerne i korova) silirane mase nakon otvaranja bala te je utvrđeno da je DTS sadržavala 51 % klupčaste oštrice, 31 % lucerne i 18 % širokolisnih korova. Uz pomoć sonde iz svake je bale uzet uzorak silirane biljne mase (po cca 500 g).

Sadržaj ST utvrđen je sušenjem uzorka u sušioniku pri temperaturi 60°C u trajanju od 48 sati. Uzorci su samljeveni u mlinu (Christi, model 11) na veličinu čestica 1 mm. Sadržaj SP, NDV, KDV, ME i POT utvrđeni su korištenjem NIR aparata (Foss, model 6500), skeniranjem u blisko-infracrvenom spektru valnih duljina 1100-2500 nm, u intervalima po 2 nm. Prije skeniranja uzorci su dosušeni u sušioniku pri temperaturi 105 °C u trajanju od 3 sata. pH uzorka utvrđen je ionometrijskom metodom korištenjem pH-metra Mettler Toledo. Rezultati su obrađeni u SAS programskom paketu (SAS, 1999) korištenjem MIXED procedure.

Rezultati i rasprava

Rezultati analize varijance (ANOVA) i prosječne vrijednosti istraživanih pokazatelja kvalitete silirane biljne mase ovijene PE folijom različite boje prikazani su u Tablici 1.

Boja folije utjecala je na sadržaj ST silirane biljne mase (Tablica 1). Biljna masa ovijena zelenom folijom imala je značajno niži sadržaj ST u usporedbi sa silažom ovijenom crnom i bijelom PE folijom ($P < 0,05$).

Nije utvrđen utjecaj boje folije na sadržaj SP, NDV, KDV, POT, ME i pH silaže DTS-a ($P > 0,05$) (Tablica 1).

Prosječni sadržaj SP za sve tretmane iznosio je 112 g kg⁻¹ ST i odraz je košnje DTS-a u kasnoj fazi rasta i razvoja. Sadržaj NDV od prosječno 515 g kg⁻¹ ST, kreće se unutar raspona poželjnih vrijednosti za silažu trava i DTS-a (Chamberlain i Wilkinson, 1996). Nešto viši prosječni sadržaj KDV (319 g kg⁻¹ ST), te niža POT (557 g kg⁻¹ ST) i sadržaj ME (9,0 MJ kg⁻¹ ST) od poželjnih vrijednosti (Chamberlain i Wilkinson, 1996) za travnu silažu ukazuje na slabiju hranidbenu vrijednost silaže DTS-a u ovom istraživanju. Posljedica je to košnje i siliranja fiziološki stare biljne mase, a ne primjene PE folije različitih boja prilikom ovijanja. Prosječna pH vrijednost silirane mase svih tretmana iznosila je pH 5,8, što također znatnije odstupa od poželjnih pH vrijednosti za travne silaže (Chamberlain i Wilkinson, 1996).

Tablica 1. Rezultati analize varijance (ANOVA) i prosječne vrijednosti istraživanih pokazatelja kvalitete silirane biljne mase ovijene PE folijom različite boje

	ST	SP	NDV	KDV	POT	ME	pH
Boja PE folije	g kg ⁻¹ SM		g kg ⁻¹ ST			MJ kg ⁻¹ ST	
Zelena	495	116,5	515,0	318,3	538	8,6	5,7
Crna	664	109,5	521,8	318,3	583	9,4	5,9
Bijela	684	110,0	509,5	319,5	550	8,9	5,8
SED	60	14,6	22,8	26,1	45	0,7	0,4
P	0,0216	0,8693	0,8675	0,9985	0,6035	0,5372	0,9354

ST-suha tvar; ME-metabolička energija; POT – probavljivost organske tvari; SP-sirovi proteini; NDV-neutralna detergent vlakna; KDV- kisela detergent vlakna; SED – standardna pogreška razlike; P – P vrijednost; SM – svježa masa

Većina radova u kojima su prezentirani rezultati istraživanja utjecaja boje PE folije na kemijski sastav i razvoj plijesni kod travnih silaža ukazuju na činjenicu da boja folije nema utjecaja na kvalitetu travne silaže. Slično kao u ovom istraživanju, izostanak utjecaja boje PE folije na kemijski sastav kao i razvoj plijesni utvrđen je u radovima na travnoj silaži siliranoj u valjkaste bale (McEniry i sur., 2011) i horizontalni silos (Snell i sur., 2003) ili na kukuruznoj silaži (Snell i sur., 2002). Štoviše, iako je u istraživanjima Snell i sur. (2002) i Snell i sur. (2003) utvrđen značajan utjecaj boje PE folije na površinsku temperaturu PE folije, utjecaj boje folije na kvalitetu silaže je izostao za sve istraživane pokazatelje kvalitete. Međutim, u tim istraživanjima istraživana je folija za pokrivanje horizontalnih silosa koja je znatno deblja (90-150 μm) u usporedbi sa folijama za ovijanje bala (25-30 μm). K tome u tim je istraživanjima vrijeme do otvaranja silosa bilo znatno kraće (104 i 107 dana) dok su u ovom su istraživanju bale otvarane 10 mjeseci nakon baliranja. Suprotno tome, uspoređujući plavu i zelenu PE foliju Vranić i sur. (2015) ukazuju na značajan utjecaj boje folije na sadržaj SP, $\text{NH}_3\text{-N}$ i KDV i NDV travne silaže 35 dana nakon siliranja, dok utjecaj boje PE folije nije bio značajan za sadržaj ST, ME, POT i pH vrijednost silaže.

Zaključak

U uvjetima dugog skladištenja travne silaže, kakav je bio u ovom istraživanju, boja folije nema utjecaja na pokazatelje kvalitete travne silaže silirane u valjkaste bale.

Napomena

Istraživanje koje je prezentirano u ovom radu provedeno je uz potporu tvrtke „Boomark ambalaža“. Autori zahvaljuju na doniranoj PE foliji koja je korištena u istraživanju.

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The effect of the colour of polyethylene film on the forage quality ensiled in big bales

Abstract

The aim of the study was to determine the effect of the colour of polyethylene (PE) film on the nutritive value of silage from grass-clover mixture (GCM) with orchardgrass and alfalfa baled in round bales. The mixture was cut in the flowering phase of orchardgrass and baled into round bales of 1.2 m in diameter. The three experimental treatments consisted of PE film of different colour - black, green, and white. After the fermentation in the covered area, the bales were opened 10 months after baling and samples of the plant material were taken. Samples were analysed for the content of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), metabolic energy (ME) and organic matter digestibility (OMD). The colour of the PE film affected only the DM content ($P < 0.05$), while the content of CP, NDF, ADF, ME as well as OMD was not affected with colour of PE film ($P > 0.05$). It can be concluded that in longer stocking period as were in this experiment, the feeding quality of grass-clover silage baled in big round bales is not affected with the colour of the PE film.

Keywords: baled silage, polyethylene film, colour, quality

Utjecaj primjene krutog stajskog gnoja na sadržaj sirovih proteina i nitrata u krmi s poluprirodnog travnjaka

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Sažetak

Cilj ovoga istraživanja bio je utvrditi sadržaj sirovih proteina (SP) i nitrata u voluminoznoj krmi nakon primjene različitih količina i vremena primjene krutog stajskog gnoja (KSG) na poluprirodnom travnjaku. Ukupno 6 gnojidbenih tretmana uključivalo je različite količine KSG (30 i 50 t ha⁻¹ god⁻¹), različito vrijeme primjene (proljeće i jesen) te dva tretmana mineralne gnojidbe kao kontrola. Biljna masa je pokošena u fazi metličanja dominantnih trava te je utvrđen sadržaj SP i NO₃-N. Primjena KSG rezultirala je 21,9 % nižim sadržajem SP i 15 % nižim sadržajem NO₃-N u usporedbi sa mineralnom gnojidbom (P<0,05). Proljetna primjena KSG rezultirala je povećanjem sadržaja NO₃-N za 25 % (P<0,05), dok vrijeme primjene KSG nije utjecalo na sadržaj SP (P>0,05). Količina primjene KSG nije utjecala na sadržaj SP i NO₃- u biljnoj masi (P>0,05). Može se zaključiti da iako primjena KSG na poluprirodnim travnjacima smanjuje sadržaj SP u usporedbi sa mineralnom gnojidbom, sadržaj SP ne ograničava primjenu takve krme u hranidbi preživača, a sadržaj nitrata zadržava se ispod granica toksičnosti.

Ključne riječi: poluprirodni travnjak, stajski gnoj, sirovi proteini, nitrati

Uvod

Kruti stajski gnoj (KSG) se smatra vrijednim resursom u poljoprivredi naročito kao izvor nutrijenata (N, P, K) i organske tvari u proizvodnji na oranama i travnjacima (Crofts i Jefferson, 1999; DEFRA, 2010). S druge strane, primjena KSG može predstavljati izvor onečišćenja NO₃⁻ (Lord i sur., 1999; Nagatake i sur., 2018) zbog neprijemnog vremena primjene i/ili prekomjerne količine primijenjenog gnojiva (Thomsen i sur., 1993; Beckwith i sur., 1998).

Unatoč činjenici da je iskoristivost N iz organskih gnojiva manja u usporedbi sa mineralnim gnojivima, visokim količinama gnojiva mogu se unijeti i vrlo visoke količine hranjiva, naročito N i K. (DEFRA, 2010; Sinclair i sur., 2013). Rezultati istraživanja ukazuju da se primjenom KSG na travnjacima može očekivati slična kvaliteta krme kao kod mineralne gnojidbe (Shiel, 1995). S druge strane, Vranić i sur. (2016) utvrdili su da primjena KSG rezultira nižim sadržajem SP u usporedbi sa primjenom mineralnog gnojiva

Nitrati su uobičajena komponenta biljnoga materijala, a u biljci se mogu nakupljati u toksičnim razinama za životinje (Anderson i sur., 2016). Akumulacija NO₃⁻ može se očekivati kod mlađih biljaka te u uvjetima stresa poput rasta pri niskom intenzitetu svjetla ili u sjeni, pri niskim temperaturama i u suši te nakon primjene herbicida (Drewnoski i sur., 2019). Toksična koncentracija NO₃⁻ u krmi rezultira pojavom methemoglobinemije kod preživača (Latham i sur., 2016), a iako rijetko i kod konja (Oruc i sur., 2010). K tome, rezultati istraživanja sugeriraju da probavljivost i razgradivost voluminozne krme kao i brojnost populacije mikroorganizama u buragu mogu biti pod utjecajem NO₃⁻ puno prije pojave simptoma toksičnosti (Marais i sur., 1988), naročito ako životinje nisu prošle period prilagodbe (Lee i Beauchemin, 2014).

S druge strane, NO₃⁻ u obroku preživača, u koncentracijama ispod toksičnih, može smanjiti formiranje metana te poslužiti kao neproteinski izvor N u hranidbi, zamjenjujući tako UREU (Lee i Beauchemin, 2014; Olijhoek i sur., 2016).

Budući da primjena KSG na travnjacima može utjecati na pokazatelje hranidbene vrijednosti voluminozne krme, cilj ovoga rada bio je utvrditi utjecaj primjene KSG na sadržaj sirovih proteina i nitrata u voluminoznoj krmi s poluprirodnog travnjaka u usporedbi sa gnojidbom mineralnim gnojivom.

Materijal i metode

Poljski pokus je bio postavljen 2002. godine na pokusnoj površini "Centra za travnjaštvo" Agronomskog fakulteta u Zagrebu (638 m n.v., 45°55'42" S, 15°58'18" I), na poluprirodnom travnjaku zajednice *Arrhenatheretum medioeuropaeum* (Br-B1-19), (Hulina, 1983).

Shema pokusa bila je slučajni blokni raspored sa 4 repeticije i 6 tretmana (tablica 1). Tretmani bez primjene KSG (0KSG-P i 0KSG-J) korišteni su kao kontrola.

Tablica 1 Tretmani istraživanja utjecaja primjene KSG na sadržaj SP i NO₃-N u krmi s poluprirodnog travnjaka

Tretman	KSG (t ha ⁻¹ god ⁻¹)	Mineralna gnojidba (kg ha ⁻¹ god ⁻¹)	Vrijeme primjene
0KSG-P	0	500 (NPK 8:26:26) + 600 (KAN, 27 % N)	proljeće
0KSG-J	0	500 (NPK 8:26:26) + 600 (KAN, 27 % N)	NPK jesen; KAN - proljeće
30KSG-P	30	0	proljeće
30KSG-J	30	0	jesen
50KSG-P	50	0	proljeće
50KSG-J	50	0	jesen

Uzorci su uzeti iz prvog porasta tratine tijekom 2014 godine. Pokusne parcele su pokošene samohodnom oscilacijskom kosom u početku metličanja (faza R2, Skinner i Moore (2007)) dominantnih trava na visinu od 5 cm. Od pokošene biljne mase uzet je uzorak mase (500 g) za utvrđivanje sadržaja SP i NO₃ u biljnoj masi i osušen u sušioniku u trajanju od 48 sati pri temperaturi od 60°C. Uzorci su samljeveni u mlinu (Christi, model 11) na veličinu čestica od 1 mm. Sadržaj sirovih proteina utvrđen je su korištenjem NIR aparata (Foss, model 6500), skeniranjem u bliskoinfracrvenom spektru valnih duljina 1100-2500 nm, u intervalima po 2 nm. Prije skeniranja uzorci su dosušeni u sušioniku pri temperaturi 105 °C u trajanju od 3 sata. Sadržaj NO₃-N utvrđen je korištenjem ion selektivne metode (Miller, 1998). Rezultati su obrađeni u SAS programskom paketu (SAS, 1999) korištenjem MIXED procedure. Nakon analize varijance kod signifikantnih učinaka provedena je usporedba prosječnih vrijednosti korištenjem t-testa i kontrasta.

Rezultati i rasprava

Utvrđena je značajna razlika između istraživanih tretmana u sadržaju SP u biljnoj masi (P<0,001). Prosječni sadržaj SP prikazan je u tablici 2. Primjena KSG rezultirala je 21,9 % nižim sadržajem SP (P<0,0001) u usporedbi sa prosjekom dvije kontrole (0KSG-P i 0KSG-J). Prosječni sadržaj SP kod primjene KSG iznosila je 121 g kg⁻¹ ST dok je kod tretmana sa mineralnom gnojidbom sadržaj SP iznosio 155 g kg⁻¹ ST. Količina i vrijeme primjene KSG, te vrijeme primjene gnojiva (tablica 2) nisu utjecali na sadržaj SP u biljnoj masi (P>0,05).

Utvrđeni prosječni sadržaj SP u biljnoj masi kao reakcija na količinu, vrijeme i oblik primijenjenog gnojiva u ovom istraživanju (132 g kg⁻¹ ST) bio je sličan sadržaju SP koji su utvrdili Čop i sur. (2009) na istom tipu travnjaka. U uvjetima dovoljne opskrbe P sadržaj SP u obroku ispod 130 g kg⁻¹ ST krmi može smanjiti konzumaciju i dnevne priraste goveda u tovu (Bortolussi i sur., 1996). U ovom istraživanju sadržaj SP ispod 130 g kg⁻¹ ST utvrđen je kod količine od 30 t KSG ha⁻¹ u oba vremena primjene i 50 t KSG ha⁻¹ u jesen. K tome, sadržaj SP kod svih istraživanih tretmana kretao se iznad sadržaja od 119 g kg⁻¹ ST što se navodi kao potrebni udio SP u obroku tovnje junadi mase 270 kg za očekivani prirast od 1,2 kg dan⁻¹ (NRC, 1996) što ukazuje na činjenicu da će samo kod primjene 30 t ha⁻¹ KSG god bi potreban dodatni izvor SP u obroku tovnih goveda za očekivanu razinu prirasta.

Tablica 2 Sadržaj SP i NO₃-N u biljnoj masi poluprirodnog travnjaka gnojenog KSG i mineralnim gnojivom

Tretman	NO ₃ -N ppm (u ST)	SP g kg ⁻¹ ST
0KSG-P	630ab	159a
0KSG-J	786a	151a
30KSG-P	714ab	116b
30KSG-J	463c	115b
50KSG-P	625abc	130b
50KSG-J	607bc	121b
SED	59	6,6
Kontrasti	Signifikantnost	
KSG vs mineralni gnoj	*	***
30 t KSG vs 50 t KSG	NS	NS
KSG proljeće vs KSG jesen	**	NS
gnojidba proljeće vs gnojidba jesen	NS	NS

a, b, c – prosječne vrijednosti označene istim slovom statistički se ne razlikuju (P>0,05);

, **, * - signifikantno pri P=0,05, P=0,01 i P=0,001, respektivno*

NS- nije signifikantno

SED – standardna pogreška razlike

Prosječni sadržaj NO₃-N za sve istraživane tretmane iznosio je 638 ppm. Najveći sadržaj NO₃-N utvrđen je kod tretmana 0KSG-J te 30KSG-P i 0KSG-P između kojih nije bilo statističke razlike (P>0,05), dok je najmanji sadržaj NO₃-N utvrđen kod tretmana 30KSG-J (tablica 2). Primjena KSG rezultirala je sa smanjenjem sadržaja NO₃-N u biljnom materijalu za 15 % u usporedbi sa prosjekom mineralne gnojidbe (P=0,0103). Prosječni sadržaj NO₃-N kod primjene KSG iznosio je 602 ppm, dok je prosjek kod tretmana sa mineralnim gnojivom (0KSG-P i 0KSG-J) iznosio 708 ppm. Količina primijenjenog KSG nije utjecala na sadržaj NO₃-N u biljnom materijalu (P>0,05). Proljetna primjena KSG rezultirala je povećanjem sadržaja NO₃-N za 25 % (P=0,0056) u usporedbi sa jesenskom primjenom KSG (tablica 2). Prosječno je kod proljetne primjene KSG utvrđeno 670 ppm NO₃-N, dok je kod jesenske primjene KSG sadržaj NO₃-N iznosio 535 ppm.

Prema preporukama za hranidbu ovisno o sadržaju nitrata u krmi (Drewnoski i sur., 2019) krma sa manje od 1000 ppm NO₃-N (<4400 ppm NO₃⁻) smatra se sigurnom u hranidbi preživača. Prema nešto oštrijim kriterijima (Cash i sur., 2002), sigurnom za sve kategorije goveda se smatra krma sa manje od 350 ppm NO₃-N, dok bi krmu sa više od 350 ppm NO₃-N trebalo ograničiti na maksimalno 50 % u obrocima za gravidne životinje. Sadržaj NO₃-N u krmi utvrđen u ovom istraživanju kao reakcija na različito vrijeme i količinu primijenjenog KSG na poluprirodnom travnjaku iznosio je manje od 1000 ppm NO₃-N što se može smatrati sigurnom razinom za hranidbu preživača.

Zaključci

U usporedbi sa mineralnom gnojivom primjena KSG na poluprirodnim travnjacima rezultira 21,9 % nižim sadržajem SP i 15 % nižim sadržajem NO₃-N u voluminoznoj krmi. Sa aspekta hranidbe goveda u tovu, prihvatljiva razina SP u voluminoznoj krmi može se postići različitim količinama i različitim vremenom primjene KSG. Iako je sadržaj NO₃-N u voluminoznoj krmi kod svih tretmana bio ispod kritične razine za hranidbu preživača, proljetna primjena KSG rezultirala je povećanjem sadržaja NO₃-N za 25 %. Sadržaj SP i NO₃-N u voluminoznoj krmi kao rezultat primjene KSG na poluprirodnom travnjaku nisu čimbenici koji bi ograničavali primjenu takve krme u hranidbi preživača.

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The effect of farmyard manure application on the crude protein and nitrate content of forage from semi-natural grassland

Abstract

The aim of this study was to determine the crude protein (CP) and nitrate content in forage after application of different amounts of farmyard manure (FM) applied in spring or fall on semi-natural grassland. A total of 6 treatments included different amounts of FM (30 and 50 t ha⁻¹ yr⁻¹), different application times (spring and fall), and two mineral fertilization treatments as controls. The plant mass was cut in the floral development stage (R2) and the CP and NO₃-N content was determined. The FM application resulted in 21.9% lower CP content and 15% lower NO₃-N content compared to mineral fertilization (P <0.05). Spring FM application resulted in a 25% increase in NO₃-N content (P <0.05), without affecting SP content (P > 0.05). The amount of FM application did not affect the forage CP and NO₃- content (P >0.05). It can be concluded that although the FM application of on semi-natural grasslands reduces the CP content compared to mineral fertilization, the CP content does not limit the use of such forage in ruminant feeding and the nitrate content is kept below toxicity limits.

Keywords: semi-natural grassland, farmyard manure, crude protein, nitrates

Jogurt u funkciji zdravlja starijih osoba

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Sažetak

Cilj istraživanja je potaknuti veću konzumaciju jogurta u obrocima starijih osoba koje borave u domovima za starije i nemoćne osobe. U istraživanju je sudjelovalo 50 osoba (54% žena, 46% muškaraca) između 60 i 80 i više godina starosti. Istraživanje je provedeno u 4 faze: I) anketa o stavovima i navikama konzumacije jogurta, II) stručno predavanje o važnosti konzumacije jogurta u starijoj životnoj dobi, III) kušanje običnog, voćnog i probiotičkog jogurta, IV) anketa o stavu o jogurtu. Na temelju rezultata prve i četvrte faze istraživanja dan je prijedlog za korekciju udjela tih vrsta mliječnih proizvoda u jelovnicima domova za starije i nemoćne osobe.

Ključne riječi: jogurt, osobe starije životne dobi, konzumacija

Uvod

Broj starijih osoba u svijetu u stalnom je porastu. Prema podacima EUROSTAT-a za 2017. godinu gotovo petina (19%) stanovnika EU-a imala je 65 ili više godina. Isti izvor navodi da će se udio osoba od 80 ili više godina do 2080. udvostručiti i činiti 13% ukupnog stanovništva. Zbog toga, produženje životnog vijeka starijih osoba te zadovoljenje njihovih potreba predstavljaju veliki izazov za današnje društvo. Uravnotežena prehrana je jedan od najvažnijih faktora zdravog procesa starenja (Turconi i sur., 2012.).

Razvojem medicine, prehrambene industrije i prirodnih znanosti koje proučavaju odnos između prehrane i zdravlja doveo je do popularizacije namirnica koje imaju dokazani pozitivan učinak na ljudsko zdravlje. Primjer takvih namirnica jesu fermentirana mlijeka, skupina mliječnih proizvoda koja danas zauzima važnu ulogu u prehrani čovjeka. Najpopularnije fermentirano mlijeko je jogurt i njegove različite varijante poput voćnog i probiotičkog jogurta. Konzumacija jogurta ima povoljan učinak na probavni sustav, ublažavanje simptoma nekih vrsta dijareja, poboljšava metabolizam laktoze, pozitivno djeluje na kardiovaskularni sustav regulacijom koncentracije kolesterola i krvnog tlaka, antitumornu aktivnost, urogenitalan trakt, pravilan razvoj kosti i olakšavanje regulacije optimalne tjelesne mase (Tudor i Havranek, 2009.).

Jogurt ima visoku prehrambenu i funkcionalnu vrijednost, lako je probavljiv, a zbog pozitivnog djelovanja na zdravlje čovjeka svrstava se u funkcionalnu hranu. Osim toga, tijekom proizvodnje jogurta moguće je povećati njegovu prehrambenu i fiziološku vrijednost dodatkom mlijeka i/ili sirutke u prahu, voćnih dodataka, vlakana ili vitamina dok dodatak probiotičkih bakterija povećava njegovu zdravstvenu vrijednost.

Brojnim znanstvenim istraživanjima utvrđeno je pozitivno djelovanje probiotičkog jogurta na probavni sustav. Jogurt u svom sastavu ima približno devet različitih vrsta proteina, osam vrsta lipida, veliki broj nutritivno važnih makoelemenata i mikroelemenata, vitamine topljive u vodi, laktozu i glukozu (Samaržija, 2015.). Zbog visokog udjela bioaktivnih peptida, lipida, nutraceutika i gotovo idealnog omjera kalcija i fosfora koji uz magnezij imaju primarnu funkciju u mineralizaciji kostiju, jogurt se smatra osobito prikladnom hranom u prehrani starijih osoba. Primjerice, konzumacijom jedne jedinice serviranja (150 ml) jogurta s 3,2% mliječne masti u organizam se unosi 8,6 g proteina, 27 µg folne kiseline, 2,3 mg niacina, 0,4 mg riboflavina, 0,09 mg tiamina, 0,3 µg vitamina B12, 300 mg kalcija, 255 mg fosfora, 29 mg magnezija i 1,1 mg cinka (Samaržija, 2015.). Preporuka je da osobe starije životne dobi trebaju konzumirati od 2-3 jedinica serviranja jogurta/dan.

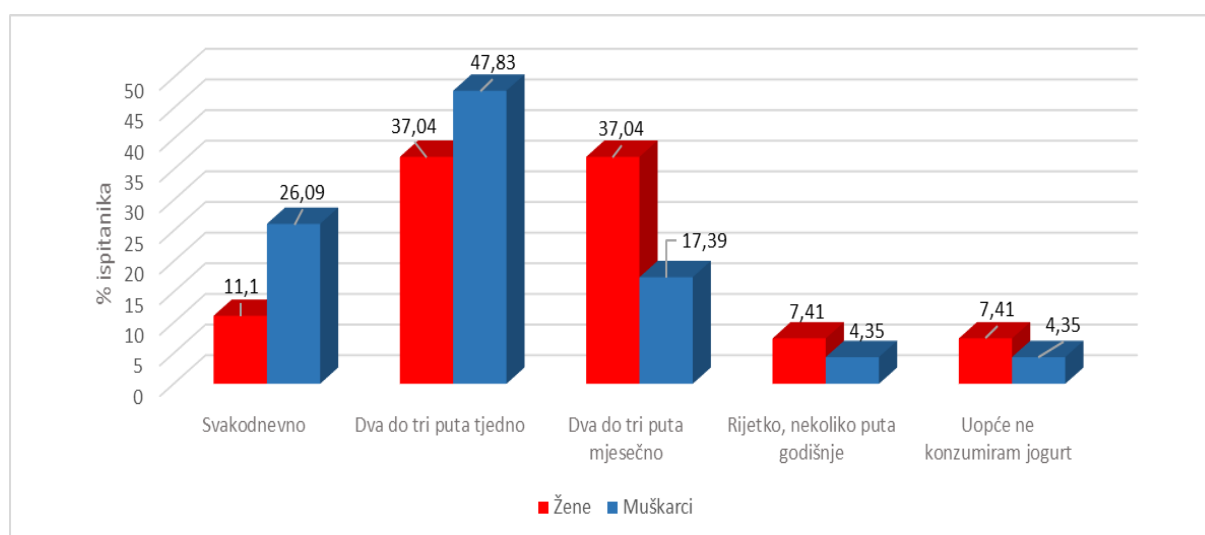
Cilj istraživanja je potaknuti veću konzumaciju jogurta u obrocima starijih osoba koje borave u domovima za starije i nemoćne osobe. Također, svrha istraživanja bila je ispitati njihove stavove i naviku konzumacije jogurta te uz pomoć stručne edukacije upoznati ispitanike o pozitivnim učincima jogurta na njihovo zdravlje.

Materijali i metode

Istraživanje je provedeno u domu za starije i nemoćne sobe na području grada Zagreba. U istraživanju je sudjelovalo 50 osoba (54% žena, 46% muškaraca) u dobi između 60 i 80 i više godina. Istraživanje je provedeno u 4 faze: I) anketa o stavovima i navikama uzimanja jogurta, II) stručno predavanje o važnosti konzumacije jogurta u starijoj životnoj dobi, III) kušanje običnog, voćnog i probiotičkog jogurta, IV) anketa o stavu o jogurtu.

Rezultati i rasprava

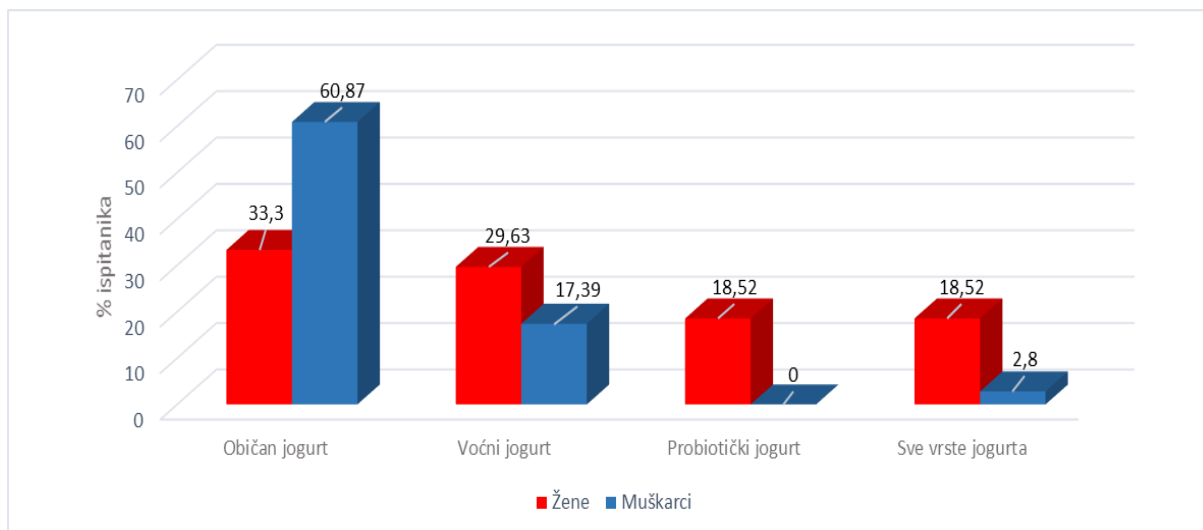
Dosadašnjim istraživanjima utvrđeno je da konzumacija jogurta ima pozitivno djelovanje na zdravlje, te blagotvoran učinak na različite organske sustave u ljudskom organizmu. Konzumacija jogurta doprinosi smanjenju pojave i simptoma bolesti u osoba starije životne dobi poput primjerice Alzheimerove bolesti, dijareje i opstipacije, dijabetesa, kardiovaskularnih bolesti i povišenog kolesterola, te osteoporoze. Starenje ima utjecaj i na humanu mikrobiotu radi promjena u prehrani, smanjene pokretljivosti crijeva, uporabe antibiotika i promjena u probavnom sustavu (O'Toole i sur., 2010.). Jogurt se pokazao idealnom namirnicom i za osobe sklone pothranjenosti koja može biti posljedica kroničnih i zaraznih bolesti, smanjenja tjelesne aktivnosti i metabolizma, tjelesne invalidnosti, poteškoća sa žvakanjem i gutanjem hrane te polifarmacije (El-Abadi i sur., 2014.). Unatoč tome, rezultati provedenog istraživanja ukazuju na nedovoljnu konzumaciju i zastupljenost jogurta u svakodnevnoj prehrani osoba starije životne dobi koje borave u domu za starije i nemoćne osobe. Istraživanjem je utvrđeno da najveći broj ispitanika (37,04% žena i 47,83% muškaraca) konzumira jogurt dva do tri puta tjedno (slika 1).



Slika 1. Učestalost konzumacije jogurta

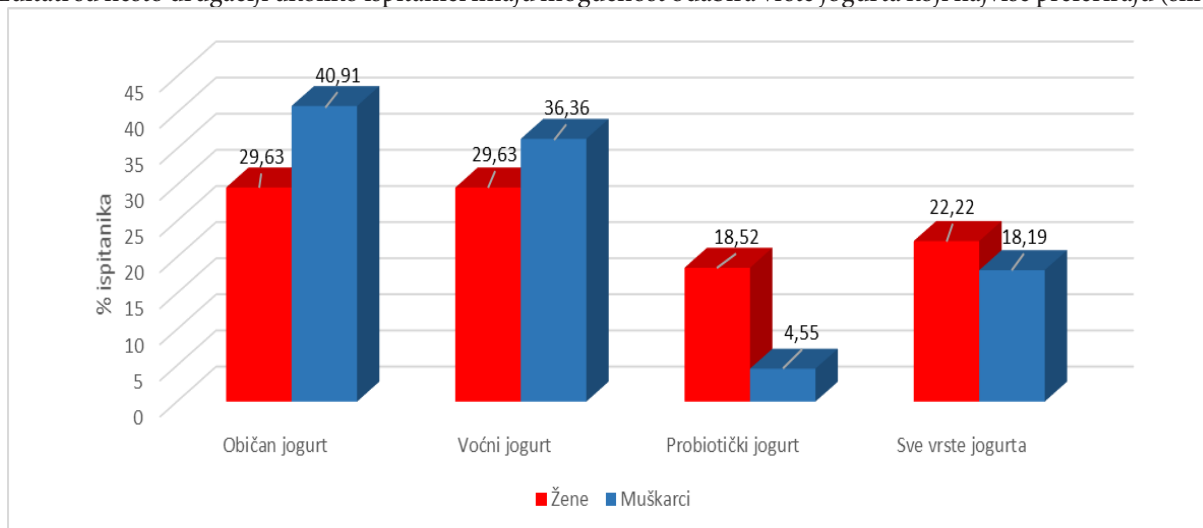
Najveći udio ispitivane populacije, 33,30% žena i 60,87% muškaraca odgovorio je da najčešće konzumiraju običan jogurt koji je i najčešće na jelovniku doma za starije i nemoćne osobe u kojem borave. Voćni jogurt najčešće konzumira 29,63% žena i 17,39% muškaraca. Probiotički jogurt najčešće konzumira 18,52% žena dok muškarci ne konzumiraju takvu vrstu jogurta. Sve vrste jogurta najčešće konzumira 18,52% žena i 2,8% muškaraca (slika 2).

Jogurt u funkciji zdravlja starijih osoba



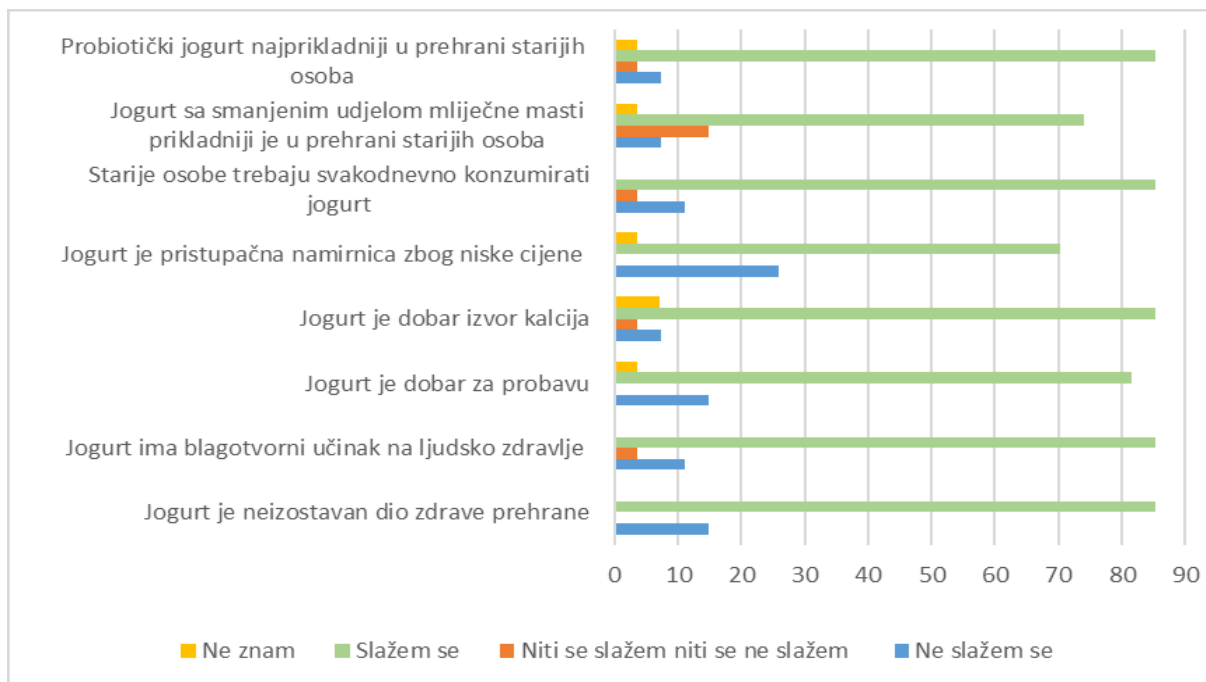
Slika 2. Najčešće konzumirane vrste jogurta

Rezultati su nešto drugačiji ukoliko ispitanici imaju mogućnost odabira vrste jogurta koji najviše preferiraju (slika 3).

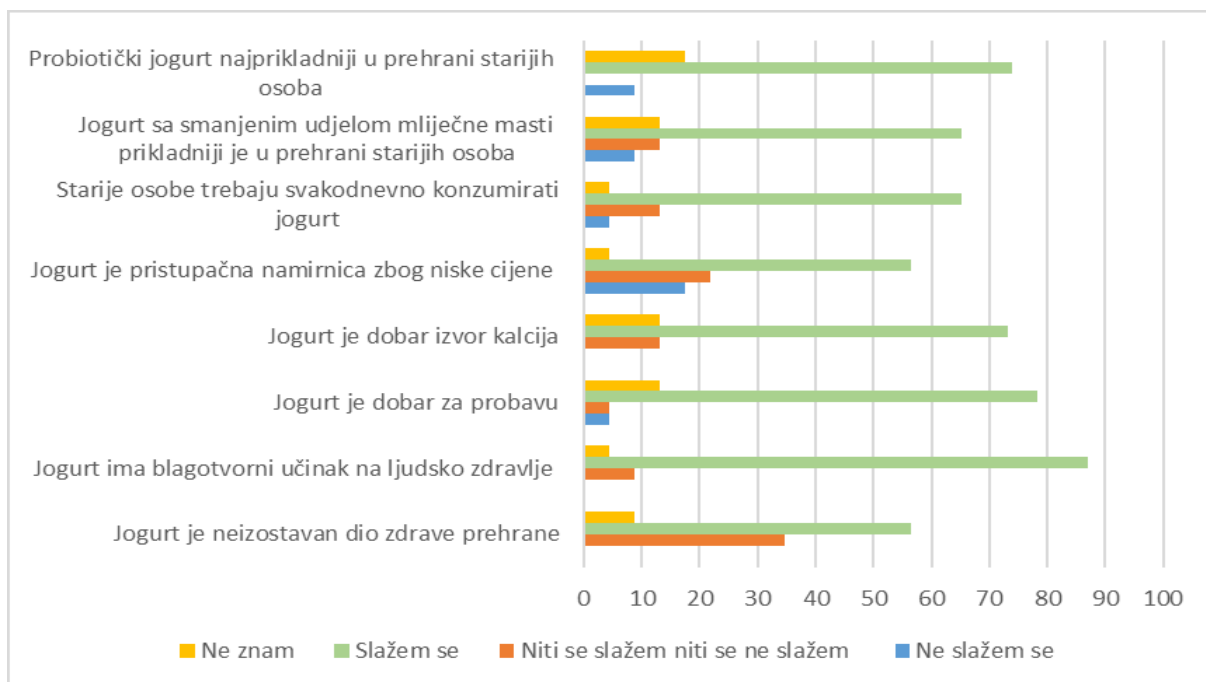


Slika 3. Preferirane vrste jogurta ispitanika

Za potrebe istraživanja ispitanici su upoznati sa pozitivnim djelovanjem jogurta na zdravlje osoba starije životne dobi. Stavove o jogurtu ispitanici su iznijeli nakon kratkog predavanja o važnosti jogurta u funkciji zdravlja starijih osoba te kušanja običnog, voćnog i probiotičkog jogurta. Najveći broj žena i muškaraca slažu se da je jogurt neizostavan dio zdrave prehrane te podupire stavove da jogurt ima blagotvoran učinak na ljudsko zdravlje, da je dobar za probavu i da predstavlja dobar izvor kalcija (slike 4 i 5). Također, ispitanici jogurt smatraju cjenovno pristupačnom namirnicom koju bi trebalo svakodnevno konzumirati pri čemu prednost daju jogurtu sa smanjenim udjelom mliječne masti i probiotičkom jogurtu kao najprikladnijima u prehrani starijih osoba.



Slika 4. Stavovi ispitanica o jogurtu



Slika 5. Stavovi ispitanika o jogurtu

Zaključak

Jogurt je gotovo savršena namirnica u prehrani osoba starije životne dobi što je potvrđeno brojnim znanstvenim istraživanjima. Istraživanje provedeno u domu za starije i nemoćne osobe ukazuje na pozitivne stavove o važnosti jogurta u funkciji zdravlja starijih osoba, s nešto većim naglaskom na pozitivne stavove ženskog djela ispitanika.

Osobe starije životne dobi vole jogurt iako ga konzumiraju u nedovoljnoj količini. U skladu s tim, preporuka je u jelovnik uvrstiti različite vrste jogurta te im dati na izbor.

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Yoghurt as a function of health in the elderly people

Abstract

The aim of the study is to encourage greater consumption of yogurt in the meals of the elderly people living in nursing homes. The study included 50 persons (54% women, 46% men) between 60 and 80 years and over. The research was conducted in four phases: I) a survey on the attitudes and habits of yogurt consumption, II) a lecture of the importance of yogurt consumption in the elderly, III) a tasting of plain, fruit and probiotic yoghurt, IV) a survey on the attitude of yogurt. Based on the results of the first and fourth phases of the research, a proposal was made for the correction of the share of these types of dairy products in menus of nursing homes.

Keywords: yogurt, elderly people, consumption

Polimorfizmi MTNR1A gena u populaciji istarske ovce

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Sažetak

U umjerenom klimatskom pojasu ovce karakterizira sezonska reproduktivna aktivnost. Cilj ovog istraživanja bio je utvrditi polimorfizme MTNR1A gena i pojavu izvansezonskih janjenja istarske ovce. Na uzorku od 30 jedinki istarske ovce PCR-RFLP metodom određeni su polimorfizmi na lokusu 606 i 612 MTNR1A gena. Utvrđena su sva tri genotipa na oba istraživana lokusa (606: CC 0,17; CT 0,40; TT 0,43; 612: GG 0,64; GA 0,33; AA 0,03), a frekvencije alela iznosile su: C 0,37; T 0,63; G 0,80 i A 0,20. U istraživanom uzorku istarske ovce utvrđena je visoka frekvencija genotipa GG i alela G koji su svojstveni za pasmine s mogućnošću izvansezonskog janjenja. Iako istarske ovce odlikuje genetska predispozicija za izvansezonska janjenja, ona su teže ostvariva uporabom trenutnih tehnoloških postupaka podređenih proizvodnji mlijeka.

Ključne riječi: istarska ovca, lokus 606, lokus 612, MTNR1A, PCR-RFLP

Uvod

Pasmine ovaca koje potječu s područja umjerenog klimatskog pojasa odlikuje sezonska reproduktivna aktivnost ovisna o fotoperiodu. Ovisno o duljini trajanja mraka, reproduktivna aktivnost regulirana je različitim stupnjem sekrecije hormona melatonina (Karsch i sur., 1984). Na ciljna tkiva i organe melatonin djeluje putem specifičnih G protein spregnutih receptora (GPCR) MT1 i MT2 od kojih je samo MT1 uključen u regulaciju reproduktivne aktivnosti (Dubocovich i sur., 2003). MTNR1A gen kodira MT1 receptor i aktivan je u regulaciji reproduktivne aktivnosti ovaca (Mura i sur., 2010) te se smatra kandidat genom u selekciji za poboljšanje izvansezonske pojave estrusa u ovaca. Polimorfna mjesta MTNR1A gena na pozicijama 606 i 612 (Reppert i sur., 1994) drugog egzona omogućila su detekciju poželjnih alela u različitim pasmina ovaca (Chu i sur., 2006; Carcangiu i sur., 2009; Saxena i sur., 2014; Držaić i sur. 2016, 2017, 2019). Istarska ovca smatra se sezonski poilestričnom pasminom u koje se estrus javlja uglavnom tijekom jeseni, a glavina ovaca se janji od siječnja do ožujka (MP, 2019). Primarno se uzgaja radi proizvodnje mlijeka koje se prerađuje u sir. Tijekom lakatacije od 179 dana ovce proizvedu u prosjeku oko 170 kg mlijeka od kojeg oko 33% posiše janjad tijekom prvih 55 dana laktacije (MP, 2019). Cilj predmetnog istraživanja bio je utvrditi frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena i pojavu izvansezonskih janjenja u istarskih ovaca tijekom standardnog proizvodnog procesa u proizvodnji ovčjeg mlijeka.

Materijal i metode

Ovim istraživanjem bilo je obuhvaćeno 30 ovaca koje su nasumično odabrane iz osam stada s područja istarskog poluotoka. Podatci o datumu rođenja pojedine jedinke, kao i podatci o janjenjima (redoslijed, datum janjenja) ustupljeni su od strane Ministarstva poljoprivrede Republike Hrvatske. Iz dlake je izolirana DNK prema preporuci proizvođača (Sigma-Aldrich, Saint Louis, MO, USA), a uspješnost izolacije i kvaliteta DNK provjereni su elektroforezom na 1%-tnom gelu agaroze. Umnažanje ciljnog odsječka DNK drugog egzona MTNR1A gena duljine 824 bazna para (bp) provedeno je postupkom lančane reakcije polimerazom (PCR) s oligonukleotidnim početnicama: 5'-TGTGTTTGTGGTGAGCCTGG-3'; 5'-ATGGAGAGGGTTTTCGTTTA -3' (Messer i sur, 1997). Uvjeti PCR reakcije za umnažanje glavnog dijela drugog egzona MTNR1A gena (GenBank Acc. No. U14109) bili su identični onima opisanim u ranije provedenim istraživanjima (Držaić i sur. 2016, 2017, 2019). Provjera uspješnosti

PCR reakcije obavljena je pomoću elektroforeze na 1%-tnom gelu agaroze. Genotipizacija istraživanih jedinki za lokus 606 i lokus 612 MTNR1A gena provedena je PCR-RFLP metodom s *RsaI* i *MnII* restrikcijskim endonukleazama (New England Biolabs, Beverly, MA, USA). Očitanje genotipova izvršeno je na 3% gelu agaroze. Genotipizacija uzoraka na lokusu 606 bila je: CC (267 bp, 23 bp), CT (290 bp, 267 bp, 23 bp) i TT (290 bp), a na lokusu 612: GG (236 bp, 67 bp), GA (303 bp, 236 bp, 67 bp) i AA (303 bp; Carcangiu i sur., 2009; Držaić i sur., 2016, 2017, 2019).

Frekvencije alela i genotipova, frekvencije janjenja po sezonama za utvrđene genotipove izračunati su korištenjem statističkog programa R (R Core Team, 2008). Sezone su definirane na sljedeći način: proljeće (21.3-20.6), ljeto (21.6-20.9.), jesen (21.9-20.12.) i zima (21.12-20.3). Testiranje odstupanja frekvencija utvrđenih genotipova od očekivanih za populaciju u Hardy-Weinbergovoj ravnoteži u promatranom uzorku izvršeno je Hi-kvadrat testom (χ^2).

Rezultati i rasprava

Analizom polimorfizama na osnovu dužine restrikcijskih fragmenata (RFLP) identificirani su polimorfizmi MTNR1A gena na poziciji 606 i poziciji 612 referentne sekvence (U14109) u svih istraživanih uzoraka.

U tablici 1. prikazane su utvrđene frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena i rezultati Hi-kvadrat testa. Na lokusu 606 MTNR1A gena utvrđene su C i T alelne varijante s frekvencijama 0,37 i 0,63, kao i zastupljenost sva tri genotipa: CC (0,17), CT (0,40) i TT (0,43). Nadalje, na lokusu 612 utvrđene su alelne varijante G (0,80) i A (0,20) te sva tri genotipa s frekvencijama GG (0,64), GA (0,33) i AA (0,03). Frekvencije alela i genotipova na lokusima 606 i 612 MTNR1A gena uvelike se razlikuju između dosad istraživanih pasmina ovaca. Tako primjerice, više frekvencije C alela (0,48-0,87) i genotipa CC (0,24-0,75) od frekvencija utvrđenih predmetnim istraživanjem navode Chu i sur. (2006), Carcangiu i sur. (2009) i Saxena i sur. (2014). Isti autori navode podjednake ili niže frekvencije alela G i genotipa GG od onih utvrđenih u istarske ovce (G 0,55-0,88; GG 0,19-0,77). Slične vrijednosti G alelne varijante i genotipa GG utvrđene su i u drugih pasmina ovaca koje slove kao sezonski poliestrične, kao na primjer travnička premenka (G 0,88; GG 0,76) i cigaja (G 0,92 i GG 0,84; Držaić i sur. 2016, 2019). Međutim, u pravilu, visoke frekvencije alela C i G, kao i genotipova CC i GG utvrđene su u pasmina čija reproduktivna aktivnost nije sezonskog karaktera (Chu i sur., 2006; Carcangiu i sur. 2009; Saxena i sur., 2014). Sa sezonskom poliestričnosti ovaca povezane su više frekvencije alela T i A, odnosno homozigota TT i AA na lokusima 606 i 612 MTNR1A gena (Chu i sur., 2006; Carcangiu i sur. 2009). Navedeno je potvrđeno i istraživanjem Carcangiu i sur. (2010) koji navode više frekvencije alela A (0,66) i genotipa AA (0,50) u ovaca muflona (*Ovis Gmelini Musimon*). Na osnovu navedenog, u istraživanom uzorku istarske ovce postoji genetska preddispozicija za izvansezonsku pojavu estrusa (a samim time i janjenja) na lokusu 612 MTNR1A gena, dok isto nije potvrđeno na lokusu 606.

Tablica 1. Utvrđene frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena te rezultati χ^2 -testa

Frekvencija genotipova						Frekvencija alela				HWE	
Lokus 606			Lokus 612			Lokus 606		Lokus 612		Lokus 606	Lokus 612
CC	CT	TT	GG	GA	AA	C	T	G	A	χ^2	χ^2
0,17	0,40	0,43	0,64	0,33	0,03	0,37	0,63	0,80	0,20	0,578 ^{nz}	0,052 ^{nz}

HWE – Hardy-Weinberg equilibrium; $\chi^2_{0,05,1} = 3,84$; nz – nije značajno

Rezultati χ^2 -testa pokazuju da utvrđeni genotipovi MTNR1A gena u istraživanoj populaciji istarske ovce na lokusu 606 i lokusu 612 statistički ne odstupaju značajno od očekivanih frekvencija genotipova populacije u Hardy-Weinbergovoj ravnoteži (Tablica 1). Distribuciju genotipova na lokusu 606 i 612 MTNR1A gena unutar Hardy-Weinbergove ravnoteže navode Saxena i sur. (2014) za Chokla pasminu, Držaić i sur. (2016) za travničku pramenku i Držaić i sur. (2019) za cigaju.

Distribucije frekvencija janjenja ovaca temeljem genotipa na istraživanim lokusima MTNR1A gena po sezonama prikazane su u tablici 2. U istraživanoj populaciji istarske ovce evidentirano je ukupno 75 janjenja tijekom tri sezone. Najveći udio janjenja evidentiran je tijekom zime (>80%), zatim tijekom jeseni (12%) i tijekom proljeća (6,66%), dok u ljetnoj sezoni nije bilo evidentiranih janjenja. Distribucija janjenja, bez obzira na genotip, rezultat je tehnološkog procesa proizvodnje mlijeka kao osnovne namjene uzgoja istarske ovce. Naime, otkup ovčjeg mlijeka započinje u ožujku ili travnju stoga uzgajivači planskim pripustom tempiraju glavninu janjenja u zimskom periodu (21.12.-20.3).

Navedenim postupkom osigurano je da do početka otkupa mlijeka janjad bude odbijena s dobi od oko 55 dana (MP, 2019) te uzgajivači mogu započeti s prodajom i/ili preradom mlijeka.

Tablica 2. Distribucija frekvencija janjenja istarskih ovaca po sezonama obzirom na genotipove lokusa 606 i 612 MTNR1A gena

Genotip	Sezona janjenja										UKUPNO	
	Proljeće		Ljeto		Jesen		Zima					
	n	%	n	%	n	%	n	%	n	%		
Lokus 606	CC	3	4,00	0	0,00	2	2,67	8	10,67	13	17,33	
	CT	1	1,33	0	0,00	2	2,67	34	45,33	37	49,33	
	TT	1	1,33	0	0,00	5	6,67	19	25,33	25	33,33	
	Σ	5	6,66	0	0,00	9	12,01	61	81,33	75	100,00	
Lokus 612	GG	0	0,00	0	0,00	8	10,67	30	40,00	38	50,67	
	GA	5	6,67	0	0,00	1	1,33	37	36,00	33	44,00	
	AA	0	0,00	0	0,00	0	0,00	4	5,33	4	5,33	
	Σ	5	6,67	0	0,00	9	12,00	61	81,33	75	100,00	

Dominantna sezona janjenja za ovce svih genotipova utvrđenih na lokusima 606 i 612 MTNR1A gena bila je zima. Gotovo polovicu svih evidentiranih janjenja ostvarile su jedinke CT genotipa (49,33%), odnosno jedinke GG genotipa (50,67%). Ovce sva tri genotipa lokusa 606, kao i ovce GA genotipa 612, imale su evidentirana janjenja u tri sezone (proljeće, jesen, zima), dok su ovce GG genotipa lokusa 612 imale evidentirana janjenja u dvije sezone (jesen, zima), a ovce AA genotipa samo zimi.

Menadžment uzgoja istarske ovce u proizvodnji mlijeka prakticira glavninu janjenja tijekom zimskog perioda što je, u ovom istraživanju, rezultiralo s manjim brojem ili izostankom janjenja u pojedinim sezonama. Uz navedeno distribucija pojedinih genotipova na istraživanim lokusima varirala je od 0,03 (AA) do 0,64 (GG) što dodatno otežava interpretaciju rezultata te određivanje smjera i intenziteta djelovanja istraživanih polimorfizama. Međutim, povezanost polimorfizma na lokusima 606 i 612 MTNR1A gena s pojavom estrusa, a posljedično i janjenja, izvan uobičajene sezone u različitim pasmina ovaca utvrdili su Chu i sur. (2006) i Carcangiu i sur. (2009). Chu i sur. (2006) navode povezanost CC i GG genotipova s nesezonskom pojavom estrusa te TT i AA genotipova i sezonske pojave estrusa u kineskih pasmina ovaca. Slične rezultate navode i Carcangiu i sur. (2009) naglašavajući utjecaj genotipa GG koji je uslijed visoke frekvencije (0,81) u Sarda pasmine djelovao na pojavu estrusa u proljeće te janjenje u jesen.

Zaključci

U populaciji istarske ovce PCR-RFLP metodom utvrđeno je postojanje polimorfizama MTNR1A gena na oba istraživana lokusa. Na lokusu 606 dominirao je alel T (0,63) i genotip TT (0,43), dok je na lokusu 612 dominirao alel G (0,80) i genotip GG (0,64). Dominantna sezona janjenja svih genotipova bila je zima (>80%), a najveći broj evidentiranih janjenja imale su jedinke CT (49,33%) i GG (50,67%) genotipa. Učestalost janjenja u zimskim mjesecima dominantno uvjetovana tehnologijom uzgoja istarske ovce otežava donošenje zaključka o utjecaju MTNR1A gena na mogućnost izvansezonskih janjenja. Ipak, uvažavajući rezultate prethodno objavljenih istraživanja, utvrđene frekvencije genotipova i alela na lokusu 612 MTNR1A gena upućuju na dobru genetsku predispoziciju istarske ovce za izvansezonsku spolnu aktivnost.

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MTNR1A gene polymorphisms in Istrian sheep population

Abstract

In the temperate climate zone, sheep are characterized by seasonal reproductive activity. The aim of the present study was to identify polymorphisms of MTNR1A gene and frequency of out-of-season lambing in Istrian sheep. On a sample of 30 Istrian sheep polymorphisms at the locus 606 and 612 of the MTNR1A gene were determined by PCR-RFLP method. All three genotypes were determined in both loci (606: CC 0.17; CT 0.40; TT 0.43; 612: GG 0.64; GA 0.33; AA 0.03) and allele frequencies were: C 0.37; T 0.63; G 0.80 and A 0.20. In investigated sample of Istrian sheep high frequency of genotype GG and allele G that are characteristic for out-off-season lambing breeds was determined. Although Istrian sheep are characterized by a genetic predisposition for out-off-season lambing, they are more difficult to achieve by using current technological procedures subordinated to milk production.

Keywords: Istrian sheep, locus 606, locus 612, MTNR1A, PCR-RFLP

Utjecaj alternativnih sustava držanja na oštećenja prsnih kostiju kokoši nesilica

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Sažetak

Napuštanjem držanja kokoši nesilica u klasičnim kavezima te prijelazom na nove alternativne sustava držanja i obogaćene kaveze očekivalo se da će doći do znatnog poboljšanja dobrobiti nesilica. Omogućen im je veći prostor za boravak i kretanje unutar kaveza i samog objekta te im je u slobodnom sustavu osiguran izlaz u ispuste. No, ipak pojavilo se i nekoliko problema za koje još nema rješenja i tek se na njima započelo raditi. Jedan od tih problema je i oštećenje prsne kosti koje se javlja kod kokoši nesilica, a može biti iskazano devijacijom prsne kosti što je blaži oblik i lomovima prsne kosti koje je teži oblik i prouzrokuje životinji veliku bol i patnju. Cilj ovog rada bio je prikazati dosadašnje spoznaje vezane uz pojavnost ovih poteškoća u držanju kokoši nesilica u alternativnim uzgojima i obogaćenim kavezima. Iz dosadašnjih je spoznaja vidljivo da su oštećenja prsnih kostiju najviše izražena kod nesilica koje su držane u volijerama i podnom uzgoju, dok je znatno manje oštećenja zabilježeno u obogaćenim kavezima. Neka od mogućih rješenja ovog problema usmjerena su prema promjenama u opremi za držanje nesilica, genetici odnosno stvaranju novih robusnijih hibrida te hranidbi s ciljem ojačavanja koštanog sustava.

Ključne riječi: kokoši nesilice, oštećenja prsne kosti, alternativni uzgoj, obogaćeni kavezi

Uvod

Začetak moderne proizvodnje konzumnih jaja datira još u davnim dvadesetim i tridesetim godinama dvadesetog stoljeća kada su u SAD-u razvijeni prvi žičani baterijski kavezi za smještaj kokoši nesilica kako bi se kokoši zaštitile od grabežljivaca, bolesti i ozljeda. Takvi su kavezi pojednostavili uzgoj povećavajući učinkovitost kokoši nesilica i smanjujući potrebu za prostorom u kojem su kokoši držane (Leaders, 2012). Sustavi za smještaj kokošima moraju osigurati prostor, hranu, vodu, svjetlo, kvalitetu zraka i sanitarne uvjete koji promiču dobro zdravlje i dobrobit kokoši te sigurnost hrane. Krajem osamdesetih i devedesetih godina prošlog stoljeća, kada su međunarodni propisi počeli ograničavati uporabu konvencionalnih kaveza, započela su razmišljanja o prijelazu na nove sustave smještaja kokoši nesilica koji su za cilj imali uravnotežiti potrebe životinja za povećanjem njihove dobrobiti te i dalje osigurati profitabilnost proizvodnje konzumnih kokošjih jaja. Alternativni sustavi proizvodnje su: slobodno držanje, polu-intenzivno držanje, držanje na dubokoj prostirci i etažno držanje u staji. Oni su sa sustavom držanja kokoši nesilica u obogaćenim kavezima obavezni načini držanja kokoši nesilica u zemljama EU od 01.01.2012. godine kada je stupila na snagu Direktiva 1999/74/EC (Čížmak i Bišćan, 2017). Te promjene su se možda čak i najviše odrazile na proizvodnju konzumnih jaja u RH, u odnosu na ostale proizvodnje jaja u EU. Ulaskom RH u članstvo EU 01.07.2013. godine hrvatski proizvođači konzumnih jaja su nakon turbulentnih 90- tih gotovo preko noći stavljeni pred zid te su svoju dosadašnju proizvodnju u klasičnim baterijskim kavezima morali zamijeniti jednim od sustava dopuštenih gore navedenom Direktivom. Neke proizvodnje su se tada naprasno ugasile, a ostali su krenuli s opremanjem i adaptacijom postojećih objekata a drugi od temelja s novim objektima i opremom najčešće kreditnim zaduživanjem i jednim dijelom uz potporu EU sredstava iz različitih tada dostupnih fondova. Između ostaloga, to je rezultiralo i time da smo od nekadašnje samodostatne proizvodnje još i danas uvoznici gotovo 20 % konzumnih kokošjih jaja (Bobetić, 2019).

Napuštanjem držanja kokoši nesilica u klasičnim kavezima te prijelazom na alternativne sustava držanja i obogaćene kaveze očekivalo se je da će doći do znatnog poboljšanja dobrobiti nesilica. Omogućen im je veći prostor za boravak

i kretanje unutar kaveza i samog objekta te im je u slobodnom sustavu osiguran izlaz u ispuste. Međutim, sve se više ukazuje na probleme koji se u tim sustavima proizvodnje pojavljuju. Jedan od tih problema su i oštećenja prsnih kostiju kod kokoši nesilica u vidu devijacija i lomova koja mogu biti bolna za kokoši te time ugrožavaju dobrobit i smanjuju produktivnost (Harlander i sur. 2015).

Stoga je cilj ovog rada bio ukazati na pojavnost oštećenja prsnih kostiju kokoši nesilica u alternativnom uzgoju i obogaćenim kavezima te prikazati neka od rješenja da tih pojava bude što manje.

Dosadašnje spoznaje o pojavnosti oštećenja prsnih kostiju

Jedan od najvećih izazova s kojim se suočavaju proizvođači konzumnih kokošjih jaja je pojavnost oštećenja prsnih kostiju. (FAWC, 2010; 2013). U posljednjem desetljeću brojne studije, koje su uglavnom provedene u zemljama EU, bilježe devijacije i lomove prsnih kostiju koje se kreću od 5 % pa do čak 97 %, ovisno o sustavu držanja i dobi nesilica. Stvarni raspon broja zahvaćenih kokoši jako varira i vjerojatno ovisi o nizu čimbenika, uključujući između ostalog: dob, okoliš uzgoja, hranidbu, genetsku liniju i sistem smještaja. Iako ptice sa ili bez prijeloma mogu djelovati slično (osim ako koristite posebne metode promatranja), prijelomi se obično mogu lako identificirati palpacijom. Vjeruje se da prijelomi, pogotovo kada su svježi i u stanju u kojem kosti nisu imobilizirane već su u zasebnim komadima, kod kokoši uzrokuju bolove i smanjuju živahnost i proizvodnost. Čini se da su učinci na proizvodnost posebno veliki u kokoši starijih od 50 tjedana.

Tako su Rodenburg i sur. (2008) proveli istraživanje koje je za cilj imalo provesti usporedbu dobrobiti kokoši nesilica u obogaćenim kavezima i držanju na dubokoj prostirci. U dobi kokoši od 60 tjedana kokoši držane u obogaćenim kavezima imale su uz smanjenu smrtnost i znatno nižu učestalost prijeloma prsnih kostiju. Cilj istraživanja koji su proveli Wilkins i sur. (2011) bio je između ostalog procijeniti oštećenost prsnih kosti kod kokoši nesilica držanih u obogaćenim kavezima i volijerama. Na kraju proizvodnog razdoblja procijenjeno je da se u obogaćenim kavezima 36 % kokoši nesilica imalo oštećenja prsnih kostiju, dok je kod volijera taj broj iznosio više od 80 %.

Riber i Hinrichsen (2016) su proveli istraživanja u Danskoj s ciljem procjene oštećenja prsne kosti u komercijalnim jatima kokoši nesilica držanih u različitim sustavima i pri različitoj dobi. Zabilježili su veća oštećenja prsnih kosti kod nesilica starih 62 tjedna u odnosu na one stare 32 tjedna. Kokoši u volijerama imale su veća oštećenja prsnih kostiju u odnosu na one držane podnim načinom držanja; 11,6 : 4,9 %. Heerkens i sur. (2016) su kod 47 jata kokoši nesilica starih 60 tjedana, a držanih u volijerama u Belgiji utvrdili 82,5 % lomova i 58,9 % devijacija prsnih kosti. Nasr i sur (2013) zaključuju da kokoši nesilice bez oštećenja u odnosu na one s lomovima prsnih kostiju nesu veći broj jaja (91,7 % : 84,9 %), jaja su teža (61,9 g : 60,2 g), jedu manje hrane (139 g : 151 g) i piju manje vode (212 ml : 237 ml). Kappeli i sur. (2011) iznose da je pojavnost oštećenja prsnih kosti na švicarskim farmama iznosila i do 83 % u višetažnim sustavima uzgoja - volijerama.

Struelens i Tuytens (2009) ukazuju na prečke za sjedenje kokoši koje propisuje Direktiva EU 1999/74, čiji dizajn i izvedba mogu utjecati na veću pojavnost oštećenja prsnih kosti. U istraživanju koje su proveli Đukić Stojčić i sur. (2017) je zaključeno da je pojavnost oštećenja prsne kosti kod obogaćenih kaveza bez prečki 4,3 % dok je kod obogaćenih kaveza sa prečkama ona iznosila 39 %. Slično su zaključili i Rodenburg i sur. (2008), te Wilkins i sur. (2011). Hester i sur. (2013) su izvijestili da je većina problema sa skeletnim sustavom povezana s osteoporozom koja je posebno naglašena kod držanja kokoši nesilica u obogaćenim kavezima, a pojačan udio oštećenja je zabilježen kod nesilica koje su u kavezima imale metalne prečke. Stratmann i sur. (2015) zaključuju da mekana poliuretanske prečke značajno smanjuju pojavnost oštećenja prsnih kosti kod višetažnih sustava držanja kokoši nesilica u odnosu na metalne prečke. Heerkens i sur. (2016) su nakon provedenog istraživanja zaključili da ugrađivanje rampi između prečaka značajno smanjuje oštećenja prsnih kosti te da je potrebno razmišljati o modifikaciji dosadašnjih sustava višetažnog držanja kokoši nesilica. Cilj istraživanja koje su proveli Rufener i sur. (2018). bio je istražiti utjecaj lomova prsne kosti na produktivnost kokoši nesilica. Zaključili su da su kokoši s prijelomima u 61. tjednu nesivosti imale za 16,2 % nižu nesivost nego kokoši bez prijeloma.

Riber i sur. (2018) u svom revijalnom radu iznose trenutne spoznaje o utjecaju oštećenja prsnih kosti na dobrobit kokoši nesilica. Zaključuju da su oštećenja prsnih kosti zabilježena u svim tipovima komercijalne proizvodnje konzumnih kokošjih jaja, s različitim postotkom pojavnosti ovisno o sistemu, dobi i hibridu kokoši nesilica. Kokoši nesilice s slomljenim prsnim kostima pokazuje razlike u ponašanju koje je vidljivo u korištenju gnijezda, prpošenju i smanjenom mobilnosti. Strogo sugeriraju na činjenicu da je lom prsne kosti izvor boli i tijekom više tjedana nakon što je do njega došlo. Negativan utjecaj lomova prsne kosti vidljiv je u smanjenoj proizvodnji jaja. Zbog manjeg broja

provedenih istraživanja, utjecaj devijacija prsne kosti na kondiciju kokoši nesilica još nije dovoljno jasan. No, nađeno je da devijacija prsne kosti svakako ima negativan utjecaj na dobrobit kokoši nesilica. Preporučuju da je potrebno vršiti daljnja istraživanja koja će se fokusirati na utjecaj genetskih linija i genetske selekcije, držanja i hranidbe na razvoj, pojavnost oštećenja prsnih kosti kod kokoši nesilica.

Intervencije za smanjenje pojavnosti oštećenja prsnih kostiju

Iz dosadašnjih spoznaja izrađen je letak za držaoce kokoši nesilica kojim se želi ukazati na mogućnosti smanjenja pojavnosti oštećenja prsnih kostiju (CA 15224, 2018). Upute su fokusirane na aktivnosti tijekom uzgoja pilenki, periodu nesenja jaja a najveći je naglasak dan hranidbi. Poznato je da pilenke kontinuirano razvijaju svoje koštane i kognitivne mehanizme potrebne za svoj daljnji život tako da je razdoblje uzgoja vrlo značajno za osiguranje jakih kostiju kod kokoši nesilica kako bi se mogle samostalno kretati kroz sisteme proizvodnje bez ozljeda. Kada je moguće, okoliš uzgoja i nesenja jaja treba biti što sličniji, osobito kod kaveznog i ne kaveznog sistema proizvodnje jaja. Tijekom uzgoja dodatnu opremu treba uvoditi postupno tako da se kokoši mogu naučiti kako ih koristiti na odgovarajući način što dovodi do preciznijih pokreta. Generalno je poznato da povećana aktivnost vodi ka boljem zdravlju kostiju. Proizvođači bi također trebali razmotriti specifične intervencije uključujući raniji pristup prečkama i/ili rampama. Poznato je da kokoši nisu dobri letači te da su bolje u hodanju i izvođenju kratkih skokova kako bi se okomito kretale. Iz tog razloga kokošima treba osigurati alternativne načine za okomito kretanje pomoću rampi ili prečki postavljenim blizu jedne drugima no opet da se ograniči nakupljanje izmeta ili spavanje (na rampama). Tijekom nesenja kokoši se sudaraju s opremom u njihovom okruženju što dovodi do prijeloma. Sudaranja se događaju u kaveznim i ne kaveznim sustavima gdje uzroci mogu biti panika ili promet visoke gustoće, ili u sumrak dok se ptice kreću prema gornjoj etaži. Također se može sumnjati da su uzroci u ubrzanom mahanju krilima tijekom panike ili dok se ptice pokušavaju vratiti u svoj prvobitni položaj nakon pada. Neke specifične preporuke za smanjenje panike uključuju postepeno kretanje uzgajivača kroz svoje nastambe u odjeći različite obojanosti, iz različitih smjerova i u različito doba dana. U početku bi se proizvođači trebali polako kretati, a nakon što se kokoši priviknu, započeti s bržim i redovitijim kretanjem. Idealno, to bi trebalo započeti što je ranije moguće, uključujući i period tijekom uzgoja i/ili unutar prvog tjedna nakon useljenja. Radio aparati ili neki drugi izvori buke također mogu biti od pomoći, poglavito ako se koriste od početka još u uzgojnim objektima. Za pomoć pri kretanju (više se odnosi na ne kavezni sustav) od početka uzgoja potrebno postaviti prečke tako da pomažu kretanje u okomitom pravcu. Generalno, prečke trebaju biti postavljene jedna od druge pod kutom manjim od 45 ° i s razmacima manjim od 50 cm okomito i 75 cm vodoravno. Pokazalo se da rampe povećavaju broj kontroliranog kretanja kokoši što dovodi do smanjenja sudaranja tijekom ključnog perioda - sumraka. Pojavnost prijeloma prsnih kostiju bila je manja za 9 % u komercijalnim volijerama s rampama (64 %) u odnosu na volijere bez rampi (73 %), a također je utvrđeno je da su veće koristi od rampi u smanjenju prijeloma ukoliko ih se uključi i u uzgoju i u periodu nesenja.

Hranidba je vrlo zahtjevna glede davanja preporuka s obzirom da su se potrebe hibridnih nesilica tijekom vremena značajno promijenile kao što su i proizvodnja jaja i duljina ciklusa nesenja dramatično porasle. Osim toga, različiti sustavi držanja poput slobodnog držanja imaju veće zahtjeve za hranjivim tvarima dok će varijacije u izloženosti sunčevoj svjetlosti, sadržaju kalcija u vodi i drugi okolišni čimbenici imati utjecaj na specifične hranidbene zahtjeve. S obzirom na mnogobrojne čimbenike konačni obrok treba biti sastavljen u suradnji s stručnjakom za hranidbu. Opće preporuke temeljene na provedenim istraživanjima ukazuju da tijekom uzgoja pilenke trebaju dobivati minimalno 9 g/kg između 1. i 14. tjedna starosti i tada slijedi povećanje do 35 g/kg kalcija u obroku, a dodatak vitamina K (12 mg/kg) dovodi do povećanja volumena kostiju što koristi boljem skladištenju kalcija. Ističe se da tijekom nesenja krmna smjesa s 0,11 do 0,26 % nefitinskog fosfora dopunjena s fitazom može poslužiti boljem zadržavanju kalcija. Korištenje linolenske kiseline (npr. iz lanenog sjemena) od 23. do 30. tjedna starosti je pokazalo da poboljšava čvrstoću kostiju i rezultira s manje prijeloma iako proizvođači trebaju biti oprezni zbog učinaka na kvalitetu jaja. Premda je malo vjerojatno da će dnevno povećanje kalcija u obroku rezultirati da kokoši apsorbiraju više kalcija postoje dokazi da bi davanje većih čestica kalcija u periodu od 2 do 3 sata neposredno prije sutona dovelo da kokoš apsorbira više kalcija (CA 15224, 2018).

Zaključak

Lomovi i devijacije prsne kosti smanjuju dobrobit kokoši nesilica jer kod njih izazivaju bol, a primjetni su i slabiji proizvodni pokazatelji. Iz dosadašnjih je spoznaja vidljivo da su oštećenja prsnih kosti zabilježena u svim tipovima

komercijalne proizvodnje konzumnih kokošnjih jaja, s različitim postotkom pojavnosti ovisno o sistemu, dobi i hibridu kokoši nesilica. Isto tako se može zaključiti da su lomovi i devijacije prsnih kosti izraženije kod ne kaveznih sustava držanja (volijere i podni uzgoj), dok je znatno manje oštećenja zabilježeno u obogaćenim kavezima. Neka od mogućih rješenja ovog problema usmjerena su prema promjenama u opremi za držanje nesilica, genetici odnosno stvaranju novih robusnijih hibrida te hranidbi s ciljem ojačavanja koštanog sustava.

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The influence of alternative rearing systems on breast bone damages of laying hens

Abstract

By leaving the rearing of laying hens in classic cages and moving to new alternative systems and enriched cages rearing, it was expected that there would be a significant improvement in the welfare of the laying hens. They have a larger space for staying and moving inside the cage and in the free range system they have exit to open area. However, there have been several problems that still have no solution and it just started to work on them. One of these problems is the damage of the keel bones that occurs in the laying hens and can be expressed by the bone deviation which is a milder form and fractures of the keel bone which is a heavier form and causes great pain and suffering of laying hens. The aim of this paper was to present the knowledge about the prevalence of these difficulties in keeping laying hens in alternative rearing and enriched cages. From the results so far, it can be seen that the damage of the bones is most pronounced in the laying hens held in barn and floor rearing, while significantly less damage was recorded in enriched cages. Some of the possible solutions to this problem are geared towards changing equipment for keeping of laying hens, genetics or creating new more robust hybrids and nutrition with the aim of strengthening the bone system.

Keywords: laying hens, keel bone damage, alternative rearing, enriched cages

Utjecaj dodatka mikroalgi u hranu kokoši nesilica na senzorska svojstva jaja

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Sažetak

Cilj ovog rada bio je utvrditi utjecaj dodatka mikroalgi (*Schizochytrium limacinum*) u hranu kokoši nesilica na senzorska svojstva jaja. U istraživanju je korišteno 36 TETRA-SL brown nesilica koje su bile podijeljene slučajnim odabirom u tri hranidbena tretmana (kontrolni tretman A, pokusni tretmani B s dodatkom 0,5% mikroalgi i tretman C s dodatkom 1% mikroalgi). Senzorska analiza provedena je primjenom triangl i hedonističkog testa na žumanjcima kuhanih jaja. Uočljivu razliku između tretmana A i C iskazalo je 12 od 19 ocjenjivača što predstavlja i statistički značajnu razliku ($P < 0,01$), a razlika je najčešće pripisana okusu žumanjka. Izraženost boje žutanjka tretmana C bila je statistički značajno veća nego kod kontrolnog tretmana A. Prihvatljivost mirisa i ukupna dopadljivost nisu bili značajno različiti između tretmana. Ovim istraživanjem utvrđen je pozitivan učinak dodatka 1% mikroalgi na senzorska svojstva jaja što upućuje na opravdanost dodavanja u hranu za kokoši nesilice.

Ključne riječi: jaja, senzorska analiza, mikroalge, *Schizochytrium*, dopadljivost

Uvod

Mikroalge su u posljednje vrijeme probudile zanimanje širom svijeta zbog njihovog potencijala za primjenu u industriji obnovljivih izvora energije, bio-farmaceutskih proizvoda i prehrambenih proizvoda, jer predstavljaju obnovljiv, održiv i ekonomičan izvor biogoriva, bioaktivnih lijekova i hranjivih tvari (Khan i sur., 2018). Mikroalge se uglavnom sastoje od proteina, ugljikohidrata, lipida, vitamina, minerala i bioaktivnih spojeva kao što su karotenoidi, ali ih karakterizira promjenjivi sastav hranjivih tvari koji ovisi o uvjetima uzgoja i vrsti mikroalge. Mikroalge iz roda *Schizochytrium* sp. okarakterizirane su velikim sadržajem lipida u suhoj tvari (do 65%) kako navode Li i sur. (2017) i poboljšanim utjecajem na sastav masnih kiselina u svinjskom i peradarskom mesu zbog visokog sadržaja dokosaheksaenske kiseline (DHA; 22:6 n3) čiji udio u ukupnim masnim kiselinama može biti veći od 35% (Aki i sur., 2003). Usprkos tome što je jedan od problema koji ograničava upotrebu mikroalgi u stočnoj hrani slabija probavljivost zbog celuloznih staničnih stijenki (Becker, 1994), pojedina istraživanja pokazuju izrazito zadovoljavajuće rezultate. Tako su Ao i sur. (2015) te Park i sur. (2015) utvrdili da dodatak mikroalgi *Schizochytrium* u hranidbu kokoši nesilica ima pozitivan učinak na proizvodnju jaja, masu jaja, boju žumanjka, profile lipida u krvi, koncentraciju DHA i eikosapentaenske kiseline u jajima i povoljniji odnos n-6 / n-3 masnih kiselina. Međutim, postoje istraživanja koja pokazuju da dodatak mikroalgi može umanjiti senzorske odlike jaja i mesa zbog pojačane percepcije aroma koje podsjećaju na ribu (Madeira i sur., 2017; Ribeiro i sur., 2014; Walker i sur., 2012). Stoga je cilj istraživanja bio utvrditi utjecaj dodatka 0,5 i 1% mikroalge *Schizochytrium limacinum* u hranu kokoši nesilica na senzorske karakteristike jaja.

Materijal i metode

U istraživanju je korišteno 36 TETRA-SL brown nesilica koje su bile slučajnim raspored podijeljene u tri hranidbena tretmana. Svaki tretman proveden je na četiri kokoši u tri ponavljanja držanih pojedinačno u kavezima na podu od žičane mreže. Kokoši kontrolnog tretmana A bile su hranjene komercijalnom smjesom, dok su kokoši u pokusnim tretmanima bile hranjene istom smjesom uz dodatak 0,5% (tretman B) i 1% (tretman C) pripravka mikroalgi *Schyzochytrium limacinum* (All-G-RichTM, Alltech Algae, USA) s 11% proteina, 64% masti i 16% DHA. Istraživanje je trajalo 8 tjedana, a starost kokoši na početku istraživanja bila je 35 tjedana. Jaja za senzorsku analizu skupljena su na kraju hranidbenog dijela istraživanja, a starost jaja pri sakupljanju bila je od jednog do tri dana. Jaja su do analize čuvana u hladnjaku na temperaturi 4 °C.

Senzorska analiza provedena je primjenom triangl i hedonističkog testa (Lawless i Heymann, 2010.) na tvrdo kuhanim jajima. Priprema jaja sastojala se od stavljanja jaja u hladnu vodu, zagrijavanja do vrenja i kuhanja 8 minuta, nakon čega su jaja ohlađena na temperaturu 50 do 60 °C, oljuštena te podijeljena na četvrtine. Jedna četvrtina jajeta je predstavljala jedan uzorak, a pripremljeni uzorci čuvani su na temperaturi oko 50 °C do serviranja. U senzorskoj analizi je sudjelovalo 19 educiranih ocjenjivača (djelatnika Agronomskog fakulteta) s ujednačenim odnosom spolova (54% muški : 46% ženski). Pri provedbi triangl testa ocjenjivačima su u jednom nizu predstavljena tri uzorka označena nasumičnom troznamenkastom šifrom te su bili upitani da nakon kušanja svih uzoraka izaberu uzorak koji smatraju različitim od preostala dva. Ocjenjivači su usput upitani odrediti u čemu percipiraju razliku (boji, mirisu, okusu ili teksturi) te je bilo moguće iskazati više odgovora. Svakom ocjenjivaču su prezentirana tri triangl niza koji su predstavljali odnose kontrolnog tretmana A i pokusnih tretmana B i C, kao i između pokusnih tretmana B i C. Redosljed nizova kao i redosljed uzoraka unutar niza određen je kao potpuno slučajni dizajn. Ocjenjivači su zamoljeni da nakon uzimanja svakog pojedinačnog niza konzumiraju kruh i vodu radi neutralizacije usta i odmora osjetila.

U hedonističkom testu ocjenjivači su ocijenili izražajnost boje i prihvatljivost mirisa na strukturiranoj skali od 1 do 5 te ukupnu dopadljivost na skali od 1 do 9 gdje je 1 označavalo neizraženost boje, neprihvatljiv miris ili potpuno nesviđanje, a 5 izrazito izraženu boju i izrazito prihvatljiv miris, odnosno 9 izrazito dopadljivost. Ocjenjivači su bili upućeni u konzumaciju kruha i vode prije svakog uzorka za neutralizaciju usta, dok su uzorci prezentirani kao slučajni potpuno izbalansirani dizajn.

Dobiveni podaci obrađeni su pomoću statističkog programa SAS Studio University Edition 3.71 (SAS Institute, 2018). U analizi podataka hedonističkih testova korištena je procedura GLM uz primjenu Least Significant Difference (LSD) post-hoc testa za utvrđivanje značajnosti razlika između tretmana ($P < 0,05$). Podaci dobiveni triangl testom obrađeni su izračunom statističke značajnosti bazirane na broju točnih odgovora, pri čemu je za odbacivanje H_0 hipoteze uz razinu značajnosti $P < 0,05$ od ukupno 19 odgovora trebalo točno odabrati različit uzorak u barem 11 slučajeva (Lawless i Heymann, 2010.).

Rezultati i rasprava

Obradom podataka prikupljenih u triangl testu prikazani su u Tablici 1. U triangl paru kontrolnog tretmana A i pokusnog B dano je 9 točnih odgovora, u triangl paru tretmana kontrolnog A i pokusnog C dano je 12 točnih odgovora dok je u triangl paru pokusnih tretmana B i C dano je 9 točnih odgovora. S obzirom na broj točnih odgovoru u odnosu na ukupan broj odgovora utvrđeno je senzorski analitičari nisu uočili značajnu razliku u paru tretmana A : B ni u paru tretmana B : C, dok je u paru tretmana A : C utvrđena statistički značajna razlika ($P < 0,01$). Temeljem toga možemo zaključiti da dodatak 1% mikroalge *Schyzochytrium limacinum* u hranu kokoši nesilica značajno utjecao na senzorska svojstva jaja.

Tablica 1. Rezultati triangl testa na kokošnjim jajima

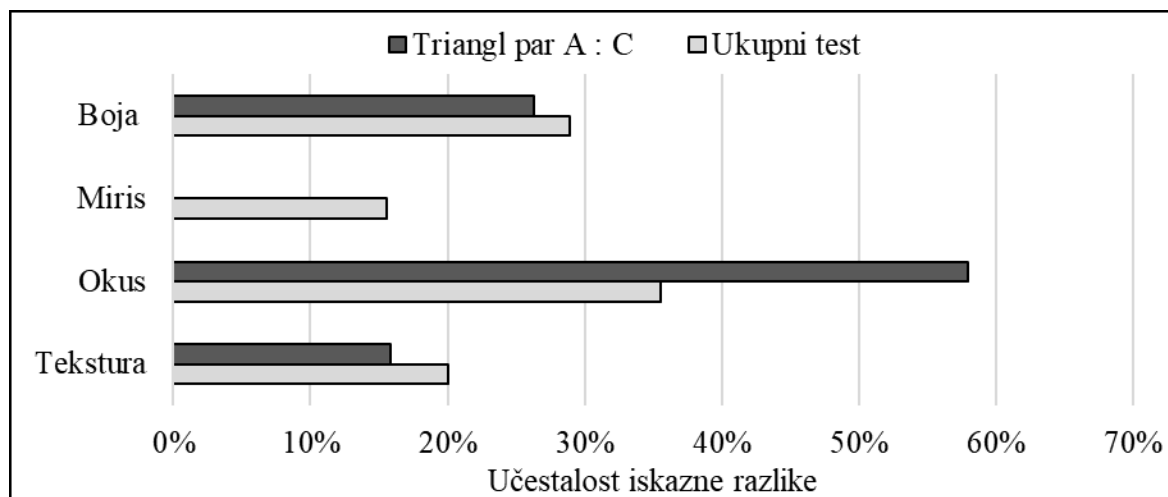
Parovi tretmana ¹	Broj ukupnih odgovora	Broj točnih odgovora	Postotak točnih odgovora	Značajnost ²
A : B	19	9	47,37%	ns
A : C	19	12	63,63%	*
B : C	19	9	47,37%	ns

¹A: kontrolni tretman s komercijalnom krmnom smjesom; B: pokusni tretman s dodatkom 0,5% pripravka mikroalge *Schyzochytrium limacinum*; C: pokusni tretman s dodatkom 1% pripravka mikroalge *Schyzochytrium limacinum*

²ns: nije statistički značajno; *: statistički značajno uz razinu $P < 0,01$

Detaljnija analiza rezultata triangl testa ukazuje na svojstva koja su doprinijela razlikovanju tretmana (Slika 1), a u izračunu su korišteni samo odgovori ocjenjivača koji su točno odabrali različit uzorak. Utvrđeno je da su ocjenjivači razliku između kontrolnog tretmana A i pokusnog tretmana C najčešće pripisali razlikama u okusu (57,89% odgovora), zatim boji (26,32% odgovora) i teksturi (15,79% odgovora), dok razlika u mirisu nije iskazana. Izvori razlika utvrđeni u cijelom testu razlikuju se u odnosu na izvore razlika triangl para A : C, jer su razlike u svojstvima iskazane s manjom učestalosti, a prisutna je razlika i u mirisu jaja. Navedeno bi mogli pripisati djelovanju topline, usitnjavanja i mastikacije uzorka u ustima, što je moglo pojačati intenzitet i percepciju osjeta u ustima, umanjujući pritom intenzitet promjene u mirisu. Vidljivo je da je okus bio izraženiji diskriminator između tretmana.

Boja jaja bila je označena kao razlikovno svojstvo između tretmana kod gotovo trećine ocjenjivača (26,32 do 33,33% odgovora). Navedeno se može povezati s velikim prijenosnim kapacitetom karotenoida iz mikroalgi u žumanjak jaja kako su utvrdili Kotrbáček i sur. (2013). Prethodna istraživanja potvrđuju visoke koncentracije karotenoida u *Schyzochytrium* mikroalgi (Aki i sur., 2003), a što je utvrđeno i kod drugih mikroalgi (Fredriksson i sur., 2006; Lemahieu i sur., 2013) iz čega proizlazi pigmentacijski učinak kako su utvrdili Ao i sur. (2015), Fredriksson i sur. (2006), Herber-McNeill i Van Elswyk (1998), te Lemahieu i sur. (2013).



Slika 1. Učestalost iskazane razlike u triangl testu

(A: kontrolni tretman s komercijalnom krmnom smjesom; C: pokusni tretman s dodatkom 1% pripravka mikroalge *Schyzochytrium limacinum*)

Triangl testom je utvrđeno postojanje značajnih razlika između tretmana kao i svojstva koja su doprinijela razlikovanju uzoraka, dok je hedonističkim testom ustanovljeno kakvo je sviđanje takvih promjena (Tablica 2). Utvrđeno je da je izražajnost boje žumanjka tretmana C (dodatak 1% mikroalge) bila značajno veća ($P < 0,05$) nego kontrolnog tretmana A. Izražajnost boje tretmana B (dodatak 0,5% mikroalge) nije se značajno razlikovala u odnosu na tretmane A i C, ali je vrijednošću bila znatno bliže tretmanu C.

Tablica 2. Rezultati hedonističkog testa na kokošjim jajima (prosjeak ± standardna devijacija)

Svojstvo	Tretman ¹		
	A	B	C
Izražajnost boje žumanjka	3,05 ± 1,08 ^b	3,63 ± 0,76 ^{a,b}	3,74 ± 0,83 ^a
Prihvatljivost mirisa	3,58 ± 0,77	3,63 ± 0,96	3,63 ± 0,83
Ukupna dopadljivost	6,89 ± 0,99	6,53 ± 1,68	6,53 ± 1,98

¹ A: kontrolni tretman s komercijalnom krmnom smjesom; B: pokusni tretman s dodatkom 0,5% pripravka mikroalge *Schyzochytrium limacinum*; C: pokusni tretman s dodatkom 1% pripravka mikroalge *Schyzochytrium limacinum*

a,b vrijednosti označene različitim slovima unutar reda značajno se razlikuju ($P < 0,05$)

U prilog dobivenim rezultatima je istraživanje Ao i sur. (2015.) koji su u hranidbu kokoši nesilica dodali mikroalge *Schyzochytrium limacinum* u iznosu od 1, 2 i 3%. Vrijednost a* (intenzitet/prisustvo crvene boje) žumanjka bila je značajno veća, a vrijednost L* (svjetlina) značajno manja kod skupina koje su hranjenje dodatkom 2 i 3% mikroalge ($P < 0,01$) dok kod kokoši hranjenih dodatkom 1% mikroalge nije bilo značajne razlike u boji žumanjka u odnosu na kontrolnu skupinu. Slično tome, Herber-McNeill i Elswyk (1998.) navode kako su a* vrijednosti žumanjka značajno povećane pri dodatku 2,8% i 4,8% mikroalge *Schyzochytrium limacinum* u usporedbi s kontrolnom grupom. Slična istraživanja na drugim mikroalgama, poput *Nannochloropsis oculata* (Fredriksson i sur., 2006), *Haematococcus pluvialis* (Walker i sur., 2012), *Phaeodactylum triconutum*, *Isochrysis galbana* or *Chlorella fusca* (Lemahieu i sur. 2013) pokazuju snažan pigmentacijski učinak mikroalgi na žumanjak jajeta zbog bogatog sastava različitih karotenoida. U predmetnom istraživanju postignut je pozitivan učinak na izražajnost boje već pri dodavanju 1% mikroalgi, što je različito od rezultata koje iskazuju Park i sur. (2015) koji nisu utvrdili značajnu razliku u instrumentalno određenoj boji žumanjka pri dodavanju 0,5 i 1% mikroalge *Schyzochytrium limacinum*.

Prihvatljivost mirisa i ukupna dopadljivost jaja iz predmetnog istraživanja nije se značajno razlikovala između tretmana, iako su prosječne vrijednosti ukupne dopadljivosti bile veće kod kontrolnog tretmana. Navedeno je u skladu s rezultatima koje su prezentirali Herber-NcNeill i Van Elswyk (1998) u kojima navode da dodatak 2,4 ili 4,8% mikroalge *Schyzochytrium* nije utjecao na svojstva okusa jaja. Do sličnih zaključaka dolaze Parpinello i sur. (2006) koji su proveli senzorska ispitivanja jaja iz hranidbenih tretmana s različitim izvorima n-3 masnih kiselina te zaključili da dodatak algi ne dovodi do povećanja prisustva nepoželjnih okusa i mirisa. Mnogi autori ukazuju kako rezultati senzorskih analiza proizvoda koji u sebi sadrže dodatke mikroalgi poput tjestenine (Abril i Barclay, 1998), kolačića (Batista i sur., 2017) ili jogurta (Dubey i Kumari, 2011) pokazuju da su ovi proizvodi uglavnom cijenjeni od strane potrošača. Međutim, pojedina istraživanja ukazuju na potencijalni problem pri upotrebi mikroalgi koji se očituje u pojavi nepoželjnih okusa „na ribu“ kako su ustanovili Walker i sur. (2012) na jajima, a Ribeiro i sur. (2013) na mesu brojlera.

Prijašnja istraživanja pokazuju da dodatak mikroalgi u iznosu 1, 2 i 3% u hranu nesilica značajno povećava udio DHA i ukupnih n-3 masnih kiselina jaja (Ao i sur., 2015; Park i sur., 2015) iz čega može proizaći veći stupanj oksidacija i potencijalno razvoj nepoželjnih okusa i aroma. Temeljem svojih rezultata Herber-NcNeill i Van Elswyk (1998) zaključuju da mikroalge sadrže spojeve koji osiguravaju oksidativnu stabilnost što pripisuju karotenoidima. Navedeno je potvrđeno istraživanjima Ao i sur. (2015) koji nisu utvrdili narušavanje oksidacijske stabilnosti jaja uz dodatak mikroalgi. Razlog zaštitnom učinku karotenoida mogao bi biti veliki prijenosni kapacitet karotenoida iz mikroalgi u žumanjak jajeta kako su zaključili Kotrbáček i sur. (2013).

Zaključak

Provedbom triangl testa na jajima iz istraživanja u kojem su u hranu nesilica dodani različiti udjeli mikroalge *Schyzochytrium limacinum* utvrđeno je da dodatak 1% mikroalge značajno utječe na senzorska svojstva jaja ($P < 0,01$). Slično tome, analizom rezultata hedonističkog testa je utvrđeno da je izražajnost boje žumanjka bila značajno veća u jajima s dodatkom 1% mikroalgi u odnosu na kontrolni tretman ($P < 0,05$). Međutim, Pritomrazlike u prihvatljivosti mirisa i ukupnoj dopadljivosti nisu bile statistički značajne. Stoga je ovim istraživanjem utvrđen pozitivan učinak dodatka 1% mikroalgi na senzorska svojstva jaja što upućuje na opravdanost njihovog dodavanja u hranu za kokoši nesilice.

Napomena

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Effect of microalgae addition in laying hen diet on egg sensory traits

Abstract

The aim of this study was to determine the effect of the addition of microalgae (*Schyzochytrium limacinum*) in laying hens' feed on the egg sensory properties. In this study 36 TETRA-SL brown laying hens were used and randomly divided into three nutritional treatments (control treatment A, trial treatments B with 0.5% microalgae and treatment C with 1% microalgae). Sensory analysis was performed using triangle and hedonistic test on boiled egg yolks. A noticeable difference between treatments A and C was reported by 12 of 19 evaluators, which was treated as statistically significant difference ($P < 0.01$), and the difference was most frequently ascribed to yolk taste. The yolk colour of treatment C was statistically significantly higher than that of control treatment A. Overall likeability and odour acceptability were not significantly different between treatments ($P > 0.05$). With this research a positive effect of the addition of 1% microalgae was determined indicating that the addition to the feed for laying hens is justified.

Keywords: eggs, sensory analysis, microalgae, *Schyzochytrium*, likeability

Mišljenje potrošača u Hrvatskoj o konzumaciji omega-3 obogaćenih jaja

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Sažetak

U radu se istražuju mišljenja konzumenata o značajkama omega-3 jaja koja se nalaze na hrvatskom tržištu. Anketno istraživanje provedeno je u Osječko-baranjskoj županiji. Ukupno su uključena 272 ispitanika oba spola u dobi od 18-65 godina. Anketni listovi popunjeni su pomoću intervjua. Uvodno pitanje glasilo je: Da li ste upoznati s omega-3 jajima - obogaćenim proizvodom? Od ukupnog broja pozitivno su odgovorile 144 osobe (52,9%) i to 76 muških (M) i 68 ženskih (Ž) ispitanika, a 128 osoba (47,1%) nije bilo upoznato s funkcionalnim proizvodima, posebice omega-3 jajima. Za vrednovanje odgovora upotrijebljena je Likertova skala od 1 (min) do 7 (max) ocjena. Oba spola ispitanika smatraju omega-3 jaja sigurnim proizvodom (4,74 M: 4,76 Ž) i vjeruju podacima na deklaraciji (4,12 M : 4,17 Ž). Ženske osobe više vjeruju od muških osoba (4,35 Ž : 4,28 M) u deklarirane karakteristike omega-3 jaja koje se odnose na prirodno povećani sadržaj omega-3 masnih kiselina za više od 30% u odnosu na konvencionalna jaja (EPA + DHA 80 mg) te doprinose normalnoj funkciji srca. U znanstvenu provjeru deklariranih karakteristika jaja i njihove konzumacije od strane zdravih osoba, više povjerenja imaju muški (3,05 i 3,68) od ženskih ispitanika (2,85 i 3,47). Oba spola voljna su koristiti omega-3 jaja u svojoj prehrani (4,66 M i 4,69 Ž) ukoliko im mogućnosti to dozvoljavaju.

Ključne riječi: anketa, ispitanici, omega-3 jaja, konzumacija, deklaracija

Uvod

Omega-3 jaja smatraju se funkcionalnim proizvodom. Koncept funkcionalne hrane prvi puta je spomenut u Japanu 80-tih godina prošlog stoljeća. Razvijen je program FUFOS (Functional Food Science in Europe) koji obuhvaća proizvode s posebnim učinkom na ljudsko zdravlje na osnovi sadržaja specifičnih sastojaka – nutricina (Ashwell, 2002.). Istraživanja su pokazala da je prehrana ljudi u zapadnoeuropskim zemljama deficitarna na omega-3 polinezasićenim kiselinama (n-3 PUFA), a suficitarna na n-6 polinezasićenim masnim kiselinama (n-6 PUFA). Omjer n-6/n-3 PUFA nepovoljan je za ljudsko zdravlje i kreće se od 15/1 do 27/1, a trebao bi biti od 9/1 do 4/1 (Simopoulos, 2002.). U razvijenim zemljama dnevno se po osobi konzumira 0,15 g/dan dugolančanih omega-3 kiselina, što se smatra nedostatnim (Kolanowski i sur., 2004.). Jaja se mogu obogatiti s n-3 PUFA modificiranjem obroka za nesilice - uporabom biljnih i ribljeg ulja (Simopoulos, 2002.). U izvješću istraživanja transparentnosti tržišta (TMR) za tvrtku Global Nutraceuticals navodi se da će vrijednost proizvoda ove tvrtke koja se bavi proizvodnjom funkcionalne hrane i pića, dodataka prehrani i proizvodima za osobnu njegu, narasti s 182,60 milijardi USD, (podatci za 2015. godinu) na 278,96 milijardi USD (do 2021. godine). S obzirom na navedeni rastući trend u proizvodnji i prodaji funkcionalnih proizvoda, neophodno je kontinuirano pratiti mišljenje konzumenata o različitim funkcionalnim proizvodima koji se nalaze na našem tržištu. Cilj istraživanja bio je anketiranjem dobiti mišljenje konzumenata o kvalitativnim značajkama omega-3 jaja kao funkcionalnom proizvodu.

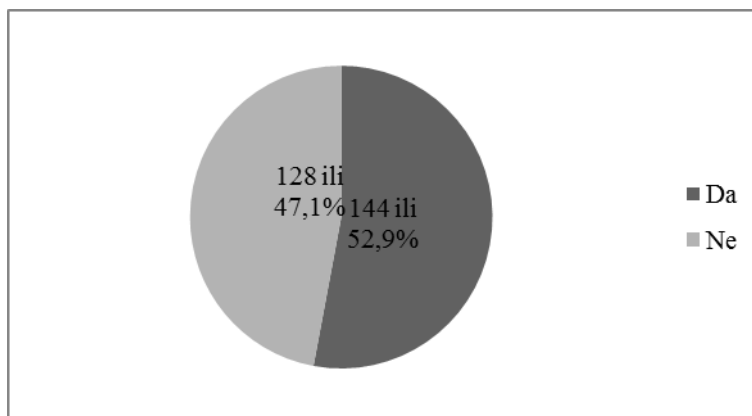
Materijal i metode

Ukupno su anketirane 272 osobe muškog i ženskog spola u dobi od 18 do 65 godina. Anketa je provedena na području Osječko-baranjske županije. Anketni listovi sadržavali su uvodno pitanje „Da li ste upoznati s omega-3

jajima - obogaćenim proizvodom?“, a ostala pitanja odnosila su se na sigurnost uporabe proizvoda, povjerenje odnosno nepovjerenje u deklaraciju, da li omega-3 jaja trebaju konzumirati zdrave osobe, kao i voljnost korištenja obogaćenih jaja u svojoj prehrani. Za vrednovanje odgovora upotrebljena je Likertova skala (1-min., 7-max. ocjena). Prema Likertovoj skali razina slaganja s ponuđenim upitom procijenjena je brojevima koji označavaju: 1 = potpuno se ne slažem, 2 = uglavnom se ne slažem, 3 = djelomično se ne slažem, 4 = nema mišljenja, 5 = djelomično se slažem, 6 = uglavnom se slažem, 7 = potpuno se slažem. Odgovori ispitanika obrađeni su statističkim paketom Statistica for Windows version 13.3. (StatSoft Inc., 2017.). U obradi rezultata korištena je deskriptivna statistika.

Rezultati i rasprava

Na grafikonu 1 prikazani su rezultati uvodnog pitanja u anketi, gdje je vidljivo da 128 ispitanika ili njih 47,1% nisu upoznati s postojanjem omega-3 jaja, dok 144 ispitanika odnosno njih 52,9% znaju što su to omega-3 jaja. Za daljnje anketiranje uzeti su samo oni ispitanici koji su odgovorili pozitivno, odnosno znaju što su to omega-3 jaja. Iz tablice 1 vidljivo je da od ukupno 144 ispitanika koji su upoznati s pojmom omega-3 jaja njih 76 (52,77%) bili su muškarci, a 68 su žene (47,22%). Da među potrošačima vlada zapravo „neznanje“ vezano za prisutnost na tržištu i benefit obogaćenih i funkcionalnih proizvoda, u svom istraživanju potvrđuju i Sass i sur. (2018.). Autori navode da su ispitanici u njihovom istraživanju, a sukladno našim rezultatima, pokazali nedostatak znanja o obogaćenim jajima, koristima njihove konzumacije i preporučenom unosu. Oni postavljaju da je uzrok ovakvih odgovora u anketi zapravo povezan s oskudnim deklaracijama na ambalaži proizvoda, ali i slabom marketinškom promidžbom.



Grafikon 1. Odgovori ispitanika na pitanje „Da li ste upoznati s omega-3 jajima- obogaćenim proizvodom?“

Prosječnu ocjenu na pitanje o sigurnosti proizvoda muške osobe vrednovale su s 4,74, a ženske s 4,76. Na ovo pitanje od ukupnog broja ispitanika 38.8% u potpunosti se slaže da su omega-3 jaja u potpunosti siguran proizvod, a svega 1,38% njih sumnja u sigurnost omega-3 jaja. Na pitanje o povjerenju u deklaraciju od ukupno 144 anketiranih osoba oba spola, svega 11,1% u potpunosti vjeruje deklariranim vrijednostima za omega-3 jaja, a 4,2% ispitanika uopće ne vjeruje u podatke s deklaracije.

Tablica 1. Prikaz spolne strukture ispitanika koji su upoznati s postojanjem omega-3 jaja

Odgovor / ispitanici	Da	
	Broj (kom)	(%)
Broj ispitanika	144	100,00
Muškarci	76	52,77
Žene	68	47,22

Na Tablici 2 prikazane su prosječne ocjene ispitanika prema spolu, na postavljena pitanja putem intervjua.

Tablica 2. Ocjena ispitanika (± s)

Pitanja	Muški ispitanici	Ženski ispitanici
Da li su omega-3 jaja siguran proizvod?	4,74±0,16	4,76±0,12
^b Da li vjerujete podacima na deklaraciji?	4,12±0,19	4,17±0,14
Da li su omega-3 jaja znanstveno provjeren proizvod?	4,28±0,17	4,35±0,13
Da li su omega-3 jaja znanstveno neprovjeren proizvod?	3,05±0,13	2,85±0,15
Da li omega-3 jaja trebaju koristiti zdrave osobe?	3,68±0,13	3,47±0,16
^c Da li ste voljni koristiti omega-3 jaja u svojoj prehrani?	4,66±0,24	4,69±0,19

U Likertovoj skali brojevi označavaju: 1 = potpuno se ne slažem, 2 = uglavnom se ne slažem, 3 = djelomično se ne slažem, 4 = nema mišljenja, 5 = djelomično se slažem, 6 = uglavnom se slažem, 7 = potpuno se slažem.

b1 = potpuno ne vjeruje, 2 = uglavnom ne vjeruje, 3 = djelomično ne vjeruje, 4 = nema mišljenja - ni da ni ne, 5 = djelomično vjeruje, 6 = uglavnom vjeruje, 7 = potpuno vjeruje.

c1 = potpuno nije voljan, 2 = uglavnom nije voljan, 3 = djelomično nije voljan, 4 = nema mišljenja - ni da ni ne, 5 = djelomično je voljan, 6 = uglavnom je voljan, 7 = potpuno je voljan

U odgovoru na ovo pitanje ne postoji bitna razlika između spolova ($m=4,12$; $ž=4,17$). Na kutiji u kojoj su pakirana jaja navodi se deklaracija o hranjivim vrijednostima u 100 g jestivog jajeta i to: energetska vrijednost 535 KJ, sadržaj masti 8,4 g, zasićene masne kiseline 3 g, mononezasićene masne kiseline 3,6 g, polinezasićene masne kiseline 1,8 g te EPA + DHA 80 mg. Od ukupnog broja ispitanika 25% se potpuno slaže da su omega-3 jaja znanstveno dokazan proizvod, misleći prvenstveno na sadržaj deklaracije u pogledu hranjive vrijednosti i sastava masnih kiselina, a sumnju u navedeno iskazuje 2,08% anketiranih osoba. U prosjeku veće povjerenje prema znanstvenoj provjeri kvalitete jaja iskazuju ženske od muških osoba (4,35: 4,28). Sumnju u znanstvenu provjeru proizvoda muški ispitanici ocjenjuju s ocjenom 3,05, a ženski ispitanici s ocjenom 2,85, što znači da se djelomično ne slažu s tvrdnjom da su omega-3 jaja znanstveno neprovjeren proizvod. Deklaraciju proizvoda provjerava inspeksijska služba. Prema Commission Regulation (EU) No 116/2010, da bi se jaja deklarirala kao izvor omega-3 PUFA, trebaju sadržavati EPA+DHA 80 mg/100 g jestivog dijela. Na pitanje da li zdrave osobe trebaju koristiti omega-3 jaja u prehrani, anketirani ispitanici odgovorili su različito. U prosjeku 22,2% ispitanika u potpunosti se slaže i smatra da omega-3 jaja trebaju koristiti zdrave osobe, što znači da su upućeni u činjenicu da omega-3 jaja djeluju preventivno na normalan rad srca i krvožilnog sustava, a čak 48% ih u potpunosti smatra da zdrave osobe ne trebaju konzumirati omega-3 jaja. Ova činjenica upućuje na zaključak da ovi ispitanici nisu dovoljno upoznati s funkcionalnim djelovanjem omega-3 jaja u ljudskom organizmu. Prosječna ocjena muških ispitanika za ovo pitanje iznosila je 3,68, a ženskih 3,47. Hayat i sur. (2010.) također navode kako konzumenti na području Pakistana nisu dovoljno informirani o mogućnosti kupovine i konzumacije obogaćenih jaja na njihovom tržištu. Navedeni autori također ističu da su muški ispitanici bili nešto više informirani o mogućnostima kupovine i konzumacije obogaćenih-dizajniranih jaja na pakistanskom tržištu u odnosu na ženske ispitanice. Ukoliko bi imali mogućnost koristiti omega-3 jaja u svojoj prehrani, 41,6% ispitanika odgovorilo je da je u potpunosti spremno koristiti jaja obogaćena omega-3 PUFA u prehrani, dok 13,9% uopće nije zainteresirano za konzumaciju omega-3 jaja. U svom istraživanju o percepciji pakistanskih potrošača o kupnji i konzumaciji dizajniranih jaja, Hayat i sur. (2010.) navode da je 88% potrošača pokazalo spremnost za kupovinom obogaćenih jaja, te da je od njih čak 22% bilo je spremno platiti za 20-30% višu cijenu za dizajnirano jaje u odnosu na jaja iz konvencionalne proizvodnje.

Zaključak

U radu se istražuju mišljenja muških i ženskih ispitanika u dobi od 18 do 65 godina, o konzumiranju omega-3 jaja. Rezultati istraživanja upućuju da na postavljeno pitanje o upoznatosti o postojanju omega-3 jaja postoje minimalne razlike u odgovorima između muških i ženskih ispitanika. Ispitanici oba spola imali su na sva postavljena pitanja u anketi u prosjeku odgovore između 4 i 5, što znači da ili nemaju točno definirano mišljenje o postavljenom pitanju ili su djelomično sigurni u neku tvrdnju. Ovom anketom utvrđeno je da, iako ispitanici znaju za postojanje omega-3 jaja i spremni su ih koristiti u prehrani, oni još uvijek nisu dovoljno informirani o korisnosti ovog proizvoda.

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Consumers' opinion in Croatia on consumption of omega-3 enriched eggs

Abstract

The paper examines consumer opinions on the characteristics of omega-3 eggs found on the Croatian market. The survey was conducted in the Osijek-Baranja County. A total of 272 examinees of both gender, aged 18-65, were included. Questionnaires were filled out using interviews. The introductory question was: Are you familiar with the omega-3 egg-enriched product? From the total number, 144 individuals (52.9%) replied positive, 76 male (M) and 68 female (F), respectively, and 128 individuals (47.1%) were unaware of functional products, particularly omega-3 eggs. A Likert scale from 1 (min) to 7 (max) ratings was used to evaluate the response. Both gender consider omega-3 eggs a safe product (4.74 M: 4.76 F) and believe the information on the declaration (4.12 M: 4.17 F). Females are more likely to believe than males (4.35 F: 4.28 M) in the declared characteristics of omega-3 eggs related to naturally increased omega-3 fatty acids by more than 30% over conventional eggs (EPA + DHA 80 mg), and their contribution to normal heart function. Males have more confidence in the scientific verification of the declared characteristics of eggs and their consumption by healthy individuals (3.05 and 3.68, respectively) compared to females (2.85 and 3.47, respectively). Both gender are willing to use omega-3 eggs in their diet (4.66 M and 4.74 F) if their possibilities allow.

Keywords: survey, respondents, omega-3 eggs, consumption, declaration

The effect of age on semen quality of Holstein-Friesian bulls

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Abstract

The aim of this research was to investigate the influence of the bull age on production and some semen quality traits. The semen was collected during 2014. The LSD test (Least Significant Difference) was used to compare the bulls of different age groups and the results of this test showed in some cases significant differences. This test showed that there was no significant difference in ejaculate volume between six-year-old and four-year-old bulls, but in two-year-old bulls a significant difference to older bull groups was determined. The results showed that there was a significant effect of the bull age ($p < 0.001$) on the ejaculate volume and the number of doses per ejaculate.

Keywords: bulls, LSD test, sperm, age, Holstein-Friesian breed

Introduction

The production and quality of bull semen is strongly influenced by genetic factors such as breed and individual traits. However, sperm production and quality are also conditioned by other factors, such as diet, housing method and care of the bulls, body size and weight, age of the bull, environment conditions, frequency of ejaculation, and the skill to collect, preserve and store sperm (Fuerst-Waltl et al., 2006; Fiaz et al., 2010). The impact of bull age on semen quality will be discussed in more detail in this paper.

It is widely acknowledged that the age of a bull at collection affects semen characteristics (Brito et al., 2002a; Mandal et al., 2010), with older mature bulls having greater semen volume and quality than younger bulls (Brito et al., 2002a).

The quantity of ejaculate and the number of spermatozoa in them increase with age of the bull regardless of the season and the interval between collections. This is explained by the fact that the major factor affecting sperm production is testicular mass, which increases at least five years after puberty. All semen traits (ejaculate volume, total volume per day, sperm motility, sperm concentration per ml and per ejaculate) were significantly influenced ($P < 0.01$) by bull age and season (Bhakat et al., 2011). The same authors state that the ejaculate trait values such as ejaculate volume and sperm concentration increase by the age of five, and then these values decrease. The highest fertility of bull has been observed at around 2-4 years of age and started declining once bull attained more than 4 years of age (Smith Thomas, 2009).

The age of the bull at semen collection affects the volume of the ejaculate, its concentration, and sperm motility (Mathevon et al., 1998). In general, the literature shows that all of these ejaculate characteristics increase as bulls age (Fuente et al., 1984; Siratskii, 1987). During the puberty, bull ejaculates contain an increased number of immature and abnormal sperm whose progressive motility is very poor. Bulls aged less than 1 year had the poorest semen production and sperm motility values for all parameters compared with bulls older than 1 year ($P < 0.01$) (Murphy et al., 2018).

As the bulls mature, their body weight increases, and the testicles also develop rapidly. This leads to a rapid decrease in the number of immature sperm and increase in the amount of semen and the number of mature sperm with good motility (Balić et al., 2012). The simultaneous development of the testis and accessory glands post puberty and during sexual maturation consequently leads to an increase in semen production (Almquist, 1978). Spermatogenous maturation results in the formation of mature sperm. Most bulls reach this condition from 3 to 5 years of age,

which again depends on breed, but also on diet, housing and care. After the sperm maturation is achieved, bulls maintain this level of semen production with minor variations over the next few years, followed by a gradual decline in the quantity and quality of semen as a result of atrophy of the seminal ducts (senile period). The reproductive performance of young bulls depends on the onset of puberty, and that period can vary greatly between breeds and within breeds (Barth et al., 2008). Under practical conditions, bulls are kept in production until they are 7-8 years old. After that, they are soon replaced by younger and better bulls. The exceptions are bulls of remarkable superiority over the population average, which are maintained in production as long as they produce sufficient semen.

Material and Methods

Research was carried out at the Livestock Center of PKB Corporation in Belgrade and included the results of 9 Holstein-Friesian bulls during 2014. Bulls were divided into three age categories: two-year, four-year and six-year bulls. The entire process of producing seed doses at the Center was monitored, beginning with the ejaculation of bulls and ending with the quality check of the semen after thawing. The ejaculates from majority of bulls were taken twice a week, usually on Mondays and Fridays. The ejaculate from bulls was taken once a day, while some bulls were used twice a day for collection. Collection of ejaculates was performed by an artificial vagina while another bull was used to induce a sexual reflex or jump.

A general examination of sperm involved an assessment of colour, odour and consistency. Ejaculates that were dirty or with blood/pus had to be discarded but recorded in the current year. If the sperm received positive evaluation in the general examination, the volume of ejaculate, density and motility of the sperm were evaluated. The ejaculate volume was determined volumetrically in a graduated sperm collector. Ejaculate density (sperm count in 1 ml of ejaculate) was measured using a photometer while motility was estimated based on the number of sperm exhibiting progressive motility. Observation was performed using a microscope (magnification 20 - 40x) and ratings were given for motility from 1 to 5.

The ejaculate that met the basic criteria was then diluted and divided into a number of doses, which were packed in paillette form. The most common was the mean degree of dilution (1:10 - 1:15) and the doses obtained had up to 20 million sperm. The most commonly used diluent was AndroMed, and the diluted semen was vacuum packed into 0.22mL straws and then sealed with ultrasound. Subsequently, the usual procedure of deep freezing semen doses was applied. The first control of deep-frozen semen was carried out 24 hours after freezing when the percentage of progressively motile spermatozoa was determined, which should be at least 50% in a dose.

The semen quality properties analyzed were: ejaculate volume (in mL), sperm concentration (in 10⁶/mL), sperm motility in the native ejaculate (score 1 to 5), degree of sperm dilution (sperm: diluent ratio), number of doses from one ejaculate and sperm motility after thawing (%). For these properties, the basic parameters of the descriptive statistics (average, minimum, maximum, standard deviation) were calculated. The method of analysis of variance (F test) was used to examine the influence of bull age on semen quality, and the least significant difference test (LSD - test) was used to test the significance of differences in average values between different age groups. The number of ejaculates whose quality parameters were analyzed was not the same for all traits tested. The minimum values that the semen had to satisfy in order to be used for artificial insemination were: ejaculate volume - 2mL, sperm concentration - 800 x 10⁶/mL, motility score - 4 (75-80% progressively motile sperm), motility after thawing - 50 %. Statistical processing of the obtained data was performed with the software package "STATISTICA 6.0 StatSoft, 2001".

Results and discussion

Tables 1 and 2 show the average values for semen traits tested depending on the age of the bull. All bulls from which the data were collected were divided by age into three groups: two-year-olds (2 bulls), four-year-olds (3 bulls) and six-year-olds (4 bulls). The analysed data showed that the age of the bull had a significant effect on the volume of ejaculates and the number of doses but not on semen quality traits.

Table 1. Effect of bull age on some semen quality traits

Age, year	Ejaculate volume, Average	Std. error	N	Dilution ratio Average	Std. error	N	No. of doses Average	Std. error	N
2	4.6	0.15	156	16.4	0.38	81	299.0	13.81	81
4	5.8	0.19	103	17.0	0.51	45	474.9	18.53	45
6	5.3	0.23	67	15.3	0.62	32	397.5	22.33	31
Fexp.	11.948 ^{***}		326	2.456 ^{n.s.}		158	30.000 ^{***}		157

^{***}($p < 0.001$); ^{**}($p < 0.01$); ^{*}($p < 0.05$); ^{n.s.}($p > 0.05$)

Table 2. Effect of bull age on some semen quality traits

Age, year	Concentration (10 ⁶ /ml), Average	Std. error	Motility, Average	Std. error	N	Post thawing motility Average (%)	Std. error	N
2	1092.1	39.30	3.36	0.07	156	51.65	1.24	79
4	1076.7	48.37	3.39	0.09	103	49.44	1.64	45
6	997.9	59.97	3.30	0.11	67	51.72	2.05	29
Fexp.	0.886 ^{n.s.}		0.218 ^{n.s.}		326	0.645 ^{n.s.}		153

^{***}($p < 0.001$); ^{**}($p < 0.01$); ^{*}($p < 0.05$); ^{n.s.}($p > 0.05$)

The average ejaculate volume values were higher in 4-year and 6-year-old bulls than in 2-year-old bulls. These values are expected as two-year-old bulls have yet to achieve full sperm maturation. Also, older bulls have higher body weight, which means more developed testicles and other genital organs, which contributes to producing ejaculates of greater volume than in younger bulls. Analysis of the data using the F test revealed that the age of the bull significantly affected the volume of the ejaculate. This allowed the LSD test to be conducted to examine the significance of differences between bulls of different ages (Table 3). The results showed that there was no significant difference between six-year-old and four-year-old bulls. The two-year-old bulls had lower ejaculate volume.

Table 3. Effect of bull age on ejaculate volume (LSD test)

Bulls' age	Two-year	Four-year	Six-year
Two-year		***	*
Four-year	0.000002		n.s.
Six-year	0.022210	0.076180	

^{***}($p < 0.001$); ^{**}($p < 0.01$); ^{*}($p < 0.05$); ^{n.s.}($p > 0.05$)

Another trait that was significantly influenced by the bull's age is the number of doses per ejaculate. This result was expected because the number of doses depends on the volume of ejaculates.

The LSD test showed a significant differences between bulls of different ages on the number of doses per ejaculate (Table 4). Two-year-old bulls gave significantly lower ($p < 0.001$) doses in relation to older bulls. Four-year-old bulls gave a significant more ($p < 0.01$) number of doses in relation to six-year-old bulls.

Age of bull significantly affected all traits (ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate) ($P < 0.01$ to $P < 0.001$) except motility score (Fuerst-Waltl et al., 2006). The same authors state that the ejaculate volume and total number of spermatozoa increased with age of bull while sperm concentration was lower in higher age classes.

Table 4. Effect of bull age on number of doses per ejaculate (LSD test)

Bulls' age	Two-year	Four-year	Six-year
Two-year		***	***
Four-year	0.000000		**
Six-year	0.000245	0.008510	

***($p < 0.001$); **($p < 0.01$); *($p < 0.05$); *n.s.*($p > 0.05$)

For other tested ejaculate properties (sperm concentration, sperm motility in native ejaculate, degree of dilution, and sperm motility after thawing), the F test results showed that there was no significant effect of bull age on the manifestation of these traits.

Conclusion

Based on the obtained results it could be concluded that the bull age has a strong effect on volume of ejaculate and semen quality traits.

Especially great differences were found in bulls, with a 4 year age difference. The results are expected, as with the maturation of the bull, an increase in body weight, including testes, results in higher volume ejaculates and better quality. The four-year-old bulls had the highest volume of ejaculate with the high semen quality with greatest potential in semen production.

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Utjecaj automatskog sustava mužnje na proizvodnju mlijeka

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Sažetak

Za procjenu utjecaja automatskog sustava mužnje (ASM) analizirani su podatci parametra mliječnosti i reprodukcije baze podataka Govedo. Statistički značajne razlike provjerene su t-testom. Proučavane tehnološke parametre upotrijebili smo i kod ekonomske analize. Zbog nedostatka podataka napravili smo anketu. Trenutačno je u Sloveniji 98 aktivnih ASM. Uz investicije u ASM došlo je do povećanja broja muzara na gazdinstvu (+23%). Značajno se povećao i broj izlučivanja muzara zbog bolesti nogu i papaka, loše plodnosti i drugih uzroka. Značajne razlike su i u manjem sadržaju mliječne masti, većem sadržaju mliječnih bjelančevina, i skraćenom periodu među teljenja (-16 dana). Troškovi proizvodnje mlijeka sa ASM su slični troškovima kod klasičnog sustava mužnje. Trošak rada je poslije uvođenja ASM manji za 30%, međutim je trošak amortizacije veći za više od 35%.

Ključne riječi: automatski sustav mužnje, proizvodnja mlijeka, ekonomika

Uvod

Poslije ulaza Slovenije u Evropsku uniju, proizvodnja mlijeka je pod stalnim pritiskom ukupnoga evropskog i povremeno svjetskog tržišta mlijekom. Zbog toga je došlo u posljednja dva desetljeća do jakih strukturnih promjena i poboljšanju efikasnosti proizvodnje mlijeka. Povećanjem proizvodnje mlijeka, te istovremenim nedostatkom radne snage, neka gospodarstva su krenula ulagati financijska sredstva u automatski sustav za mužnju (ASM). ASM je sustav dobrovoljne mužnje, koja se potpuno podržava uz kompjuterski vođen proces, i sa brojnim sensorima stvara manji informacijski sustav. Po Steeneveld i sur. (2012) se uz ASM stvara nova dimenzija uzgoja, koja zahtijeva više znanja za bolje razumijevanje i upravljanje brojnim tehnološkim informacijama i upravljanjem ekonomike proizvodnje mlijeka. Svennersten-Sjaunja i Pettersson (2008) navode, da brojni zadaci nisu više potrebni, ali sa ASM pokazale su se potrebe za novim, pošto su kontroliranje i čišćenje ASM, češća dnevna kontrola podataka, češća kontrola muzara, tjeranje muzara na mužnju u razdoblju najveće mliječnosti, itd. Unatoč tome Mathijs (2004) kaže, da se uz ASM prosječno potroši manje rada za 20%, ali kritično pri tome je razdoblje prilagodbe. Slično govore i Bijl i sur. (2007), osim što je prosjek veći, 29 %. Dalje Bijl i sur. (2007) upozoravaju na visoko povećanje troška kapitala, osobito zbog veće amortizacije i troškova za funkcioniranje (voda, električna) i održavanje ASM. Znači, poboljšanje tehnološke efikasnosti smanjeno je za veću vrijednost uloženoga kapitala (Svennersten-Sjaunja i Pettersson, 2008). Uz studije o ekonomskoj efikasnosti, neke studije pokazale su, da ima ASM utjecaj i na parametre kvalitete mlijeka. Tako Abeni i sur. (2003) kažu, da se kod ASM sadržaj mliječne masti i bjelančevina u ukupnom uzorku smanjuje, vjerojatno uz veću frekvenciju mužnje. Zbog brojnih utjecaja na mliječnost jasni rezultati u studijama nisu prikazivani, ali Hogeveen i sur. (2001) pokazali su značajnu vezu između duljine intervala među mužnjama i količinu mlijeka i upozoravaju na veliku varijabilnost broja prelaza pojedinačnih muzara na mužnju. O povećanju dnevne količine mlijeka (u prosijeku za 3,4%) zbog veće frekvencije mužnje kod pojedinačnih muzara pišu i De Marchi i sur. (2017). Suprotno kažu Steeneveld i sur. (2012), koji su usporedili cjelokupne količine proizvedenog mlijeka pojedinačnih stada i nisu otkrili značajne razlike. Da bi se procijenile ekonomske posljedice prijelaza mužnje na ASM, potrebno je uzeti u obzir specifične uvjete proizvodnje mlijeka u Sloveniji, koji kao takvi jako utječu na tehnološke parametre i ekonomiju proizvodnje mlijeka. Radi toga smo odlučili opširnije analizirati stanje kod slovenskih proizvođača mlijeka.

Material i metode

Osnova za studiju su podatci baze Govedo na Kmetijskem inštitutu Slovenije. U prvom koraku evidentirani su aktivni ASM. Kod analize mliječnosti i reprodukcije uzeli smo u obzir razdoblje prilagodbe 6 mjeseci. Prije analize podataka smo za proučavane značajke pogledali njihovu distribuciju, normalnost i varijabilnost. Proučavane tehnološke i proizvodne parametre prikazujemo u Tablici 1. Kod svake značajke smo najprije napravili jednostavni t-test, sa kojim smo provjeravali statističku značajnost. Statistička analiza podataka napravljena je sa statističkim programom R (R Development Core Team).

Tablica 1. Popis procijenjenih tehnoloških i proizvodnih parametra proizvodnje mlijeka

Tehnološki parametri i parametri proizvodnje	
Broj muzara	Razdoblje između koncepcije
Mliječnost	Servis perioda
Sadržaj mliječne mast	Servis interval
Sadržaj mliječnih bjelančevina	Indeks oplodnje
Broj somatskih stanica	Razlozi izlučivanja muzara
Razdoblje između teljenja	

Na osnovu parametra iz Tablice 1 napravili smo model za proizvodnju mlijeka, koji je ugrađen u sustav kalkulacija (simulacijski model) za poljoprivrednu proizvodnju. Bazna kalkulacija napravljena je za proizvodnju mlijeka slobodnog uzgoja sa tandemskim prostorom za mužnju i 8 jedinica za mužnju. Kalkulacija za ASM generalno napravljena je uz jednake parametre, osim pretpostavljenog muznog robota sa kapacitetom između 55 i 80 muzara i analiziranih tehnoloških (proizvodnih) parametra. Zbog nedostataka empiričkih podataka pojedinačnih faza rada u primjeru ASM, provjerili smo faze rada kod manjeg broja gazdinstava sa ASM. Kod vrijednosti rada uzeti su u obzir svi porezi za mirovinsko i zdravstveno osiguranje, a osnova je prosječna plata u Republici Sloveniji.

Rezultati i rasprava

U Sloveniji, tijekom mjeseca rujna 2019, bilo je aktivnih 98 ASM, koji su evidentirani u kontroli proizvodnje mlijeka. Studija je pokazala, da su gazdinstva usporedno sa investicijom u robote planirala i povećanje proizvodnje. Prosječno se je broj muzara povećao za 25%, na nekim farmama čak i za više od 30%. Sa ASM ukupni broj izlučivanih muzara povećao se za 77 %, što ukazuje na probleme sa prilagodbu muzara na robotsku mužnju i prilagođavanjem uzgajivača na novu tehnologiju. Najčešći uzroci prikazani su u tabeli 2. Prema podacima za razdoblje od 2 godine prije i poslije prelaska na ASM najčešće su muzare izlučivane zbog loše plodnosti, a zatim zbog bolesti nogu i papaka. Razlika je logična, muzare zbog problema sa nogama i papcima ne žele dolaziti na mužnju, posljedično gube mlijeko, podložne su i ostalim bolestima. Značajno povećanje izlučivanja pokazalo se i kod uzroka „neprimjernost za uzgoj“, što ukazuje na precizniji odabir životinja za stado. Trend povećavanja izlučivanja događa se i zbog niske proizvodnje, te ozlijede vimena. Uzroke za povećavanje izlučivanja možemo pripisivati i aktivnijem praćenju podataka, koje omogućava ASM.

Tablica 2. Broj muzara, prosječni broj i distribucija najčešćih izlučivanja

	Prije ASM	ASM	Indeks	Prosječni broj izlučivanja na stado		
Broj muzara	5.590	6.827				
Broj muzara/gazdinstvo	56	69	123,2			
Broj izlučivanja	1.780	3.152	177,1			
Razlozi izlučivanja	Distribucija izlučivanja (%)		Indeks	Prije ASM	ASM	P-test*
Loša plodnost	23,2	23,2	100,0	6,88	12,00	P<0,001
Bolesti nogu i papaka	12,0	13,7	114,2	4,55	7,43	P≤0,01
Bolesti vimena (mastitis)	12,6	9,5	75,4	4,77	5,38	P=0,49
Uginuća - nepoznat uzrok	10,6	9,6	90,6	4,50	5,96	P=0,14
Nije primjerna za uzgoj	1,2	2,5	208,3	1,31	3,08	P≤0,05
Niska proizvodnja	4,7	6,3	134,0	2,96	4,30	P=0,06
Ozlijede vimena	3,0	3,6	120,0	2,00	3,03	P=0,08

* statistička značajnost procijene t-testa

Razlika u prosječnoj mliječnosti po muzari (izračunato iz cjelokupne količine mlijeka i broja krava muzara) prije i poslije ASM nije statistički značajna. Planiranje izlučivanja muzara znači i prilagođavanje stada novijem načinu mužnje, što ima za posljedice i smanjenje sadržaja mliječne masti. Analiza je pokazala, da razlog tome nije ASM, nego proces zamjene stada i promijene u strukturi pasmina.

Tablica 3. Tehnološki i proizvodni parametri proizvodnje mlijeka prije i poslije ASM

	Prije ASM	ASM	Indeks	P
Mliječnost (kg/krava)	7.988	7.977		P=0,78
Sadržaj mliječne masti (%)	4,10	3,97		P<0,001
Sadržaj mliječnih bjelančevina (%)	3,36	3,39		P<0,001
Povećan broj somatskih stanica (%)	14,7	15,9		P<0,001
Razdoblje između teljenja (dana)	431	415	96,3	P<0,001
Razdoblje između oplodnja (dana)	55	50	90,9	P=0,13
Servis interval (dana)	98	88	89,8	P<0,001
Servis perioda (dana)	145	130	89,7	P<0,001
Indeks oplodnje	1,98	1,97	99,5	

Suprotno stranim studijama, u našoj se pokazalo povećanje sadržaja mliječnih bjelančevina. Detaljnija analiza pokazala je, da ima kod ovog tehnološkog parametra najveći utjecaj pasmina i razina mliječnosti. Do sličnih zaključaka došli su i neki drugi istraživači (Abeni i sur., 2003). Rezultati plodnosti ukazuju na poboljšanje poslije uvođenja ASM, što ima utjecaj i na poboljšanje ekonomike proizvodnje mlijeka. Kraća razdoblja su kod: razdoblje između teljenja za 16 dana, razdoblje između oplodnja za 5 dana, servis interval za 10 dana, servis perioda za 15 dana. Indeks oplodnje, koji ukazuje koliko oplodnja je potrebno za koncepciju, ostaje nepromijenjen. Poboljšanje parametra reprodukcije možemo pripisivati bržemu prilagođavanju hranidbenim potrebama muzara na osnovi dnevnog kontroliranja mliječnosti. Pored toga imaju neki sustavi ASM uključeno i elektronsko praćenje gonjenja sa takozvanima pedometrima. Analize troškova proizvodnje mlijeka napravljene su uz intenzivnost proizvodnje 8.200 l/muzara, što je ekvivalentno 7.960 kg/muzara. Procijene pojedinačnih skupina troškova ukazuju na neka znatna odstupanja između dva sustava mužnje. Tako je trošak zamjene muzara zbog intenzivnoga prilagođavanja stada kod ASM, u usporedbi sa klasičnim sustavom mužnje, veći za oko dvije trećine. Između troška ostalog materijala i usluga razlika nema. Ali potrebno je reći, da se trošak strojnog rada zbog hranjenja po muzari smanjio zbog većeg broja

muzara (primjer ASM), suprotno pa su se važno povećali troškovi energije, vode, osiguranje opreme i održavanja. Očekivano je trošak amortizacije kod ASM veći (za 36%) od amortizacije klasičnog sustava za mužnju. Razlozi su u većoj investicijskoj vrijednosti i kraćem razdoblju amortizacije opreme. Suprotno trošku amortizacije, trošak rada je kod ASM manji, što su pokazivale i strane studije. Rada je manje za oko 30%, iako se uz ASM stvaraju nove faze rada, kao što su traženje i tjeranje muzara na mužnju, praćenje poruka muznog robota i dnevno čišćenje robota. Zbog većeg broja muzara naše analize pokazuju, da je trošak rada kod AMS manji za oko 20%.

Tablica 4. Procijenene troškova proizvodnje mlijeka prije i poslije ASM (cijene: god. 2018)

	Prije ASM	ASM	
Broj muzara	56	69	
Mliječnost (l/muzara)	8.200	8.200	
Troškovi	EUR/muzaro	EUR/muzaro	Indeks
Junica	286,4	477,3	166,7
Ishrana	1.434,0	1.434,4	100,0
Ostali materijal i usluge	574,4	573,4	99,8
Amortizacija	219,0	297,6	135,9
Rad	611,1	491,7	80,5
Ostali troškovi	324,4	333,1	102,7
Ukupni troškovi	3.449,2	3.607,4	104,6
Ostali proizvodi i poticaji	851,3	962,1	113,0
Proizvođačka cijena (EUR/l)*	0,317	0,323	101,8

* sa uključenim vrijednostima ostalih proizvoda i poticaja

U usporedbi sa klasičnim sustavom mužnje ukupni troškovi proizvodnje mlijeka su kod ASM veći za 5%. Ali pošto je zbog veće izmjene stade vrijednost ostalih proizvoda (meso izlučivanih muzara, teladi) veća za 13%, trošak na jedinicu proizvoda (proizvođačka cijena mlijeka) je veći samo za 2%. Na kraju znači, da zbog brojnih utjecaja na ekonomiko proizvodnje mlijeka ne možemo jasno prikazivati prednosti jednog sustava mužnje pred drugim. Opravdanje investicije u ASM tehnologiju je kod gospodarstava, koja imaju dosta tehnološkog i ekonomskog znanja u proizvodnji mlijeka. Automatizacija radnih procesa vezana je na relativno visok ulog kapitala, koji je opravdan samo uz značajni porast produktivnosti muzara, poboljšanjem kvalitete mlijeka i održive zamjene stada. Za racionalno upravljanje robotom važna je i težnja rasporeda teljenja preko cijele godine, što bi olakšalo i poboljšalo kvalitetu menadžmenta proizvodnje mlijeka. Prije uvođenja ASM potrebno je temeljito razmišljanje o optimalnoj veličini stada, uz očekivanu proizvodnju mlijeka i o utjecajima na poboljšanje radnih uvjeta i kvalitete života uzgajivača muzara.

Zaključak

Poslije uvođenja ASM su stada muzara u prosijeku veće za 23%, međutim mliječnost ostaje na sličnoj razini. Iako se sadržaj mliječne masti u mlijeku nešto smanjio i sadržaj mliječnih bjelančevina povećao, direktno promjena tehnologije mužnje nema utjecaja na kvalitetu mlijeka. Zdravstveno stanje mliječnih žlijezda, kojeg smo prezentirali sa indeksom prekomjernoga broja somatskih stanica, se je pogoršalo, što pripisujemo predugim intervalima među mužnjama, osobito muzara u početnom razdoblju laktacije. Broj izlučivanja muzara se uz promijene tehnologije mužnje povećao za više od 77%. Najčešći razlozi za izlučivanje muzara ostaju isti kod oba dva sustava mužnje, međutim se kod ASM značajno povećaju oni, za koje uzgajivači ne žele da se, jer njim nije omogućeno plansko izlučivanje zbog selekcijskih uzroka. Sa ekonomskom analizom saznajemo, da ukupni troškovi proizvodnje mlijeka po uvođenju ASM ne odstupaju značajno. Međutim, postoje značajne razlike unutar pojedinih skupina troškova, poput većih troškova remonta i amortizacije i nižih troškova rada. Iznad svega, promjene u troškovima rada i amortizacija otvaraju dodatna društveno-ekonomska pitanja, poput pitanja zadržavanja plaćenih radnih mjesta na gazdinstvima usredotočenih na proizvodnju mlijeka.

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The influence of the automatic milking system on milk production

Abstract

To estimate the impact of the automatic milking system (AMS) dairy and reproduction parameters from the livestock base Govedo were analysed. By using t-test, statistically significant differences were verified. Analysed technological parameters were also used in the economic research. Due to lack of data, a survey was conducted. In September 2019, there were 98 active AMSs in Slovenia. By investing in AMS, there was an increase in the number of dairy cows per household (+23%). The rate of culling cows also increased significantly with the most significant causes as fett and leg diseases, reproductive disorders and other causes. Detected were lower milk fat content, higher milk protein content, shortening of calving period (-16 days), and improvement in other reproduction parameters. Economic analysis has shown that, with the introduction of AMS, milk production remains at a similar cost level. The cost of labour is lower because of lower labour consumption (-30%) after the introduction of AMS, while the cost of depreciation increases by more than 35%.

Keywords: automatic milking system, dairy production, economic efficiency

Klaonički pokazatelji i kakvoća mesa tovljenika banijske šare iz poluotvorenog sustava držanja

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Sažetak

Cilj rada bio je utvrditi klaoničke pokazatelje i najvažnija svojstva kakvoće mesa tovljenika banijske šare svinje iz poluotvorenog sustava držanja. U dobi od 16 mjeseci svinje su prosječno bile teške 162 kg. Klaonički pokazatelji tovljenika banijske šare pokazali su dobru iskoristivost trupa i visoku preradbenu vrijednost. Vrijednosti pH_{45} od 6,32 izmjerene u *m. longissimus dorsi* te pH_{24} u *m. longissimus dorsi* (5,49), *m. semimembranosus* (5,46) i *m. gracilis* (5,56), kao i boje mesa ne ukazuju na nedostatke u kakvoći mesa, dok je sposobnost vezanja vode određena EZ metodom nešto iznad očekivanih vrijednosti (> 5%). Dobiveni pokazatelji klaoničke kvalitete polovica i kakvoće mesa ukazuju na dobru mogućnost prerade mesa banijske šare u mesne proizvode s dodanom vrijednošću.

Ključne riječi: svinje, banijska šara, klaonička svojstva, kakvoća mesa, poluotvoreni sustav

Uvod

Krajem 2018. godine u Hrvatskoj je priznata treća autohtona pasmina svinja – banijska šara. Iako je u prošlosti, osobito početkom 20. stoljeća bila vrlo raširena na području današnje Banovine i predstavljala temelj za sirovinu mesne industrije Gavrilović, nakon 2. svjetskog rata dolazi do stagnacije, a potom i skoro nestanka ove pasmine tijekom ratnih zbivanja 90-tih. Salajpal i sur. (2017.) navode da je banijska šara nastala križanjem krmača domaće bijele svinje s klopavim ušima (landras) i/ili turopoljske svinje i nerasta berkšir pasmine. Banijska šara svinja pripada u kombinirane pasmine svinja. Zahvaljujući aktivnostima stručnjaka u području svinjogojstva s više institucija izvršen je obilazak terena s ciljem pronalaska grla u tipu banijske šare, što je konačno dovelo i do stvaranja udruge uzgajivača svinja, priznavanja pasmine te povećanja broja svinja (Škorput i sur., 2019). Već u početku rada na revitalizaciji uzgoja banijske šare svinje kao jedan od ciljeva zadano je i utvrđivanje tovnih, klaoničkih i svojstava kakvoće mesa, uz pretpostavku da bi upravo specifičan način držanja, genotip i kakvoća mesa trebali dati osnovu za korištenje mesa banijske šare u preradi u mesne proizvode s dodanom vrijednošću, čime bi se pokrenula svinjogojaska proizvodnja na području Banovine, a i šire.

Cilj rada bio je utvrditi klaoničke pokazatelje i svojstva kakvoće mesa te procijeniti klaoničku vrijednost tovljenika banijske šare i mogućnosti prerade mesa u visokokvalitetne proizvode.

Rad je dijelom izvod iz diplomskog rada Dominika Petričevića, mag. ing. agr., naslova „Kakvoća mesa tovljenika banijske šare iz poluotvorenog sustava držanja”

Materijal i metode

Istraživanje je provedeno na 10 tovljenika (4 kastrata i 6 nazimica) pasmine banijska šara. Tov svinja je proveden na obiteljskom poljoprivrednom gospodarstvu Čačić u Taborištu pored Petrinje u razdoblju od listopada 2016. godine do studenog 2017. godine. Prva faza tova provedena je od listopada 2016. do travnja 2017. godine u zatvorenom objektu u boksu na punom podu, uz prosječnu površinu od 1,5 m²/životinji. Prosječna masa svinja na početku prve faze tova iznosila je 32 kg. Svinje su u prvoj fazi hranjene gotovom krmnom smjesom (16 % sirovih bjelančevina, 13 MJ metaboličke energije/kg), u količini od 1 kg/dan na početku do 3 kg/dan na kraju prve faze. Druga faza tova provedena je od travnja 2017. do studenog 2017. godine. Tijekom druge faze tova svinje su držane na površini od 1 ha gdje su hranjene gotovom krmnom smjesom s 14 % sirovih bjelančevina i 13 MJ metaboličke energije/kg, u količini od 2 kg po danu. Voda je bila osigurana po volji. Na kraju tova svinje su prevezene u klaonicu. Udaljenost od farme do klaonice je bila 15 km, a prijevoz je trajao oko 30 minuta. Nakon pojedinačnog vaganja svinje su premještene u boks gdje su se odmarale 1 h prije klanja. Omamljivanje je izvršeno električnom strujom jakosti 1,3 A. Nakon iskrvarenja šurenje je provedeno u vodi temperature od 60 do 70° C tijekom 5 do 10 minuta. Nakon evisceracije, trupovi su rasječeni na polovice. Zabilježeni su podaci o klaoničkoj masi, te izmjerene mjere debljine leđne slanine (S) i dubine mišića (M) radi procjene mesnatosti metodom dvije točke te izračuna randmana. Izmjerena je duljina trupa kao najkraća mjera od stidne kosti (*os pubis*) do prednjeg ruba prvog rebra te duljina polovice kao udaljenost od *os pubis* do prvog vratnog kralješka (*atlasa*).

Mjerenje pH vrijednosti mesa banijske šare svinje je provedeno pomoću digitalnog pH metra IQ 150 (Texas Instruments, SAD) opremljenog s ubodnom pH sondom (BlueLine 21pH, SI Analytics, Njemačka). Izmjerena je pH₄₅ vrijednost dugog leđnog mišića (MLD – *musculus longissimus dorsi*), te vrijednost pH₂₄ dugog leđnog mišića (MLD) i dva mišića buta (MS – *musculus semimembranosus* i MG – *musculus gracilis*). Boja mesa banijske šare svinje je utvrđena uređajem Konica Minolta Chromameter CR-410 određivanjem vrijednosti CIE L*, a* i b* (CIE, 1976). Boja mesa izmjerena je za MLD (*musculus longissimus dorsi*), MS (*musculus semimembranosus*) i MG (*musculus gracilis*). Ispitivanje sposobnosti vezanja vode je provedeno pomoću EZ metode (Christensen, 2003) određivanjem gubitka mesnog soka (*engl. drip loss*). S desnih polovica nakon 24-satnog hlađenja (+4° C) u visini zadnjeg rebra prikupljeni su uzorci MLD-a za utvrđivanje gubitka mesnog soka 24 i 48 sati *post mortem*. Sa svakog uzorka pomoću standardne inox sonde (Ø 25 mm) izuzeti su uzorci mišićnog tkiva debljine oko 2 cm. Pojedinačni uzorci mesa su stavljeni u plastične spremnike, nakon čega su pohranjeni u hladnjak na temperaturu od +4° C te izvagani nakon 24 h i 48 h. Nakon vaganja spremnika s uzorkom mesa i mesnim sokom, izvađen je uzorak mesa kako bi se izvagao spremnik samo s mesnim sokom. Sposobnost vezanja vode mesa izračunata je nakon 24 h i 48 h prema formuli:

$$\text{EZ gubitak mesnog soka (\%)} = \frac{(M_t - M_p) + 100}{M_{mt} - M_p}$$

U navedenoj formuli: M_p predstavlja masu praznog spremnika; M_{mt}, masu spremnika s mesom i mesnim sokom; M_t, masu spremnika s tekućinom, odnosno mesnim sokom. Opisni statistički pokazatelji (aritmetička srednja vrijednost (\bar{x}), standardna devijacija (SD), minimum (Min), maksimum (Max) i koeficijent varijabilnosti (CV) izračunati su primjenom MEANS procedure statističkog programa SAS STAT (2013).

Rezultati i rasprava

Prosječna završna masa tovljenika banijske šare u dobi od 16 mjeseci iznosila je 162,2 kg, a klaonička masa 133,7 kg (Tablica 1.). Randman polovica je bio zadovoljavajući i iznosio je prosječno 82,3 %. Dužina trupa prosječno je iznosila 88,2 cm, a dužina polovica 106,8 cm. Prosječna debljina leđne slanine iznosila je 35,3 mm, a dubina mišića 62,4 mm. Udio mišićnog tkiva prosječno je iznosio 45,15 ± 4,59 %.

Tablica 1. Klaonička svojstva tovljenika banijske šare svinje

Svojstvo	\bar{x}	SD	Min	Max	CV, %
Završna masa, kg	162,20	17,37	145,00	196,00	10,71
Klaonička masa, kg	133,76	16,30	115,40	165,40	12,19
Randman, %	82,35	1,90	79,59	84,39	2,30
Duljina trupa, cm	88,22	5,70	80,00	97,00	6,53
Duljina polovice, cm	106,89	7,80	97,00	120,00	7,29
Debljina leđne slanine, mm	35,33	7,21	26,00	52,00	20,40
Dubina mišića, mm	62,44	5,70	52,00	70,00	9,13
Udio mišićnog tkiva, %	45,15	4,59	37,04	48,86	10,16

Tovljenici banijske šare su imali značajno veći kapacitet rasta u odnosu na crnu slavonsku svinju (Senčić i sur., 2016), a pogotovo turopoljsku svinju podjednake dobi (Leščić, 2017). Mjere duljine trupa, odnosno polovice su također veće u odnosu na crnu slavonsku svinju (Luković i sur., 2007).

Tablica 2. Vrijednosti pH mesa banijske šare svinje

Svojstvo	\bar{x}	Min	Max	SD	CV, %
pH ₄₅ (MLD)	6,32	6,20	6,43	0,07	1,17
pH ₂₄ (MLD)	5,49	5,44	5,55	0,04	0,80
pH ₂₄ (MS)	5,46	5,38	5,50	0,04	0,66
pH ₂₄ (MG)	5,56	5,47	5,61	0,05	0,85

* MLD (*musculus longissimus dorsi*), MS (*musculus semimembranosus*), MG (*musculus gracilis*)

Prosječna vrijednost pH₄₅ izmjerena u MLD-u bila je 6,32, dok je vrijednost pH₂₄ u MLD-u iznosila 5,49, u MS-u 5,46 te u MG-u 5,56 (Tablica 2.). Dobivena vrijednost pH₄₅ pokazala je da meso nije imalo karakteristike razvoja blijedog, mekog i vodnjikavog (BMV) mesa. Navedene vrijednosti pH₂₄ nam ukazuju da meso nema značajke tamnog, čvrstog i suhog mesa (TČS). Senčić i sur. (2011) su istraživali kakvoću mesa crne slavonske svinje te su utvrdili vrlo slične vrijednosti pH. Karolyi i sur. (2006) navode pH vrijednosti MLD-a kod crne slavonske svinje, domaće bijele svinje te križanaca domaće bijele i duroka. Srednja vrijednost pH₄₅ crne slavonske svinje je iznosila 6,18 ± 0,05, a srednja vrijednost pH₂₄ je iznosila 5,87 ± 0,02. Srednja vrijednost pH₄₅ domaće bijele svinje je iznosila 6,65 ± 0,05, a srednja vrijednost pH₂₄ 5,46 ± 0,02. Križanci domaće bijele svinje i duroka su imali srednju vrijednost pH₄₅ 6,83 ± 0,06, a srednja vrijednost pH₂₄ je iznosila 5,42 ± 0,02.

Boja mesa tovljenika banijske šare svinje je promatrana na različitim mišićima (Tablica 3.) Tako je MLD imao najizraženiju svjetlinu, a najmanju vrijednost razvoja crvene boje. MG je imao najmanju svjetlinu, te najizraženiji razvoj crvene boje mesa, dok je imao srednju vrijednost razvoja žute boje. Usporedbom rezultata boje mesa, dugog leđnog mišića, banijske šare svinje i crne slavonske svinje (Senčić i sur., 2011) iz poluotvorenih sustava držanja, uočavaju se slične vrijednosti parametra L*, između banijske šare (51,25 ± 2,43) i crne slavonske svinje (51,15 ± 2,41) te vrijednosti parametra a*, banijske šare (19,27 ± 1,57) i crne slavonske svinje (18,43 ± 1,22). Usporedbom rezultata banijske šare svinje i rezultata istraživanja Karolyi i sur. (2006.), boja mesa MLD-a, prema parametru L*, je imala manju vrijednost od domaćih bijelih svinja (56,08 ± 1,19), a veću od crne slavonske (49,93b ± 1,19) i križanaca domaće bijele i durok pasmine (48,74 ± 1,33). Vrijednosti parametra a* banijske šare svinje (19,27 ± 1,57) su bile manje nego kod crne slavonske (20,02 ± 0,42) i križanaca domaće bijele i durok pasmine (22,40 ± 0,47), a veće nego kod domaće bijele (18,22 ± 0,42). Vrijednosti parametra b* banijske šare svinje (6,69 ± 0,45) su veće nego kod crne slavonske (4,67 ± 0,44) i domaće bijele pasmine (5,75 ± 0,44), a manje nego kod križanaca domaće bijele i durok pasmine svinja (9,68 ± 0,49). Promatrajući određene mišiće banijske šare svinje, uočene su razlike u boji mišića, ali i općenito nešto tamnija boja mesa što se može objasniti dijelom utjecajem genotipa, a dijelom kretanja svinja na otvorenom tijekom druge faze tova.

Tablica 3. Boja mesa banijske šare svinje na presjeku MLD-a

Svojstvo	Srednja vrijednost	Najmanja vrijednost	Najveća vrijednost	Standardna devijacija (SD)	Koeficijent varijabilnosti (CV(%))
L* MLD	51,25	48,88	56,21	2,43	4,73
a* MLD	19,27	16,25	20,45	1,57	8,13
b* MLD	6,69	5,99	6,92	0,45	6,77
L* MS	47,05	42,83	53,68	3,52	7,49
a* MS	21,82	20,77	22,84	0,76	3,47
b* MS	5,94	5,03	6,84	0,94	15,89
L* MG	39,84	37,76	42,72	1,57	3,95
a* MG	23,68	21,82	25,95	1,18	5,00
b* MG	6,61	5,88	7,76	0,74	11,20

Prosječna vrijednost gubitka mesnog soka nakon 24 sata u MLD-u je iznosila $5,76 \pm 2,18$ % (Tablica 4). Najmanja izmjerena vrijednost je iznosila 2,66 %, a najveća vrijednost 10,75 %. Koeficijent varijabilnosti gubitka mesnoga soka 24 sata *post mortem* je iznosio 37,79 %. Gubitak mesnoga soka nakon 48 sati iznosio je $7,02 \pm 1,94$ %. Najmanja izmjerena vrijednost je iznosila 4,18 %, a najveća vrijednost 11,89 %. Koeficijent varijabilnosti gubitka mesnoga soka 48 sati *post mortem* je iznosio 27,59 %, što predstavlja visoke vrijednosti.

Tablica 4. Gubitak mesnoga soka - EZ metoda

Svojstvo	Srednja vrijednost	Najmanja vrijednost	Najveća vrijednost	Standardna devijacija (SD)	Koeficijent varijabilnosti (CV(%))
Mesni sok 24 h	5,76	2,66	10,75	2,18	37,79
Mesni sok 48 h	7,02	4,18	11,89	1,94	27,59

Srednja vrijednost gubitka mesnoga soka poslije 24 h na uzorcima MLD-a je bila veća od 5 %. Žilić i sur. (2016.) u istraživanju o sposobnosti vezanja vode turopoljske svinje navode da je nakon 24 sata *post mortem*, gubitak mesnog soka u prosjeku iznosio $1,40 \pm 0,18$ %, dok je najmanja izmjerena vrijednost iznosila 0,65 %, a najveća 3,39 %. Srednja vrijednost gubitka mesnog soka nakon 48 sati je iznosila $1,94 \pm 0,23$ %, dok je najmanja vrijednost iznosila 0,98 %, a najveća vrijednost 4,98 %. Koeficijent varijabilnosti za gubitak mesnog soka nakon 24 sata je iznosio 60,15 %, a za gubitak nakon 48 sati, 55,08 %. Kao moguće uzroke velike varijabilnosti autori navode djelovanje pre mortalnih i *post mortalnih* čimbenika te manipulaciju s mesom tijekom uzorkovanja. Senčić i sur. (2011) u istraživanju o kakvoći mesa crne slavonske svinje u poluotvorenom sustavu držanja navode da je meso crne slavonske svinje izgubilo $4,65 \pm 1,64$ % mesnog soka.

Zaključak

U tovu svinja banijske šare u poluotvorenom sustavu utvrđena je prosječna završna masa od 162 kg u dobi od 16 mjeseci. Klaonički pokazatelji tovljenika banijske šare pokazali su dobru iskoristivost trupa i visoku preradbenu vrijednost. Vrijednosti pH i boje mesa banijske šare su zadovoljavajuće, dok je sposobnost vezanja vode određena EZ metodom nešto iznad očekivanih vrijednosti (> 5%). Za potpuno sagledavanje svojstava kakvoće mesa potrebno je nadalje analizirati i udio intramuskularne masti koja je važan kriterij u preradi mesa u suhomesnate proizvode.

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Slaughtering performance and meat quality of Banija spotted fatteners from semi-outdoor system

Abstract

The aim of the study was to determine slaughtering performance and the most important quality traits of fattening pigs from a semi-outdoor system. At 16 months, pigs weighed 162 kg on average. The slaughtering performance of carcasses showed good carcass utilization and high processing value. The pH and color values of meat are satisfactory, while the water holding capacity obtained by drip loss showed slightly higher values than expected (> 5%). Obtained measurements of slaughtering quality of carcasses and meat quality indicate a good possibility of processing raw meat into added value meat products.

Keywords: pigs, Banija spotted pig, slaughtering traits, meat quality, semi-outdoor system

Porodna masa i odlike rasta muške jaradi izvornih i inozemnih pasmina koza u Hrvatskoj

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Sažetak

Cilj rada bio je utvrditi породnu masu i odlike rasta muške jaradi izvornih (hrvatska šarena koza i hrvatska bijela koza) i inozemnih pasmina koza (alpina, sanska koza, srnasta koza i burska koza) u Hrvatskoj. Prikupljeni su podaci dobiveni provedbom performance testa 650 odabrane muške jaradi (od 2011. do 2017. godine). Utvrđen je značajan ($P < 0,001$) utjecaj pasmine na odlike rasta, pri čemu je jarad inozemnih pasmina ostvarila veću ($P < 0,001$) prosječnu породnu masu, veći prosječni dnevni prirast i završnu tjelesnu masu nego jarad izvornih pasmina. Međutim, jarad izvornih pasmina je ostvarila veći ($P < 0,001$) relativni prirast. Utvrđen je značajan ($P < 0,001$) utjecaj mjeseca jarenja na породnu masu, relativni prirast i završnu masu jaradi.

Ključne riječi: pasmina, performance test, dnevni prirast, relativni prirast, tjelesna masa

Uvod

Proizvodnja kozjeg, odnosno jarećeg mesa u Hrvatskoj temelji se na izvornim pasminama koza, ali i na inozemnim (prvenstveno mliječnim) pasminama koza, kao što su alpina, sanska te srnasta koza. Posljednjih godina u sve više stada uzgaja se burska koza (Boer). Njihova neraspodna muška i ženska jarad temelj su ove proizvodnje, a o njihovoj proizvodnosti (porodnoj masi, intenzitetu rasta i slično) u našim uvjetima malo je znanstvenih spoznaja. Naime u zemljama s razvijenim kozarstvom suvremena znanost prvenstveno je bila usmjerena na istraživanje njihovih mliječnih svojstava, fiziologije i hranidbe te reproduktivnih odlika (Kezić i sur., 2005).

Promatrajući globalno stanje, proizvodnja i potrošnja kozjeg mesa je relativno skromna, posebice u odnosu na neke druge vrste mesa (Madruga i Bressan, 2011). U sjevernoj Europi, kozje meso je vrlo slabo cijenjeno, a kao glavni razlog navodi se velika količina 4-metiloktanske i 4-metilnanonske kiseline pronađene u kozjem mesu koje nisu utvrđene u drugim vrstama mesa, a poznato je da upravo one doprinose njegovoj karakterističnoj aromi. Veliki problem u proizvodnji i plasmanu kozjeg mesa također predstavlja i nepostojanje tipičnih mesnih pasmina (genotipova) koza (izuzev burske koze), zatim slabije izražene tovnne odlike jaradi (u odnosu na, primjerice, janjad), zarazne bolesti (bruceloza), sezonska pojava jaretine na tržištu, navike potrošača i postojanje određenih predrasuda prema ovoj vrsti mesa (Kegalj i sur., 2011).

Pretpostavka ovog rada je postojanje značajnih razlika u odlikama rasta (porodna masa, prosječni dnevni prirast, relativni prirast te završna tjelesna masa) jaradi najzastupljenijih pasmina koza u Hrvatskoj. Stoga je cilj rada bio utvrditi neke odlike rasta odabrane muške jaradi izvornih (hrvatska šarena koza i hrvatska bijela koza) i inozemnih pasmina (alpina, sanska koza, srnasta koza i burska koza) koza u Republici Hrvatskoj.

Materijal i metode

U predmetnom istraživanju prikupljeni su podaci dobiveni provedbom performance testa (organiziranom od strane Hrvatske poljoprivredne agencije) odabrane muške jaradi različitih pasmina koza uzgajanih u Hrvatskoj, u razdoblju od 2011. do 2017. godine. Istraživanjem je obuhvaćeno ukupno 650 muške jaradi izvornih hrvatskih pasmina (hrvatska šarena koza - 99 grla; hrvatska bijela koza - 17 grla) i inozemnih pasmina koza uzgajanih u Hrvatskoj (alpina koza - 413 grla; sanska koza - 102 grla; srnasta koza - 12 grla; burska koza - 17 grla).

Provedbom performance testa u field uvjetima, odnosno testa na vlastitu proizvodnost prikupljeni su podaci o slijedećim odlikama jaradi: porodna masa, prosječni dnevni prirast i završna tjelesna masa jaradi na kraju performance testa, odnosno pri prosječnoj dobi jaradi od 105 dana. Na temelju prikupljenih podataka izračunat je relativni prirast istraživane jaradi izražen u postocima prirasta mase ili određene tjelesne mjere na kraju ispitivanog razdoblja u odnosu na početni (Pond i sur., 2004)

Prvi odabir muške jaradi izvršen je na temelju vanjštine i podataka pedigreea odmah nakon jarenja. Sljedeći odabir bio je pri odbiću, a treći u dobi od 105 dana (HSC, 2004).

Opisni statistički pokazatelji rasta janjadi (aritmetička srednja vrijednost (\bar{x}), standardna devijacija (SD), standardna greška (SE), minimum (Min), maksimum (Max) i koeficijent varijabilnosti (CV) izračunati su primjenom MEANS procedure statističkog programa SAS STAT (2013). Statistička obrada dobivenih podataka provedena je primjenom procedure GLM istog programskog paketa. U navedeni model je, osim fiksnog utjecaja pasmine (6 kategorija, odnosno pasmina), uključen i utjecaj mjeseca jarenja (pet kategorija, ovisno o pasmini), od prosinca do travnja.

Rezultati i rasprava

U tablici 1 prikazani su opisni statistički pokazatelji istraživanih odlika rasta jaradi. Utvrđena prosječna porodna masa odabrane muške jaradi bila je 3,35 kg, a tjelesna masa na kraju performance testa (pri prosječnoj dobi jaradi od 105 dana) iznosila je 26,22 kg. Pritom je istraživana jarad u prosjeku dnevno priraštala 217 grama, da bi na kraju testa prosječno povećala vlastitu porodnu masu za oko 7 puta. Najvarijabilnije od istraživanih svojstava bio je relativni prirast (koeficijent varijabilnosti 26,37%) što je i očekivano s obzirom da je ovisan ne samo o ostvarenom dnevnom prirastu jaradi, već i o njihovoj porodnoj masi.

Tablica 1. Opisni statistički pokazatelji porodne mase i odlika rasta jaradi (n=650)

	\bar{x}	SD	Min	Max	C.V. (%)
Porodna masa (kg)	3,35	0,77	1,90	5,9	23,10
Prosječni dnevni prirast (kg)	0,217	0,04	0,090	0,420	20,32
Završna tjelesna masa (kg)	26,22	4,87	17,50	38,5	18,58
Relativni prirast (%)	704,03	185,66	322,10	1151,10	26,37

U tablici 2 prikazan je utjecaj pasmine na porodnu masu te tjelesnu masu jaradi na kraju performance testa (u prosječnoj dobi od 105 dana). Istraživanjem je utvrđen značajan utjecaj pasmine na porodnu masu jaradi ($P < 0,001$), što je u skladu s navodima Morand-Fehr-a (1981) da porodna masa jaradi prije svega ovisi o pasmini, odnosno o tjelesnoj masi odrasle koze – majke. Najveća prosječna porodna masa utvrđena je u jaradi srnaste pasmine (3,83 kg), iako tek neznatno ($P > 0,05$) veća u odnosu na jarad alpina, burske i sanske koze. Sličnu prosječnu porodnu masu muške i ženske jaradi (3,77 kg) srnaste koze utvrdili su Prpić i sur. (2015). Prosječna porodna masa muške i ženske jaradi alpina i sanskih koza u istraživanju Gökdal i sur. (2017) iznosile je od 3,26 kg do 3,63 kg što je u skladu s utvrđenim rezultatima muške jaradi u predmetnom istraživanju. Slično rezultatima ovog istraživanja, Đuričić i sur. (2012) su utvrdili prosječnu porodnu masu jaradi pasmine Boer od 3,46 kg. Međutim, Prpić i sur. (2010) su utvrdili manju prosječnu porodnu masu muške jaradi hrvatske šarene koze (2,33 kg) u odnosu na predmetno istraživanje.

Tablica 2. Utjecaj pasmine na porodnu masu i završnu tjelesnu masu jaradi ($\bar{x} \pm S.E.$)

Pasmina	Porodna masa (kg)	Završna tjelesna masa (kg)
Hrvatska šarena koza	2,66 ± 0,07 ^a	23,98 ± 0,48 ^a
Hrvatska bijela koza	2,48 ± 0,17 ^a	23,62 ± 1,15 ^a
Alpina	3,54 ± 0,03 ^b	26,94 ± 0,23 ^b
Sanska koza	3,35 ± 0,07 ^b	25,82 ± 0,47 ^b
Boer	3,54 ± 0,17 ^b	26,33 ± 1,15 ^b
Srnasta koza	3,83 ± 0,20 ^b	27,04 ± 1,37 ^b
Razina značajnosti	P<0,001	P<0,001

a, b Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju.

\bar{x} = aritmetička srednja vrijednost; S.E. = standardna greška.

Iz podataka u tablici 2 vidljivo je da je jarad izvornih hrvatskih pasmina koza (hrvatska šarena koza i hrvatska bijela koza) imala najmanju prosječnu porodnu masu između istraživanih pasmina, ali međusobno vrlo sličnu ($P>0,05$) prosječnu porodnu masu (2,66 kg : 2,48 kg). U skladu s navedenim rezultatima, Kezić i sur. (2005) su utvrdili znatne razlike između pasmina u prosječnoj porodnoj masi jaradi, pri čemu je najveću prosječnu porodnu masu imala alpina jarad (3,41 kg), a najmanju jarad hrvatske bijele koze (2,96 kg).

Također, prema podacima prikazanim u tablici 2 vidljive su značajne ($P<0,001$) razlike u završnoj tjelesnoj masi jaradi različitih pasmina. Pritom je najveću prosječnu završnu tjelesnu masu ostvarila jarad srnaste koze (27,04 kg), a neznatno ($P>0,05$) manju prosječnu završnu tjelesnu masu imala je jarad ostalih inozemnih pasmina obuhvaćenih istraživanjem (alpina, sanska i burska koza). Slično tome, jarad izvornih hrvatskih pasmina imala je značajno ($P<0,001$) manju prosječnu završnu tjelesnu masu u dobi od 105 dana nego jarad inozemnih pasmina (23,98 kg jarad hrvatske šarene koze te 26,62 kg jarad hrvatske bijele koze).

Tablica 3. Utjecaj pasmine na prosječni dnevni prirast i relativni prirast jaradi ($\bar{x} \pm S.E.$)

Pasmina	Dnevni prirast (kg)	Relativni prirast (%)
Hrvatska šarena koza	0,204 ± 0,004 ^a	819,47 ± 17,98 ^a
Hrvatska bijela koza	0,200 ± 0,011 ^a	843,07 ± 44,51 ^a
Alpina	0,224 ± 0,002 ^b	678,71 ± 8,76 ^b
Sanska koza	0,218 ± 0,004 ^b	686,16 ± 17,72 ^b
Boer	0,221 ± 0,011 ^b	676,25 ± 43,18 ^b
Srnasta koza	0,225 ± 0,012 ^b	637,16 ± 51,39 ^b
Razina značajnosti	P<0,001	P<0,001

a, b Vrijednosti unutar istog stupca tablice označene različitim slovima statistički značajno se razlikuju.

\bar{x} = aritmetička srednja vrijednost; S.E. = standardna greška.

U tablici 3 prikazan je utjecaj pasmine na prosječni dnevni i relativni prirast jaradi. Istraživanjem je utvrđen značajan ($P<0,001$) utjecaj pasmine na dnevni prirast i relativni prirast jaradi. Najveći prosječni dnevni prirast utvrđen je u jaradi srnaste (0,225 kg) i alpina pasmine (0,224 g). Iz tablice 3 je uočljivo da jarad hrvatskih izvornih pasmina koza ima neznatno ($P>0,05$) različite prosječne dnevne priraste (oko 200 g), što je znatno više nego su ranijim istraživanjem muške jaradi hrvatske šarene koze (125 g/dan) utvrdili Mioč i sur. (2011). Međutim, relativni prirast istraživane jaradi izvornih pasmina (Tablica 3) bio je značajno ($P<0,001$) veći u odnosu na jarad inozemnih pasmina. Dakle, unatoč manjem prosječnom dnevnom prirastu jarad izvornih pasmina je ostvarila veći relativni prirast zahvaljujući značajno manjoj porodnoj masi u odnosu na jarad izvornih pasmina koza (Tablica 2).

U tablici 4 prikazan je utjecaj mjeseca jarenja na porodnu masu te tjelesnu masu jaradi na kraju performance testa (u dobi od 105 dana). Istraživanjem je utvrđen značajan ($P<0,001$) utjecaj mjeseca jarenja na porodnu masu jaradi. Najveća prosječna porodna masa jaradi utvrđena je u jaradi ojarane u siječnju i veljači (3,51 kg), a najmanja prosječna

porodna masa u jaradi ojarane u ožujku (3,01 kg), odnosno travnju (3,08 kg). Utvrđen je, također, značajan ($P < 0,05$) utjecaj mjeseca jarenja na tjelesnu masu jaradi na kraju performance testa. Najveća prosječna završna tjelesna masa utvrđena je u jaradi ojarane u prosincu, a najmanja u jaradi ojarane u ožujku (razlika veća od 2 kg).

Tablica 4. Utjecaj mjeseca jarenja na porodnu masu i završnu tjelesnu masu jaradi ($\bar{x} \pm S.E.$)

Mjesec jarenja	Porodna masa (kg)	Završna tjelesna masa (kg)
Siječanj	3,51 \pm 0,05 ^a	26,80 \pm 0,33 ^a
Veljača	3,51 \pm 0,05 ^a	26,34 \pm 0,32 ^a
Ožujak	3,01 \pm 0,06 ^b	25,07 \pm 0,42 ^b
Travanj	3,08 \pm 0,10 ^b	26,67 \pm 0,66 ^a
Prosinac	3,33 \pm 0,22 ^{ab}	27,21 \pm 1,40 ^a
Razina značajnosti	$P < 0,001$	$P < 0,05$

a, b Vrijednosti u istoj koloni tablice označene različitim slovima statistički se značajno razlikuju.

\bar{x} = aritmetička srednja vrijednost; S.E. = standardna greška.

U tablici 5 prikazan je utjecaj mjeseca jarenja na prosječni dnevni i relativni prirast jaradi pri čemu nije utvrđena statistički značajna razlika između prosječnih dnevnih prirasta jaradi u odnosu na mjesec njihova jarenja. Međutim, utvrđen je značajan ($P < 0,001$) utjecaj mjeseca jarenja na relativni prirast jaradi, pri čemu je najveći relativni prirast ostvarila jarad ojarana u travnju (813,16%), a tek nešto manji jarad ojarana u ožujku (770,02%). Razlike u utvrđenom relativnom prirastu između jaradi ojarane u proljeće u odnosu na jarad ojaranu zimi mogu se objasniti činjenicom da je jarad ojarana u ožujku i travnju ostvarila manju ($P < 0,001$) prosječnu porodnu masu nego jarad ojarana zimi (Tablica 4).

Tablica 5. Utjecaj mjeseca jarenja na prosječni dnevni prirast i relativni prirast jaradi

Mjesec	Prosječni dnevni prirast (kg)	Relativni prirast (%)
Siječanj	0,22 \pm 0,003	682,77 \pm 12,16 ^a
Veljača	0,22 \pm 0,003	669,77 \pm 12,08 ^a
Ožujak	0,21 \pm 0,004	770,02 \pm 15,68 ^b
Travanj	0,22 \pm 0,006	813,16 \pm 24,61 ^b
Prosinac	0,20 \pm 0,011	692,67 \pm 52,21 ^a
Razina značajnosti	NZ	$P < 0,001$

a, b Vrijednosti označene različitim slovima statistički značajno se razlikuju. NZ = nije statistički značajno.

\bar{x} = aritmetička srednja vrijednost; S.E. = standardna greška.

Zaključak

Rezultati ovog istraživanja upućuju na važnost i potencijal inozemnih pasmina koza, osobito mliječnih pasmina, s obzirom da je njihova muška jarad imala značajno veću prosječnu porodnu masu od jaradi izvornih pasmina te je ujedno ostvarila veći prosječni dnevni prirast i, posljedično, značajno veću prosječnu završnu tjelesnu masu. Navedeno ide u prilog činjenici da jarad inozemnih (većinom mliječnih) pasmina koza koja nije namijenjena daljnjem rasplodu, uz jarad izvornih pasmina, čini temelj proizvodnje jarećeg mesa u Hrvatskoj.

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Birth weight and growth traits of male goat kids of indigenous and foreign goat breeds in Croatia

Abstract

The aim of the study was to determine birth weight and growth traits of male goat kids of indigenous Croatian (Croatian spotted goat and Croatian white goat) and foreign breeds of goats (Alpine, Saanen, German Improved Fawn and Boer). Data were collected from the performance test of a total of 650 selected male goat kids (during period from 2011 to 2017). A significant ($P < 0.001$) effect of the breed on birth weight and growth traits was found, with the goat kids of foreign breeds achieved significantly higher average birth weight, higher average daily gains and average final body weight than the goat kids of the indigenous breeds. However, goat kids of indigenous breeds achieved a higher ($P < 0.001$) relative gain. Significant ($P < 0.001$) effect of the month of kidding on birth weight, relative gain and final body weight of the goat kids was determined.

Keywords: breed, performance test, daily gain, relative gain, body weight

Potential gain of genome editing for improved animal breeding

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Abstract

Genome editing is a modern technology for modifying or manipulating the genome. The initial molecular techniques like Zinc-Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) had a relatively low resolution in a splicing site recognition and thus suffered from a lower specificity due to their off-target side effects. The contemporary method that involves Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has recently gained a wide acceptance among researchers due to its speed, simplicity and ability for modifying genes. In this paper, the potential gain of genome editing with an accent on application in animal breeding will be discussed.

Keywords: genetic variation, genome editing, animal breeding, CRISPR, Cas9.

Introduction

Genetic variability is the main precondition that enables both natural and artificial selection. It occurs through spontaneous mutations, recombination processes during meiosis, gamete combinations during fertilization or is induced by mutagenesis (Kawall, 2019). Mutations are the main tool of natural selection and represent any change in genetic material that is not a consequence of two individuals crossing. Genome editing is also related to DNA sequence change by deletions, mRNA processing and post-transcriptional modifications that results in altered gene expression and functional behaviour of proteins (Khan, 2019). It is based on highly specific and programmable nucleases, which produce specific changes in regions of interest in the genome by introducing double-strand breaks (DSBs) that are later repaired by cellular mechanisms. Repair permits the genesis of insertions, deletions or substitutions in the target area. These mutations may prevent, eliminate or correct the defects in genes (Rodriguez-Rodriguez et al., 2019). The recent expansion and advancements in the field of biotechnology, especially in animal breeding, have provided more information and insight into the biochemical and molecular mechanisms to edit DNA. The new techniques seem promising, but the earlier ones have also been updated and improved. For simplicity and consolidation, an overview of genome editing techniques as well as their potential use in animal breeding will be presented below.

Genome editing techniques

Genome editing techniques are an advanced tool that can be used to introduce site specific modifications into the genome. Currently, there are three well-defined techniques for gene editing: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Palindromic Repeats (CRISPRs) with CRISPR-associated (Cas) nucleases (table 1).

Table 1: Main differences among genome-editing techniques

Feature	CRISPR-Cas	TALEN	ZFN
Ease of design	simple	slightly complex	moderate
Multiplex genome editing	high-yield multiplexing	few models	few models
Specificity	high	intermediate	low
Cost	low	high	low
Target DNA recognition	RNA-DNA	Protein-DNA	Protein-DNA
Key components	Guide RNA and Cas9 protein	TALE FokI fusion protein	ZF FokI fusion protein
Advantages	Highly efficient, editing multiple sites simultaneously	Highly efficient and specific	Highly efficient and specific
Disadvantages	PAM ¹ motif next to target sequence required	Tedious and time consuming to be constructed	Largescale screening, time consuming

¹PAM – Protospacer Adjacent Motif, a short DNA sequence (usually 2-6 base pairs in length) that follows the DNA region targeted for cleavage by the CRISPR system, such as CRISPR-Cas9

Each of these systems is defined by an adaptable sequence-specific DNA binding domain and a nuclease domain that creates a double-strand cleavage. The site specific DNA binding domains of the ZFN and TALEN systems are based on chimeric protein, while the CRISPR-Cas system utilizes an RNA molecule. Developing a genome editing tool requires engineering endonucleases that can create highly efficient and accurate DSBs at a user defined location in the genome and subsequently activate the cellular pathways involved in DSB repair processes via Homologous Recombination (HR) - mediated gene repair and Non-Homologous End Joining (NHEJ). HR uses homologous DNA sequences as templates for precise repair. It involves strand invasion and requires a homologous DNA template to precisely edit a genomic sequence or insert exogenous DNA that results in gene knock out or gene knock in (Shafie et al., 2014). NHEJ is an error-prone ligation process that results in small insertions or deletions (indel mutations). This process involves the re-ligation of the two broken ends at the cleavage sites and is catalyzed by DNA ligases (Gratz et al., 2014). CRISPR is capable of modifying the chromosomal target by indel mutations at high frequency (Cradick et al., 2013). In addition, CRISPR-Cas9 allows the simultaneous targeting of several sequences for multiplexed gene editing (Cong et al., 2013) and has the potential for gene replacement by concurrently targeting the sequences upstream and downstream from a given locus (Auer et al, 2014).

Zinc-finger nucleases (ZFNs)

ZFNs are purely artificial structures generated by a combinatorial approach where restriction endonucleases are joined with zinc-finger-binding domain protein. The nuclease part of ZFNs is normally a *FokI* nuclease, which cuts the DNA. Two *FokI* molecules come together to make a cut in the DNA, so a pair of ZFNs are made, one binding to each strand. The engineered DNA binding protein domain specifically localizes the ZFN at a predetermined location in the genomic sequence of interest and facilitates targeted genome editing by creating double-strand cleavage by the catalytic domain of the *FokI* endonuclease. Being the early version of artificial engineered nucleases, ZFN opened a new possibility for gene targeting manipulation in many livestock species, although this technology still suffers from a complicated construction process and unpredictability of targeting activity. In general, a rational design and assembly of ZFN is somewhat a tough task for many laboratories (Chandrasegaran and Carroll, 2016).

Transcription Activator-Like Effector Nucleases (TALENs)

TALENs were discovered in 2010 (Boch and Bonas, 2010) and gained a great interest among researchers because these nuclease molecules permit more predictable and specific binding to target DNA (Beumer et al., 2013). TALENs almost resemble ZFNs in terms of manufacturing and mode of action. A TALEN has two functional domains: a transcription activator-like effector domain, which is an oligopeptide array of modules (each module constitutes

33~35 amino acids) from the bacterium *Xanthomonas* spp., and a cleavage domain of *FokI* nuclease. Because there are four different modules, one for each nucleotide base, constructing customizable sequence-specific TALENs is a convenient method to target nearly any sequence of interest (Boch et al., 2009). Compared with ZFNs, TALENs are easy to manufacture, several times cheaper, and functionally better than ZFNs (table 1).

CRISPR-Cas9 systems

The first report of CRISPR system was in 1987 (Ishino et al., 1987). It is an adaptive immune mechanism present in many bacteria and the majority of characterized Archaea. The Eubacteria and Archaea possess a defense system that adapts, through RNA, to recognize and destroy external DNA and RNA. This provides acquired immunity against invading plasmids and viruses (Horvath and Barrangou, 2010) and therefore it is successfully adapted as a molecular biotechnology tool. It is used to edit DNA and RNA or to alter the epigenetic landscape of chromatin. The simplicity and flexibility of the system come from its targeting component, the guide RNA (gRNA), which directs the endonuclease Cas9 to the intended DNA region. The gRNA determines the specificity for a target DNA sequence through base-pair mediated binding to complementary DNA sequence. The binding of the gRNA then co-localizes Cas9 at the same site, which leads to cuts in the DNA backbone and the generation of DSB at the site (Hsu et al., 2014). This system is easy to design, highly specific, efficient, and well-suited for high-throughput and multiplexed gene editing for a variety of cell types and organisms.

Recent application of genome editing in animal breeding

Genome editing offers an opportunity to make specific and precise changes to the genome of an animal to increase productivity and disease resistance. The myostatin gene was an early target for gene editing in farmed animal species as disruption of this single gene has significant effects on a trait of economic importance. To date, the farmed animals in which the myostatin gene has been edited include pig, cattle, sheep, goat and Channel Catfish (Tait-Burkard et al, 2019). Beyond growth phenotypes, there has been a focus on more-efficient farming practices such as cattle dehorning, introducing reduced risk of injury, reduced competition for feeding-through space and less aggressive behaviors. Therefore, Carlson et al. (2016) used TALEN to introduce the Pc POLLED allele into the genome of bovine embryo fibroblasts which represents a potential approach for reducing physical dehorning in dairy cattle without a loss of productivity. In another area of genome editing application, some attempts were observed in a KISS1R gene knock-out to reduce aggressive behavior and to avoid the accumulation of androstenone and skatole in non-castrated male pigs (Sonstegard et al., 2016). Many studies are focused on the potential of genome editing for control of infectious diseases such as porcine reproductive and respiratory syndrome virus – PRRS (Whitworth et al., 2015), African swine fever (Lillico et al., 2016) and some epizootic and enzootic pneumonias (Shanthalingam et al., 2016). Finally, there is a huge potential of livestock use in the production of organs for human transplantation. Genome editing has a great role here, especially in removing or inactivating porcine endogenous retroviruses (PERVs) found within the genome of all pigs. As they are integrated into the genome, they exist in all tissues and organs and are passed on by inheritance. Inactivating or removing PERVs, as well as glycans responsible for the defense from foreign tissue makes their organs suitable for xenotransplantation (Niu et al., 2017).

Future perspectives and obstacles of gene editing

One important consideration in improving of animal breeding through genetic manipulation is the need of precise genome editing at multiple loci. Many traits of interest are complex – that is, they are ruled by many alleles, whereat each of them has only a small effect. Growth and development of animals are influenced by multiple genes that function throughout the entire body, and different production traits are often controlled by different and/or multiple genes. Different mutation sites or mutation types in the same gene may also have a large impact on the production performance. Therefore, significant enhancement of multiple livestock production traits requires precise genome editing at multiple genomic regions or sites. Improving the efficiency in multiplexed genome editing will promote the efficiency of livestock breeding and bring livestock breeding to a new level. Simulations have shown that, even with complex traits, genome editing could have a role in livestock improvement, either by increasing the frequency of favorable alleles or removing deleterious alleles as part of a genomic selection-driven breeding programme (Tait-Burkard et al., 2018). Genome editing technology can rapidly edit key genes that affect target traits and obtain the

required genotype through a single generation of editing, thereby greatly reducing breeding time. It would not be simple to develop a sustainable line of livestock based on multiple genome edits. Many edited founders would need to be produced to maintain background genetic variation and avoid excessive inbreeding levels (Ruan et al., 2017). The current genome editing technologies (ZFNs, TALENs and CRISPR/ Cas9) all have potential to induce off-target mutations in the genome. Although these mutations may not have any impact on the health of individual animals, they still carry a potential risk and can create obstacles for the future promotion of genome editing. In most cases, off-target mutations will either be selected against, if they are deleterious, or will likely disappear by drift if they are neutral. A big concern of gene editing use are bioethical issues too, especially in human pathology. In the wrong hands, this technology could result with an abuse and misuse in multiple ways, including manipulation of germline genetics. The scientists should be very cautious with the methods that are still in the experimental phase, because it could bring out some problems we do not yet understand. While the quest for a healthy baby and right of best possible treatment choice have been acknowledged in many societies, the approaching biotechnological revolution seems inevitable and undeniable (Khan, 2019). This will need consensus in public opinion, debates among biotechnologists and bioethical experts, regulatory frameworks within legislatures, and final guidelines and oversight for the finally allowed limited application.

Conclusions

This paper considered various aspects of genome editing, starting with a short description of genome editing in general, classification of most important methods, basic explanation of biochemical mechanisms, comparison of methods and possible application in animal breeding. CRISPR-Cas technologies took the leading role over ZFNs and TALENS owing to numerous advantages such as simplicity, low cost, speed and high efficiency in genome editing. Genome editing could have a role in livestock improvement, either by increasing the frequency of favorable alleles or removing deleterious alleles as part of a genomic selection-driven breeding programme. The bioethical issues still remain actual because of possible off-target effects that stay unclear and need a serious attention.

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Utjecaj hranidbe teladi sa starterom na bazi proizvoda kvasaca na dnevne priraste i zdravstveno stanje teladi

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Sažetak

Istraživanje je provedeno na 238 teladi Holstein pasmine, po 119 teladi u kontrolnoj i pokusnoj grupi. Plan napajanja mliječnom zamjenicom bio je isti u obje skupine, dok se smjesa razlikovala po tome što su u pokusnoj skupini korišteni proizvodi na bazi kvasaca. Vrijednosti prirasta nisu se značajno razlikovale. Gledajući prema spolu, muška telad u kontrolnoj skupini imala je značajno veći prirast u odnosu na žensku telad. U kontrolnoj skupini bilo je 184 terapije pneumonije, a u pokusnoj 139. Broj terapija enteritisa je u kontrolnoj skupini bio 45, a u pokusnoj 29. Proizvodni rezultati u vidu prirasta nisu poboljšani, ali je poboljšano zdravstveno stanje.

Ključne riječi: telad, kvasci, prirast, zdravstveno stanje

Uvod

Odgoj teladi je prva faza proizvodnje, kako u proizvodnji mesa, tako i u proizvodnji mlijeka. Obzirom da se radi o najosjetljivijoj kategoriji goveda, ova faza proizvodnje je zahtjevna i bitna za ostvarenje optimalnih proizvodnih rezultata životinja u kasnijoj fazi proizvodnje. U više radova opisan je utjecaj visoko probavljivog startera za telad na razvoj buraga. Starter koji sadrži lako probavljive ugljikohidrate stimulira razvoj buraga, a što uključuje i promjene u epitelu predželudaca (Baldwin i sur. 2004; Drackley, 2008). Newman i sur. (1993) zaključili su da telad koja je uz mliječnu zamjenicu bila hranjena i MOS-om imala nižu pojavnost respiratornih bolesti nego telad koja nije konzumirala MOS. Upotreba MOS-a smanjuje pojavnost proljeva i poboljšava konzistenciju fecesa, što je utvrđeno u mnogim pokusima (Dildey i sur. 1997; Nippe, 1996; Heinrichs, 2001). Upotreba nukleotida u hranidbi teladi poboljšava funkciju crijeva teladi, osiguravajući bolje uvjete u crijevima i poboljšavajući morfologiju crijeva (Kehoe i sur. 2008). Probiotici kvasaca povećavaju konzumaciju hrane te rast teladi i janjadi (Kmet i sur., 1993.; Cole i sur., 1992; Strzetelski i sur., 1996). Cilj provedenog istraživanja bio je utvrditi da li hranidba teladi starterima sa dodatkom nukleotida, živih kvasca i mananoligosaharida ima utjecaj na povećanje bruto dnevnih prirasta te smanjenje pojavnost pneumonija i enteritisa.

Materijal i metode

Pokus je proveden na farmi za uzgoj teladi Karanac, Hrvatska (lat.:45°45'13"N, long.: 18°39'45"E), u periodu od 20.1.2015. do 20.3.2015. Telad je bila podijeljena u dvije skupine, kontrolnu (119 teladi, od čega 52 ženska i 67 muška teleta, prosječne starosti 26,1 dan i tjelesne mase 51 kg) i pokusnu (119 teladi, od čega 59 ženska i 60 muška teleta, prosječne starosti 27 dana i tjelesne mase 50 kg). U ovoj fazi telad je hranjena mliječnom zamjenicom na automatima za napajanje teladi, u koncentraciji 125 g/L otopine tijekom 58 dana (obje grupe su imale isti program napajanja: 7 dana Kalvostart Energy s 4,0 L na 4,5 L, 14 dana Kalvostart Energy s 4,5 L na 5,5 L, 21 dan Kalvolac 5,5 L, 15 dana Kalvolac s 5,5 L na 0 L), a sijeno i peletirana smjesa bili su dostupni po volji. Kontrolna skupina je

hranjena standardnom smjesom za telad, a pokusna je hranjena sa smjesama u koje su dodani nukleotidi, živi kvasci i mananoligosaharid (MOS). Na 50. dan pokusa je u obrok uključena i kukuruzna silaža, radi prilagodbe na obrok u idućoj fazi uzgoja. Udio krmiva u smjesama za telad prikazan je u Tablici 1.

Tablica 1. Udio krmiva i kemijski sastav peletiranih smjesa za telad u pokusnoj i kontrolnoj skupini

KRMIVO	JM	GT -1 18 % SB pokus	GT -1 18 % SB kontrola
KUKURUZ	%	30.98	35.5
PŠENICA	%	6.0	7.0
PŠENIČNE POSIJE	%	10.0	10.0
JEČAM	%	6.0	7.0
SOJINA SAČMA 46 %	%	16.0	17.35
SOJA PUNOMASNA (tost.)	%	10.0	10.0
REPIN REZANAC	%	10.0	5.0
MELASA ŠEĆRNE REPE	%	4.0	4.0
DEKSTROZA	%	1.0	1.0
SOL (NaCl)	%	0.3	0.5
VAPNENAC H.	%	1.0	1.1
MONOKALCIJ FOSFAT	%	1.12	1.15
ŽIVI KVASCI	%	0.2	
MANANOOLIGOSAHARIDI	%	1.0	
NUKLEOTIDI	%	2.0	
AROMA SWEET VANILIJA	%	0.05	0.05
VEZIVO ZA PELETIRANJE	%	0.1	0.1
PREMIKS 0,25% ZA TELAD ¹	%	0.25	0.25
KEMIJSKA ANALIZA			
SUHA TVAR	%	88.67	88.52
SIROVI PROTEIN	%	18.78	18.80
SIROVA MAST	%	3.73	3.88
SIROVA VLAKNINA	%	6.17	5.45
KALCIJ	%	0.89	0.88
FOSFOR	%	0.67	0.69
NATRIJ	%	0.22	0.23
MAGNEZIJ	%	0.23	0.23
PEPEO	%	5.95	6.02
ŠKROB	%	27.26	30.77
NETO ENERGIJA RASTA	MJ/kg	7.88	7.95
NDF	%	20.9	19.00

Premiks sadrži: vitamin A 4.000.000 IJ, vitamin D3 800.000 IJ, vitamin E 16.000 mg/kg, vitamin B1 400 mg/kg, vitamin B2 800 mg/kg, vitamin B12 5 mg/kg, vitamin B5 4.000 mg/kg, vitamin B6 4.000 mg/kg, niacin 12.000, mg/kg, bakar 5.000 mg/kg, cink 16.000 mg/kg, jod 300 mg/kg, selen 120 mg/kg, kolin klorid 60.000 mg/kg, željezo 20.000 mg/kg, antioksidant BHT 40.000 mg/kg

Telad je prvi dan po ulasku u pokus bila smještena u objektu koji je prethodno bio opran, dezinficiran i „odmoren“, te je raspoređena u 4 boksa po spolu i skupinama, a prosječan broj teladi u boksu je bio 29,75. Telad je vagana na ulazu

u istraživanje i na kraju istraživanja, 58. dan. Konzumacija mliječne zamjenice je praćena individualno po svakom teletu, a konzumacija smjesa i sijena dnevno, na nivou skupine, na način da se duž valova raspodijelila odvagana količina hrane i sutradan u isto vrijeme su pokupljeni ostaci hrane, koji su vagani i izračunata je konzumacija u prethodnom danu.

Rezultati i rasprava

Konzumacija hrane u kontrolnoj i pokusnoj skupini je prikazana u Tablici 2., gdje se može vidjeti da je konzumacija po hranidbenom danu bila ujednaćena, dok je telad u pokusu imala nešto bolju konverziju. Kehoe i sur. (2008) također nisu utvrdili povećanje konzumacije hrane upotrebom nukleotida dobivenih od kvasaca.

Tablica 2. Konzumacija hrane u kontrolnoj i pokusnoj skupini po hranidbenom danu i dnevnom prirastu

Parametar/ skupina	Pokus	Kontrola
Mliječna zamjenica, kg/ hranidbenom danu	0,54	0,51
Smjesa GT-1 18% SB kontrola, kg/ hranidbenom danu	1,04	
Smjesa GT-1 18% SB pokus, kg/ hranidbenom danu		1,13
Sijeno, kg/ hranidbenom danu	0,37	0,33
Ukupna konzumacija, kg/ hranidbenom danu	1,95	1,97
Mliječna zamjenica, kg/ kg prirast	0,62	0,60
Smjesa GT-1 18% SB kontrola, kg/ kg prirast	1,19	
Smjesa GT-1 18% SB pokus, kg/ kg prirasta		1,32
Sijeno, kg/ kg prirasta	0,42	0,38
Ukupna konzumacija, kg/ kg prirast	2,24	2,30

U Tablici 3. prikazane su tjelesne mase na ulazu u pokus, tjelesne mase na kraju pokusa i dnevni prirast teladi. Na početku pokusa telad je u obje skupine imala ujednaćenu tjelesnu masu, na kraju pokusa je kontrolna skupina teladi imala veću ($P > 0,05$) tjelesnu masu. Prirasti su također bili ujednaćeni, nešto veći ($P > 0,05$) u kontrolnoj skupini u odnosu na pokusnu skupinu. Dobiveni rezultati su u skladu s istraživanjem Cole i sur. (1992), koji također nisu utvrdili značajno bolje proizvodne rezultate kod teladi, upotrebom proizvoda na bazi kvasaca.

Tablica 3. Tjelesne mase i dnevni prirasti teladi kontrolne i pokusne hranidbene skupine

Parametar	Kontrolna skupina	Pokusna skupina	P
	\pm sd	\pm sd	
Početno vaganje, kg	51,18 \pm 6,99	50,40 \pm 6,56	0.391
Završno vaganje, kg	102,55 \pm 15,52	101,11 \pm 14,71	0.474
Prirast/HD, kg	0,89 \pm 0,20	0,87 \pm 0,19	0.653

\bar{x} = srednja vrijednost; sd = standardna devijacija

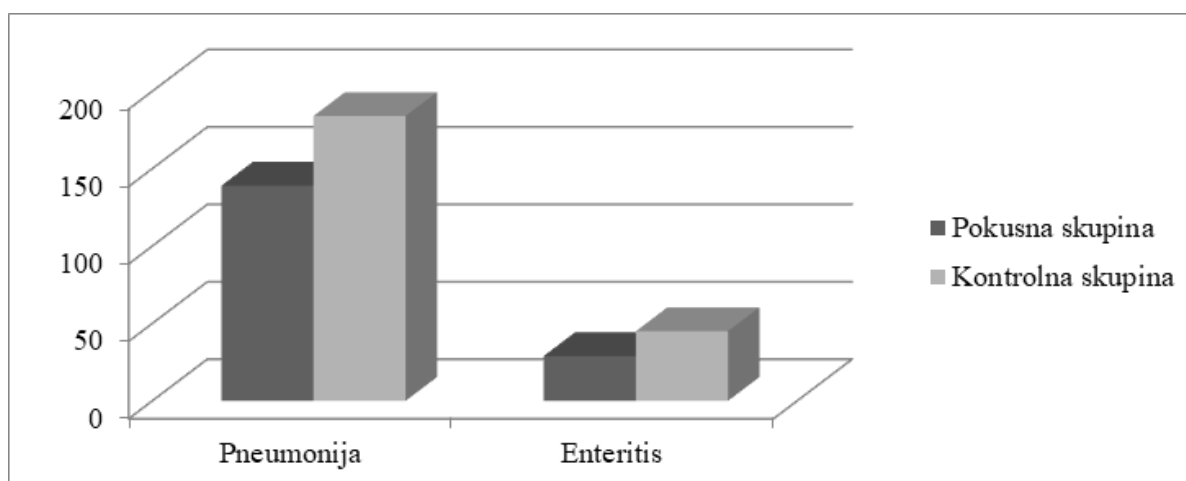
Promatrajući dnevne priraste po skupinama u odnosu na spol teladi, može se vidjeti da je muška telad u kontrolnoj skupini imala značajno ($P > 0,05$) veći prirast od ženske teladi, dok su u ženske teladi pokusne skupine prirasti u odnosu na spol bili ujednaćeni.

Tablica 4. Dnevni prirasti i tjelesne mase kontrolne i pokusne skupine prema spolu teladi

Parametar	1	2	3	4	P
	± sd	± sd	± sd	± sd	
Početno vaganje, kg	53.34 ^{2,4} ± 6.96	48.45 ^{1,3} ± 6.06	52.38 ^{2,4} ± 6.59	48.50 ^{1,3} ± 6.00	0.001
Završno vaganje, kg	107.73 ^{2,4} ± 16.60	96.00 ^{1,3} ± 11.09	104.16 ² ± 15.58	98.16 ¹ ± 13.30	0.001
Prirast/ hranidbeni dan, kg	0.94 ² ± 0.21	0.82 ¹ ± 0.16	0.89 ± 0.21	0.86 ± 0.16	0.008

\bar{x} = srednja vrijednost; sd = standardna devijacija; 1 = kontrolna skupina muški; 2 = kontrolna skupina ženski; 3 = pokusna skupina muški; 4 = pokusna skupina ženski; značajnost ^{1,2,3,4} (P < 0,05)

U provedenom istraživanju utvrđeno je smanjenje pojavnosti pneumonija i proljeva (Grafikon 1). Telad u pokusnoj skupini imala je 24,46 % manje terapija pneumonije, što je slično rezultatima studije Newman i sur. (1993), koji su također utvrdili manje respiratornih problema. Heinrichs i sur. (2003) su utvrdili da mananoligosaharidi (MOS) imaju pozitivan utjecaj na zdravlje i rast teladi, osobito značajno poboljšanje zdravlja crijeva. U predmetnom istraživanju je utvrđeno 35,56 % manje slučajeva enteritisa u teladi u pokusnoj skupini.



Grafikon 1. Ukupan broj terapija pneumonije i enteritisa

Zaključak

Korištenjem smjese za telad sa dodatkom živih kvasaca nisu utvrđeni značajno veći dnevni prirasti u teladi u odnosu na telad hranjenu smjesom bez tih dodatka. Smjese za telad sa dodatkom živih kvasaca su imale pozitivan utjecaj na zdravstveno stanje teladi, čime je djelomično potvrđena postavljena hipoteza o upotrebi proizvoda kvasaca u hranidbi teladi.

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Influence of feeding calves with starter based on yeast products on daily weight gain and calf health

Abstract

The study was conducted on 238 Holstein breed calves, 119 calves per control and experimental group. The plan for feeding the milk pronoun was the same in both groups, while the mixture differed in that the yeast-based products were used in the experimental group. The values of the average daily weight gain were not significantly different. Looking at gender, male calves in the control group had significantly higher average daily gain than female calves. In the control group there were 184 pneumonia therapies and in the experimental 139. The number of enteritis therapies in the control group was 45 and in the experimental 29. The production results in the form of growth were not improved, but the health status was improved.

Keywords: calves, yeasts, growth, health status

Influence of genotype, farm, and test year on the variability of traits monitored in the performance test of gilts

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Abstract

The production traits of gilts were examined in two pig herds for three consecutive years. The study included 3,610 gilts of 3 genotypes, originating from 84 boar sires. The test included only those boar sires who had 10 or more daughters. At the end of the test, the weight and thickness of the fat (FT1 and FT2) and depth were measured by a back muscle depth-ultrasound apparatus. The aim of this study was to determine the phenotypic and genotypic variability of the observed traits in the performance test of gilts: age at the end of the test (FA – final age), lifetime daily gain (LDG), fat thickness 1 and 2 (FT1 and FT2) and back muscle depth (BMD). In the first herd there were 1228 gilts and in the second 2382 gilts tested. Within the Landrace (L) breed there were 1962 gilts, Large white (LW) 1324 and Duroc (D) 319 gilts. In the first year, 885 gilts were tested, in the second 1145 and in the third 1580 gilts. Based on the results obtained, the genotype of gilts was found to have a statistically significant effect ($P < 0.001$) on FT1, FT2, and BMD, while LDG was unaffected ($P > 0.05$). Considering the farm as a source of variation in gilt traits, it was concluded that the farm had a highly significant statistical effect on the traits tested ($P < 0.001$). The year of testing of gilts as a source of variation in their traits showed a high statistical influence ($P < 0.001$) on all traits examined. Observed traits - fat thickness 1, 2 and BMD were highly statistically dependent ($P < 0.001$) on the weight at the end of the test.

Keywords: pigs, boars, genotype, gilts

Introduction

The performance test represents a significant measure in pig selection. The aim of on-farm selection is to improve the performance of the herd by increasing the frequency of desirable genes. Selection based on performance test results brings the improvement of economically important traits (life gain, backfat thickness, back muscle depth), by 2-3% per year (Schinkel, 1999). Szyndler-Nędzka et al. (2010) have concluded that the differences between the studied genotypes of performance tested gilts (LW, L, D, and P) were statistically very significant ($P \leq 0.01$) for all traits tested except for the BMD ($P \leq 0.05$). Brkić et al. (2001) have found statistically highly significant ($P < 0.01$) differences between breeds for all traits studied except for FT1 and BMD where the results obtained were not statistically significant ($P > 0.05$). While Radović et al. (2011) and Petrović et al. (1999) cited gilt genotype as a statistically highly significant ($P < 0.01$) source of variation of all analysed traits of tested gilts (FA, FT1, and FT2). The effect of year on fat thickness and muscle depth of the performance tested F1 gilts of Large White and Landrace breeds (PLW \times PL and PL \times PLW) was investigated by Nowachowicz et al. (2009). In these studies, a significant and highly significant influence of the year on the fat thickness P2 (measured behind the last rib 3 cm from the medial plane) was found. Petrović et al. (2009) have found in their study that the average weight of gilts at the end of the test varied significantly between herds ($P < 0.01$) at all years. Also, the same author states that the year of testing has a statistically significant influence on the manifestation of all traits tested. The gilts studied were of the Swedish Landrace and Large White sires (Petrović et al., 1999). Brkić et al. (2001) have examined the carcass quality of gilts on two farms at an average age of 209.25 days, weighing 103.81 kg (with an average daily gain of 0.491 kg), at the end of the test, on live animals

with an ultrasonic apparatus (Piglog 105). The average thickness of the loin fat (FA1) was 20.09 mm, of the back fat (FT2) 15.25 mm, the average depth of the long back muscle (BMD) was 45.25 mm and the average lean meat content was 53.64%.

Material and methods

The production traits of gilts were examined in two pig herds in three consecutive years. The study included 3,610 gilts from 3 genotypes, originating from 84 boar sires. The test included only those sires who had 10 or more daughters. At the end of the test, the weight and thickness of the fat (FT1 and FT2) and depth were measured with a MLD ultrasound apparatus. In the first herd, there were 1228 gilts and in the second 2382 gilts tested. Within the Landrace (L) breed there were 1962 gilts, Large White (LW) 1324 and Duroc (D) 319 gilts. In the first year, 885 gilts were tested, in the second 1145 and in the third 1580 gilts. The study included the following traits: age at end of test (FA – final age), fat tissue thickness between 3rd and 4th lumbar vertebra, 7 cm lateral to dorsal line (FT1), fat tissue thickness between 3rd and 4th rib, 7 cm lateral from dorsal line (FT2) and back muscle depth (BMD). The values of statistical indicators for the phenotypic expression of the traits tested were calculated using the least squares method and applying the GLM procedure using the software package “SAS/STAT” (SAS Inst. Inc., 2010) using the following models:

$$\text{Model 1: } Y_{ijklm} = \mu + F_i + G_j + R_k + b_1 (X_l - \bar{X}_l) + \varepsilon_{ijklm}$$

Model 1 was used to determine the significance ($P < 0.05$) of systematic influences on the fat thicknesses 1 and 2 and back muscle depth of the performance tested gilts, where: Y_{ijklm} = observation i.e. the manifestation of the trait of the m individual, the i farm, the j year of testing, k = genotype, μ = general population average, F = farm, G = year of testing, R = animal genotype, b_1 = linear regression effect of body weight at the end of the test, ε = random error, i = farm subscript ($i = 1, 2$), j = test-year subscript ($j = 1, 2, 3$), k = animal genotype subscript ($l = 1, 2, 3$), m = offspring subscript.

$$\text{Model 2: } Y_{ijklm} = \mu + F_i + G_j + R_k + \varepsilon_{ijklm}$$

Like the previous one, model 2 was used to determine the significance ($P < 0.05$) of systematic effects on trait age at the end of the test of the performance tested gilts, where: Y_{ijklm} = observation i.e. manifestation of the trait of the m individual, the i farm, the j year of testing, k = genotype, μ = general population average, F = farm, G = year of testing, R = animal genotype, ε = random error, i = farm subscript ($i = 1, 2$), j = subscript for the test year ($j = 1, 2, 3$), k = subscript for the animal genotype ($l = 1, 2, 3$), m = subscript for the offspring.

The fixed part of the model for the age at the end of the gilt test (FA) did not include a linear regression effect of the weight on the variability of age at the end of the test, since a previous correction of this trait for age at a weight of 100 kg was performed. The correction was made using the following expression:

$$\text{Corrected age at end of test} = (\text{Actual age} \times 100\text{kg}) / \text{Weight achieved}$$

Results and Discussion

Table 1 presents descriptive statistical indicators for the studied growth and carcass quality traits determined at the end of the gilt performance test.

Table 1. Average values and variability of tested traits

Variable	N	\bar{x}	SD	Min	Max
FA (day)		180.75	19.17	122.22	273.81
FT1(mm)	3610	9.83	2.32	5.20	23.00
FT2(mm)		8.92	1.97	4.70	24.00
BMD(mm)		53.40	5.13	34.00	72.00

FA- final age, FT1- fat tissue thickness 1; FT2- fat tissue thickness 2; BMD-back muscle depth;

Gilts at a body weight of 100 kg achieved an average age of 180.75 days. The result presented for FA is better than the results presented by Petrović et al. (2009), Gogić et al. (2012) and Popovac (2016), the cited authors have found higher values of this trait. On the basis of the results shown in Table 1, it can be seen that the average fat tissue thickness 1 and 2 was 9.83 and 8.92 mm, respectively. In terms of the average value of the fat tissue thickness, the result obtained in the present study is similar to the results presented by Szyndler-Nędza et al. (2010) and Gogić et al. (2012). The BMD at the end of the test averaged 53.40 mm, making this result close to the results of Szyndler-Nędza et al. (2010), while lower than the results presented by Gogić et al. (2012).

Table 2 shows the LSM (Least Squares Average) \pm SE values of the traits tested across the effects of the farm, year of testing and animal genotype factors.

Table 2. LSM \pm S.E. Values of tested traits for the farm, test year and genotype

Source variation	of	FA ²⁾ (day)	FT1(mm)	FT2(mm)	BMD(mm)
Farm	1	200.83 \pm 0.42	10.09 \pm 0.05	8.97 \pm 0.05	49.61 \pm 0.15
	2	171.50 \pm 0.34	8.01 \pm 0.04	7.55 \pm 0.04	54.79 \pm 0.12
Year	1	190.46 \pm 0.46	9.43 \pm 0.06	8.53 \pm 0.06	50.58 \pm 0.17
	2	184.69 \pm 0.93	8.97 \pm 0.04	8.23 \pm 0.04	51.66 \pm 0.12
	3	183.35 \pm 0.41	8.75 \pm 0.05	8.01 \pm 0.05	53.36 \pm 0.15
Genotype	1 ¹⁾	186.10 \pm 0.21	8.65 \pm 0.02	7.86 \pm 0.02	52.78 \pm 0.08
	2	185.84 \pm 0.27	8.57 \pm 0.03	7.80 \pm 0.03	52.77 \pm 0.10
	3	186.56 \pm 0.53	9.92 \pm 0.07	9.11 \pm 0.07	50.04 \pm 0.20

¹⁾ 1-L, 2- LW, 3-D, ²⁾ FA- final age, FT1- fat tissue thickness 1; FT2- fat tissue thickness 2; BMD-back muscle depth;

When the farm was observed as a source of variation, the following results were obtained: 1) The animals on farm 1 completed the test with an average of 200.83 days and were older than the animals on farm 2 which completed the test with an average age of 171.50 days, the difference between them being 29.33 days; 2) The thickness of fat tissue 1 is greater on farm 1 by 2.08mm compared to farm 2, as is the thickness of fat 2 on farm 1 compared to farm 2 by 1.42mm; 3) Farm 1 animals had less back muscle depth than animalson farm 2 by 5.18 mm.

The youngest animals were examined in the third year and the oldest in the first year. Fat tissue thickness 1 wasthe highest in the first year and the lowest in the third. The thickness of fat tissue 2, as well as 1, were the highest in the first and the lowest in the third year. Back muscle depth was the lowest in the first year and the highest in the third test year.

When the genotype of gilts is observed as a source of variation, it was found that: 1) Duroc animals were the oldest, while the youngest was the Large White (the difference between them was 0.72 days); 2) The lowest value for the thicknesses of dorsal fat tissue 1 and 2 was recorded in animals of Large White breed, and the highest in animals of Duroc breed; 3) The highest value for the depth of the back muscle was recorded in Landrace animals, and the lowest in Duroc.

Table 3 shows the statistical significance of the fixed and regression influences included in the models for the analysis of the variability of the growth and carcass quality traits of gilts and the coefficient of determination.

Influence of genotype, farm, and test year on the variability of traits monitored in the performance test of gilts

Tabela 3. Statistical significance of fixed and regression effects on FA, FT1, FT2, and BMD

Studied traits	Farm	Year	Genotype	FW		R ²
				B	p	
FA (day)	<0,0001***	<0,0001***	0.2255 NS	-	-	0,56
FT1(mm)	<0,0001***	<0,0001***	<0,0001***	0,08565	<0,0001***	0,57
FT2(mm)	<0,0001***	<0,0001***	<0,0001***	0,07869	<0,0001***	0,44
BMD(mm)	<0,0001***	<0,0001***	<0,0001***	0,07123	<0,0001***	0,52

*FW-final weight, B-regression coefficient, R²-determination coefficient, FA- final age, FT1- fat tissue thickness 1; FT2- fat tissue thickness 2; BMD-back muscle depth; NS=P>0,05; *=P<0,05; **=P<0,01; ***=P<0,001*

Using the model, the following results were obtained:

- 1) The farm had statistically significant ($P < 0.001$) effect on the manifestation of tested traits;
- 2) The year also had statistically significant ($P < 0.001$) effect on all studied gilt traits;
- 3) The gilt genotype had statistically significant effect ($P < 0.001$) on FT1, FT2, BMD, while FA was unaffected ($P > 0.05$).

The effect of year on fat tissue thickness and muscle depth of the performance tested gilts is consistent with the results obtained by Nowachowicz et al. (2009). In these studies, a significant and highly significant influence of the year on the thickness of P2 fat tissue (measured behind the last rib 3 cm from the medial plane) is found. Petrović et al. (1999) have found that gilt genotype and age have a statistically significant effect on the manifestation of traits in performance tested gilts, which was inconsistent with the results obtained in the present study. Also Petrović et al. (2009) have found in their study that the average weight of gilts at the end of the test varies significantly between herds ($P < 0.01$) in all years, which is consistent with the results obtained in the present study.

The regression effect of the final weight at the end of the test on the tested properties showed the following: by increasing the mass by 1 kg, FT1 increased by 0.085 mm and FT2 by 0.078 mm; the back muscle increased by 0.071 mm. Also, weight at the end of the test had a statistically significant effect on the properties of FT1, FT2 and BMD ($P < 0.001$). The coefficients of determination of R^2 indicated that the effects included in the model (farm, year, and genotype) explained from 0.44 to 0.57% the variability of the traits tested in the performance tested gilts.

Conclusion

The objective of this paper was to determine the phenotypic and genotypic variability of the observed traits in the performance test of gilts. Based on the test results, the following conclusions can be drawn: The gilt genotype was found to have a statistically significant ($P < 0.001$) effect on FT1, FT2 and BMD, but no effect on FA was recorded ($P > 0.05$). Considering the farm as a source of variation of gilt traits, it can be concluded that the farm had a very significant statistical effect on the traits tested ($P < 0.001$). The year of testing of gilts as a source of variation showed high statistical effect ($P < 0.001$) on all traits examined. Observed traits of fat tissue thickness 1, 2 and BMD were highly statistically dependent ($P < 0.001$) on the weight at the end of the test.

Acknowledgment

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Rotacijsko napasivanje u sustavu krava-tele

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Sažetak

Rotacijsko napasivanje u sustavu krava-tele predstavlja pomno planirano pomicanje goveda na pašnjaku tijekom cijele godine, što je jedan od glavnih ciljeva kvalitetnog upravljanja pašnjakom. Ovakvo prirodno imitirana migracija, osigurava govedima svakodnevno napasivanje svježom travnom biomasom. Pašnjak je najčešće ograđen električnom ogradom. Za zimsko napasivanje važno je osigurati raznolikost vrsta iz porodice trava, te odabrati najpovoljnije. U sustavu krava-tele koriste se i mesne i kombinirane pasmine. Ekonomske analize pokazuju isplativost ovakvog uzgoja jer su smanjeni troškovi mehanizacije, hranidbe, liječenja i ljudskog rada. Rotiranjem goveda, travne vrste se brže regeneriraju, tlo se obogaćuje humusom, izbjegava se zbijenost tla, a životinje su zdravije.

Ključne riječi: rotacijska ispaša, sustav krava-tele, pašnjak, voda, električne ograde

Uvod

Sustav krava-tele predstavlja ekstenzivni način držanja goveda, gdje je tele osnovni proizvod ovakvog uzgoja. Navedeni sustav proizvodnje podrazumijeva držanje goveda na pašnjacima, tijekom cijele godine. Kod rotacijskog napasivanja goveda su uglavnom ograđena ogradama (električnim ili drvenim). U sustavu krava-tele, u vrijeme laktacije, oteljena krava othranjuje vlastito tele (Walker i sur., 1989). U sustavu krava-tele najčešće se koriste mesne i kombinirane pasmine goveda. Za ovakav sustav od kombiniranih pasmina pogodna je simentalaska pasmina, a od mesnih pasmina: Limousin, Salers, Charolais, belgijsko plavo govedo, Hereford i Angus (Marohnić, 2008). Primjena rotacijske ispaše u sustavu-krava tele najpoznatija je u SAD-u i Kanadi (Ruechel, 2006). Ovakav način držanja podrazumijeva sustav krava-tele, gdje goveda tijekom cijele godine borave vani, pri čemu se ograda na pašnjaku pomiče svaki dan. Na taj način se osigurava održivost pašnjaka što je vrlo važno za njegovo duže razdoblje korištenja. Sve navedeno vodi jednom prirodnom, ekološkom i ekonomičnom načinu uzgoja goveda na pašnjacima. Dok se jedan dio pašnjaka napasuje, za to vrijeme drugi dio pašnjaka se regenerira. Tijekom regeneracije tratina na pašnjacima obnavlja energetske rezerve, te se produbljuje sustav korijenove mase što će omogućiti i dugoročniju proizvodnju na pašnjacima. (Undersander i sur., 2002). Rotacijsko napasivanje rijetko se spominje u stručnoj literaturi i praktičnim primjerima na terenu u R. Hrvatskoj. Zato je cilj ovog rada bio opisati primjenu rotacijskog napasivanja u sustavu krava-tele, te njezinu moguću uporabu na primjerima obiteljskih poljoprivrednih gospodarstava u Republici Hrvatskoj.

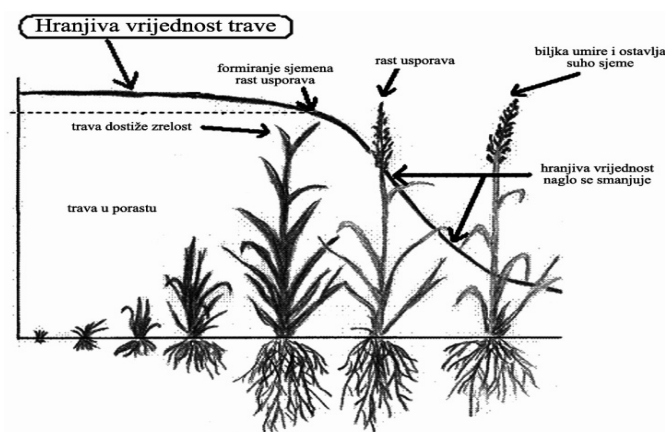
Osnovne značajke rotacijskog napasivanja

Rotacijsko napasivanje u sustavu krava-tele predstavlja pomno planirano pomicanje goveda na pašnjaku na način da goveda svaki dan pasu svježu travnu biomasu. Prema Andraeu (2008) rotacijsko napasivanje je metoda koja koristi ponavljajuća razdoblja napasivanja i odmora (regeneriranja) između dva ili više podijeljena pregona na pašnjaku. Jedan od glavnih ciljeva rotacijskog napasivanja je kvalitetno upravljanje pašnjakom. Podjelom pašnjaka na pregone pomoću pomičnih i nepomičnih ograda, ograničava se količina pojedene travne biomase. Vrlo je važno da krave ne popasu svu travnu biomasu u potpunosti do zemlje, jer će se tada pašnjak teško regenerirati i trebat će više vremena dok bude ponovno spreman za korištenje napasivanjem. Tratina pašnjaka treba ostati dovoljno visoka (u rasponu od 10 do 15 cm) kako bi se što bolje sačuvala vlažnost tla. Time će se omogućiti lakši i brži porast tratine pašnjaka. Kako je ovim sustavom predviđen boravak goveda na pašnjaku tijekom cijele godine, važno je tijekom zime na pašnjaku osigurati visoke travne vrste koje snijeg neće moći zatrpati. Ruechel (2006) navodi neke od visokih travnih vrsta: vlasulja trstikasta (*Festuca arundinacea* Schreb), vlasulja livadna (*Festuca pratensis* Huds), talijanski ljulj (*Lolium*

multiflorum Lam.), stoklasa bezosata (*Bromus inermis* Leyss), te vlasulja nacrvena (*Festuca rubra* L.) (iako je to niska trava). Rotacijski sustav napasivanja omogućuje govedima svakodnevnu ispašu svježom travom, što je zapravo imitacija prirodne migracije goveda na pašnjaku. Utrošak ljudskog rada je vrlo mali, oko pola sata dnevno, i to su najčešće poslovi vezani za pregon stoke s jednog na drugi pašnjak. Također i troškovi mehanizacije su smanjeni jer se goveda hrane isključivo livadnim vrstama trava, a zdravlje životinja je dosta dobro (Carter, 2016). Ne treba zanemariti i činjenicu obogaćivanja tla sa izmetom goveda na pašnjaku. Kao nedostatak ovakvog sustava može se navesti da goveda koja su namijenjena prodaji na tržištu borave od 2 do 3 mjeseca duže na pašnjaku od goveda koja se nalaze u intenzivnom tovu.

Važnost tratine pašnjaka i stanje tla

Svaki put kada goveda popasu travu na pašnjaku, mrtvi dijelovi korijenove mase trava se raspadaju, pri čemu stvaraju nove organske komponente tla. U samom korijenu travnih vrsta pohranjena je i određena rezerva hranjiva, što omogućava zaštitu i otpornost korijena u slučaju visokih ili niskih temperatura. Ako goveda popasu tratinu pašnjaka do same razine tla, a prije njezinog odlaska u fazu mirovanja, tada će korijenova masa imati puno manje rezervnih hranjiva za obnovu i rast tratine. Također, ako ostanu neke vlati travnih vrsta nakon prethodnog napasivanja, rezerve hranjiva u korijenu neće nestati, nego će omogućiti biljkama ponovnu regeneraciju bez prekida rasta. Ruechel (2006) navodi primjer kako goveda ne bi trebala napasivati tratinu pašnjaka ako nije bar 7 cm visine. Trave tada u sebi imaju odličan udio hranjivih tvari, a korjenova masa dovoljno snage za brzi oporavak i porast biljaka.



Slika 1. Hranjiva vrijednost trave (Ruechel, 2006)

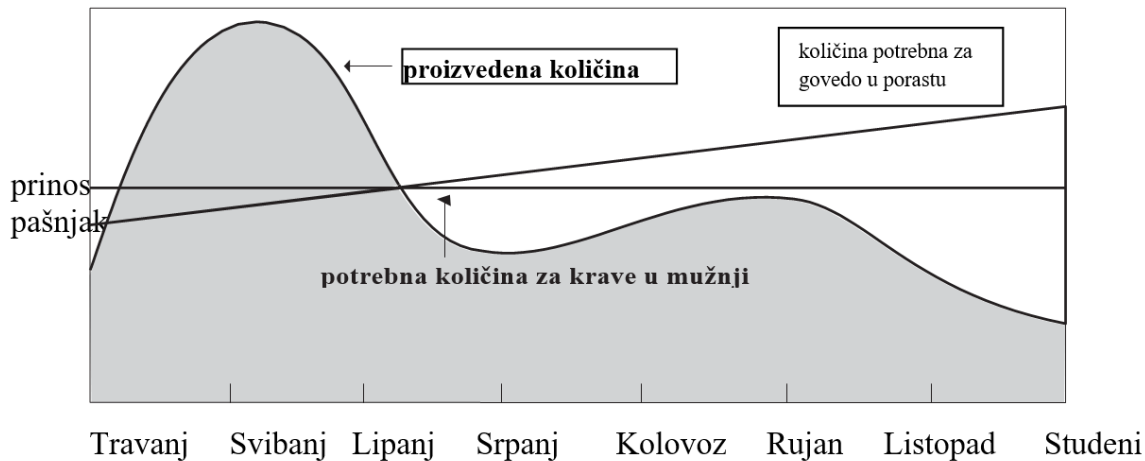
Pored usklađene migracije stada, također je važno pratiti i stanje tla na kojem raste biljni pokrov. Gornji sloj tla trebao bi biti sastavljen od 25 % vode i 25 % zraka. Ako bi tlo postalo zbijeno i kompaktno, tada zrak i voda ne bi mogli dospjeti do mikroorganizama u tlu koja bi omogućila njihovu aktivnost. U tom slučaju korijenova masa tratine pašnjaka ne bi bila u mogućnosti se proširiti i imati pristup hranjivim tvarima (Ruechel, 2006). Zato je uzgoj goveda u rotacijskom sustavu napasivanja najbolji način za stvaranje i održavanje zdravih i dobro prozračenih pašnjaka. Ova značajka se pokazala kao ključni čimbenik za opstanak velike raznolikosti biljaka, životinja i mikroorganizama koji održavaju vrijedan travni pokrov, te na taj način određuju i profitabilnost proizvodnje. Različite životinjske vrste preferiraju i različite biljne vrste u sustavu napasivanja. Uz to, životinje pasu različite krmne vrste na različite načine. Njihove hranidbene sklonosti prikazane su u Tablici 1.

Tablica 1. Hranidbeni odabir stoke ovisno o biljnom pokrovu (u %) (Undersander i sur., 2002)

Biljni pokrov	Krave i konji	Jelen obični	Ovce	Koze
Trave i leguminoze	90	70	60	20
Korovske vrste	4	20	30	20
Drvenaste biljne vrste	6	10	10	60

Cjelogodišnja strategija napasivanjem jedna je od najvažnijih stavki koja utvrđuje uspjeh i financijsku održivost cijele

produkcije na pašnjacima. Potrebna je pažljiva priprema pašnjaka tijekom vegetacijske sezone, pažljivo osmišljen plan zimskog napasivanja, strog nadzor kondicijskog stanja životinja, mjerenje sadržaja hranjiva i minerala travnih vrsta pogotovo u vrijeme vegetacije, te pomno osmišljen program za nadoknadu nutritivnih vrijednosti u slučaju nekih nedostataka samog napasivanja govedima na pašnjaku.



Grafikon 1. Primjer proizvodnje pašnjaka i životinjskih potreba tijekom napasivanja (Undersander i sur., 2002)

Važnost napajanja goveda s vodom

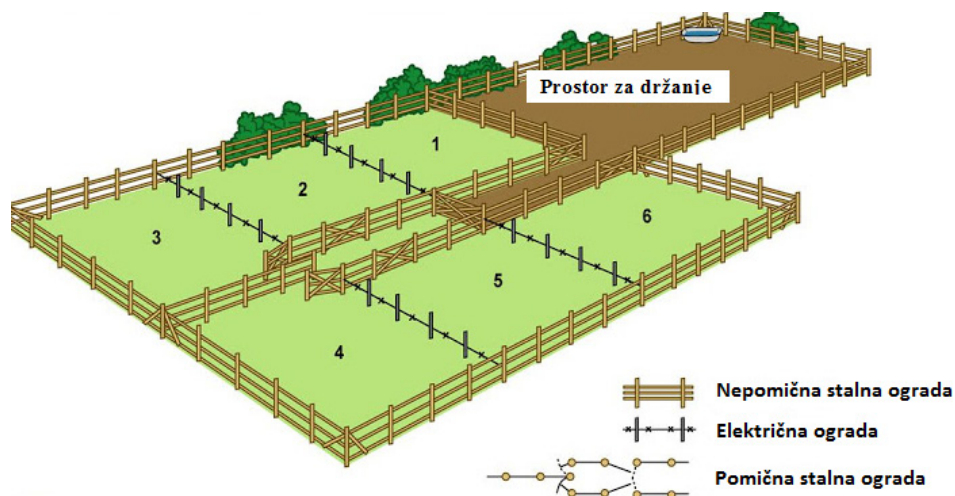
Voda je neophodna za probavu hrane i ispiranje toksina iz tijela nastalih tijekom probave, ali je značajna i za dobar prirast grla stoke. Kod goveda se tjelesna masa i dobro zdravlje vežu za količinu i kvalitetu vode koju popiju. Izvor vode za stoku može biti prirodni (rijeka, jezero, ribnjak) ili mobilni poput cisterni ili vodovodne mreže. Voda mora biti stalno dostupna životinjama jer se tada ne stvara stres kod životinja. Ako je voda iz prirodnog izvora, tada je potrebno napraviti ograde i pristupne točke. Najbolje je rotacijsko napasivanje osmisliti tako da bude pristupačna pri svakoj podjeli na pašnjaku. Količina vode koja je potrebna govedu (u litrama dnevno) dramatično se mijenja tijekom sezone jer ovisi o kategoriji životinje, postotku vlage u hrani, laktaciji, ljetnim vrućinama i pristupu hladovine. Količina vode koju stoka pije i sposobnost vode da regulira njihov probavni i imunološki sustav također ovisi o kvaliteti vode i okusu. Pored zagađivača kao što su paraziti, pesticidi, hrđa i kemikalije, postoji niz dodatnih onečišćenja koji mogu biti prisutni u vodoopskrbi.

Tablica 2. Dnevna potrošnja vode po životinji (Ruechel 2006)

Kategorija goveda	Prosječne dnevne potrebe vode u litrama
Krave u suhostaju i junice	22-56
Krave u laktaciji	41-68
Bikovi	26-72
Junice u porastu	
180 kg	13-28
270 kg	19-55
360 kg	22-66
Odrasla junad	
450 kg	32-77
540 kg	36-87
Mliječne krave	38-95

Tehnika postavljanja električne ograde

Električna ograda (električni pastir) je jeftina, učinkovita, dugotrajna, sigurnija i povoljnija opcija za životinje. Nije zamišljena kao fizička zapreka koja ograničava kretanje goveda, već kao psihološka zapreka jer joj govedo neće prići zbog straha. Prijenosna električna ograda omogućuje daljnje podjele pašnjaka po želji. Takvom ogradom može se oponašati prirodna migracija goveda, promjenom veličine i broja pregona tijekom godišnjeg doba kako bi se postiglo učinkovito rotacijsko napasivanje. Potrebni materijali za ograđivanje pašnjaka su žice (vodiči), uređaj, izolatori, šipke za uzemljenje, ograde (pokretne ili nepokretne, ovisno po potrebi). Trajne (nepokretne) ograde obrubljuju pašnjak, te unutar trajnih nalaze se privremene (pokretne) ograde koje se pomiču po potrebi.



Slika 2. Izgled rotacijskog pašnjaka s postavljenom električnom ogradom (Bozeman, 2018)

Potrebno je određeno vrijeme dok se goveda nauče na rotacijsko napasivanje. Kada svladaju ovu vještinu, tada bez teškoća prolaze kroz pomična vrata i ograde prema svježoj travi na pašnjaku.

Zaključak

Rotacijsko napasivanje u sustavu krava-tele planirano je upravljanje napasivanjem. Strategiju napasivanjem treba raditi za čitavu godinu jer je ona bitna za dobar uspjeh i financijsku održivost proizvodnje tovnih pasmina goveda na pašnjacima. Pri napasivanju pašnjaka tratina se brzo regenerira, što na godišnjoj razini omogućuje stvaranje dostatne količine hranidbenih tvari za goveda na pašnjacima. Također, prozračnost i plodnost tla su izuzetno dobri. Potrebno je voditi brigu o sastavu tratine pašnjaka, kako tijekom intenzivne vegetacije, tako isto i tijekom zime. Dostupnost i kvaliteta vode za goveda na pašnjacima je posebno važna. Zbog jednostavnije organizacije pregona, potrebno je napraviti zajedničku točku pristupa izvoru vode za goveda na što većem broju pregona na pašnjaku.

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Rotational grazing in cow-calf system

Abstract

Rotational grazing in the cow-calf system is a carefully planned movement of cattle on pasture throughout the year, which is one of the main goals of quality pasture management. This naturally imitated migration ensures that cattle are fed daily with fresh grass biomass. The pasture is usually surrounded by an electric fence. For winter grazing it is important to ensure the diversity of species from the grass family, and choose the most suitable. Meat and combination breeds are used in the cow-calf system. Economic analysis shows the cost-effectiveness of such farming as the costs of mechanization, feeding, treatment and human labor are reduced. By rotating cattle, grass species regenerate faster, soil is enriched with humus, soil compaction is avoided, and animals are healthier.

Keywords: rotational grazing, cow-calf system, pasture, grass, water, electric fences

Povezanost polimorfizma *DGAT1* gena sa mesnim odlikama janjadi

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Sažetak

Skroman je broj istraživanja na temu utjecaja genotipa na mesne odlike janjadi. Cilj ovog rada je prikazati rezultate dosadašnjih istraživanja pojavnosti frekvencija alelnih varijanti i genotipova *DGAT1* gena te povezanosti polimorfizama *DGAT1-C/T* s nekim mesnim odlikama janjadi (završna tjelesna masa, sastav trupa, kakvoća mesa). Dosadašnja istraživanja potvrdila su povezanost između *DGAT1* gena i nekih mesnih odlika janjadi. Postoji povezanost *C* alelne varijante *DGAT1* gena s većom tjelesnom masom i masom trupa janjadi, dok se alelna varijanta *T* povezuje s većim sadržajem intramuskularne masti i većim stupnjem mramoriranosti mesa.

Ključne riječi: janjad, *DGAT1* gen, polimorfizam, mesne odlike

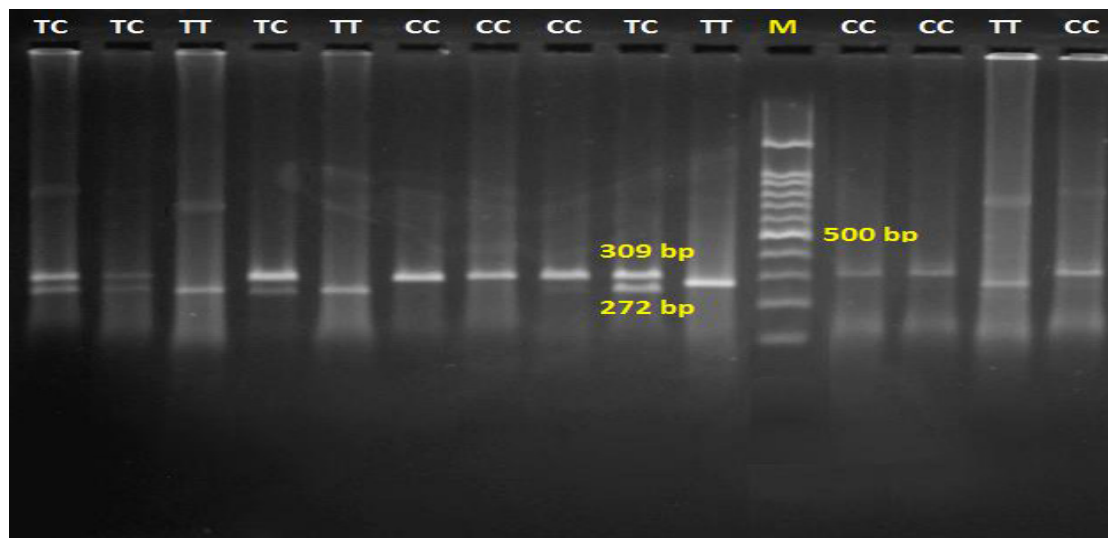
Uvod

Od početaka razvoja civilizacije ovce su bile jedna od najvrjednijih vrsta životinja koje je udomaćio čovjek, a uzgoj ovaca važna privredna grana koja se proširila na sve kontinente. Zbog naglašenog svojstva adaptabilnosti na različite klimatske i geografske uvjete uzgajaju se na područjima na kojim je smanjena mogućnost uzgoja drugih domaćih životinja. Među domaćim životinjama odlikuje ih najsvestranija korisnost i to kroz proizvodnju mesa, mlijeka, vune, kože i stajskog gnoja.

Prema podacima Jedinственог регистра ovaca i koza, u Republici Hrvatskoj se uzgaja oko 580.000 rasplodnih ovaca (MP, 2019). Brojno stanje uzgojno valjanih ovaca bilježi pozitivan trend zadnjih pet godina (MP, 2019). U posljednjih dvadesetak godina u zemljama Europske unije značaj ovčjeg, odnosno janječeg mesa kao glavnog proizvoda ovčarske proizvodnje, primjetno je porastao. U istom je razdoblju zamijećeno jače zanimanje potrošača za kvalitetom janječeg mesa (Knapik i sur., 2017). Ovčje i janjeće meso posjeduje jedinstvene kulinarske vrijednosti, poput mekoće, okusa i velike hranjive vrijednosti pa je stoga atraktivno i uživa veliku potražnju potrošača na mnogim stranim tržištima (Knapik i sur., 2017). Kvaliteta ovčjeg mesa ovisi o nizu čimbenika genetske i okolišne naravi kao što su npr. boja mesa, sposobnost zadržavanja vode, tvrdoća mesa i otpornost prema oksidacijskim promjenama (Rosenvold i Andersen, 2003). Svojstva koja određuju kvalitetu mesa su složena i kontrolira ih nepoznat broj gena tj. lokusa za kvantitativna svojstva (QTL) (Andersson i Georges, 2004). Korištenjem tradicionalnih uzgojnih programa teško je postići poboljšanje kvalitete mesa jer je stupanj nasljednosti (heritabilitet) tih svojstava nizak. Stoga je zadnjih desetaka godina uloženo mnogo rada na utvrđivanju povezanosti potencijalnih gena s kvalitetom mesa domaćih životinja. Već 80tih godina prošlog stoljeća bilo je poznato da se neki lokusi na genomu mogu koristiti kao markeri za susjedne lokuse koji utječu na kvantitativna svojstva (Tier i sur., 2007). Tako se tradicionalne metode selekcije nadopunjavaju genetskim analizama koristeći genetske markere u cilju dobivanja što točnije procjene proizvodnih osobina. Do sada, uporaba genetskih testova na molekularnom nivou u uzgojnim programima nije dovela do značajnijeg poboljšanja ekonomski važnih pokazatelja u proizvodnji janječeg mesa. Međutim, identifikacija i izravni odabir odgovarajućih gena može biti obećavajući, budući da pokazatelje proizvodnje janjadi karakterizira niska nasljednost (Koopaei i Koshkoiyeh, 2011). Posljednjih godina, otkriveno je nekoliko gena i sekvenca koji utječu na kvalitetu mesa domaćih životinja, posebice goveđeg i svinjskog mesa. Također, sve više je istraživanja koja proučavaju utjaj određenih gena na kvalitetu janječeg mesa. Stoga je cilj ovoga rada dati pregled dosadašnjih istraživanja povezanih s utjecajem *DGAT1* gena na neke odlike mesa janjadi.

Diacilglicerol O-aciltransferaza 1 gen

Diacilglicerol O-aciltransferaza 1 gen (*DGAT1*) kodira enzim diacilglicerol-0-aciltransferazu koji je ključan za metabolizam lipida. Igra ključnu ulogu u sintezi triglicerida u adipocitima (Winter i sur. 2002, Mahrous i sur., 2015) te djeluje kao modulator u taloženju masti (Cui i sur., 2011; Patel i sur., 2009, Mahrous i sur., 2015). Neka dosadašnja istraživanja pokazala su povezanost *DGAT1* gena s kvalitetom mesa različitih vrsta domaćih životinja (Giusti i sur., 2013; Borges i sur., 2014; Renaville i sur., 2015), uključujući i ovce (Xu i sur., 2009; Mohammadi i sur., 2013; Noshahr i Rafat, 2014; Nanekarani i sur., 2016; Armstrong i sur., 2017; Bayram i sur. 2019). Ovčji *DGAT1* gen veličine 8676 bp, lokaliziran je na kromosomu OAR 9. Za određivanje genotipova koristi se reakcijska smjesa s *AluI* restrikcijskom endonukleazom (Mohammadi i sur., 2013). Rezultati elektroforeze (slika 1.) pokazali su da SNP ima dvije alelne varijante *C* (309 bp) i *T* (272 i 27 bp) i tri genotipa *TT* (272 i 37 bp), *CT* (309, 272 i 37 bp) i *CC* (309 bp).



Slika 1. Uzorak elektroforeze polimorfizama unutar ovčjeg *DGAT1* gena: M – marker veličine 100 bp CC - 309 bp; CT - 309, 272, 37 bp; TT - 272, 37 bp (Nanekarani i sur, 2016).

U većini provedenih istraživanja u različitim populacijama ovaca dominira alelna varijanta *C*. Tako Mahrous i sur. (2015) navode veću frekvenciju alelna varijante *C* (0,75) u odnosu na frekvenciju alelna varijante *T* (0,25) *DGAT1* gena. Navedeni autori su u populaciji utvrdili samo *CC* i *CT* genotipove (0,50; 0,50). Bayram i sur. (2019) proveli su slično istraživanje na Akkaraman pasmini u kojem su frekvencije genotipova *DGAT1* gena iznosile: *CC* (0,91) i *CT* (0,09), dok *TT* genotip nije utvrđen. Iz navedenog je vidljivo da je frekvencija alelna varijante *T* vrlo niska (0,04) u odnosu na *C* varijantu (0,96). Suprotno, u ranijem istraživanju kineskih izvornih pasmina janjadi Xu i sur. (2009) utvrditi su veće frekvencije *TT* genotipa *DGAT1* gena (0,57) u odnosu na *CC* genotip (0,34), te sukladno veći udio frekvencije alelna varijante *T* u odnosu na frekvenciju alelna varijante *C* (0,62: 0,38).

Utjecaj *DGAT1* gena na intenzitet rasta i neke klaoničke pokazatelje janjadi

Rast je kvantitativna karakteristika koju kontroliraju mnogi geni. Mohammadi i sur. (2013) utvrdili su značajan utjecaj *DGAT1* gena na intenzitet rasta janjadi. Jedinke *CT* genotipa imale su najveću prosječnu masu trupa, a najmanji randman. Isti autori navode da su u trupovima janjadi *CC* genotipa utvrđene najveće vrijednosti za debljinu leđne potkožne masnoće i vrijednosti za randman. Slično istraživanje proveli su i Noshahr i Rafat (2014) koji su potvrdili da je alelna varijanta *C* povezana s većom masom toplog trupa i boljim randmanom. U njihovom istraživanju najveću masu toplog trupa imale su homozigotne jedinke *CC* genotipa. Armstrong i sur. (2017) slažu se s tvrdnjama Mohammadija i sur. (2013) i Noshahra i Rafata (2014). Oni u svome radu navode kako janjad *CC* genotipa Texel pasmine ima veću masu toplog trupa u odnosu na janjad *TT* genotipa. Navedeni rezultati nisu u suglasju s rezultatima istraživanja Xua i sur. (2009) koji su utvrdili da je najveća masa toplog trupa utvrđena u janjadi *TT* genotipa. Bayram i sur. (2019) su istraživali povezanost *DGAT1* gena s tjelesnom masom janjadi turske

Akkaraman pasmine pri čemu je janjad *CT* genotipa imala veću porodnu masu, dok je janjad *CC* genotipa imala veću tjelesnu masu u dobi od 30, 60 i 90 dana starosti. Međutim, razlika u tjelesnoj masi ova dva genotipa nije bila značajna.

Utjecaj DGAT1 gena na neke odlike trupa i mesa janjadi

Dosadašnja istraživanja pokazala su da je *DGAT1* gen povezan s nekim od čimbenika koji utječu na kvalitetu mesa različitih vrsta domaćih životinja (Giusti i sur., 2013; Borges i sur., 2014; Renaville i sur., 2015). Malo je istraživanja koja povezuju ovaj gen s kvalitetom janječeg mesa. Xu i sur. (2009) utvrdili su značajnu povezanost između genotipova ovog gena i odlika trupa i mesa janjadi. Isti autori navode da mutacija u egzonu 17 (Ala487Ala; *T>C*) *DGAT1* gena utječe na važne odlike mesa janjadi, odnosno da postoji povezanost alelne varijante *T* sa stupnjem mramoriranosti mesa i sadržajem intramuskularne masti. Naime, janjad *TT* genotipa ima značajno veći stupanj mramoriranosti mesa u odnosu na *CT* i *CC* genotipove. Također, janjad *TT DGAT1* genotipa ima značajno veći sadržaj intramuskularne masti, ali i nižu silu presijecanja i manji kapaoni gubitak u odnosu na janjad *CT* i *TT* genotipova. Pored toga, alel *T* imao je pozitivan učinak na mekoću mesa. Isto su potvrdili i Noshahr i Rafat (2014) koji navode da je *DGAT1* gen jedan od gena-kandidata za poboljšanje odlika trupa i mesa janjadi.

Boja je važno svojstvo mesa koje potrošač prilikom kupovine mesa može sam vizualnim putem procijeniti, odnosno, boja mesa ostavlja prvi dojam na potrošača. Xu i sur. (2009) navode kako ne postoji značajna povezanost *T* alelne varijante s bojom mesa (L vrijednost), pH vrijednosti, debljinom potkožne masnoće (mjerenom na 7. rebrenom isječku), površinom MLD-a i udjelom mesa u trupu. Mohammadi i sur. (2013) su u svom istraživanju ustanovili kako je alelna varijanta *C* povezana s debljinom potkožne masnoće. S navedenim se slažu i Armstrong i sur. (2017) koji su utvrdili povezanost *C* alelne varijante *DGAT1* gena s nekim odlikama trupa janjadi (debljina potkožne masnoće, površina MLD-a i masa plečke).

Zaključak

Razvojem molekularne genetike omogućena je izravna analiza genoma ovaca kao i identifikacija određenih gena povezanih s kvalitetom janječeg mesa. U posljednje vrijeme sve više istraživanja vezano je za utjecaj određenih gena na kvalitetu janječeg mesa. Dosadašnja istraživanja pokazala su povezanost *DGAT1* gena s nekim mesnim odlikama janjadi. Utvrđena je povezanost *C* alelne varijante s većom tjelesnom masom, masom trupa janjadi i boljim randmanom dok se alelna varijanta *T* povezuje s većim sadržajem intramuskularne masti i većim stupnjem mramoriranosti mesa, te pozitivnim učinkom na mekoću mesa. Janjad *CC* genotipa ima značajno veću završnu masu, veću masu toplog trupa, bolji randman i veću vrijednost za debljinu potkožne masnoće, ali i manji sadržaj intramuskularne masti u odnosu na janjad *TT* genotipa.

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Polymorphism of *DGAT1* gene on some meat characteristics of lambs

Abstract

There is a modest amount of studies considering the impact of certain genotypes on the meat characteristics of lambs. The aim of this paper was to present the results of studies to date frequencies of allelic variants and genotypes of *DGAT1* gene and to establish the association between *DGAT1*-C/T polymorphisms with some meat characteristics of lambs (final body weight, carcass composition, meat quality). Previous studies confirmed that the *DGAT1* gene is associated with some meat characteristics of lambs. There is an association of the C allelic variant of the *DGAT1* gene with higher final body weight and carcass weight, whereas the allelic variant T is associated with higher intramuscular fat content and higher degree of marbling.

Keywords: lambs, *DGAT1* gene, polymorphism, meat characteristics

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Utjecaj djelomične defolijacije na prinos i sastav grožđa sorte vinove loze ‘Belina starohrvatska’ (*Vitis vinifera* L.)

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Sažetak

‘Belina starohrvatska’ je sorta koja se projektom revitalizacije autohtonih sorata vraća u proizvodne nasade Hrvatskog zagorja. Kako bi se omogućio komercijalni uzgoj potrebno je definirati ampelotehničke zahvate koji će osigurati kvalitetu grožđa. Cilj ovog istraživanja je bio utvrditi tijekom dvije godine utjecaj djelomične defolijacije na prinos i kvalitetu grožđa ove sorte. Rana defolijacija je provedena u dva termina: neposredno prije cvatnje i neposredno poslije cvatnje. Tretmani defolijacije u prvoj godini nisu značajno utjecali na kemijski sastav grožđa dok je tretman defolijacije prije cvatnje rezultirao nešto većim prinosom. Međutim kod kontrolne varijante došlo je do potpune zaraze grožđa sivom plijesni. U drugoj godini ponovljena je usporedba dvije varijante defolijacije, sa istim rezultatima, te je uz njih provedena i redukcija cvatova, koja također nije značajno utjecala na kemijske parametre grožđa.

Ključne riječi: autohtone sorte, Hrvatsko zagorje, defolijacija, kvaliteta grožđa

Uvod

Područje Hrvatskog zagorja spada u vinogradarsku podregiju Zagorje-Međimurje, koje odlikuje brežuljkast teren različitih nagiba i ekspozicija. Na ovom području u povijesti se uzgajao velik broj sorata i to najčešće u mješovitim nasadima, unutar kojih su se često bijele sorte skupno nazivale „belinama“ (Maletić i sur., 2015.). Projektom revitalizacije autohtonih sorata Hrvatskog zagorja sustavno se počelo s prikupljanjem različitih sorata koje nose naziv „beline“, njihovim opisivanjem i genetičkom identifikacijom. Unutar ove grupe sorata najviše se istaknula ‘Belina starohrvatska’, poznatija pod imenom ‘Gouais blanc’, sorta koja je imala velik utjecaj u razvoju europskog sortimenta. Naime, u istraživanju Bowers i sur. (1999.) otkriveno je kako je ona zajedno s ‘Pinot crnim’ roditelj 16 francuskih sorata, među kojima je i ‘Chardonnay’. Ukupno, ‘Belina starohrvatska’ je sudjelovala u roditeljstvu kod više od 70 sorata (Boursiquot i sur., 2004.), a važna je i za kreiranje hrvatskog sortimenta, gdje je evidentirana kao roditelj šest sorata (Žulj Mihaljević, 2017.). Iako je ‘Belina starohrvatska’ krajem 20. stoljeća gotovo nestala iz proizvodnih nasada, zahvaljujući provedenim mjerama revitalizacije, danas dolazi do porasta broja trsova u uzgoju (Maletić i sur., 2015.).

‘Belina starohrvatska’ je sorta koju odlikuje visoka bujnost i visoki prinos, a zbog svoje relativno tanke kožice i zbijenog grozda, u vlažnim uvjetima tijekom dozrijevanja osjetljiva je na pojavu sive truleži (Maletić i sur., 2015.). Kako bi se omogućio komercijalni uzgoj ove sorte, potrebno je definirati ampelotehničke zahvate. Defolijacija je zahvat koji se provodi radi boljeg prozračivanja i osvjetljenosti grozdova, kako bi se smanjila podložnost gljivičnim bolestima i poboljšalo dozrijevanje grožđa. Iako se većinom provodi prije i za šare, rana defolijacija prije cvatnje mogla bi predstavljati alternativu uobičajenom provođenju defolijacije (Lemut i sur., 2015.). U pokusu na sorti ‘Pinot crni’ u Švicarskoj, rana defolijacija provedena prije cvatnje pozitivno je utjecala na smanjenje štete od sive plijesni, povećanje antocijana, smanjenje ukupnih kiselina, te smanjenje prinosa (Verdenal i sur., 2017.). Slični rezultati dobiveni su na sortama ‘Sangiovese’ i ‘Trebiano’, gdje je rana defolijacija prije cvatnje utjecala na smanjenje veličine

bobice, broja bobica po grozdu, težine grozda i zbijenosti. Također, poboljšao se sastav mošta, jer su sorte nakupile više šećera, antocijana i ostalih polifenolnih spojeva (Poni i sur., 2006.). Utjecaj rane defolijacije na bijelu sortu 'Semillon' pokazao se u smanjenom broju bobica po grozdu i zbijenosti, povećao se šećer u moštu, dok ukupna kiselost je ostala nepromijenjena. Nadalje, defolijacija je utjecala i na povećanje koncentracija hlapljivih spojeva, naročito terpena i norisoprenoida, čime su se poboljšala senzorna svojstva vina (Alessandrini i sur., 2018.). Cilj ovoga pokusa je utvrditi utjecaj djelomične defolijacije, provedene u dva termina, na prinos i sastav grožđa sorte 'Belina starohrvatska'.

Materijal i metode

Istraživanje je provedeno na lokalitetu Komor Začretski, Hrvatsko zagorje, u dvije uzastopne godine (2017. i 2018.). Utjecaj defolijacije promatrao se na sorti 'Belina starohrvatska' u tri tretmana pokusa sa tri ponavljanja na pet trsova: kontrola (K), defolijacija prije cvatnje (V1) i defolijacija poslije cvatnje (V2). U 2017. godini defolijacije se provela prije cvatnje 25.5., poslije cvatnje 6.6., dok je berba bila 22.9. U 2018. godini kontrola nije ostavljena, dok se defolijacija prije cvatnje provela 22.5., poslije cvatnje 6.6., a berba je bila 14.9. Zbog visokih prinosa u prethodnoj godini, u 2018. godini pristupilo se uklanjanju dijela cvatova (R) prije cvatnje s ciljem povećanja kvalitete grožđa. Defolijacija je izvršena u zoni grožđa, a redukcijom je ostavljen jedan cvat po mladici. Tijekom berbe na svih pet trsova iz ponavljanja izmjeren je prinos po trsu i broj grozdova po trsu. Za osnovnu kemijsku analizu mošta uzeti su uzorci od pet grozdova u tri ponavljanja po tretmanu pokusa. U 2017. godini provedeno je određivanje prosječnog broja bobica u grozdu i prosječne mase bobica. Osnovna kemijska analiza obuhvaćala je mjerenje šećera, ukupnu kiselost, organske kiseline i pH. Šećer je određen refraktometrom, ukupna kiselost titracijskom metodom, sukladno OIV-u (OIV, 2019.), dok su organske kiseline određene tehnikom tekućinske kromatografije (HPLC). Sve analize provodile su se u laboratoriju Zavoda za vinogradarstvo i vinarstvo Agronomskog fakulteta. Statistička obrada podataka provedena je korištenjem računalnog programa SAS 9.4.

Rezultati i rasprava

Rezultati osnovnih gospodarskih karakteristika ovisno o varijanti pokusa defolijacije prikazani su u Tablici 1. Defolijacija je pozitivno utjecala na nakupljanje šećera, te postoji značajna razlika između kontrole i tretmana, dok među tretmanima nema razlike. Ukupna kiselost i pH, kao i organske kiseline (tablica 2.) svih varijanta pokusa se ne razlikuju značajno. Što se tiče prinosa, najveći prinos ima varijanta V1 sa 5,94 kg/trsu, dok najmanji prinos ima V2 sa 3,68 kg/trsu. Ovi rezultati su obrnuti od rezultata istraživanja provedenog na sorti 'Sauvignon bijeli', gdje je prinos smanjen 10% kada se defolijacija provodila prije cvatnje, dok defolijacija nakon cvatnje nije imala utjecaja na prinos (Sivilotti i sur., 2017.). Iz Tablice 1. je vidljivo kako najveći broj bobica u grozdu ima kontrola, a zatim slijede defolijacija prije cvatnje i poslije cvatnje. Međutim, što se tiče mase bobica, dolazi do obrnutog slučaja, najveću masu bobice ima defolijacija poslije cvatnje, a slijede defolijacija prije cvatnje i kontrola. Bitno je istaknuti kako su u 2017. godini u vrijeme dozrijevanja grožđa nastupile izuzetno obilne i dugotrajne kiše, te je došlo do propadanja grozdova kao posljedica značajnih šteta od sive truleži. Kod kontrolnih trsova šteta od sive plijesni je bila 100% najveća, dok je najbolje zdravstveno stanje grožđa bilo kod varijanata s provedenom defolijacijom tj. ispod 10% zaraženog grožđa (rezultati nisu prikazani). Stoga, s obzirom na nepovoljne vremenske prilike, teško je utvrditi utjecaj defolijacije na prinos. Međutim, defolijacija je vrlo povoljno djelovala na zdravstveno stanje grožđa. To potvrđuje istraživanje Mosetti i sur. (2016.), gdje je defolijacija pomogla u smanjivanju šteta od sive plijesni (*Botrytis cinerea*) tijekom dozrijevanja 'Sauvignona bijelog' u kišnoj 2010. godini.

Utjecaj djelomične defolijacije na prinos i sastav grožđa sorte vinove loze 'Belina starohrvatska' (Vitis vinifera L.)

Tablica 1. Analiza varijance i usporedba srednjih vrijednosti za mjerena svojstva prinosa i kemijskog sastava mošta sorte 'Belina starohrvatska' ovisno o varijanti pokusa u prvoj godini istraživanja (2017.)

Varijanta pokusa	prinos/trsu (kg)	broj grozdova/trsu	sadržaj šećera (Oe)	sadržaj kiselina (g/L)	pH	masa bobice (g)	broj bobica u grozdu
K	4,25ab	13,10b	52,89b	9,00	2,86	2,49c	129,8a
V1	5,94b	17,42ab	64,33a	8,9	2,93	2,97b	114,6b
V2	3,68a	12,25b	64,33a	8,8	2,94	3,03a	108,4c
ANOVA	**	*	**	n.s.	n.s.	**	**

*K-kontrola, V1-defolijacija prije cvatnje, V2-defolijacija poslije cvatnje; ANOVA: n.s. – nije signifikantno, * - $p < 0,05$, ** - $p < 0,01$); usporedbu srednjih vrijednosti provedena je korištenjem Duncan's multiple range testa a srednje vrijednosti za pojedino svojstvo označene različitim slovima su statistički značajno različite uz $p < 0,05$*

Tablica 2. Analiza varijance sadržaja organskih kiselina kod sorte 'Belina starohrvatska' u prvoj godini istraživanja

Varijanta pokusa	Limunska kiselina	Vinska kiselina	Jabučna kiselina
K	0,21	6,64	1,85
V1	0,16	6,46	1,83
V2	0,15	6,06	1,62
ANOVA	n.s	n.s	n.s

*K-kontrola, V1-defolijacija prije cvatnje, V2-defolijacija poslije cvatnje; ANOVA: n.s. – nije signifikantno, * - $p < 0,05$, ** - $p < 0,05$)*

U 2018. godini istraživanje je usmjereno na tretmane defolijacije, a kako bi se povećala kvaliteta grožđa dodatno je provedena redukcija broja cvatova prije cvatnje. U tablici 3. prikazani su rezultati postavljenog pokusa. Iz rezultata je vidljivo kako među tretmanima pokusa nema značajnih razlika u kvaliteti grožđa. Prinos je, očekivano, bio manji kod varijante redukcije prinosa. Postoje određene razlike u prosječnim vrijednostima organskih kiselina, međutim statistički nisu značajne. Razlika je vidljiva u sadržaju vinske kiseline, koja je viša kod defolijacije napravljene prije cvatnje.

U 2018. godini, zbog povoljnih vremenskih uvjeta, nije bilo značajnih problema sa sivom plijesni kod svih tretmana pokusa. Redukcija broja cvatova prije cvatnje nije pokazala značajne razlike u odnosu na dva tretmana defolijacije što se tiče kemijskih parametara prikazanih u tablici 3. Slični rezultati dobiveni su u istraživanju na sorti 'Veltlinac zeleni', gdje se provodila rana defolijacija, provedena u trenutku otvaranja cvjetova, i redukcija prinosa, ostavljan jedan grozd po mladici (Smith i Centinari, 2019.). Ni rana defolijacija, ni redukcija prinosa nisu značajno utjecali na kemijski sastav grožđa. Nadalje, Smith i Centinari (2019.) uočili su manju pojavu i jačinu napada sive plijesni tijekom vegetacije. Stoga, autori preporučuju u područjima uzgoja sa visokom vlagom zraka i učestalim padalinama tijekom faze dozrijevanja grožđa provođenje zahvata defolijacije radi boljeg održavanja zdravstvenog stanja grožđa.

Tablica 3. Analiza varijance i usporedba srednjih vrijednosti za mjerena svojstva prinosa i kemijskog sastava mošta sorte 'Belina starohrvatska' ovisno o varijanti pokusa u drugoj godini istraživanja (2018.)

Varijanta pokusa	prinos/trsu (kg)	broj grozdova/trsu	sadržaj šećera (Oe)	sadržaj kiselina (g/L)	pH	vinska kiselina (g/L)	jabučna kiselina (g/L)
V1	3,00a	11,00a	78,60	9,45	3,11	6,2	2,2
V2	2,60a	11,80a	79,60	8,91	3,12	5,1	2,4
R	2,10b	8,30b	78,50	9,15	3,13	5,7	2,3
ANOVA	**	**	n.s.	n.s.	n.s.	n.s.	n.s.

*K-kontrola, V1-defolijacija prije cvatnje, V2-defolijacija poslije cvatnje; ANOVA: n.s. – nije signifikantno, * - $p < 0,05$, ** - $p < 0,01$); usporedbu srednjih vrijednosti provedena je korištenjem Duncan's multiple range testa a srednje vrijednosti za pojedino svojstvo označene različitim slovima su statistički značajno različite uz $p < 0,05$*

Zaključak

Tretmani defolijacije provedeni prije i poslije cvatnje nisu značajno utjecali na najvažnije kemijske parametre (sadržaj šećera i kiselina, pH) grožđa 'Belina starohrvatska' tijekom dvije godine pokusa. U 2017. godini tijekom dozrijevanja grožđa zbog nepogodnih vremenskih prilika došlo je do jakog napada sive truleži i propadanja grozdova kao posljedice napada, što je otežalo utvrđivanje utjecaja tretmana defolijacije na prinos. Iako se zdravstveni status grožđa nije promatrao u ovom istraživanju, primijećeno je značajno bolje zdravstveno stanje grožđa kod tretmana s provedenom defolijacijom u odnosu na kontrolu gdje nije provedena defolijacija. U 2018. godini istraživanje je usmjereno na dva tretmana defolijacije, a radi povećane kvalitete grožđa dodatno je provedeno uklanjanje, redukcija, dijela cvatova. Između tretmana defolijacije i tretmana redukcije nije bilo značajnih razlika u kemijskom sastavu grožđa. Iako su potrebna dodatna istraživanja, djelomična defolijacija se može preporučiti kao zahvat koji ne utječe na važne kemijske parametre, ali može pozitivno utjecati na zdravstveno stanje grožđa, naročito u kontinentalnoj klimi sa visokom količinom padalina tijekom dozrijevanja grožđa.

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Influence of partial defoliation on yield and grape content of grapevine cultivar 'Belina starohrvatska' (*Vitis vinifera* L.)

Abstract

'Belina starohrvatska' is a grapevine variety that is coming back to the vineyards of Hrvatsko zagorje due to the program of revitalization of indigenous varieties. To enable the commercial production, it is necessary to define the varietal canopy management practices to achieve better grape quality. The aim of this research was to determine in two years the impact of partial defoliation on yield and grape quality. Defoliation was conducted in two periods: before flowering and after flowering. Defoliation treatments had no significant effect on chemical parameters of grapes. The influence on yield was difficult to observe due to severe incidence of botrytis bunch rot. Along with defoliation treatments, reduction of flowers was conducted, which also had no significant effect on grape chemical parameters.

Keywords: indigenous varieties, Hrvatsko zagorje, defoliation, grape quality

Influence of different vinification techniques on stilbenes and total polyphenolic content in Teran wines

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Abstract

The aim of this work was to study the influence of different vinification techniques on stilbenes and total polyphenolic content in red wines made from cv. Teran (*Vitis vinifera* L.). Four different vinification techniques were carried out: control (7 days maceration), prolonged maceration (10 and 21 days), and thermovinification. Four stilbenes were determined by high-performance liquid chromatography (HPLC), and total polyphenols were determined by spectrophotometric methods. The results showed the treatment of extended maceration (21 days) had a significant effect on the increase of total (35.74 mg/L) and individual stilbenes (*trans*-piceid, *cis*-piceid, *trans*-piceatannol, *trans*-resveratrol), as well as total polyphenols (2702.88 mg GAE/L). Treatment of thermovinification resulted in significant increase of the total polyphenolic content by nearly 20% in relation to control.

Keywords: extended maceration, thermovinification, polyphenols, resveratrol, piceid

Introduction

Grapes contain a large amount of different polyphenolic compounds in skins, pulp, and seeds (Jackson, 2008). The polyphenolic composition of wines is affected by the grape variety, geographical location, soil, weather conditions and terroir components, and content depends on extraction from grapes during winemaking.

Polyphenols are directly related to wine color, astringency, bitterness and oxidative level. Their health beneficial features, particularly of stilbenes, include regulating cholesterol level, exhibiting antioxidant, anti-obesity, anti-diabetic, anti-atherosclerosis, and anti-inflammatory activities, and showing cardio and cancer-protective effects (Castillo-Sánchez et al., 2008; Gambacorta et al., 2011; Hossain et al., 2016).

Stilbenes are natural compounds occurring in many plant families including Vitaceae and *Vitis vinifera* L. cultivars (Bavaresco et al., 1999). The synthesis of these phytoalexins in grapevine is stimulated by stresses (Ribeiro de Lima et al., 1999). Resveratrol (3,5,4'-trihydroxystilbene) is considered to be the most important stilbene in wine. Red wines usually contain higher stilbene concentrations than white wines, which is because stilbenes are present in solid parts of the grapes, mainly in the grape skin, and are extracted during maceration (Atanacković et al., 2012). *Trans*-resveratrol is present in a concentration range of 0.2–13 mg/L in red and 0.1–0.8 mg/L in white wines. Concentrations of piceid (resveratrol-3-β-D-glucopyranoside) isomers were reported to be in a range of 0.3–9 and even up to 68 mg/L in red wines, and 0.1–2.2 mg/L in white wines (Moreno-Arribas and Polo, 2009). *Trans*-piceatannol (3,3',4,5'-tetrahydroxystilbene) was found in concentrations up to 6.4 mg/L in red and up to 0.2 mg/L in white wines (Moss, 2014).

Winemaking technique plays an important role in the extraction of polyphenols and their further stability in wines (Boulton, 2001). According to several authors, duration and temperature of skin maceration have the largest impact on wine polyphenols (Auw et al., 1996; Koyama et al., 2007).

Post-fermentation extended maceration usually lasts 4 days to 4 weeks and is achieved at temperatures between 15°C and 35°C. During this time water-soluble and alcohol-soluble compounds diffuse from the skin and seeds of the grapes into the fermented juice, resulting in a richer wine with greater capability of aging (Joscellyne, 2009).

Thermovinification is a pre-fermentative process of heating whole or crushed grapes to stimulate the rapid extraction of polyphenols from grape skins. The efficiency of extraction is temperature-dependent. The heat, within the range 60–80°C, destroys hypodermal cell membranes, releasing anthocyanins, and denatures polyphenol oxidase, preventing browning (Atanacković et al., 2012).

Teran (*Vitis vinifera* L.) is a traditional Croatian grapevine variety, mostly found on the Istrian peninsula. Teran wine is mainly produced by 5–10 days of maceration (Plavša et al., 2012). Its total polyphenolic content increases significantly with the length of maceration (Plavša et al., 2012), while no detailed investigation of its stilbene content has been carried out. The aim of this study was to investigate the influence of different maceration times and temperatures on stilbenes and total polyphenolic content of Teran wine.

Materials and methods

The grapes of cv. Teran (*Vitis vinifera* L.) were harvested at technological maturity (21.6° Brix, 8.3 g/L of total acidity expressed as tartaric acid, and pH 3.2) in 2018 in Western Istria (Croatia). The experiment was performed in the experimental cellar of the Institute of Agriculture and Tourism in Poreč. Grapes were destemmed and crushed and then homogeneously transferred into 110 L stainless steel vats with the addition of 30 g/hL of selected dry yeast Fermol Mediterranee (*Saccharomyces cerevisiae*, AEB). Four different vinification techniques were carried out. Three maceration periods were applied: 7 days (TM7, control treatment), 10 days (TM10), and 21 days (TM21) at the temperature of 24°C, and 10 days of maceration with heating on 45°C for the first 48 hours (thermovinification, TTV). The heating was followed by cooling down before yeast inoculation. Three replications of each vinification technique were done.

Standard physico-chemical parameters were determined according to the OIV methods. Average values with standard deviation were: relative density 0.9974±0.0008, alcoholic strength by volume (%) 12.78±0.50, reducing sugars (g/L) 3.13±0.13, pH 3.19±0.06, free SO₂ (mg/L) 8.33±2.60.

Total polyphenols were determined by the Folin-Ciocalteu colorimetric method (Singleton et al., 1999) simple, and require only common equipment and have produced a large body of comparable data. Under proper conditions, the assay is inclusive of monophenols and gives predictable reactions with the types of phenols found in nature. Because different phenols react to different degrees, expression of the results as a single number—such as milligrams per liter gallic acid equivalence—is necessarily arbitrary. Because the reaction is independent, quantitative, and predictable, analysis of a mixture of phenols can be recalculated in terms of any other standard. The assay measures all compounds readily oxidizable under the reaction conditions and its very inclusiveness allows certain substances to also react that are either not phenols or seldom thought of as phenols (e.g., proteins using a Cary 50 UV/Vis spectrophotometer (Varian Inc., Harbour City, CA, USA). The absorbance was measured against blank at the wavelength of 765 nm. Results were expressed as gallic acid equivalents in mg/L of wine (mg GAE/L).

Analysis of stilbenes was carried out by high-performance liquid chromatography (HPLC), according to the method proposed by Mark et al. (2005). The HPLC system used was an Agilent Infinity 1260 equipped with a G1311B quaternary pump, a G1329B auto sampler, a G1316A column oven, a G4212B DAD detector, and a G7121B FLD detector. Wine samples were filtered through 0.45 µm PTFE filters and 10 µL of wine were injected on a Zorbax SB-C18 column (4.6 x 250 mm, 5 µm). Two solvents were used for the separation: methanol-water-acetic acid (10:90:1, v/v) as solvent A and methanol-water-acetic acid (90:10:1, v/v) as solvent B. The flow rate was 1.5 mL/min. The solvent gradient system was as follows: 0.0–18.0 min from 0% to 40% B, 18.0–25.0 min from 40% to 100% B, and 25.0–27.0 min to 100% B. Chromatographic separations were monitored at 306 nm. Identification was performed by comparing retention times and UV/Vis spectra of wine samples with those of pure standards. Standard calibration curve was constructed for each stilbene compound and used for quantification.

The data were processed using one-way analysis of variance (ANOVA). Fischer's least significant difference test (LSD) was used to compare mean values ($p < 0.01$ and $p < 0.05$). Statistical analyses of results of one year of research were performed using Statistica 10 software (StatSoft, Inc. 1984–2008).

Results and discussion

The total polyphenolic content in Teran wines varied from 2448.33 mg to 2916.67 mg GAE/L (Figure 1). The obtained results showed that total polyphenolic content was influenced by maceration duration and that the increase was proportional according to maceration time. Similar results were reported by Budić-Leto et al. (2008), who observed an increase in the polyphenolic content in wines made by extended maceration, as extraction of monomeric and polymeric polyphenolics increases with maceration time (Kantz and Singleton, 1991).

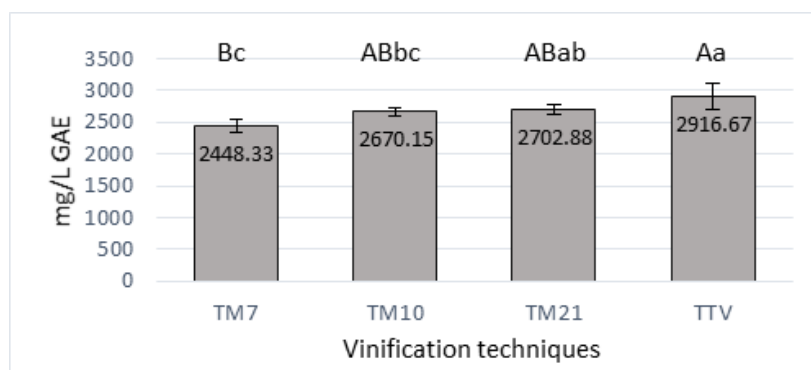


Figure 1. Influence of different vinification techniques on total phenols in Teran wines
Capital letters represent significant differences at $p < 0.01$ level, and lower-case letters represent significant differences at $p < 0.05$ level according to the LSD test.

Results showed that wines produced by TTV and extended maceration technique TM21 had significantly higher amounts of polyphenolic compounds than those from the control wine. It is important to note that there was no significant difference between TTV and TM21 treatments with respect to total polyphenolic content ($p < 0.05$). The increase of approximately 20% noted for TTV treatment, with respect to the control, similar to relatively higher amounts of polyphenolic compounds in wines produced by thermovinification than those from the control group reported by Wang et al. (2016), is due heat destroyed skin cell membranes, releasing the pigments, tannins and different polyphenolic substances into the must (Atanacković et al., 2012).

Table 1. Effect of different vinification techniques on stilbenes in Teran wines (mg/L)

	TM7 (control)	TM10	TM21	TTV
<i>trans</i> -piceid	18.16±1.54 ^{Bb}	17.09±0.17 ^{Bb}	21.54±0.50 ^{Aa}	17.69±0.60 ^{Bb}
<i>cis</i> -piceid	8.95±0.40 ^{Bb}	8.84±0.07 ^{Bb}	10.58±0.08 ^{Aa}	8.67±0.36 ^{Bb}
<i>trans</i> -piceatannol	0.58±0.05 ^{Bc}	0.64±0.02 ^{Bb}	0.78±0.02 ^{Aa}	0.63±0.03 ^{Bbc}
<i>trans</i> -resveratrol	2.84±0.06 ^{Aa}	2.41±0.07 ^{Bb}	2.85±0.08 ^{Aa}	2.48±0.13 ^{Bb}
total stilbenes	30.52±2.01 ^{Bb}	28.98±0.25 ^{Bb}	35.74±0.38 ^{Aa}	29.47±0.96 ^{Bb}

Each value is the mean ± standard deviation, $n=3$. Capital letters represent significant differences at $p < 0.01$ level, and lower-case letters represent significant differences at $p < 0.05$ level according to the LSD test.

Prolonged maceration resulted in a significant increase in total and individual stilbenes (Table 1). The prolonged maceration TM21 had significantly different ($p < 0.01$) and the highest concentration of both *trans*- and *cis*-piceid (21.54±0.50 mg/L and 10.58±0.08 mg/L, respectively) and piceatannol (0.78±0.02 mg/L). However, there was no difference between TM7 and TM21 wines in concentration of *trans*-resveratrol (2.84±0.06, 2.85±0.08, respectively). Similar results were reported by Kocabey et al. (2016) on red wines made from the *Vitis vinifera* L. Karaoglan, who observed no difference in *trans*-resveratrol content between 5, 10 and 15 days of maceration duration (2.55±0.03, 2.19±0.26, and 2.68±0.16 mg/L, respectively), suggesting that the extraction of resveratrol was complete during or at the end of alcoholic fermentation.

Conclusions

Treatment of extended maceration TM21 increased the concentration of total and individual stilbenes, as well as total polyphenols, while treatment of thermovinification TTV significantly increased concentration of total polyphenols. No significant difference in concentration of total polyphenols was found between TTV and TM21 treatment. These results suggest that the use of extended maceration and thermovinification in Teran wine production increase the polyphenolic concentration and consistently enhance the health benefits and sensory properties of these wines.

Acknowledgments

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Utjecaj različitih vinifikacijskih tehnologija na sadržaj stilbena i ukupnih polifenola u vinima sorte ‘Teran’

Sažetak

Cilj ovog istraživanja bio je utvrditi utjecaj različitih vinifikacijskih tehnologija na sadržaj stilbena i ukupnih polifenola u crnom vinu sorte ‘Teran’ (*Vitis vinifera* L.). Provedene su četiri različite tehnologije proizvodnje: kontrola (7 dana maceracije), produljena maceracija (10 i 21 dan) i termovinifikacija. Koncentracija četiri stilbena određena je korištenjem tekućinske kromatografije visoke djelotvornosti (HPLC), a analize ukupnih polifenola provedene su spektrofotometrijskim metodama. Iz dobivenih rezultata vidljiv je značajan porast ukupnih (35.74 mg/L) i pojedinačnih stilbena (*trans*-piceid, *cis*-piceid, *trans*-piceatanol, *trans*-resveratrol), kao i ukupnih polifenola (2702.88 mg GAE/L) u tretmanu produljene maceracije (21 dan). Tretman termovinifikacije rezultirao je značajnim porastom koncentracije ukupnih polifenola za gotovo 20% u odnosu na kontrolu.

Ključne riječi: produljena maceracija, termovinifikacija, ukupni fenoli, resveratrol, piceid

The influence of the vintage year on Blatina wine quality

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Abstract

The goal of this study was to determine how and at what intensity the harvest years affect the quality of the wine. Analyzes were carried out on grapes and wine of the Blatina cultivar, in two vintage years, 2014 and 2015. Based on the results of chemical analysis of must and wine, it was evident that the climatic conditions for grape production were more favourable in 2015, which was recorded as drier year compared to 2014, with higher insolation and higher temperatures at ripening time and moderate precipitation during vegetation. The adverse weather conditions in 2014 affected the quality of the wine, which is why the 2014 vintage entered the category of quality wines, unlike the 2015 vintage which entered the premium wine category.

Keywords: Blatina, climate conditions, chemical analysis, wine quality, vintage year

Introduction

Blatina is an autochthonous Herzegovina variety, whose grapes are used to produce top quality and high quality wines. Blatina must contain 86 - 95 °Oe, and even more in late harvest. Blatina wines have an average alcohol content of 11% vol. minimum, up to 13% vol. alcohol, and sometimes up to 14% vol., 5 to 7 g/L total acids, total dry extract from 21 to 32 g/L. Regarding the ripening stage in the second or third decade of September, is a late variety, which requires warm and sunny conditions to reach full technological maturity (Mirošević, Turković, 2003). The quality of the wine depends on a number of factors such as: environmental conditions, yield, technological maturity of the grapes, the amount of sugars and acids in the grape juice, the yeasts that conduct the alcoholic fermentation, the temperature during the fermentation, and certainly the way of vinification and the equipment which is used. The climatic conditions of one area may vary from year to year, so certain years are marked as good or bad wine-growing years, depending on precipitation, temperature, and other climatic factors. Grape ripening involves many physiological and biochemical processes that allow the development of optimal physicochemical properties of grapes. The changes that occur from the partial maturity (pattern) to the full maturity of the grapes do not occur simultaneously. Each compound, or group of compounds, develops differently and is influenced by genetic, climatic and geographic factors as well as technological processes during production (Esteban et al., 2001; Jones and Davis, 2000).

Anthocyanins are carriers of red grape wine colour, and they are concentrated in the skin of the berry. Their synthesis begins during the partial maturity and gradually builds up in the skin during the ripening process (Bautista-Ortín et al., 2006; Cholet and Darne, 2004). How much anthocyanin will accumulate in the berry skin depends on the technological maturity of the grapes, and especially on the climatic conditions of a particular harvest. It is well known that grapes rich in anthocyanins do not necessarily give a wine of intense colour. Considering the fact that anthocyanins are extracted into grape, must and wine during the maceration and fermentation processes, knowledge of their extractability can contribute to the quality of management at this stage of vinification and to the prediction of wine colour. Experience has shown that wines with a more stable colour and more intense purple tones are obtained from more mature grapes. The ripening process determines the quality of the grapes, and the harvesting period is an important factor in the continued production of quality wine. Harvesting time is determined by the weather of a particular year. This varies from moment to moment as the weather varies from year to year, which gives specificity to each harvest.

Materials and methods

The research was conducted in the musts and wines of the Blatina cultivar, vintages 2014 and 2015. The grapes originate from the vineyard location of Dubrava, in Mostar's vineyard. The grapes of both vintage years, 2014 and 2015 were harvested by hand in PVC crates. In the vintage 2014, 35.100 kg of Blatina was harvested. Upon receipt the grapes at the winery, the grapes were sulfurized with 10g/100kg of potassium metabisulfite. After mulching and grinding the grapes, the wine butter was placed in the Ganymede vinifier with the addition of BDX yeast in the amount of 25g/100kg and the enzyme Lallzyme OE in the amount of 1.5g/100kg.

In vintage 2015, the total amount of grapes was higher, amounting to 36.200 kg. The process of vinification was the same as in the previous year. The maceration lasted 10 days in both vintage years.

Official data of climatic conditions during these two years were provided by the Federal Hydrometeorological Institute of BiH. Climatic conditions were monitored between May and October for 2014 and 2015, including daily precipitation, insolation, average daily air temperature, minimum daily air temperature, and maximum daily air temperature. The following must and wine analyze were performed: The sugar in the musts was determined using an Oechsle must balance, and then the amount of sugar in g/L was read from the Salleron tables. The reducing sugar content in wines expressed in g/L was determined by titration, using the Rebelein method. Total acidity of must and wine is expressed in g/L of tartaric acid, determined by the method of neutralization of the sample with 0.1 M NaOH with the indicator bromothymol blue. The volatile acidity in wines, expressed in g/L of acetic acid, was determined by the method of neutralization of a sample previously distilled in a stream of water vapour, with 0.1 M NaOH and an indicator of phenolphthalein. The pH value of musts and wine was determined by measuring on a Beckman expandomatic type SS 2 pH meter. Wine alcohol was determined by the method of distillation based on the specific gravity of the distillate at 20 °C, based on water of the same temperature. From the values obtained using Reichard tables, the corresponding quantities of alcohol in g/L and vol.% were read. Total dry extract in wines was determined densimetrically from the rest of the distillation, and the corresponding amount in g/L was read from Reichard tables. The sugar-free extract in wines was obtained by subtracting the amount of reducing sugar from the value of the total extract. Ash in wines was determined by combustion of dry matter in a muffle furnace at 525 °C, by the method prescribed by O.I.V. (1990). Free and total SO₂ was determined by the Ripper iodometric method. Sensory evaluation of wines for both years of research was carried out using the 100 positive points method including five members of tasting panel. (O.I.V. 1995).

Results and discussion

Meteorological data showed that rainfall was more abundant in 2014, especially at the time of grapes ripening and before harvest, as opposed to 2015 when they were more properly distributed, especially in August and September. There were also fewer of them throughout the vegetation. The number of hours of sunshine, ie insolation, was higher in 2015, throughout the growing season. The mean daily air temperatures as well as the minimum and maximum daily air temperatures were higher during the 2015 vegetation compared to 2014, especially during the summer, which resulted in a decrease in humidity and a lower risk of disease.

The results of chemical analysis of the must of cultivar Blatina show higher quality of grapes in terms of sugar content for 2015 with 223 g/L, compared to 2014 with 204 g/L of sugar. This results are similar to previous report by Kovačina et al., (1987) in which the concentration of sugar in Blatina grapes obtained from different positions in Mostar winegrowing hill, was in a range 180 and 220 g/L. For this range of total acidity between two years, adverse climatic events in 2014 were responsible for the precipitation at the time of ripening, when the total acidity was higher by 1 g/L compared to 2015. Authors Šupica and Kaljužni, (1958) state that the general average of acids from 4 different positions of Mostar vineyard was 6.75 g/L. The pH value of Blatina must recorded in 2014 was lower 3.25 in comparison to 2015 when the pH was 3.34 what is in correlation with total acidity in each year.

Table 1. Chemical composition of Blatina must in 2014 and 2015

	Vintage 2014	Vintage 2015
Sugar °Bx	19	20.5
Sugar g/L	204	223
Total acidity g/L	6.8	5.8
pH	3.25	3.34

Table 2. Chemical composition of Blatina wines in 2014 and 2015

	Vintage 2014	Vintage 2015
Alcohol vol.%	12.1	13.2
Total extract g/l	24.3	31.1
Reducing sugar g/l	2.0	2.8
Sugar-free extract g/l	23.3	29.3
Total acidity g/l	6.0	5.2
Volatile acidity g/l	0.6	0.54
Ash g/l	2.7	2.5
Free SO ₂ g/l	0.02	0.02
Total SO ₂ g/l	0.09	0.08

In 2014, the alcohol concentration was 12.1 vol.% and in 2015 it was 13.2 vol.%, what is in accordance with previous investigation...

In wines of Blatina obtained from different locations of Mostar winvgrowing hill indicate alcohol content of 12 to 13vol.% (Kovačina et al. 1987).

According to Radovanovic (1986) within the same variety, the content of total dry extract in wine is influenced by climatic and geomorphological factors. Due to climatic conditions in 2015, the total extract was extremely high. At the time of grapes ripening, there was sufficient rainfall, unlike in 2014 when they were much more abundant, and the number of sundials in the final stage of ripening was higher in 2015.

Radovanovic(1986) states that the value of the extract in our wines is usually between 20 and 30 g/L; it goes up to 35 and 40 g/L and can sometimes reach up to 50g/L, as is the case with overripped grapes, for example.

The difference in the amount of reducing sugar negligible and both wines are in the category of dry wines containing up to 4 g/L of unfermented sugar. Sugar-free extract was lower in 2014 Blatina wine compared to 2015 vintage. The total acidity of 6.0 g/L in 2014 was due to climatic factors during the growing season. In 2015, with more sunshine at the time of grape ripening, it brought lower total acidity and amounted to 5.2 g / L. Lower total acids were also observed in the must.

The obtained results are consistent with the literature cited by Kovačina et al. (1987) in Blatina wines. These are lower total acid concentrations than normal or average acids, and can be attributed to above average quality harvesting when there were extremely high sugar concentrations and lower acid concentrations in must. The volatile acidity was slightly higher in 2014. A lower concentration of volatile acids indicates a well-conducted vinification and good health of the wine. The concentration of volatile acids in wines is influenced by a number of factors. First of all, the concentration of volatile acids depends on the health status of the grapes, the type or breed of the yeast that conducts the alcoholic fermentation, the temperature during the alcoholic fermentation, the mode of vinification and the access of oxygen.

According to 2014 data, the ash was 2.7 g/L, which is higher than in 2015 when the ash concentration was 2.5 g/L. The year 2015, as drier with less precipitation during vegetation, reduced the flow of minerals from the soil to the berry, resulting in a lower ash content compared to 2014 which had more precipitation during the growing season. Radovanovic(1986) states that the total ash in the wine ranges from 1.5 to 3.0 g/L. Because minerals are largely contained in the solid parts of the grapes, the ash in the wine will be even more if the must during fermentation was

longer in contact with the hat.

Sensory evaluation of wines was performed using the 100 positive points method. The 2014 and 2015 vintage wines from Dubrava were evaluated nine months after the harvest, that is, three months after bottling with the participation of five tasters, good wine connoisseurs of Blatina cultivar. Five tasters gave their ratings for both wines in each year, and eventually averaged the scores of all the results. According to the results of the evaluation of the Blatina wine by the 100 points method, 2015 vintage wine was rated with a higher average score of 86 points. The average Blatina vintage 2014 wine rating was 82 points. According to the number of points, the wine from the 2014 vintage was categorized as quality, and the wine from the 2015 vintage entered into the premium wine category.

Conclusions

After the research, based on the results of chemical analysis of must and wine, it was evident that the climate conditions for grape production was more favorable in 2015, which was recorded as a drier year than the 2014, with higher hours of sunshine and higher temperatures during ripening and moderate precipitation during the growing season. The adverse weather conditions of 2014, in the form of heavy rainfall, especially during ripening and pre-harvesting, affected the quality of the wine, which was reflected in higher acid content and lower sugar content, lower extract, and as a result, the 2014 vintage entered the category of quality wines, unlike the 2015 vintage wines, which entered the category of premium wines and had a better rating in sensory wine judging. This research is of practical value because it is possible to conclude that the quality of Blatina wine is not influenced only by the quality of the grapes, technological maturity and method of vinification, but also by the climatic conditions of each year, and how those conditions can play a crucial role in the production of premium wine. Blatina from the same locality, treated in the same way, varied over two years due to different climatic conditions.

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Utjecaj godine berbe na kvalitetu vina Blatina

Sažetak

Cilj ovog istraživanja bio je utvrditi kako i kojim intenzitetom godina berbe utječe na kvalitetu vina. Analize su provedene na grožđu i vinu kultivara Blatina, u dvije godine berbe, 2014. i 2015. Na temelju rezultata kemijske analize mošta i vina, vidljivo je bilo da su klimatske prilike za proizvodnju grožđa bile povoljnije 2015. godine, koja je zabilježena kao sušnija godina u odnosu na 2014. godinu, s većim brojem sunčanih sati i višim temperaturama u vrijeme dozrijevanja, te umjerenim padalinama za vrijeme vegetacije. Nepovoljnije vremenske prilike 2014. godine, utjecale su na kvalitetu vina, te je zbog toga vino berbe 2014. godine ušlo u kategoriju kvalitetnih vina, za razliku od vina berbe 2015. godine koje je ušlo u kategoriju vrhunskih vina.

Ključne riječi: Blatina, klimatske prilike, kemijska analiza, kvaliteta vina, godina berbe

Impact of cluster thinning on Merlot and Cabernet Sauvignon (*Vitis vinifera* L.) must quality

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Abstract

The aim of this study is to present the influence of yield reduction on the chemical composition of must of black grapevine varieties (Merlot and Cabernet Sauvignon). The study was conducted in the vineyard and laboratory of the Polytechnic of Požega in 2019. The thinning of the clusters was done prior to veraison the on set of the phenophase of the ripening of the grapes. Each shoot was left with one cluster, resulting in a 25% yield reduction in Cabernet Sauvignon and 34% in Merlot. Statistical analysis revealed that the ampelotechnical procedure had a significant effect on the average sugar content, total acids and pH value of Cabernet Sauvignon variety. Although the differences between the average values of the other parameters among the treatment are visible, they are not statistically significant. The analysis of the obtained paramatars shows that in 2019, under the given environmental conditions, the operation of thinning clusters on the cultivars in question was not justified.

Keywords: Merlot, Cabernet Sauvignon, Cluster thinning, chemical composition of must, Kutjevo vineyards

Introduction

Under the environmental conditions of the Slavonia sub-region (ZOI Slavonija), winegrowing zone C1 (Maletić et al. 2003), the operation of thinning grapes on black varieties (*Vitis vinifera* L.) Merlot and Cabernet Sauvignon plays an important role in achieving high quality grapes and wines. Thinning usually leaves one cluster on the shoot so that the cluster is cut with scissors in the upper part of the petiol (Mirošević and Karoglan Kontić 2008). Canopy management is generally viewed as positioning and maintaining bearing (growing) shoots and their fruit in a microclimate optimal for grape quality. Cluster thinning comprises the removal of whole or parts of inflorescence and fruit clusters to improve the microclimate of fruit zone and leaf area and fruit balance (Jackson 2014). Under the environmental conditions of the Slavonia sub-region (ZOI Slavonija), winegrowing zone C1, the operation of thinning grapes on black varieties (*Vitis vinifera* L.) Merlot and Cabernet Sauvignon plays an important role in achieving high quality grapes and wines. Targeted sugar content is around 100 °Oe. The second principal components in most fruits is the acids. Two main acids found in grapes are malic and tartaric, which together constitute over 90% of the acidity of the grapes (Bird 2014; Jackson 2014). For the majority wines, a range of between 5.5 and 8.5 g/L total acidity is desirable. White wines are typically preferred at the higher end of the scale, whereas red wines are preferred at the lower end a pH range of between 3.1 and 3.4 is suitable for most white wines, and between 3.3 and 3.6 for most red wines (Jackson 2014, Beslić 2016). Must acidity, an important element of enological, is essentially constituted by tree acids: tartaric, malic and citric. They depending on the cultivar, the climate and grape maturity (Ribereau – Gayon 2006).

Clusters removing improve the quality of 'Riesling x Silvaner' berries, retaining 66% of the clusters per plant is recommended, because it generated the highest fruit production per area, mass of clusters and fruits, and total solid soluble content, as well as considerable values of total titratable acids and technical maturity index with a low pH value (Almanza-Merchán et. al 2011). Leaf removal and cluster thinning were carried out prior to veraison to evaluate the effects on berry quality of two *Vitis vinifera* cultivars (Cabernet Sauvignon and Ugni Blanc) in the Weibei Dryland of China in 2013 and 2014, and comprehensive analysis of the chemical and volatile composition in berries was performed. The results showed that content of soluble solids in both varieties was not affected while total acid was generally decreased by leaf removal and cluster thinning. The pH of berry juice was correspondingly higher in most treatment groups (Song et.al. 2018).

Material and methods

The research was conducted on the grapes of the vineyards of the Polytechnic of Pozega. The vineyard is located in the position of Gradina (Podgorje) in the Kutjevo Vineyard, the Slavonia wine-growing sub-region, the Slavonia region and the Croatian Danube region. The altitude is 350 m, it is a plantation of southern exposure and moderate inclinations, suitable for vineyard sites. The rows of the test plantation extend from north to south. The subject of the study are grapevine varieties of the vine Merlot and Cabernet Sauvignon, which were introduced to vineyards in the 1990s. Both cultivars were grafted on Kober 5BB in 2007. The training system is Guyot. The number of shoots per vine is 10, two of which are on the spur and the other eight are located on the cane.

The thinning of the clusters is a summer pruning that is usually done on the black cultivars of the plantation in order to continuously achieve the highest quality of grapes and wine. Although 2019 was ultimately dry and favorable during the ripening phase, heavy rainfall by June and July caused significant damage in many plantations due to the harsh conditions of protecting grapes from disease and pests.

The removal of the clusters was done at stage of fruit development (according to BBCH scale berries beginning to touch - 77) by leaving one cluster per shoot. The experiment was set up according to a randomised block design for each cultivar with two treatments in four repetitions. The repetition consists of one gap of 8 vines. Between the two pillars in the row. The treatment labels are: MK - Merlot, control; MO - Merlot, cluster thinning; CSK - Cabernet Sauvignon, control and CSO - Cabernet Sauvignon, clusters removed.

During the harvest, on October 2, 2019, data were collected: cluster number per vine and yield per vine, average yield per vine and the average cluster weight. The extracted must was analyzed immediately after harvesting on Gibertini wineflow according to the manufacturers instructions for nitrogen compounds (α -amino and ammonium nitrogen) and total acidity and individual organic acids (tartaric, malic, lactic, citric). The results of the sugar content were measured on a Gibertini superalcomat hydrostatic balance. All data were processed variationally statistically.

Results and discussion

Tables 1 and 2 show the number of clusters and the average yield per vine in the vines undergoing cluster thinning treatment and in the control vines. The average yield of Merlot on vines where part of the clusters were removed was 34 lower, whereas for Cabernet Sauvignon the yield reduction per vine was 25% lower.

The average sugar content of the must of both cultivars is higher in the treatment with the cluster thinning, the total acidity is reduced while the pH is slightly increased in the grapevines with the removed clusters. Similar results are reported by other authors (Almanza Merchán et al. 2011; Jackson 2014; Song et al. 2018). The sugar content of Merlot's must is higher than 4,9% and is 103 °Oe, while for Cabernet Sauvignon (CSO) the sugar content is higher by 6,7% Climate conditions had a significant influence on the above-average warm and dry period after the operation in the grape ripening phase.

Table 1: Average number of clusters per vine, average yield per vine (g), average yield per hectare (kg/ha), Merlot 2019

	The number of clusters per vine	Average yield per vine (g/vine)	Average yield (kg/ha)
MK	10	1524	8992
MO	8	1098	6478

MK – Merlot - control = without cluster thinning, MO – Merlot = cluster thinning

Table 2: Average number of clusters per vine, average yield per vine (g), average yield per hectare (kg/ha), Cabernet Sauvignon, 2019.

	Number of clusters per vine	Average yield per vine (g/vine)	Average yield (kg/ha)
CSK	13	1569	9257
CSO	9,5	1170	6903

CSK – Cabernet Sauvignon - control = without cluster thinning, CSO – Cabernet Sauvignon = cluster thinning

Table 3: Average sugar content in must (°Oe), total acidity expressed as tartaric acid (g/L), pH of must, Merlot, 2019.

	Sugar (°Oe)	Total acidity (g/l)	pH
MK	98 ^a	6,7 ^a	3,50 ^a
MO	103 ^a	6,2 ^a	3,66 ^a

MK – Merlot - control = without cluster thinning, MO – Merlot = cluster thinning, ^{a,b} Values in the same column of the table marked with different letters are statistically different.

Table 4: Average sugar content in must (°Oe), total acidity expressed as tartaric acid (g/L), pH of must, Cabernet Sauvignon, 2019.

	Sugar (°Oe)	Total acidity (g/l)	pH
CSK	96 ^a	7,1 ^a	3,22 ^a
CSO	103 ^b	6,3 ^b	3,24 ^b

CSK – Cabernet Sauvignon - control = without cluster thinning, CSO – Cabernet Sauvignon = cluster thinning, ^{a,b} Values in the same column of the table marked with different letters are statistically different.

Table 5: Average content of tartaric, malic, lactic and citric acids in must (g/l), Merlot, 2019

	Tartaric acid	Malic acid	Lactic acid	Citric acid
MK	4,9 ^a	1,7 ^a	0,043 ^a	0,018 ^a
MO	4,2 ^a	1,9 ^a	0,045 ^a	0,020 ^a

MK – Merlot - control = without cluster thinning, MO – Merlot = cluster thinning, ^{a,b} Values in the same column of the table marked with different letters are statistically different.

Table 6: Average content of tartaric, malic, lactic and citric acids in must (g/l), Cabernet Sauvignon, 2019.

	Tartaric acid	Malic acid	Lactic acid	Citric acid
CSK	5,8 ^a	2,4 ^a	0,029 ^a	0,06 ^a
CSO	4,9 ^a	2,6 ^a	0,031 ^a	0,04 ^b

CSK – Cabernet Sauvignon - control = without cluster thinning, CSO – Cabernet Sauvignon = cluster thinning, ^{a,b} Values in the same column of the table marked with different letters are statistically different.

Tables 5 and 6 show the average values of the most represented organic acids in must. Although the differences between the average values of tartaric and malic acid are not statistically significant, it can be seen that in the case of vine varieties with reduced yield, the tartaric acid content is smaller and that of males slightly higher compared to the grapevines with full yield. Significant differences caused by the removal of the clusters were found in Cabernet Sauvignon cultivars in the parameters of sugar content, total acidity and pH. The average sugar content is higher with CSO treatment while the content is acidic and pH lower than CSK treatment. is 5 °Oe and is 103 °Oe, while for Cabernet Sauvignon (CSO) treatments the sugar content is higher by 6,7%. On all parameters were significant influence on the above-average warm and dry period during the grape ripening.

Conclusion

Taking into account the favorable climatic conditions during the grape ripening period in the year of the experiment, differences in the tested parameters do not represent a significant shift in improving the quality of the grapes, although the differences in individual parameters are significant. For a complete picture of the success of the experiment itself, research should be extended to the final product (wine).

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Utjecaj prorjeđivanja grozdova kultivara Merlot i Cabernet Sauvignon (*Vitis vinifera* L.) na kakvoću mošta

Sažetak

Cilj rada je prikazati u utjecaj redukcije uroda na kemijski sastav mošta crnih kultivara vinove loze (Merlot i Cabernet Sauvignon). Istraživanje je provedeno u vinogradu i laboratoriju Veleučilištu u Požegi tijekom 2019. godine. Prorjeđivanje grozdova obavljeno je pred početak fenofaze dozrijevanja grožđa. Na svakoj rodnoj mladici ostavljen je po jedan grozd iz čega proizlazi da je urod reduciran za 25% na kultivaru Cabernet sauvignon odnosno 34% na kultivaru Merlot. Statističkom obradom podataka utvrđeno je da je provedeni ampelotehnički zahvat imao značajan utjecaj na prosječni sadržaj šećera, ukupnih kiselina i pH vrijednost kod kultivara Cabernet Sauvignon. Iako su vidljive razlike među prosječnim vrijednostima ostalih parametara među tretmanima one nisu statistički značajne. Analizom dobivenih parametara može se reći da u 2019. godini u danim okolinskim uvjetima nije bio opravdan zahvat prorjeđivanja grozdova na predmetnim kultivarima.

Ključne riječi: Merlot, Cabernet Sauvignon, prorjeđivanje grozdova, kemijski sastav mošta, Vinogorje Kutjevo

Pregled novih tehnologija za praćenje vinogradarske proizvodnje i primjenu preciznog vinogradarstva

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Sažetak

Ovaj rad daje kratki pregled alata i tehnologija koje se koriste u praćenju vinogradarske proizvodnje, a s ciljem praćenja varijabilnosti unutar vinograda i primjene preciznog vinogradarstva. Geoprostorne tehnologije (GPS), sustavi daljinskog i senzorskog praćenja vinograda te sustavi optimizacije vinogradarske proizvodnje već su testirani i primjenjivi ili su u istraživačkoj fazi, a cilj je pokazati njihova osnovna svojstva, način rada i mogućnost primjene u preciznom vinogradarstvu. Navedene tehnologije daju preciznije podatke za učinkovitije upravljanje vinogradom, kontrolu varijabilnosti i potrošnje inputa, održivost proizvodnje i postizanje visoke kvalitete grožđa.

Ključne riječi: GPS, mreže senzora, nove tehnologije, precizno vinogradarstvo, sustav daljinskog praćenja

Uvod

Digitalizacija poljoprivrede u posljednjih tridesetak godina doprinosi brzom razvoju novih tehnologija za praćenje poljoprivredne proizvodnje, pa tako i vinogradarstva. Precizna poljoprivreda može se objasniti kao novi način upravljanja proizvodnjom koji omogućava donošenje odluka na temelju precizno prikupljenih podataka pomoću raznih alata/novih tehnologija. Slikovito, preciznu poljoprivredu opisuju Pierce i Novak (1999) te Pierpaoli i sur. (2013), kao mogućnost da se napravi što je potrebno, točno kada i gdje je potrebno i to na „pravi“ način. Precizno vinogradarstvo omogućava različito upravljanje proizvodnjom na različitim parcelama unutar vinograda uz pomoć novih tehnologija, koje omogućavaju prikupljanje i obradu podataka sa visokim stupnjem točnosti na osnovu kojih se mogu donijeti kvalitetnije odluke pri upravljanju vinogradarskom proizvodnjom. Sve ovo, u konačnici, rezultira prilagođenom pristupu vinogradarskoj proizvodnji koja, uz pomoć novih tehnologija, omogućava učinkovitiju i kvalitetniju proizvodnju grožđa uz održivo korištenje proizvodnih resursa i vlastitog rada, dok istovremeno utječe na smanjenje troškova proizvodnje te minimiziranje negativnog utjecaja na okoliš (Fraigneau 2009; Matese i Di Genaro, 2015; Barnard, 2018).

Tehnologije za praćenje vinogradarske proizvodnje

Za precizno vinogradarstvo ključni su specifični podaci koji omogućavaju praćenje vinogradarske proizvodnje. U današnje vrijeme, alati i tehnologije koje se koriste za praćenje različitih parametara u vinogradu mogu se podijeliti u nekoliko kategorija (Tisseyre i Taylor, 2005; Fraigneau, 2009; Matese i Di Genaro, 2015; Ozdemir i sur., 2017):

Geoprostorne tehnologije

Najviše korištena tehnologija za geo referenciranje je GPS (engl. *Global Positioning System*) koji se bazira na većem broju satelita raspoređenih u orbiti zemlje. GPS je svemirski satelitski navigacijski sustav koji korisnicima pruža vrlo precizne 3D položaje te brze i pravovremene informacije. Njegova točnost lociranja je 3-15 metara, dok uz zemaljske

referentne stanice postiže centimetarsku točnost lociranja, što je vrlo bitno kod korištenja u preciznoj poljoprivredi i vinogradarstvu za npr. mapiranje vinograda, preciznu primjenu gnojiva i zaštitnih sredstava i sl. (Tisseyre i Taylor, 2005; Krstić, 2012; Matese i Di Genaro, 2015).

Praćenje stanja lisne mase i jačine trsa (engl. canopy and vigour monitoring system)

U preciznom vinogradarstvu, praćenje stanja lisne površine i jačine trsa smatra se vrlo važnim jer omogućava dobivanje preciznih podataka u gotovo realnom vremenu u toku vegetacijske sezone te mogu olakšati vinogradaru donošenje odluka o potrebnim agrotehničkim i ampelotehničkim zahvatima, ali i dati preciznu sliku pojedinih zona u vinogradu koje se razlikuju po promatranim parametrima (Tisseyre i Taylor, 2005). Za sada, postoje dva načina praćenja stanja lisne mase i jačine trsa:

a) Sustav daljinskog praćenja (engl. *remote sensing system*)

Sustavom daljinskog praćenja moguće je u vrlo kratkom roku dobiti opise stanja, veličine i jačine trsova u vinogradu te je na taj način moguće vrlo lako i brzo detektirati određenu varijabilnost u vinogradu. Primjena daljinskog praćenja u vinogradarstvu zasniva se uglavnom na korištenju satelitskih snimaka i bespilotnih letjelica, koji se razlikuju po razlučivosti i kakvoći snimke, ali i po cijeni koštanja. Satelitske snimke nisu pogodne za korištenje u vinogradarstvu zbog uskih redova u vinogradu te mogućih povremenih loših rezolucija snimaka uslijed oblačnog vremena (Matese i Di Genaro, 2015; Barnard 2018). Korištenje bespilotnih letjelica – dronova koji su opremljeni različitim kamerama i sensorima postaje sve više korištena tehnologija u preciznom vinogradarstvu. Njihova prednost je u visokoj rezoluciji snimanja kao i mogućnosti planiranja pravovremenog snimanja vinograda. Njima se mogu snimati i vinogradi manjih površina (1-10 ha), što za satelitske snimke nije isplativo (min 50 ha). Sensori koji se koriste rade na principu refleksne spektroskopije, optičke tehnike koja se temelji na mjerenju refleksije elektromagnetskog zračenja na različitim valnim duljinama, u vidljivom spektru (400–700 nm), blisko infracrvenom (NIR-700–1,300 nm) i toplinsko infracrvenom (7,500–15 000 nm) (Matese i Di Genaro, 2015). Ljudsko oko može vidjeti samo vidljivi spektar svjetlosti dok refleksija biljke, poput vinove loze, ovisi o valnoj duljini. Refleksija je vrlo visoka za valnu duljinu od 800 nm (NIR), a što je biljka aktivnija, to će biti veća refleksija te je taj način moguće razlikovati zone vitalnosti unutar vinograda. Refleksija tla bez pokrova puno je manja nego refleksija vinove loze pri istim valnim duljinama te snimanja sa NIR valnim pojasom mogu pomoći u razlikovanju vinove loze od tla (Fraigneau, 2009). Snimke daljinskih praćenja najčešće se koriste za izračun vegetacijskog indeksa normalizirane razlike (engl. *NDVI-Noramlised Difference Vegetative Index*) i Indeksa gustoće biljnih stanica (engl. *PCD- Plant Cell Density*) (Tisseyre i Taylor, 2005.). Indeksi su usko povezani sa razinom reflektirajućeg zračenja i koriste se za procjenu vitalnosti-jačine vinove loze u određenom vinogradu, kao i za uočavanje razlika unutar samog vinograda (Ozdemir i sur., 2017). Uporaba podataka iz daljinskog praćenja često predstavlja relevantan i cjenovno prihvatljiv izvor informacija za uočavanje varijabilnosti i provedbu zoniranja na određenoj lokaciji/vinogradu (Tisseyre i Taylor, 2005).

b) Sustav praćenja sa tla (engl. *ground-based monitoring system*)

Kao potpora ili korektiv daljinskom praćenju koristi se sustav praćenja sa tla, odnosno senzori koji se mogu montirati na postojeću opremu za obradu vinograda te tijekom ostalih aktivnosti u vinogradu (zaštita vinograda, malčiranje i sl.) mogu se prikupiti potrebni podaci o stanju trsa (količina biomase, visina trsa, fitosintetska aktivnost) (Tisseyre i Taylor, 2005). Pri daljinskom praćenju, zbog jačine rezolucije snimaka, problem mogu stvoriti biljke unutar redova, trava ili tlo te se u tom slučaju koriste sustavi praćenja sa tla.

Praćenje stanja tla

Senzorsko praćenje stanja tla važno je pri promatranju varijabilnosti različitih svojstava tla (struktura, dubina, vlažnost, sadržaj organske tvari, pH, dušik, fosfor, električna provodljivost i sl.). Poznavanje prostorne varijabilnosti karakteristika tla unutar vinograda omogućava bolje razumijevanje fiziološkog stanja i reakcija vinove loze kao i identificiranje uzroka koji utječu na varijabilnost u prinosu i/ili kvaliteti. Za praćenje stanja tla koriste se senzori koji rade po dobro utvrđenim geofizičkim metodama, a najviše se koriste senzori koji svoj rad baziraju na elektromagnetskim svojstvima tla (Tisseyre i Taylor, 2005; Matese i Di Genaro, 2015). Sensori koji se koriste su

senzor elektromagnetske indukcije- EMI (mjeri električnu provodljivost tla), a drugi je senzor električnog otpora (mjeri otpor kroz razliku potencijala između elektroda) (Fraigneau 2009).

Bežična senzorska mreža (engl. wireless sensor network (WSN))

Bežična mreža senzora danas predstavlja jednostavan i brz način praćenja određenih parametara u vinogradu u stvarnom vremenu. Postoji veliki broj senzora koji se mogu međusobno umrežiti i pratiti željene parametre kao što su vlaga tla, vlaga lisne površine, temperatura grožđa i sl. dok se kao najvažnije ipak smatra praćenje meteoroloških podataka u samom vinogradu uz postavljanje odgovarajućih mikro meteoroloških postaja (Matese i Di Genaro, 2015; Ozdemir i sur., 2017).

Praćenje prinosa i kvalitete

Stvaranje mape prinosa i kvalitete unutar vinograda se može smatrati osnovnom značajkom za daljnji razvoj preciznog vinogradarstva (Ozdemir i sur., 2017). Za sada, postoji svega nekoliko razvijenih rješenja na tržištu kao što je HarvestMaster Sensor System HM570 (Juniper Systems Inc., Logan, UT, USA), Canlink Grape Yield Monitor 3000GRM (Farmscan, Bentley, WA, Australia), i Advanced Technology and Viticulture (ATV) (Advanced Technology Viticulture, Joslin, SA, Australia) koji rade na principu senzorskog praćenja prinosa mjerenjem mase grožđa, a mogu se postaviti na ostalu opremu (berače, traktore i sl.). Također, postoje i „ručni“ senzori (Spectron (Pellenc SA, Pertuis Cedex, France)) koji rade na principu spektrofotometrije, a mogu mjeriti parametre u dozrijevanju grožđa kao što su količina šećera i kiselina, sadržaj antocijana i vode (Matese i Di Genaro, 2015).

Tehnologija promjenjive količine (engl. VRT- variable rate technology) i primijenjena robotika (engl. farmbots, agbots)

Tehnologija promjenjive količine (VRT- variable rate technology) ima za cilj optimizaciju proizvodnje uz ispravno upravljanje inputima u vinogradarstvu kao što su gnojiva, zaštitna sredstva i sl. VRT tehnologija koristi softver koji može kombinirati podatke o položaju, dobivene pomoću GPS modula, sa senzorski generiranim kartama za svaku specifičnu operaciju. Ključni čimbenici potencijala VRT-a su zasnovani na razvoju inovativnih tehnologija praćenja vegetacije i visoko-učinkovitih sustava atomizacije, a sve sa izravnim utjecajem na troškove, kvalitetu i održivost okoliša (Tisseyre i Taylor, 2005; Arno i sur. 2009; Matese i Di Genaro, 2015). Primijenjena robotika još je u fazi izrade različitih prototipova strojeva/rješenja koji će jednog dana biti potpora odlučivanju u kvalitetnom upravljanju vinogradima. Postoji nekoliko inovacija na području robotike, a jedan od zanimljivih, koji je prepoznala i EU, jest VineRobot. To je projekt Televitis grupe i Sveučilišta La Rioja (Španjolska) koji je za cilj imao dizajnirati, razviti i staviti u upotrebu novi poljoprivredni robot (bespilotno terensko vozilo (UGV)), koji je opremljen sa nekoliko neinvazivnih senzorskih tehnologija za praćenje: 1) prinosa grožđa, 2) vegetativnog rasta, 3) vodnog stresa i 4) sastava grožđa kako bi se optimiziralo upravljanje vinogradima i poboljšao sastav grožđa i kvaliteta vina (Tardaguila i sur., 2017). Osim toga postoje i VINBOT projekt, Wall-Ye robot, VineGuard, Vitirover i slični, a svi svoj rad baziraju na optičkom praćenju stanja vinograda, predviđanju količine prinosa, potrebama za zaštitom vinograda i sl. (Matese i Di Genaro, 2015).

Programski paketi (Software-i/aplikacije)

Osim sustava za prikupljanje podataka koriste se i različiti programski paketi (software-i) za organizaciju, obradu i jednostavniji prikaz podataka. Danas je na tržištu dostupan veliki broj programskih rješenja za upravljanje vinogradarskom proizvodnjom, ali isključivo na razini praćenja meteoroloških podataka (iz dostupnih izvora) te praćenja unosa pojedinih inputa. Ono što je kod programskih sustava uočeno kao problem jest količina vremena koju vinogradar mora izdvojiti za unos svih potrebnih podataka koje bi sustav trebao obraditi, ali i nedovoljna informatička pismenost poljoprivrednika općenito, za korištenje naprednijih aplikacija.

Zaključak

Precizno vinogradarstvo u samom je začetku u Republici Hrvatskoj dok je u ostalim vinogradarskim zemljama prilično korištena metoda za postizanje veće učinkovitosti i kvalitete vinogradarske proizvodnje. Varijabilnost unutar proizvodnih površina važan je faktor za postizanje veće kvalitete, ali i optimizaciju proizvodnje i korištenje inputa kao što su gnojiva i zaštitna sredstva. Svi navedeni alati i tehnologije mogu se smatrati korisnima za praćenje varijabilnosti i identifikaciju svih važnih parametara u proizvodnji grožđa. Sve navedene tehnologije koriste različite sustave (GPS, daljinsko praćenje, mreže senzora) za prikupljanje podataka u vinogradarstvu koji mogu doprinijeti ciljevima preciznog vinogradarstva.

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Overview of new technologies for monitoring viticulture production and the application of precision viticulture

Abstract

This paper gives a brief overview of the tools and technologies used in monitoring viticulture production, with the aim of monitoring variability within the vineyard and applying precision viticulture. Geospatial technologies (GPS), vineyard remote sensing and monitoring systems and optimization systems for viticulture have already been tested and applied or are in the exploratory phase, and the aim is to show their basic characteristics, the way they work and the possibility of application in precision viticulture. These technologies provide more accurate data for more efficient vineyard management, variability control and input consumption, sustainable and high-quality grapes production.

Keywords: GPS, new technologies, precision viticulture, remote sensing system, sensors network

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Pomološka svojstva sorata šljive na lokalitetu Jazbina

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Sažetak

Cilj rada bio je utvrditi prikladnost za uzgoj sorata 'Topfirst', 'Topfive', 'Top 2000' i 'Jojo' cijepljenih na podlozi WaxWa određivanjem pomoloških značajki plodova. Sorte su zasađene u voćnjaku pokušališta Jazbina Agronomskog fakulteta u Zagrebu (lok. 45°51.320' SGŠ, 16°00.217' IGD) bez sustava za navodnjavanje i zaštitnih mreža. Na temelju provedenog istraživanja sorta 'Top 2000' imala je najsitnije, najbrojnije plodove i najmanju košticu, no pokazala je najveću osjetljivost utvrđenu najvećim brojem oštećenih plodova. Sorta 'Topfirst' imala je najkrupnije plodove, dok je randman plodova bio najveći kod sorata 'Top 2000' i 'Topfive'. Sorte 'Jojo' i 'Topfive' su imale najveću količinu suhe tvari.

Ključne riječi: pomološke karakteristike, *Prunus domestica*, sorte

Uvod

Šljiva je važna voćarska kultura zbog njezine sortne raznolikosti, rasprostranjenosti i prilagodljivosti različitim edafskim i klimatskim uvjetima (Blažek, 2007). Proizvodnja šljive u Hrvatskoj, unatoč povoljnim ekološkim uvjetima i bogatoj tradiciji, ne zadovoljava potrebe domaćeg tržišta zbog nedostatnih količina. U svijetu se uzgaja više od 3.000 različitih sorata šljive, no za koju sortu će se proizvođač odlučiti ovisi ne samo o ekološkim čimbenicima budućeg voćnjaka već i o zahtjevima tržišta odnosno o namjeni ploda.

Najzastupljenije sorte u Hrvatskoj su: 'Bistrica', 'Čačanska ljepotica', 'Čačanska najbolja', 'Čačanska rana' i 'Čačanska rodna'.

Početkom 2000.-tih godina smatralo se da je uzgoj šljive zapušten radi korištenja neadekvatnih sorata, podloga i uzgojnog oblika nepogodnog za intenzivni uzgoj. Tada se započelo s introdukcijom tadašnjih novih, boljih i otpornijih sorata na prikladnoj podlozi uz dobar sustav uzgoja. Introdukcijski pokusi sa sortama 'Topfirst', 'Topfive', 'Topstar Plus', 'Topgigant Plus', 'Haganta', 'Jojo', 'Elena', 'Top 2000' i 'Tophit' cijepljenih na podlozi WaxWa kao osnovica za uvođenje u komercijalnu proizvodnju provedeni su tijekom nekoliko godina (Čmelik i sur., 2007a, 2007b, 2007c, Gadže i sur., 2011.). Spomenute sorte su nešto bujnije, izrazito visoke rodnosti, povoljne za uzgoj u intenzivnim nasadima, nisu osjetljive na transport, podnose duže vrijeme skladištenja te nisu osjetljive na virus šarke šljive. Očekivalo se da će se odnos prema šljivi promijeniti i da će se početi podizati novi nasadi šljiva, što se nije ostvarilo. U periodu 2000.-2012. ukupan godišnji prinos šljiva iznosio je u prosjeku 38.510,33 t (Statistički ljetopis 2007., 2013.) da bi od 2012. godine do danas ukupan godišnji prinos šljiva u prosjeku iznosio 15.489,33 t.

Rad je izvod iz diplomskog rada Maja Zagorc, mag. ing. agr. naslova „Vegetativna razvijenost, generativne osobine te pomološka svojstva plodova sorata šljiva“

Cilj ovog istraživanja je utvrditi prikladnost za uzgoj sorata 'Topfirst', 'Topfive', 'Top 2000' i 'Jojo' cijepljenih na podlozi WaxWa u voćnjaku bez sustava navodnjavanja i zaštitnih mreža. Prikladnost sorata utvrđena je određivanjem slijedećih pomoloških značajki plodova: masa ploda s košticom (g), masa koštice (g), širina i visina ploda (mm), širina i visina koštice (mm), dužina peteljke (mm), broj oštećenih i neoštećenih plodova kao i određivanjem kakvoće plodova pomoću postotka suhe tvari.

Materijal i metode

Istraživanje je provedeno na četiri sorte šljive: 'Topfirst', 'Topfive', 'Top 2000' i 'Jojo' u voćnjaku pokušališta Jazbina Agronomskog fakulteta u Zagrebu (lok. 45°51.320' SGŠ, 16°00.217' IGD) dvije godine nakon sadnje. U voćnjaku su posađene dvogodišnje sadnice cijepjene na podlozi WaxWa, sa razmakom između redova 3 m, a u redu 2,5 m. Uzgojni oblik voćaka je piramidalna krošnja. Voćnjak nije navodnjavao niti zaštićen zaštitnom mrežom, zaštita od bolesti i štetočina provodila se sukladno integriranoj proizvodnji.

Praćen je početak (10% otvorenih cvjetova) i trajanje cvatnje (kraj cvatnje – otvoreno do 100% cvjetova) kod svake sorte. Navedena zapažanja su provedena vizualno. Svaka voćka zasebno je obrana, određen je prinost i ukupan broj plodova po voćki. Kod svake sorte odbrano je 45 plodova približno iste veličine, s peteljka, na kojima su obavljena slijedeća mjerenja: masa ploda s košticom (g), masa koštice (g), širina i visina ploda (mm), širina i visina koštice (mm), dužina peteljke (mm) te broj oštećenih i neoštećenih plodova. Randman ploda (%) izračunat je kao odnos mase mesa ploda i ukupne mase ploda pomnožen sa 100. Postotak suhe tvari (°brix) izmjeren je pomoću ručnog refraktometra (PAL-1, Atago, Tokyo) tijekom mjerenja morfoloških parametara.

Meteorološki parametri u istraživanoj godini prikazani su u tablici 1.

Tablica 1. Meteorološki parametri: srednja, minimalna i maksimalna temperatura (°C) te ukupna količina oborina (mm) za postaju Zagreb - Maksimir u istraživanoj godini (izvor: Državni hidrometeorološki zavod, ustupljeni podaci za istraživačku 2012. godinu).

Mjesec	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Srednja												
temperatura	2,5	-1,9	9,4	12,5	16,7	22,0	24,2	24,0	18,1	11,8	9,2	1,5
Minimalna temperatura	-8,0	-17,1	-3,1	-2,3	3,3	7,0	11,8	9,4	4,9	-1,5	1,0	-15,9
Maksimalna temperatura	12,5	18,3	23,9	30,5	29,3	35,3	35,3	38,6	31,2	24,8	23,1	14,9
Oborine	19,4	26,3	4,5	51,3	81,8	127,9	56,3	9,8	120,0	85,4	112,4	66,0

Pokus je postavljen po slučajnom bloknom rasporedu u tri repeticije, s tri voćke po repeticiji. Broj odabranih plodova za pomološka svojstva u repeticiji iznosio je 15.

Podatci su obrađeni analizom varijance (ANOVA) gdje su nakon signifikantnog F-testa srednje vrijednosti uspoređene odgovarajućim testovima na razini signifikantnosti $P \leq 0,05$ pomoću računalnog programa StatView (SAS Institute Inc. Version 5.0.1).

Rezultati i rasprava

Fenologija cvatnje prikazana je u tablici 2. Na početak i trajanje cvatnje utječe prvenstveno temperatura, odnosno suma aktivnih temperatura nakon završetka zimskog mirovanja. Zbog vrlo povoljnih temperatura u mjesecima ožujku i travnju istraživane godine, nastupila je izuzetno kratka cvatnja.

Tablica 2. Fenologija cvatnje istraživanih sorti šljive na Jazbini.

Sorta	Početak cvatnje	Puna cvatnja	Završetak cvatnje
'Jojo'	02.04	05.04	09.04.
'Top 2000'	04.04.	06.04.	10.04.
'Topfirst'	04.04.	06.04	10.04.
'Topfive'	06.04.	08.04.	13.04.

Morfologija ploda i koštice sorata prikazana je u tablici 3. Najmanju masu ploda s košticom imala je sorta 'Top 2000', dok je najveća bila kod sorte 'Topfirst' (Tablica 3.). Masa koštice bila je najmanja također kod sorte 'Top 2000', a najveću masu koštice su imale sorte 'Jojo' i 'Topfirst'. Masa plodova po vočki (g) nije se bitno razlikovala između sorata (Tablica 3.). Prema randmanu ploda (%) najveću iskoristivost ploda imale su sorte 'Top 2000' i 'Topfive', a najmanju sorta 'Jojo' (Tablica 3.). Gadže i sur. (2011) utvrdili su značajne razlike u prosječnoj masi ploda između sorata 'Topfive', 'Topgigant', 'Topstar', 'Haganta', 'Jojo', 'Top 2000', 'Elena', i 'Tophite', koja je bila različita u odnosu na prosječnu masu plodova istih sorata u ovom istraživanju. Odnosi prosječnih vrijednosti mase plodova kod sorata bili su različiti, iako su oba pokusa bila na istoj lokaciji, ali se očitovao utjecaj različite starosti voćaka kao i utjecaj klimatskih čimbenika. Masa koštice i randman ploda imali su slične vrijednosti i odnose između sorata kod oba istraživanja.

Dužina peteljke kod sorata nije se bitno razlikovala (Tablica 4.). Širina i visina ploda bila je najmanja kod sorte 'Top 2000', dok je najveća širina i visina ploda bila kod sorata 'Jojo' i 'Topfirst' (Tablica 4.). Širina i visina koštice bila je najmanja kod sorte 'Top 2000' (Tablica 4.), a najveća kod sorata 'Jojo' i 'Topfirst'.

Tablica 3. Utjecaj sorte na masu ploda s košticom (g), masu koštice (g), masu plodova po vočki te randmanu ploda (%).

Sorta	Masa ploda s košticom (g)	Masa koštice (g)	Masa plodova po vočki (g)	Randman ploda (%)
'Jojo'	34,19 a	1,69 a	6637,76	94,80 c
'Top 2000'	18,80 b	0,74 c	7132,88	95,56 a
'Topfirst'	40,88 a	1,83 a	5556,33	95,22 b
'Topfive'	34,27 a	1,44 b	3297,39	95,73 a
ANOVA	***	***	NS	***

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$. Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05$.

Tablica 4. Utjecaj sorte na dužinu peteljke (mm), širinu i visinu ploda (mm), širinu i visinu koštice (mm).

Sorta	Dužina peteljke (mm)	Širina ploda (mm)	Visina ploda (mm)	Širina koštice (mm)	Visina koštice (mm)
'Jojo'	16,49	45,52 a	34,62 b	28,75 a	15,26 a
'Top 2000'	19,62	34,63 c	29,43 c	19,32 c	11,84 c
'Topfirst'	17,95	47,53 a	39,43 a	28,30 a	14,39 a
'Topfive'	18,88	39,01b	36,67 ab	22,03 b	13,39 b

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$. Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05$.

Najveći broj plodova po vočki zabilježen je kod sorte 'Top 2000' (Tablica 5.). Mogli bi pretpostaviti da je to jedan od razloga najmanje mase i najmanje veličine plodova kod te sorte (Tablica 3. i Tablica 4.).

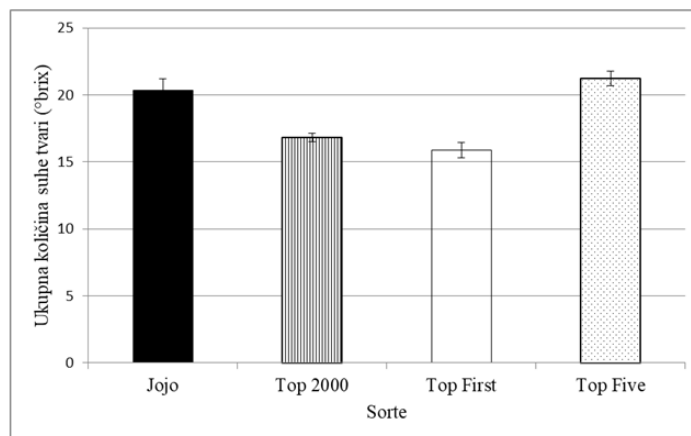
Sorta 'Top 2000' pokazala je najveću osjetljivost utvrđenu najvećim brojem oštećenih plodova (Tablica 5.). Oštećenja plodova nastala su najvećim dijelom pod utjecajem klimatskih čimbenika (tuča i suša). Šljiva najbolje uspijeva u područjima s godišnjom sumom oborina od 700 do 1100 mm i vegetacijskom sumom oborina od 350 do 600 mm te relativnom vlagom zraka od 75 do 85 %. Najosjetljivija je prema suši u vrijeme formiranja koštice (oko 30 dana nakon završetka cvatnje) te ako se u to vrijeme javi nedostatak vlage može doći do masovnog otpadanja zametnutih plodova kao i do nenormalnog razvoja plodova koji ostaju na grani (Mišić, 2006).

Tablica 5. Utjecaj sorte na ukupan broj plodova i broj neoštećenih i oštećenih plodova po vočki.

Sorta	Ukupno plodova po vočki	Neoštećeni plodovi po vočki	Oštećeni plodovi po vočki
'Jojo'	181,11 b	40,67 b	144,44 b
'Top 2000'	373,11 a	94,11 a	293,11 a
'Topfirst'	137,56 b	53,11 b	81,11 b
'Topfive'	107,78 b	37,44 b	68,78 b
ANOVA	**	*	***

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$. Značajnost je označena kao: NS, *, **, *** pri $P \leq 0,05$.

Sadržaj suhe tvari sortna je osobina koja se povećava dozrijevanjem plodova. Plodovi sorte 'Jojo' i 'Topfive' imali su najveću količinu suhe tvari ('brix) dok se ista nije razlikovala kod plodova sorte 'Top 2000' i 'Topfirst' (Slika 1.). Pomološki parametri plodova svih istraživanih sorata u skladu su sa istraživanjima Gadže i sur. (2011) i Čmelik i sur. (2007).



Slika 1. Utjecaj sorte na ukupnu količinu suhe tvari.

Zaključak

Na temelju provedenog istraživanja u početnoj fazi plodonošenja na sortama 'Topfirst', 'Topfive', 'Top 2000' i 'Jojo' možemo zaključiti slijedeće:

- plodovi sorte 'Top 2000' bili su najsitniji te su imali i najmanju košticu u odnosu na druge sorte,
- sorta 'Top 2000' imala je najveći broj plodova (razlog najsitnijim plodovima) po vočki te najveći broj oštećenih plodova
- plodovi sorte 'Topfirst' bili su najkrupniji,
- najveća iskoristivost ploda (randman) bila je kod sorata 'Top 2000' i 'Topfive', a najmanja kod sorte 'Jojo' i
- plodovi sorte 'Jojo' i 'Topfive' imali su najveću količinu suhe tvari (% brix).

Iz navedenih zaključaka proizlazi da sve navedene sorte, iako su na početku rodnosti te zasađene u voćnjaku bez navodnjavanja i zaštitne mreže, imaju zadovoljavajuću kvalitetu plodova.

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Pomological properties of plum varieties at the Jazbina site

Abstract

The aim of the study was to determine the cultivation suitability of 'Topfirst', 'Topfive', 'Top 2000' and 'Jojo' varieties grafted on WaxWa by determining the pomological characteristics of the fruits. The varieties were planted in the orchard of the Jazbina Trial Grounds at the Faculty of Agriculture in Zagreb (loc. 45°51.320' N, 16°00.217' E) without irrigation systems and protective nets. Based on the research conducted, the 'Top 2000' variety had the smallest, most numerous fruits and the smallest pit, but showed the highest sensitivity determined by the highest number of damaged fruits. The 'Topfirst' variety had the largest fruit, while the flesh percentage in the fruit weight was the highest in 'Top 2000' and 'Topfive' varieties. The 'Jojo' and 'Topfive' varieties had the highest amounts of dry matter.

Keywords: pomological characteristics, *Prunus domestica*, varieties

Dinamika porasta mladice kruške sorata 'Williams' i 'Santa Maria'

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Sažetak

U radu je praćena dinamika porasta mladica dviju sorata kruške: 'Williams' i 'Santa Maria'. Cilj istraživanja bio je utvrditi i usporediti dinamiku porasta mladice istraživanih sorata kruške u 2019. godini. Na svakoj krošnji zasebno praćen je porast mladica u gornjem i donjem dijelu krošnje. Rast mladice pratio se u pet rokova tijekom vegetacije u 2019. godini (20. 4., 4. 5., 19. 5., 31. 5. i 15. 6.). Nakon provedenih istraživanja može se zaključiti kako je sorta 'Williams' imala veći ukupni porast u donjem dijelu krošnje u odnosu na sortu 'Santa Maria', a u gornjem dijelu krošnje ukupni porast je bio veći kod sorte 'Santa Maria'. Također je utvrđeno kako sorta 'Williams' ima ujednačeniju dinamiku porasta tijekom praćenog vegetacijskog perioda. Kod obje istraživane sorte najintenzivniji vegetativni porast mladice bio je tijekom petog mjeseca, kada su mladice narasle više od 70% od svog ukupnog rasta.

Ključne riječi: kruška, porast, dinamika, mladica, sorta

Uvod

Danas su intenzivni moderni voćnjaci zasađeni u sklopu sa 2.000 - 5.000 stabala/ha (nasad visoke gustoće – high density planting; HDP) cijepljenih na slabobujnim podlogama dunje s prirodom od barem 40-50 t/ha (Sansavini et al., 2008).

U Republici Hrvatskoj ukupna proizvodnja kruške (*Pyrus communis* L.) za 2017. godinu je iznosila 2.796 tona, od čega je 2.381 tona bilo u intenzivnoj proizvodnji (Statistički ljetopis, 2018., www.dzs.hr). Proizvodna površina pod kruškom u 2017. godini iznosila je samo 714 ha, s prosječnim prinosom od 3,3 t/ha (Statistički ljetopis, 2018., www.dzs.hr), što je nedovoljna proizvodnja s obzirom na ekološke uvjete i potrebe u RH. Sličan trend se uočava u svim zemljama regije.

Dva glavna čimbenika utječu na veličinu voćke, odnosno porast mladica kao i na mogućnost formiranja uzgojnog oblika. To su vrsta i/ili sorta (Lauri et al. 1995) i korijenov sustav odnosno podloga (Seleznova et al. 2008). Sosna i Czaplicka (2008) su istraživali dva različita sustava uzgoja kod tri sorte kruške na porast, prinos i kvalitetu ploda i zaključili kako sorta nema statistički značajnu ulogu u ukupnoj dužini jednogodišnje mladice, ali je pokazala značajnu razliku u broju mladica. Studija koju su proveli Milošević i Milošević (2010.) je pokazala kako je na rani rast kruške, u prvim godinama nakon cijepjenja pupoljaka, utjecaj sorte neusporedivo veći nego utjecaj podloge.

Cilj ovog istraživanja bio je pratiti dinamiku rasta mladica dviju različitih sorata kruške, u donjem i gornjem dijelu krošnje u jednoj vegetacijskoj godini, kako bi se utvrdile razlike u vegetativnoj aktivnosti između sorata.

Materijal i metode

Voćnjak u kojem je proveden istraživački dio završnog rada smješten je u Žepču (Bosna i Hercegovina), katastarska općina Brankovići, na području srednje Bosne. Podignut je 2015. godine na jugozapadnom položaju. Voćnjak se prostire na površini od 0,80 ha i nalazi se na 256 m nadmorske visine. U voćnjaku su zasađene sorte kruške: 'Williams' i 'Santa Maria'. Razmak sadnje iznosi 3,5 m x 2,5 m, a kao podloga je korištena dunja MA U proljeće, prije početka vegetacije, obavljena je rezidba krušaka. Nakon rezidbe provedena je prihrana mineralnim gnojivom NPK 15:15:15 u količini 100 g do 120 g po stablu. Krajem zime obavljeno je prskanje plavim uljem. U voćnjaku se redovno obavlja

košnja i malčiranje. Porast mladica praćen je na sortama 'Williams' i 'Santa Maria' na šest stabala krušaka (svaka sorta po tri stabla, slučajnim odabirom), s tim da je na svakoj krošnji zasebno praćen porast mladica u gornjem i donjem dijelu krošnje. Svaka praćena mladica je bila pravilno označena. Rast mladice pratio se u pet rokova tijekom vegetacije u 2019. godini (20. 4., 4. 5., 19. 5., 31. 5. i 15. 6.).

Rezultati i rasprava

U tablici 1 prikazan je porast mladica na donjem dijelu krošnje za sortu 'Williams'. Početna dužina mladice izmjerena 20. 4.2019. prosječno je iznosila 9,3 mm. Nakon dva tjedna mladice su u prosjeku porasle za 4,3 mm. Nakon toga se može vidjeti nagli prosječni porast mladica između 4. svibnja i 19. svibnja, te su u tom periodu mladice prosječno porasle za 65,7 mm. Sljedećih dva tjedna mladice su prosječno rasle oko 31,3 mm, te nakon toga se porast smanjuje u sljedećih četrnaest dana na prosječno 14,0 mm. Iz tablice je vidljivo da je u donjem dijelu krošnje kod sorte 'Williams' najveći porast mladice bio od 4. 5.2019. do 19. 5.2019., odnosno tokom mjeseca svibnja.

Tablica 1: Porast mladice kruške 'Williams' u donjem dijelu krošnje (u mm)

Datum	20. 4.2019.	4. 5.2019.	19. 5.2019.	31. 5.2019.	15. 6.2019.
Stablo 1	9,1	12,3	78,6	115	123
Stablo 2	10,0	15,1	70,4	109,5	131
Stablo 3	8,9	13,6	88,9	107,3	119
Prosjeck	9,3	13,7	79,3	110,6	124,3

U tablici 2 prikazan je porast mladica na donjem dijelu krošnje za sortu 'Santa Maria'. U prvom terminu (20.4.2019.) izmjerena je prosječna duljina mladice od 8,8 mm. Nakon dva tjedna mladice su u prosjeku porasle za 1,8 mm. Između 4. 5. i 19. 5. mladice su prosječno porasle za 61,7 mm. Sljedećih dva tjedna mladice su prosječno rasle oko 29,4 mm, te nakon toga se porast smanjuje na prosječno 12,8 mm.

Tablica 2: Porast mladice kruške 'Santa Maria' u donjem dijelu krošnje (u mm)

Datum	20. 4.2013.	4. 5.2019.	19. 5.2019.	31. 5.2019.	15. 6.2019.
Stablo 1	8,1	10,3	71,3	105	115,2
Stablo 2	10,1	11,5	70	101,3	119
Stablo 3	8,3	10,2	75,7	98,8	109,6
Prosjeck	8,8	10,7	72,3	101,7	114,6

U tablici 3 prikazan je porast mladica u gornjem dijelu krošnje za sortu 'Williams'. Na početku mjerenja (20. 4.) utvrđena je početna duljina mladica koja je prosječno iznosila 7,9 mm. Nakon dva tjedna mladice su u prosjeku porasle za 2,9 mm. Nakon toga se može vidjeti nagli prosječni porast mladica između 4. 5. i 19. 5., te su u tom periodu mladice prosječno porasle za 92,2 mm. Sljedećih dva tjedna mladice su prosječno rasle oko 27,8 mm, te se nakon toga porast opet povećao na prosječno 52,5 mm.

Tablica 3: Porast mladice kruške 'Williams' u gornjem dijelu krošnje (u mm)

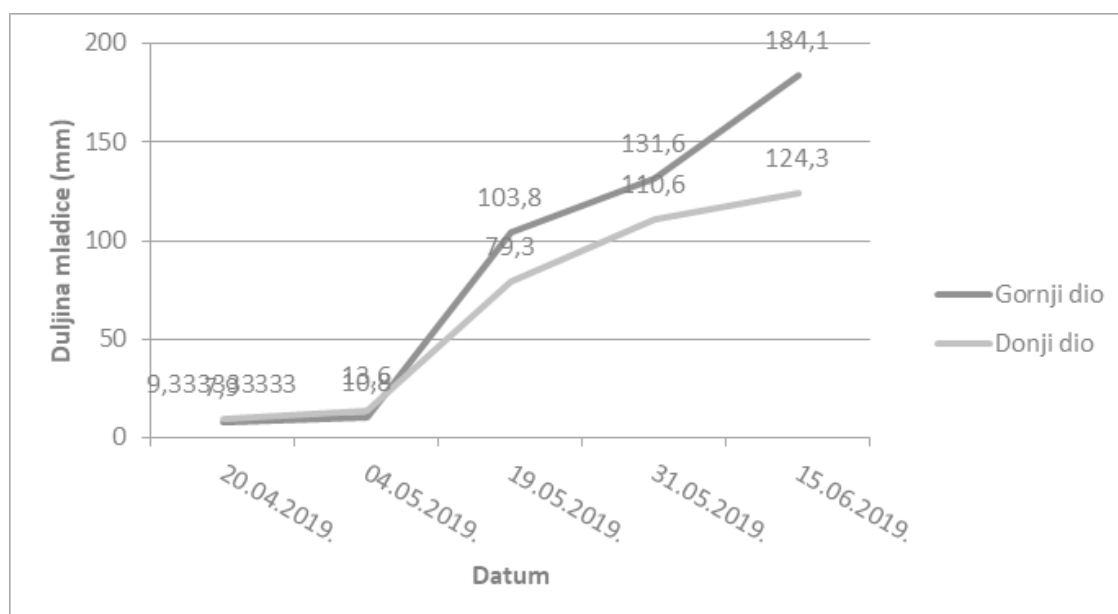
Datum	20. 4.2013.	4. 5.2019.	19. 5.2019.	31.05.2019.	15.06.2019.
Stablo 1	7,5	10,8	100,1	132,6	174,4
Stablo 2	8,3	11,4	98,6	121	184,9
Stablo 3	8,1	10,3	112	141,3	193,2
Prosjeck	7,9	10,8	103,8	131,6	184,2

U tablici 4 prikazan je porast mladica u gornjem dijelu krošnje za sortu 'Santa Maria'. U terminu 20. 4.2019. izmjerena je početna dužina mladice koja je prosječno iznosila 8,7 mm. Nakon dva tjedna mladice su u prosjeku porasle za 3,7 mm. Nakon toga se može vidjeti nagli prosječni porast mladica između 4. 5. i 19. 5., te su u tom periodu mladice prosječno porasle za 102,8 mm. Sljedećih dva tjedna mladice su prosječno rasle oko 44,1 mm, te nakon toga se porast smanjuje u sljedećih četrnaest dana na prosječno 39,6 mm.

Tablica 4: Porast mladice kruške 'Santa Maria' u gornjem dijelu krošnje (u mm)

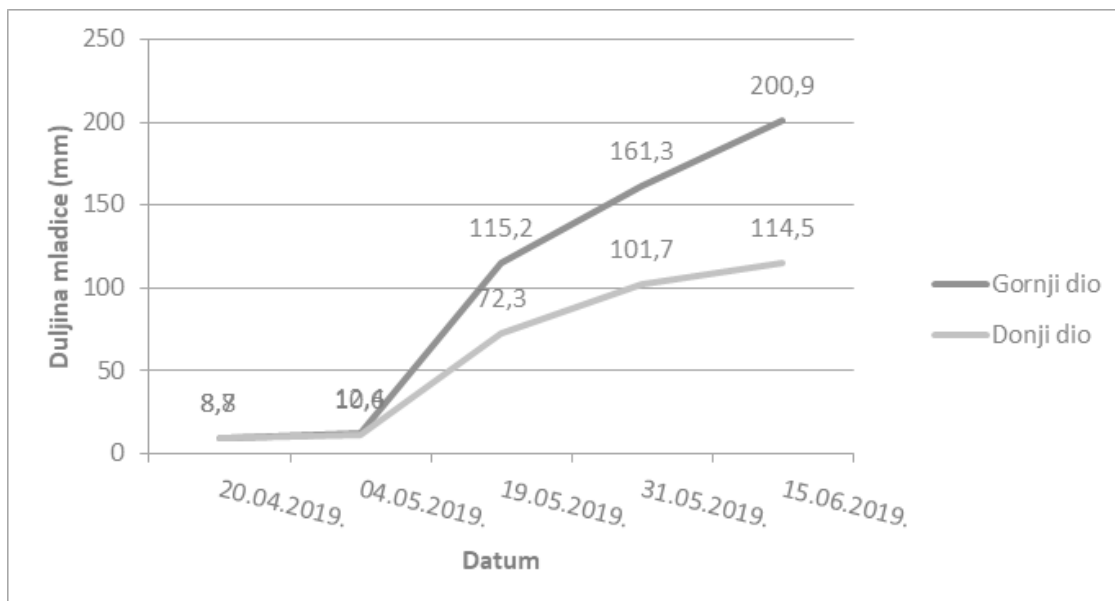
Datum	20.04.2013.	04.05.2019.	19.05.2019.	31.05.2019.	15.06.2019.
Stablo 1	8,3	13,6	110,4	157,6	198,9
Stablo 2	9,2	12,3	115,2	148,9	198,4
Stablo 3	8,7	11,4	120	176,6	205,4
Prosjeck	8,7	12,4	115,2	161	200,9

Grafikon 1 prikazuje dinamiku porasta mladica sorte 'Williams' u gornjem i donjem dijelu krošnje. Uočljivo je da mladice u prva dva tjedna praćenja imaju približno jednaku brzinu porasta, a potom je tijekom petog mjeseca intenzivniji rast mladice u gornjem dijelu krošnje. Ako se uspoređi porast mladice na donjem i gornjem dijelu krošnje uočljivo je kako je sorta 'Williams' imala prosječno veći porast mladice u gornjem dijelu krošnje.



Grafikon 1: Dinamika prosječnog porasta mladica u gornjem i donjem dijelu krošnje stabala sorte 'Williams'

Na grafikonu 2 prikazan je porast mladica na gornjem i donjem dijelu krošnje stabala kod sorte 'Santa Maria'. Uočljiv je podjednak porast mladice u prva dva tjedna praćenja. Usporedbom donjeg i gornjeg dijela krošnje uočljivo je kako je sorta 'Santa Maria' imala prosječno veći porast mladice u gornjem dijelu krošnje u periodu praćenja.



Grafikon 2: Dinamika prosječnog porasta mladica u gornjem i donjem dijelu krošnje stabala kod sorte 'Santa Maria' (u mm)

Rezultati dobiveni u ovom istraživanju se slažu s rezultatima drugih autora koji su istraživali porast grana na stablima voćnih vrsta (Topolovec et al, 2014., Miljković, 2006.).

Zaključak

Tijekom praćenja rasta mladice u 2019. godini utvrđeno je kako u donjem dijelu krošnje sorta 'Williams' ima veći ukupni porast, a u gornjem dijelu krošnje sorta 'Santa Maria' ima nešto veći ukupni porast. Također je utvrđeno kako sorta 'Williams' ima ujednačeniju dinamiku porasta tijekom praćenog vegetacijskog perioda, dok je porast mladice kod sorte 'Santa Maria' slabiji nakon petog mjeseca u odnosu na sortu 'Williams'. Kod obje istraživane sorte najintenzivniji vegetativni porast mladice bio je tijekom petog mjeseca, kada su mladice narasle više od 70% od svog ukupnog rasta.

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The growth dynamics of branches on pears cultivars 'Williams' and 'Santa Maria'

Abstract

The study evaluated the growth dynamics of branches in two cultivars of pears, 'Williams' and 'Santa Maria'. The aim of the study was to determine and compare the growth dynamics of the increase branches of pears in 2019 year. Branches growth was monitored from 20th April 2019 till the 15th June 2019, in five terms. Following these studies, it can be concluded that in the lower part of the crown the cultivar 'Williams' had a greater overall increase of branches. In the upper part of the crown an overall increase branches were higher in the cultivar 'Santa Maria'. It was also found that the cultivar 'Williams' had more balanced dynamic of growth branches during the studied period. In both research cultivars the most intense increase in vegetative growth was during the fifth month, when the branches have grown more than 70% of its overall growth.

Keywords: pear, growth, dynamics, branch, cultivars

Evolution of phenolic compounds and quality parameters after storage of Istarska bjelica and Buža cv. extra virgin olive oil

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Abstract

This study aimed to evaluate changes in the quality parameters and phenolic compounds profile, determined by HPLC-DAD, after a 6-month storage period of Istarska bjelica and Buža cv. virgin olive oil. Obtained results showed diverse susceptibility to oxidative degradation in the two investigated monovarietal olive oils. Istarska bjelica virgin olive oil preserved its phenol content during the storage period, while there was a slight decrease in Buža secoiridoid content. Based on the obtained results it can be concluded that the oxidative stability and evolution of phenolic compounds in virgin olive oil are dependent on the initial phenolic composition of the selected olive cultivar.

Keywords: monovarietal virgin olive oil, quality parameters, phenolic content, storage

Introduction

Extra virgin olive oil (EVOO) is largely consumed mainly due to its health benefits. It is promising as a supplementary food product in preventing the risk of cardiovascular diseases and cancer (Fernandes et al., 2019) interleukin-6 (IL-6). The source of these health benefits is not only from the fatty acid profile, abundant in monounsaturated acids, but also from diverse bioactive components, primarily phenols (Khalifa et al., 2018). Even in small quantities, phenol compounds are crucial for oxidation protection of lipid substrate (Kotsiou and Tasioula-Margari, 2016). Consequently, they are liable to deteriorate resulting with the reduction of the nutritional value and quality of the oil during storage (Daskalaki et al., 2009) due to its polyphenol content. Virgin olive oils were subjected to heat treatment simulating common domestic processing, including boiling, frying and storage. These processes can affect the phenolic compounds content of oils to a certain degree, depending on each one treatment. Thermal oxidation of oils at 180 °C (frying). The rate of the deterioration depends not only on the storage conditions (temperature, oxygen, light) but also on the quality characteristics of the initial fresh virgin olive oil (Gutiérrez and Fernández, 2002; Kotsiou and Tasioula-Margari, 2016; Valli et al., 2019). Furthermore, there is a difference between cultivars susceptibility to oxidative degradation as a result of its unique chemical profile (Brkić Bubola et al., 2014; Daskalaki et al., 2009) phenolic contents and volatile profiles of Buža, Črna and Rosinjola monovarietal virgin olive oils after 12 months of storage was investigated in this study. Virgin olive oils stored at low temperatures maintained better quality parameters than oils stored at room temperature. A negligible decrease in the total phenols was detected after 12 months of storage at all investigated temperatures. The total volatile compounds, aldehydes, alcohols and esters in almost all stored samples were unchanged compared to fresh oils. Total ketones increased after storage, although at a lower temperature these changes were less notable. An increase in the oxidation indicators hexanal and hexanal/E-2-hexenal ratio was the lowest in oils stored at +4 °C. Storage at temperatures lower than room temperature could help to prolong the shelf-life of extra virgin olive oil by maintaining high quality parameters and preserving the fresh oil's volatile profile. © 2014 CSIC, author: [{"dropping-particle": "", "family": "Brkić Bubola", "given": "K.", "non-dropping-particle": "", "parse-names": false, "suffix": ""}], [{"dropping-particle": "", "family": "Koprivnjak", "given": "O.", "non-dropping-particle": "", "parse-names": false, "suffix": ""}], [{"dropping-particle": "", "family": "Sladonja", "given": "B.", "non-dropping-particle": "", "parse-names": false, "suffix": ""}], [{"dropping-particle": "", "family": "Belobrajić", "given": "I.", "non-dropping-particle": "", "parse-names": false, "suffix": ""}], container-title: "Grasas y Aceites", id: "ITEM-1", issue: "3", issued: [{"date-parts": [{"2014"}]}, title: "Influence of storage temperature on quality parameters, phenols and volatile compounds of Croatian virgin olive

oils";type:"article-journal";volume:"65"},uris":["http://www.mendeley.com/documents/?uuid=69b9b5d5-e96b-4a5c-a7f4-296783815fa2"]},{id:"ITEM-2";itemData":{"ISBN":"2651098345";ISSN:"13368672";abstract:"Virgin olive oil is oil with a high biological value, due to its polyphenol content. Virgin olive oils were subjected to heat treatment simulating common domestic processing, including boiling, frying and storage. These processes can affect the phenolic compounds content of oils to a certain degree, depending on each one treatment. Thermal oxidation of oils at 180 °C (frying. Due to these findings, this study aimed to evaluate changes in the quality parameters, phenol composition and content during the 6-month storage of Croatian autochthonous virgin olive oils obtained from Istarska bjelica and Buža cultivars. The two cultivars were chosen for this purpose as the most commonly used autochthonous cultivars whose oil is characterized by a different initial phenolic composition and sensory properties (Brkić Bubola et al., 2017; Lukić et al., 2018)total phenols, sensory profile.

Materials and methods

Samples of Istarska bjelica and Buža olive fruit were harvested from the same orchard located in Vodnjan, Istria region of Croatia on the 10th October 2018. Two-phase extraction was performed within 12 hours from the harvest and the obtained oils were immediately filtered using filter press system (Euro 20, TEM, Italy) accompanied by 19 cellulose filter plate (OV110, Omniafiltra, Italy). The obtained samples were analysed instantly after the production (3 bottles per monovarietal oil), while the other 3 bottles per monovarietal oil were kept in the dark, at room temperature (20 ± 2 °C) in one liter dark green glass bottles for the 6-month storage period and then analysed.

Quality parameters, such as peroxide value (PV) and spectrophotometric values (K_{232} , K_{270} , and ΔK), were determined according to the standard analytical methods established by the European Commission Regulation (EEC, 1991).

Extraction and analysis of virgin olive oil phenol compounds were performed using the method proposed by Jerman Klen et al. (2015) and modified by Lukić et al. (2018). The analysis was completed using an Agilent Infinity 1260 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a G1311B quaternary pump, a G1329B autosampler, a G1316A column oven, and a G4212B DAD detector. A Kinetex PFP column (2.6 µm, 100 mm × 4.6 mm) with a guard was used (Phenomenex, Sydney, Australia). The identification of peaks was performed by comparing the retention times and UV/Vis spectra with those of pure standards when available, and from the literature (Jerman Klen et al., 2015). For quantification, standard calibration curves were constructed for tyrosol, hydroxytyrosol, vanillic acid, vanillin, p-coumaric acid, luteolin, apigenin, pinoresinol, and oleuropein. For other compounds semi-quantitative analysis was accomplished: secoiridoids were expressed in mg/kg as oleuropein, and acetoxypinoresinol as pinoresinol equivalents. Statistica version 13.2 (Stat-Soft Inc., Tulsa, OK, USA) was used for statistical analyses.

Results and discussion

The analysis of the quality parameters showed that both fresh monovarietal oil samples were of EVOO category and their initial quality characteristics were similar based on the analysed parameters (EEC, 1991). It is notable that even after the 6-month storage the samples from both investigated cultivars remained within the EVOO category (Table 1). Peroxide value (PV), an indicator of the initial phase of oxidation, slightly increased in both cultivars. Differently, Méndez and Falqué (2007) reported an increase above the extra virgin stated limits after 6-month storage, however the used samples had higher initial PV than oils used in our study. Daskalaki et al. (2009) due to its polyphenol content. Virgin olive oils were subjected to heat treatment simulating common domestic processing, including boiling, frying and storage. These processes can affect the phenolic compounds content of oils to a certain degree, depending on each one treatment. Thermal oxidation of oils at 180 °C (frying, with lower initial PV determined in Linolia and Koroneiki cv. Greek monovarietal EVOO, reported that the samples did not exceed the prescribed limits even during 15-month of storage.

Values of the coefficients K_{232} , K_{268} , and ΔK remained unchanged after the storage of Buža EVOO. However, in the case of Istarska bjelica EVOO there was an increase in both K_{232} and K_{268} , indicating that the hydroperoxides partially converted into secondary oxidation products (Table 1). Several studies conducted on different monovarietal olive oils reported the increase of K values (Méndez and Falqué, 2007; Valli et al., 2019) while others showed no change after 6-month storage in dark conditions (Caponio et al., 2005). As expected, ΔK parameter remained unchanged (Caponio et al., 2005).

Evolution of phenolic compounds and quality parameters after storage of Istarska bjelica and Buža cv. extra virgin olive oil

Table 1. Quality parameters (peroxide value – PV, spectrophotometric indices – K_{232} , K_{268} , and ΔK) of fresh Buža and Istarska bjelica cv. virgin olive oil and after 6 months of storage.

	Istarska bjelica		Buža		EVOO*
	Fresh	Stored	Fresh	Stored	
PV (meq O ₂ /kg)	6.22 ± 0.15 b	7.54 ± 0.07 a	6.74 ± 0.07 b	8.23 ± 0.05 a	≤ 20.0
K_{232}	1.81 ± 0.04 b	2.12 ± 0.02 a	1.98 ± 0.03 a	2.00 ± 0.04 a	≤ 2.50
K_{268}	0.12 ± 0.00 b	0.18 ± 0.01 a	0.12 ± 0.00 a	0.12 ± 0.00 a	≤ 0.22
ΔK	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	≤ 0.01

Results are expressed as mean values ± standard deviation of three independent repetitions. Mean values labelled with a different letter within the same row are statistically different (Tukey's test, $p < 0.05$). *Certain limits for extra virgin olive oil category (EEC, 1991).

Changes in the phenol content during virgin olive oil storage depend on the initial quality characteristics of the fresh samples (Kotsiou and Tasioula-Margari, 2016). Istarska bjelica oil is known for its high total phenolic content (TPC) when compared to other cultivars, especially Buža (Lukić et al. 2018). In our case, no such differences between the two cultivars TPC were found (Table 2), while higher secoiridoid content was detected in Buža oil (Figure 1). This anomaly could be attributed to the cellulose filtration process applied on the investigated oils immediately after production, in which a part of the initial phenol content could be lost due to retention of phenolic compounds in the filtration aid (Brkić Bubola et al., 2017 total phenols, sensory profile). After the 6-month period TPC of Istarska bjelica oil remained stable, while the oil from Buža cultivar showed a significant reduction in TPC of 26 % (Table 2).

Table 2. Phenolic compounds concentration (mg/kg) in fresh Buža and Istarska bjelica cv. virgin olive oil and after 6 months of storage.

Phenolic compounds	Istarska bjelica		Buža	
	Fresh	Stored	Fresh	Stored
Hydroxytyrosol	1.41 ± 0.38 a	1.93 ± 0.24 a	2.81 ± 0.12 a	3.17 ± 0.39 a
Tyrosol	3.08 ± 0.51 a	3.58 ± 0.28 a	5.71 ± 0.51 a	6.08 ± 0.76 a
Vanillic acid	0.47 ± 0.07 a	0.47 ± 0.02 a	0.48 ± 0.02 a	0.43 ± 0.03 a
Hydroxytyrosol acetate	0.13 ± 0.05 a	0.19 ± 0.02 a	0.30 ± 0.03 a	0.26 ± 0.02 a
3,4-DHPEA-EDA	95.07 ± 12.82 a	84.35 ± 3.50 a	100.87 ± 5.54 a	72.41 ± 8.85 b
Oleuropein aglycone (isomer I)	71.53 ± 8.95 a	66.46 ± 2.57 a	65.11 ± 2.16 a	45.86 ± 2.30 b
<i>p</i> -HPEA-EDA	51.12 ± 6.90 a	44.71 ± 2.85 a	88.94 ± 4.80 a	60.17 ± 9.91 b
Oleuropein + ligstroside aglycones	24.42 ± 2.93 a	21.82 ± 1.33 a	57.37 ± 3.04 a	43.79 ± 5.52 b
Pinoresinol	6.84 ± 1.34 a	6.81 ± 0.57 a	5.37 ± 0.40 a	4.53 ± 0.63 a
Acetoxypinoresinol	9.44 ± 1.48 a	9.36 ± 0.63 a	12.16 ± 0.99 a	10.88 ± 1.48 a
Oleuropein aglycone (isomer II)	28.37 ± 4.43 a	27.60 ± 2.23 a	35.30 ± 2.48 a	27.86 ± 3.25 a
Ligstroside aglycon (isomer III)	9.61 ± 1.99 a	11.14 ± 2.10 a	21.55 ± 0.97 a	15.04 ± 3.08 b
Oleuropein aglycone (isomer III)	5.72 ± 0.86 a	4.70 ± 0.58 a	15.12 ± 0.41 a	12.90 ± 2.04 a
Vanillin	0.17 ± 0.03 a	0.16 ± 0.01 a	0.13 ± 0.01 a	0.12 ± 0.01 a
<i>p</i> -Coumaric acid	1.63 ± 0.24 a	1.63 ± 0.09 a	0.96 ± 0.05 a	0.87 ± 0.07 a

Luteolin	2.78 ± 0.49 a	2.63 ± 0.35 a	3.37 ± 0.60 a	2.97 ± 0.50 a
Apigenin	0.65 ± 0.10 a	0.58 ± 0.09 a	1.00 ± 0.21 a	0.75 ± 0.13 a
Total phenol content	312.43 ± 43.37 a	288.11 ± 17.45 a	416.92 ± 14.16 a	308.09 ± 38.84 b

Results are expressed as mean values ± standard deviation of three independent repetitions. Total phenol concentration was calculated as a sum of all analysed phenols. Mean values labelled with a different letter, within the same row are statistically different (Tukey's test, $p < 0.05$).

After 6-months the concentrations of individual phenols in Istarska bjelica cv. oil remained unchanged. On the other hand, the oil obtained from Buža cv. showed a decrease in some components, mainly in the secoiridoid group of phenols (3,4-DHPEA-EDA, oleuropein aglycones, *p*-HPEA-EDA, ligestroside aglycones) (Table 2). The reason for this diversity in phenol stability may be in the higher initial content of the secoiridoids in Buža oil. This was first reported by Krichene, Salvador, and Fregapane (2015) when comparing four different Tunisian varieties. It is important to note that the secoiridoids were the only group that showed a significant decrease during storage, while simple phenols showed a slight, even not significant, rise in concentration (Figure 1). This was expected due to the transformation process in which complex derivatives of tyrosol and hydroxytyrosol (secoiridoids) are converted into their simple forms (Kotsiou and Tasioula-Margari, 2016).

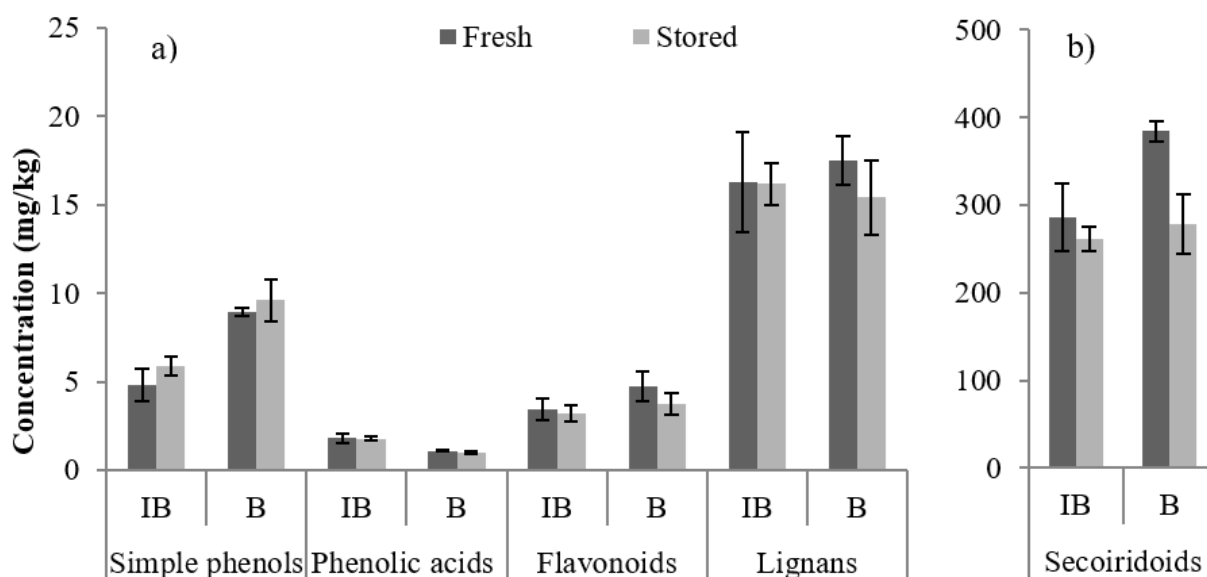


Figure 1. Concentration of main groups of phenols present in fresh Buža (B) and Istarska bjelica (IB) cv. virgin olive oil and after 6-month storage period (stored).

Results are expressed as mean values ± standard deviation of three independent repetitions, calculated as a sum of individual phenols: simple phenols (hydroxytyrosol, tyrosol, vanillin, hydroxytyrosol acetate), phenolic acids (vanillic acid, *p*-coumaric acid), flavonoids (luteolin, apigenin), lignans (pinoresinol, acetoxypinoresinol) (Figure 1a) and secoiridoids (3,4-DHPEA-EDA, oleuropein aglycones, ligestroside aglycones, *p*-HPEA-EDA) (Figure 1b).

*Represents statistically significant difference between fresh and stored monovarietal olive oil samples, for the same group of phenols (Tukey's test, $p < 0.05$).

Conclusions

During the 6-month storage period, Istarska bjelica and Buža monovarietal virgin olive oils have showed good oxidative stability and remained within the extra virgin category based on the quality parameters. Under the applied

storage conditions, total phenol content in Buža oil slightly decreased due to the oxidation of secoiridoid derivatives. On the other hand, Istarska bjelica oil preserved its phenol content during the same storage period. Based on the obtained results it can be concluded that the oxidative stability and evolution of phenolic compounds in virgin olive oil during storage are dependent on the initial phenolic composition of the selected olive cultivar. In this regard, this study contributes to the knowledge useful for predicting the shelf life of Croatian monovarietal olive oils.

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Utjecaj skladištenja na parametre kvalitete i fenolni sastav ekstra djevičanskih maslinovih ulja sorti Istarska bjelica i Buža

Sažetak

Cilj istraživanja bio je utvrditi promijene u parametrima kvalitete i fenolnom sastavu nakon 6 mjeseci skladištenja djevičanskih maslinovih ulja sorti Istarska bjelica i Buža. Rezultati su pokazali kako su ulja dviju sorti različito oksidacijski degradirala u navedenom razdoblju. Ukupni fenolni sastav ulja Istarske bjelice ostao je nepromijenjen nakon skladištenja, dok se u ulju Buže koncentracija ukupnih fenola snizila, ponajviše zbog oksidacije sekoiridoida. Temeljem dobivenih rezultata može se zaključiti da je utjecaj skladištenja na parametre kvalitete i fenolni sastav izravno ovisan o početnom sastavu fenolnih spojeva odabranog sortnog djevičanskog maslinovog ulja.

Ključne riječi: sortno djevičansko maslinovo ulje, parametri kvalitete, fenolni sastav, skladištenje

The strategies for antioxidant enrichment of Buža cv. virgin olive oil

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Abstract

The aim of this study was to investigate two strategies for the improvement of the antioxidant content of Buža cv. virgin olive oil: (i) leaf addition (2.5%) during oil extraction and (ii) prolongation of malaxation duration (from 30 to 45 min). The results showed that the addition of leaves increased the content of pigments in oil, while the total phenolic content slightly decreased. Extended malaxation increased the content of pigments, main groups of phenols, and total phenols. Both strategies could be potentially applied for the antioxidant enrichment of Buža cv. virgin olive oil, but the extended malaxation duration improved its nutritional value in a higher degree.

Keywords: virgin olive oil, antioxidants, malaxation, leaf addition

Introduction

Virgin olive oil (VOO) has nutritional and healthy properties, beside high unsaturated fatty acid composition, mainly associated to biologically active minor components, such as phenols and oil pigments. As well, the higher concentration of antioxidants contributes to VOO stability (Velasco and Dobarganes, 2002). VOO is obtained exclusively by mechanical and physical processes which include collecting and crushing of olive fruits, malaxation of olive paste, centrifugation, decantation or filtration, storage and oil packaging. Different production conditions, such as temperature and duration of malaxation, can modify qualitative and quantitative composition of antioxidants in VOO (Clodoveo, 2012). Olive leaves are by-products of farming of the olive grove, can be found in high amounts in the olive oil industries (up to 10% of the total weight of the olives), and could be considered as a potential antioxidant source of natural origin (Bouaziz et al., 2008) with low cost and high availability (Tabera et al., 2004). Virgin olive oils obtained from fruits of a Croatian autochthonous cultivar Buža often have relatively low phenol content (Škevin et al. 2003), especially if made from fruits in advanced maturity stage, when total phenol, chlorophyll and carotenoid concentrations decrease (Brkić Bubola et al., 2012). Therefore, the aim of this study was to study two strategies for improving the antioxidant content of Buža cv. virgin olive oil obtained from ripe fruits: the addition of olive leaves during oil extraction and prolongation of malaxation duration.

Materials and methods

For this study olive fruits from cv. Buža were selected from the experimental fields of the Institute of Agriculture and Tourism in Poreč, Croatia. The fruits were harvested in an advanced stage of maturity (maturity index 4.04) with olive skin color black and green flesh according to method proposed by Beltran et al. (2004). The VOO samples were extracted using an Abencor laboratory oil mill (MC2 Ingeniería y Sistemas, Sevilla, Spain). For all the samples, fruits without and with the addition of leaves in the rate of 2.5% were crushed by a hammer mill and olive paste was malaxed at $25\pm 1^\circ\text{C}$ for 30 and 45 minutes. The olive paste was centrifuged for 1 minute at 3500 rpm and extracted oil was decanted after natural sedimentation. The oil samples were bottled in 50-mL dark glass bottles and stored at 4°C until analysis. The oil extraction was done in triplicate for each treatment. Phenolic compounds were extracted from VOO samples according to the method based on ultrasound assisted liquid-liquid extraction with methanol reported by Jerman Klen et al. (2015) with some modifications proposed by Lukić et al. (2017). Ten grams of VOO were dissolved in 10 mL of n-hexane, and 5 mL of methanol were added. The mixture was vortexed for 2 min, and then sonicated for 10 min, and centrifuged at 5000 rpm for 5 min. The procedure was repeated 2 more times.

Methanol extracts were unified, and defatted by 3 portions of 10 mL n-hexane. The extracts were evaporated to dryness at 35°C using a vacuum evaporator, the dry residue was re-dissolved in 2 mL of a mixture of HPLC eluents (A(95:5 water:acetic acid (v/v)):B(methanol) = 90:10 (v/v)), and filtered through 0.45-µm PTFE filters (Macherey-Nagel, Düren, Germany). The extract (20 µm) was injected into a HPLC DAD system Agilent Infinity 1260 (Agilent Technologies, Palo Alto, CA, USA) consisting of an autosampler, a pump, a column (PFP, 2.6 µm, 100 mm x 4.6 mm), and a diode array detector - DAD, at 27°C. Identification was performed by comparing retention times and UV/Vis spectra with those of pure standards when available, and with UV/Vis spectra from the literature (Jerma Klen et al., 2015). The standard calibration curves were constructed for tyrosol, hydroxytyrosol, vanilin, vanillic acid, *p*-coumaric acid, luteolin, apigenin, pinosresinol, oleuropein. For other phenol compounds semi-quantitative analysis was applied. Results were expressed as the sums of particular phenols pertaining to the following main groups: simple phenols (hydroxytyrosol, tyrosol, vanillin, hydroxytyrosol acetate), phenolic acids (vanillic acid, *p*-coumaric acid), flavonoids (luteolin, apigenin), lignans (pinosresinol, acetoxypinosresinol), secoiridoids (3,4-DHPEA-EDA, *p*-HPEA-EDA, oleuropein aglycones, ligstroside aglycones) and total phenols. Chlorophylls and carotenoids were determined according to Minguez-Mosquera et al. (1991) using a UV/Vis spectrophotometer (Varian Cary 50, Varian USA). The samples were dissolved in 25 mL cyclohexane and concentration of pigments was measured by spectra from 470 nm to 670 nm. The adsorption was measured for chlorophylls on 670 nm as mean component *pheophytin* also for carotenoids on 470 nm as mean component *lutein*. Statistical data elaboration was performed using Statistica v.13.2 software (Stat-Soft. Inc., Tulsa, OK, USA). The differences among the oil samples obtained with and without leaf addition and by different malaxation durations were tested using two-way ANOVA at 0.1%; 1% and 5% significance levels, and mean values were compared using the Fisher LSD test ($p < 0.001$, $p < 0.01$, $p < 0.05$).

Results and discussion

Influence of leaf addition and extended malaxation on phenolic compounds

Leaf addition during extraction of Buža VOO caused a slight decrease in the concentration of particular groups of phenols, such as phenolic acids, secoiridoids and total phenols, while simple phenols, flavonoids and lignans did not change (Table 1). Some authors reported that leaf addition induced an increase in the phenolic content (Ammar et al., 2017; Tarchoune et al., 2019).

Table 1: Results of two-way analysis of variance (ANOVA) for the concentrations (mg/kg) of the main groups of phenol compounds and total phenols in Buža cv. virgin olive oils, produced with and without leaf addition and by different malaxation durations

	Leaf addition			Malaxation duration			
	no leaf	leaf	Sign. ^a	30 min	45 min	Sign. ^a	Int. ^b
Simple Phenols	10.14	9.00	n.s.	9.79	9.34	n.s.	*
Phenolic acids	3.41	2.92	**	2.95	3.38	*	*
Flavonoids	5.53	5,87	n.s.	4.82	6.59	**	n.s.
Lignans	22.16	19.29	n.s.	19.50	21.95	n.s.	n.s.
Secoiridoids	350.64	284.62	*	258.24	377.03	***	n.s.
TOTAL PHENOLS	391.88	321.70	*	295,29	418.29	***	n.s.

^a Data were analyzed by two-way ANOVA in randomized blocks design (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

^b Interaction between leaf addition and malaxation time (n.s., not significant; *, $p \leq 0.05$).

Decrease in the concentration of some of the main groups of phenols induced by leaf addition could be caused by different factors, including prooxidative activity of metals from leaves (Choe and Min, 2006), or activity of endogenous enzymes such as peroxidase originating from leaf chloroplast (Vierhuis et al., 2001). Extended duration of Buža olive paste malaxation caused an increase in the concentration of almost all the main groups of phenols: phenolic acids, flavonoids, secoiridoids and total phenols (Table 1), while the concentration of lignans did not change. Such

results are not in agreement with other authors who have determined a decrease in phenolic compound content by increasing malaxation duration (Di Giovacchino et al., 2002; Migliorini et al., 2006). The interaction of leaf addition and malaxation duration was significant for simple phenols and phenolic acids, meaning that for these groups of phenols the effects of the investigated factors were dependent on the level of the other factor.

Influence of leaf addition and extended malaxation on pigment content

Chlorophylls and carotenoids concentrations increased by the addition of olive leaf (Table 2), which is in agreement with the results reported by other authors (Di Giovacchino et al., 2002; Choe and Min, 2006). Malheiro et al. (2013) reported that the addition of leaves turned the olive oils greener, which was probably associated to the increase in the pheophytin a content. Being aware that such compounds act as prooxidants in the light, more care in preservation of olive oils produced by the addition of olive leaves should be taken by using adequate bottles and storage in the dark. Extended malaxation duration also influenced the concentration of pigments in Buža oils (Table 2), confirming the results obtained by Inarejos-García et al. (2008) who reported an increase of pigment composition in Cornicabra VOO by extending malaxation duration from 30 to 90 minutes. The interaction of the two factors was significant for both chlorophylls and carotenoids. When leaf is added, longer malaxation time has effect on increase of pigment content (data not showed), probably due to longer contact of olive leaf and oil particles during malaxation of olive paste.

Table 2: Results of two-way analysis of variance (ANOVA) for the concentrations (mg/kg) of pigments in Buža cv. virgin olive oils, obtained with and without leaf addition during extraction and by different malaxation duration

	Leaf addition			Malaxation duration			Int. ^b
	no leaf	leaf	Sign. ^a	30 min	45 min	Sign. ^a	
chlorophylls	1.35	7.27	***	3.92	4.71	***	*
carotenoids	1.73	5.00	***	3.21	3.51	***	*

^a Data were analyzed by two-way ANOVA in randomized blocks design (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

^b Interaction between leaf addition and malaxation time (n.s., not significant; *, $p < 0.05$).

Conclusion

Since virgin olive oils obtained from ripe fruits generally have lower antioxidant content and therefore exhibit shorter shelf life, the application of new strategies for antioxidant and nutritive enrichment of such olive oils are desirable. The obtained results indicate that the strategy which includes the addition of olive leaf during oil extraction has no influence on the main groups of phenols and total phenols, while the influence of the extended malaxation may be significant. Both strategies resulted with an enriched content of pigments in the investigated VOO samples. The application of both the investigated strategies could be potentially useful for the antioxidant enrichment of Buža cv. virgin olive oil; however, the extended malaxation duration improved its nutritional value in a higher degree.

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Strategije antioksidacijskog obogaćivanja djevičanskog maslinovog ulja sorte Buža

Sažetak

Cilj ovog rada bio je istražiti dvije strategije za poboljšanje sadržaja antioksidanasa u djevičanskom maslinovom ulju sorte Buža: (i) dodavanje lišća (2,5%) tijekom ekstrakcije ulja i (ii) produženje trajanja malaksacije (od 30 do 45 min). Rezultati su pokazali da je dodavanje lišća povećalo sadržaj pigmenata u ulju, dok se sadržaj ukupnih fenola neznatno smanjio. Produljena malaksacija povećala je sadržaj pigmenata, te glavnih skupina fenola i ukupnih fenola. Obje strategije potencijalno bi se mogle primijeniti za antioksidacijsko obogaćivanje djevičanskog maslinovog ulje sorte Buža, međutim produljeno trajanje malaksacije je poboljšalo njegovu hranjivu vrijednost u većoj mjeri.

Ključne riječi: djevičansko maslinovo ulje, antioksidansi, malaksacija, dodatak lišća

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Sažetak

U Hrvatskoj proizvodnja ekološkog voća nije osobito razvijena, ali ima veliku budućnost u globalnoj poljoprivredi. Cilj rada bio je utvrditi preferencije dijela potrošača Požeško-slavonske županije o konzumaciji ekološkog voća. U tu svrhu provedena je anketa na uzorku od 104 nasumično izabranih ispitanika. Anketa se sastojala od 25 pitanja, a provedena je aplikacijom „Google obrasci“. Dobiveni rezultati pokazali su da su ispitanici uglavnom upoznati sa terminom ekološki uzgojeno voće, ali gotovo polovica njih smatra da je ponuda ekološki uzgojenog voća na tržištu nedovoljno dostupna. Ispitanici uglavnom smatraju da je ekološki uzgojeno voće zdravije, a jedna trećina se slaže da je cijena ekoloških proizvoda prihvatljiva. Ispitanici žele imati više informacija o ekološki uzgojenom voću, te misle da će ekološki uzgojeno voće imati budućnost u globalnoj poljoprivredi.

Ključne riječi: ekološko voće, anketa, potrošači, preferencije, Požeško –slavonska županija

Uvod

Ekološka poljoprivreda se zasniva na korištenju obnovljivih resursa i nekorištenju kemikalija u proizvodnji hrane. Republika Hrvatska ulazi u skupinu rijetkih europskih zemalja u kojima je ekološka poljoprivreda još uvijek slabo i nedovoljno razvijena. Prirodni resursi za razvoj ekološke poljoprivrede nedvojbeno postoje, no nažalost postoje i brojni ograničavajući čimbenici. Potražnja ekoloških proizvoda premašuje proizvodnju čak i u doba recesije, tako da zemlje Europske unije uvoze eko-proizvode (Batelja Lodeta i sur., 2011). Ekološka poljoprivreda drži se globalno usvojenih načela, te se uklapa u koncept održivog razvoja pri čemu teži ekološki čistoj i gospodarski isplativoj poljoprivrednoj proizvodnji. Ekološka je proizvodnja sustav upravljanja poljoprivrednim gospodarstvima i proizvodnjom hrane koja je najbolja u zaštiti okoliša, očuvanja prirodnih resursa, dobrobit životinja i proizvodne metode koje su prikladne s obzirom na to da neki potrošači prednost daju proizvodima uz primjenu prirodnih tvari i procesa. (Šnajder, 2015). Sve je više proizvoda koji se deklariraju kao bio, organsko, zeleno, stoga je potrošačima teško odabrati onaj proizvod kojem mogu vjerovati. Od ukupnih površina u ekološkoj poljoprivredi u Republici Hrvatskoj samo 9% čine voćnjaci, od čega su najzastupljeniji nasadi jabuka, krušaka, trešanja i šljiva. Razlog tome je nedovoljno razvijena tržišna infrastruktura, neodgovarajuća organiziranost tržišta ekoloških proizvoda te nedostatak odgovarajućih znanja i vještina. Kao razvojni potencijali se ističu mladi educirani ljudi, ljudi zainteresirani za ekološku poljoprivredu i sve veći broj zainteresiranih i osviještenih potrošača za ekološke proizvode (Megla, 2017)

Dorotić (2016) smatra da Hrvatsko tržište ekoloških proizvoda još nije dovoljno organizirano zbog nedostatnosti proizvoda, ograničenih preradbenih kapaciteta i nedovoljne informiranosti potrošača, a cijena ekoloških proizvoda je, u odnosu na konvencionalne, veća za 20 – 50 %.

Pokos Nemec, (2012), navodi da ekološko voće, uz povrće ima najbolju perspektivu u sveukupnoj ekološkoj poljoprivredi jer je ekološka svijest ljudi upravo najveća kod voća jer se ono u većini slučajeva unosi u organizam izravno bez prerade. U Hrvatskoj proizvodnja ekološkog voća je u svojim počecima, ali ima veliku budućnost u globalnoj poljoprivredi. Konzumacija ekološkog voća još nije općeprihvaćena jer potrošači nemaju dovoljno informacija o ekološkom voću i ekološkoj poljoprivredi (Brčić Stipčević i Petljak, 2011). Tržište ekološke hrane u Hrvatskoj je slabije razvijeno, a da bi povećali potrošnju potrebno je educirati potrošače o prednostima ekološkog voća u odnosu na konvencionalno proizvedeno. Naravno da bi još više ekološko voće razvijalo potrebno je smanjiti maloprodajnu cijenu i povećati njegovu dostupnost.

Materijal i metode

U istraživanju je korištena metoda ispitivanja, a podaci o preferencijama potrošača o ekološkom voću su prikupljeni anketnim ispitivanjem. Anketno ispitivanje potrošača je provedeno na uzorku od 104 slučajno odabranih ispitanika. Ispitivanje je provedeno pomoću strukturiranog anketnog upitnika koji se sastojao od 3 skupina pitanja. Istraživanje o konzumaciji ekološkog voća provedeno je na Veleučilištu u Požegi, a odvijalo se od 5. do 15. lipnja 2019. godine. U istraživanju su sudjelovali nasumično izabrani ispitanici, a provedeno je metodom ankete u aplikaciji „Google obrasci“. Anketa se sastojala od 25 pitanja. Prvi dio ankete obuhvaćao je pitanja o sociodemografskim obilježjima ispitanika, a pitanja iz tog djela vezana su za spol, dob, mjesto življenja, stručnu sprema i vrstu zaposlenja. Drugi dio ankete sadržavao je pitanja o ponašanju potrošača pri kupnji i potrošnji eko voća, a pitanja su bila: gdje najčešće kupujete plodove voća?, koliko često konzumirate plodove voća?, koje plodove voća najčešće konzumirate? i sl.

Treći dio ankete činilo je 16 pitanja vezanih za ekološko voće, npr. da li ste upoznati s terminom ekološki uzgojeno voće; konzumirate li ekološki uzgojenu hranu; želite li imati više informacija o ekološkom voću; mislite li da ekološki uzgojeno voće ima budućnost u globalnoj poljoprivredi i sl.

Rezultati i rasprava

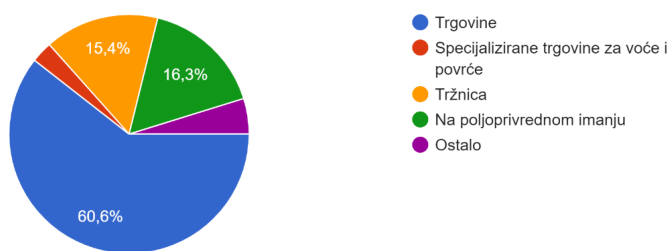
Anketi je pristupilo 104 ispitanika od čega je 69 (66,3%) bilo žena te 35 (33,7%) muškaraca. U provedenoj anketi sudjelovale su osobe različite dobi. Najviše je bilo ispitanika u dobi od 18 do 25 godina (49%), zatim od 35 do 55 ili više godina (27,9%). Ispitanika u dobi od 25 do 35 godina bilo je 22,1%, a najmanje ispitanika bilo je u dobi do 18 godina, samo 1%. 57,7% ispitanika stanovali su u gradskim područjima, dok je 42,3% bilo sa sela. Najviše ispitanika (52,9%) bilo je sa srednjom stručnom spremom, nešto manje (43,3%) sa visokom te samo (3,8%) sa osnovnom. Zaposlenih je bilo najviše (61,5%), zatim su slijedili studenti i učenici kojih je bilo 24%, a značajno manje je bilo nezaposlenih (9,6%) te umirovljenika (4,8%).

Ponašanje potrošača u kupnji i potrošnji voća

Od svih anketiranih najviše ispitanika kupovinu obavlja u trgovinama 60,6%, značajno manje izravno od proizvođača na OPGu 16,3% i tržnicama 15,4% (grafikon 1.)

Gdje najčešće kupujete plodove voća:

104 odgovora

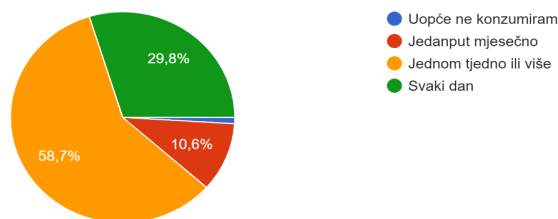


Grafikon 1. Mjesta kupovine voća

Najviše ispitanika konzumira voće jednom ili više puta tjedno (58,7%). 29,8% ispitanika konzumira voće svaki dan. 10,6% konzumira plodove voća jedanput mjesečno, a 1% uopće ne konzumira plodove voća (grafikon 2.)

Koliko često konzumirate plodove voća:

104 odgovora

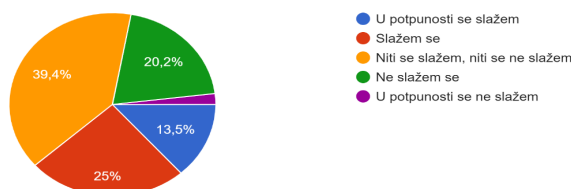


Grafikon 2. Učestalost konzumiranja plodova voća

Najviše se konzumiraju, i to u jednakim postotcima, banana i jabuka 37,5%. Pri kupnji voća ispitanicima je najvažnije kvaliteta (52,9%), a zatim cijena (21,2%), a tek onda slijedi oblik i boja ploda (14,4%) te vrsta i sorta (11,5%). Prema anketi 87,5 % ispitanika upoznato je sa terminom ekološki uzgojeno voće, ali 46,2 % ispitanika smatra da je ponuda ekološki uzgojenog voća na tržištu nedovoljno dostupna. Da su ispitanici dosta dobro educirani pokazuje i podatak da 89,4 % ispitanika smatra da je ekološki uzgojeno voće zdravije, dok ih samo 10,6% smatra da nije. To je u suprotnosti sa istraživanjem Brčić Stipčević i Petljak (2011) koje navode da potrošači u Republici Hrvatskoj još uvijek nisu u potpunosti upoznati s pojmom eko proizvedene hrane, a polovica ispitanika izjavila da nikada ne kupuje organsku hranu. Najveći broj ispitanika (38,5%) se slaže da je cijena ekoloških proizvoda prihvatljiva, dok se 22,1 % ne slaže (grafikon 3.). I u istraživanju Padel i Foster, (2005), glavni motivi za kupnju ekološki uzgojene hrane su povezani sa zdravljem, ali, kao glavni razlog zbog kojeg ispitanici ne kupuju ekološki uzgojenu navode njezinu visoku cijenu.

Mislim da je cijena ekoloških proizvoda u skladu s vrijednostima koje ti proizvodi imaju:

104 odgovora



Grafikon 3. Analiza cijene u skladu s vrijednostima.

Čak 74% ispitanika želi imati više informacija o ekološki uzgojenom voću, dok ih 26% ne želi. Zanimljivo je da čak je 80,8% ispitanika misli da će ekološki uzgojeno voće imati budućnost u globalnoj poljoprivredi 19,2% ispitanika misli da neće.

Zaključak

Ekološki uzgojeno voće i njegova kupnja posljednjih godina doseže veću popularnost kod potrošača nego prije. Većinom je uvriježeno mišljenje da je ono zdravije od konvencionalnog. Nakon provedenog istraživanja na uzorku od 104 stanovnika Požeško – slavonske županije utvrđeno je da najviše ispitanika konzumira plodove voća jednom ili više puta tjedno te im je pri kupnji voća najvažnija kvaliteta. Ispitanici su vrlo dobro upoznati sa terminom ekološki uzgojeno voće što ukazuje na dobru informiranost iako žele imati više informacija o ekološki uzgojenom voću. Ispitanici su dobro educirani i većinom smatraju da je ekološki uzgojeno voće zdravije. Najveći broj ispitanika se slaže da je cijena ekoloških proizvoda u skladu s vrijednostima koje oni imaju te da će ekološki uzgojeno voće imati budućnost u globalnoj poljoprivredi. Dobiveni podaci ukazuju na velike mogućnosti ekološkog uzgoja voća i plasmana na tržište.

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Consumer preferences on Organic Fruit Consumption in Požega Slavonia County

Abstract

Organic fruit production in Croatia is not particularly developed, but it has a great future in global agriculture. The paper aims to determine the views of consumers of Požega-Slavonia County on the consumption of organic fruits. For this purpose, a survey was conducted on a sample of 104 randomly selected respondents. The survey consisted of 25 questions and was conducted by the Google Forms application. The results show that 87.5% of the respondents are familiar with the term organic fruit, but 46.2% of the respondents believe that the supply of organic fruit on the market is not readily available. 89.4% of respondents believe that organic fruits are healthy and 38.5% agree that the price of organic products is in line with the values they have. 74% of respondents want to have more information about organic fruits, and as many as 80.8% think that organic fruits will have a future in global agriculture.

Keywords: organic fruits, survey, consumers, preferences, Požega Slavonia County

Biološki potencijal ploda kivija s različitih lokacija

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Sažetak

Cilj ovog rada bio je utvrditi osnovne fizikalno-kemijske parametre i biološki potencijal svježih plodova kivija sorte 'Hayward' s različitih lokacija (Sisk, Galdovo, Čapljina, otok Murter i Rovinj). Analizom uzoraka utvrđene su značajne razlike u sadržaju bioaktivnih spojeva plodova ubranih na pet navedenih lokacija. U uzorcima uzgojenim na otoku Murteru utvrđen je značajno veći sadržaj vitamina C (103,14 mg/100 g svježe tvari) u odnosu na plodove s ostalih istraživanih lokacija. S obzirom na dobivene rezultate može se zaključiti da je plod kivija bogat izvor različitih bioaktivnih spojeva značajnog antioksidacijskog djelovanja. Kvaliteta samog ploda kivija značajno ovisi o lokaciji uzgoja.

Ključne riječi: vitamin C, ukupni fenoli, antioksidacijski kapacitet, kemijski sastav, plod kivija

Uvod

U posljednje vrijeme povećan je interes za konzumacijom ploda kivija zbog njegove značajne nutritivne vrijednosti kao i biološkog potencijala (Li i sur. 2018). Jedan od najznačajnijih nutrijenata kada je riječ o plodu kivija je vitamin C. Nishiyama i sur. (2004) u svom istraživanju navode za sortu 'Hayward' sadržaj vitamina C od 65,5 mg/100 g svježe tvari. Ako se to usporedi sa sadržajem vitamina C u naranči ili jabuci može se zaključiti da sorta 'Hayward' sadrži čak 50 % više vitamina od naranče ili čak 10 puta više od jabuke. Karakteristično svjetlo zeleno obojenje mezokarpa kivija rezultat je prisutnosti pigmenata klorofila koji dokazano pokazuju značajnu antitumorsku, ali i antioksidacijsku aktivnost zbog čega su vrlo važni za zdravlje ljudi (Pal i sur. 2015). Prema drugim literaturnim navodima prosječne vrijednosti klorofila a i klorofila b u plodu kivija kreću se u rasponu od 1,7-1,9 i 0,9-1,0 mg/100 g, te od 0,6-0,8 i 0,2-0,4 mg/100 g (Li i sur. 2018), dok sadržaj ukupnih karotenoida između 0,6 i 0,8 mg/100 g (Gross, 1982). I upravo zbog vrlo dobrog nutritivnog sastava kivi se posljednjih godina sve više uvodi u prehranu (Singletary, 2012). Prirodni je antioksidans, antitrombotik, antipiretik, diuretik i sedativ. Kao dio zdrave prehrane može smanjiti koncentraciju triglicerida u krvi, agregaciju trombocita i povišeni krvni tlak (Stonehouse i sur., 2012). Danas znanost preporučuje konzumaciju dva do tri ploda kivija kao dobru preventivu za očuvanje kardiovaskularnog sustava ili kao zamjenu za aspirin. Istraživanja su pokazala da je svakodnevno konzumiranje barem dva ploda kivija otprilike sat vremena prije spavanja dobro za kvalitetan san, a što se prvenstveno može pripisati visokoj razini antioksidansa i serotonina zastupljenih u plodu kivija (Li i sur. 2018). Kemijski sastav ovisi o nizu čimbenika među kojima se ističu sortiment, klimatski uvjeti, pedološke osobine, primijenjene agrotehničke mjere, stupanj zrelosti i dr. Stoga je cilj ovog rada bio utvrditi osnovne fizikalno-kemijske parametre i biološki potencijal svježih plodova kivija sorte 'Hayward' s različitih lokacija.

Materijal i metode

Istraživanje je provedeno na plodovima kivija sorte 'Hayward' uzgojenim na području Siska, Galdova, Čapljine, otoka Murtera i Rovinja. Lokacije su izabrane slučajnim odabirom da se općenito dobije bolji uvid u sastav plodova kivija koji raste u RH. Plodovi su ubrani u optimalnom roku berbe, krajem listopada. Odmah nakon ubiranja prikupljeni plodovi (prosječno 20 plodova za svaku lokaciju) dopremljeni su u laboratorij gdje su skladišteni 12 dana u tamnoj prostoriji pri temperaturi od 13 °C i relativnoj vlažnosti zraka od 86 %. Za svaku lokaciju uzete su po tri repeticije, a analizirana su slijedeća svojstva kvalitete ploda: suha tvar (%), ukupna kiselost (%), topljiva suha tvar (%), pH vrijednost (AOAC, 1995), sadržaj vitamina C (mg/100 g svježe tvari) titrimetrijski s 2,6-p-diklorindofenolom (AOAC, 2002), ukupni fenoli, flavonoidi i neflavonoidi (mg GAE/100 g svježe tvari) spektrofotometrijski (Ough i Amerine,

1988), ukupni klorofili i karotenoidi ($\mu\text{g/g}$) spektrofotometrijski (Holm, 1954; Wettstein, 1957) te antioksidacijski kapacitet ($\mu\text{mol TE/L}$) ABTS metodom (Miller i sur., 1993; Re i sur., 1999). Dobiveni rezultati statistički su obrađeni u programu SAS/STAT verzija 9.3 (2010). Korišten je Duncanov test signifikantnosti razlika (1 %).

Rezultati i rasprava

U Tablici 1 prikazan je fizikalno-kemijski parametri plodova kivija sorte 'Hayward' s različitih lokacija. Između svih istraživanih parametara utvrđene su visoko signifikantne statističke razlike. Sadržaj suhe tvari kretao se od 14,03 % u plodovima s područja Murtera do 17,58 % u plodovima s područja Rovinja. Topljiva suha tvar bila je najveća u uzorcima s područja Galdova i iznosila je 13,57 %. Rezultati ukupne kiselosti također su se značajno razlikovali, od najmanje utvrđene vrijednosti (1,53 %) u plodovima s područja Galdova do najveće (1,94 %) u plodovima s područja otoka Murtera. Najveću vrijednost omjera topljive suhe tvari i kiselosti (TST/UK) imali su uzorci s područja Galdova (9,01), dok je najmanja (4,92) određena u uzorcima s područja otoka Murtera. pH vrijednosti bile su u rasponu od 3,07 (Murter) do 3,34 (Galdovo). Temeljem svih istraživanih fizikalno-kemijskih parametara u plodovima kivija ubranih na pet različitih lokacija utvrđene su značajne razlike, a što ukazuje na značajan utjecaj geografskog područja i klimatskih uvjeta na samu kvalitetu ploda. Popović (1990) navodi da prosječna vrijednost suhe tvari ploda kivija iznosi oko 14,35 %, dok su u ovom istraživanju utvrđene veće vrijednosti osim za polodove s područja otoka Murtera (14,03 %). Dobiveni rezultati za navedena svojstva suhe tvari, ukupnih kiselina i pH vrijednosti pokazuju malo veće vrijednosti od onih koje navodi Popović (1990) u svom istraživanju.

Tablica 1. Fizikalno-kemijski parametri plodova kivija sorte 'Hayward's različitih lokacija

Lokacija	Suha tvar (%) ***	TST (%) ***	Ukupne kiseline (%) ***	TST/UK ***	pH ***
Sisak	15,75 ^c ±0,06	11,63 ^b ±0,06	1,54 ^{cb} ±0,13	7,57 ^b ±0,63	3,31 ^b ±0,02
Galdovo	16,76 ^b ±0,20	13,57 ^a ±0,29	1,53 ^c ±0,01	9,01 ^a ±0,16	3,34 ^a ±0,01
Čapljina	14,42 ^d ±0,05	11,70 ^b ±0,01	1,64 ^b ±0,02	7,12 ^b ±0,09	3,36 ^a ±0,01
Murter	14,03 ^e ±0,17	9,57 ^d ±0,40	1,94 ^a ±0,01	4,92 ^d ±0,20	3,07 ^d ±0,02
Rovinj	17,58 ^a ±0,26	10,77 ^c ±0,06	1,91 ^a ±0,01	5,64 ^c ±0,06	3,14 ^c ±0,01

***- $p \leq 0,0001$, različita slova pridodana prosječnim vrijednostima upućuju na značajne razlike između sorata po pojedinom kemijskom parametru ploda nakon provedenoga *t* testa. TST/UK označava omjer topljive suhe tvari i ukupne kiselosti

U Tablici 2 prikazani su rezultati analize bioaktivnih spojeva i antioksidacijskog kapaciteta plodova kivija sorte 'Hayward' s različitih lokacija. Vitamin C je vrlo važan antioksidacijski spoj. Esencijalna je tvar te ju ljudsko tijelo ne može samostalno sintetizirati već je mora unijeti hranom (Li i sur. 2018). Sadržaj vitamina C kod sorte 'Hayward' s pet različitih lokacija kretao se u rasponu od 54,13 mg/100 g svježe tvari (Rovinj) do 103,14 mg/100 g svježe tvari (Murter) što ukazuje na značajnu varijaciju vitamina C s obzirom na područje uzgoja, odnosno na geografske i klimatske uvjete koji su specifični za svaki geografski položaj. Literaturni podaci također ukazuju na razlike u vrijednostima vitamina C. Nishiyama i sur. (2004) u svom radu navode da najčešće komercijalno raspoloživa sorta kivija 'Hayward' sadrži oko 65,5 mg/100 g svježe tvari vitamina C, dok Celik i sur. (2007) u svom istraživanju navode vrijednosti vitamina C za navedenu sortu od 108 mg/100 g svježe tvari. Sadržaj askorbinske kiseline može biti pod utjecajem različitih čimbenika kao što su sortiment, klimatski uvjeti prije branja, zrelost i načini branja, kao i postupci poslije branja (Lee i Kader, 2000). Sadržaj ukupnih fenola kretao se u rasponu od 92,30 (Rovinj) do 140,28 mg GAE/100 g svježe tvari (Galdovo), ukupnih flavonoida od 52,85 (Čapljina) do 80,18 mgGAE/100 g svježe tvari (Murter) i sadržaj ukupnih neflavonoida od 38,67 (Rovinj) do 64,88 mgGAE/100 g svježe tvari (Galdovo). Za sve navedene istraživane fenolne spojeve utvrđene su visoko signifikantne statističke razlike ($p \leq 0,0001$) s obzirom na područje uzgoja plodova. Kod sorte 'Hayward' s područja Galdova zabilježen je najveći sadržaj ukupnih fenola (140,28 mg GAE/100 g svježe tvari) i ukupnih neflavonoida (64,88 mg GAE/100 g svježe tvari). Najmanji sadržaj ukupnih fenola (92,30 mg GAE/100 g svježe tvari), ali i ukupnih neflavonoida (38,67 mg GAE/100 g svježe tvari)

zabilježen je kod sorte 'Hayward' s područja Rovinja što ukazuje na signifikantne razlike u sadržaju fenolnih spojeva s obzirom na utjecaj geografskog područja. Pal i sur. (2015) su također utvrdili više vrijednosti ukupnih fenola i niže vrijednosti ukupnih flavonoida u usporedbi s rezultatima ovog istraživanja.

Tablica 2. Sadržaj bioaktivnih spojeva i antioksidacijski kapacitet plodova kivija sorte 'Hayward' s različitih lokacija

Lokacija	Vitamin C (mg/100g)	Ukupni fenoli (mgGAE/100 g)	Ukupni flavonoidi (mgGAE/100 g)	Ukupni neflv. (mgGAE/100g)	Antioksid. kapacitet (μ molTE/L)
	***	***	***	***	***
Sisak	69,60 ^b ±1,59	111,06 ^c ±0,60	62,52 ^c ±0,61	48,54 ^c ±0,31	870,03 ^d ±14,25
Galdovo	66,24 ^c ±2,19	140,28 ^a ±1,27	75,30 ^b ±1,77	64,88 ^a ±0,78	1727,43 ^c ±4,82
Čapljina	62,66 ^d ±1,31	93,83 ^d ±1,00	52,85 ^d ±0,94	40,98 ^d ±0,08	871.37 ^d ±12,28
Murter	103,14 ^a ±0,21	134,49 ^b ±1,11	80,18 ^a ±1,56	54,11 ^b ±0,32	2025.53 ^a ±4,30
Rovinj	54,13 ^e ±0,66	92,30 ^b ±0,79	53,63 ^d ±0,99	38,67 ^e ±0,96	1996.29 ^b ±2,51

** - $p \leq 0,001$; NS - nije signifikantno, različita slova pridodana prosječnim vrijednostima upućuju na značajne razlike između sorata po pojedinom kemijskom parametru ploda nakon provedenoga t testa.

Antioksidacijski spojevi predstavljaju inhibitore oksidacijskih procesa u organizmu koji mogu dovesti do razvoja kroničnih bolesti kao što su bolesti krvožilnog sustava ili karcinoma (Li i sur. 2018). Antioksidansi su ključni za zdravlje i normalno djelovanje ljudskog organizma. Istraživanja su pokazala kako antioksidansi, poput vitamina C, fenolnih kiselina, karotenoida, antocijana i drugih spojeva, pozitivno utječu na ljudsko zdravlje (Istrati i sur., 2013). Antioksidacijski kapacitet uzoraka plodova kivija kretao se u rasponu od 870,03 μ mol TE/L do 2025,53 μ mol TE/L (Tablica 2). Najveće vrijednosti antioksidacijskog kapaciteta (2025,53 μ mol TE/L) utvrđene su kod plodova prikupljenih s područja Murtera, dok je najmanji (870,03 μ mol TE/L) utvrđen kod plodova s područja Siska. Dobivene vrijednosti veće su od onih koje navodi Drummond (2013) osim kod plodova s područja Siska i Čapljine gdje su dobivene manje vrijednosti. No kao i za sve istraživane parametre odstupanja od literaturnih vrijednosti mogu biti uzrokovane pedo-klimatskim faktorima.

Tablica 3. Sadržaj pigmentnih spojeva plodova kivija sorte 'Hayward' s različitih lokacija

Lokacija	Klorofil A (μ g/g)	Klorofil B (μ g/g)	Ukupni klorofili (μ g/g)	Karotenoidi (μ g/g)
	***	**	***	**
Sisak	5,30 ^c ±0,15	6,35 ^b ±0,37	11,65 ^b ±0,53	2,50 ^c ±0,07
Galdovo	5,84 ^b ±0,44	5,33 ^c ±0,81	11,74 ^b ±1,63	3,34 ^b ±0,31
Čapljina	8,09 ^a ±0,28	8,18 ^a ±0,49	16,27 ^a ±0,76	4,72 ^a ±0,11
Murter	5,53 ^{cb} ±0,15	6,10 ^{cb} ±0,27	11,75 ^b ±0,27	3,08 ^b ±0,04
Rovinj	3,91 ^d ±0,03	5,47 ^c ±0,07	9,40 ^c ±0,05	3,34 ^b ±0,04

** - $p \leq 0,001$; *** - $p \leq 0,0001$, različita slova pridodana prosječnim vrijednostima upućuju na značajne razlike između sorata po pojedinom kemijskom parametru ploda nakon provedenoga t testa.

Vrijednosti ukupnih klorofila, klorofila a, klorofila b i karotenoida u plodovima kivija prikazane su u Tablici 3. Iz dobivenih rezultata vidljivo je da su utvrđene signifikantne razlike između plodova prikupljenih s različitih uzgojnih područja. Vrijednosti analiziranih pigmentnih spojeva značajno variraju ovisno o lokaciji. Najveći sadržaj ukupnih klorofila (8,09 μ g/g) i karotenoida (4,72 μ g/g) utvrđen je kod plodova prikupljenih s lokacije Čapljina, dok je najmanji sadržaj ukupnih klorofila (9,40 μ g/g) utvrđen kod plodova prikupljenih s lokacije Rovinj, a karotenoida (3,08 μ g/g) s područja Murtera. Dobiveni rezultati manji su od onih koje navodi Li i sur. (2018). Razlog utvrđenih varijacija sadržaja istraživanih pigmentnih spojeva su karakteristike geografskog područja.

Zaključak

Područje uzgoja, važan je čimbenik utjecaja na kemijska svojstva kao i sadržaj bioaktivnih spojeva ploda kivija. Tako je najveći sadržaj ukupne suhe tvari utvrđen u plodovima s područja Rovinja, dok najveći sadržaj topljive suhe tvari i ukupnih fenola u plodovima s područja Galdova. Najveći sadržaj ukupnih kiselina, vitamina C te antioksidacijskog kapaciteta utvrđen je kod plodova s područja Murtera. U sadržaju pigmentih spojeva (klorofila i karotenoida) istaknuli su se plodovi s područja Čapljine. Zbog izuzetne nutritivne vrijednosti ploda značenje ove voćne vrste postaje sve veće. U Hrvatskoj kivi još uvijek nije dovoljno iskorištena voćna vrsta, dok se u svijetu sve češće upotrebljava i dobiva na vrijednosti.

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Biological potential of kiwi fruit from different locations

Abstract

The aim of this study was to determine the nutritional composition and specialized metabolites content of fresh kiwifruit from different locations (Sisk, Galdovo, Čapljina, otok Murter i Rovinj). By analysis of nutritional composition significant differences in the content of bioactive compounds of the same variety from different locations were determined. In samples from island Murter significantly higher vitamin C content (103,14 mg/100 g FW) was determined compared to the samples of kiwi fruit from other studied locations. Based on the obtained results can be concluded that kiwi fruit is a rich source of various bioactive components with significant antioxidant activity. The nutritional quality of kiwi fruit significantly depends on the cultivation location.

Keywords: vitamin C, total phenols, antioxidant capacity, chemical composition, kiwi fruit

First record of the honeysuckle whitefly *Aleyrodes lonicerae* (Hemiptera: Aleyrodidae) in Bosnia and Herzegovina and its incidence on cultivated strawberry

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Abstract

The honeysuckle whitefly *Aleyrodes lonicerae* (Hemiptera: Aleyrodidae) is a newly recorded species in the whitefly fauna of Bosnia and Herzegovina. The species was identified for the first time on cultivated strawberry (*Fragaria x ananassa* Duchesne) in 2015 at Donja Papratnica site. During 2016 specimen was found in total of 16 strawberry plantations with highest number at the sites of Donja Papratnica (Zenica-Doboj Canton) and Rauševac (Central Bosnia Canton). Strawberry is an important commercial crop in Bosnia and Herzegovina and a new pest records, such as honeysuckle whitefly, are of importance for strawberry production.

Keywords: Aleyrodidae, *Aleyrodes lonicerae*, Bosnia and Herzegovina, *Fragaria x ananassa*, first record

Introduction

Aleyrodes lonicerae Walker, 1852 (syn. *fragariae* Walker; *rubi* Signoret), (Hemiptera: Aleyrodidae), the honeysuckle whitefly is agricultural pest with a wide geographical distribution (Evans, 2007). It is an exceptionally polyphagous species found on a variety of host plants from more than 18 different families (Mound and Halsey, 1978; Lee *et al.*, 2005; Šimala, 2008). The species exhibits a preference to herbaceous and woody host plants from *Caprifoliaceae*, *Oxalidaceae*, *Ericaceae*, *Papaveraceae*, *Urticaceae*, *Violaceae* and *Rosaceae* families (Martin *et al.*, 2000; Evans, 2007). In normal circumstances, honeysuckle whitefly does not cause significant damage to host plant foliage, but in cases of strong attacks, foliage may become affected by sooty mould fungi deposits, which decrease plant transpiration and photosynthesis functions. Damage by this insect is most significant in regions with a warm climate (OEPP/EPPO, 1996). Honeysuckle whitefly may cause damages to strawberry in extensive cultivation practice (Malumphy, 2010; Petrova *et al.*, 2013). Plantations overgrown with weeds with high air humidity are favorable for the pest (Maceljski, 2002). The honeysuckle whitefly is an insect that causes direct and indirect damage to strawberry. By sucking strawberry plant sap, adults and larvae of honeysuckle whitefly directly decrease plant productivity, which reflects on the quantity and quality of yield. Heavily infested plants show a loss of vigour. Indirect damage is caused by copious excretion of honeydew that covers surfaces of leaves, and may also contaminate the fruit. The honeydew is colonized by sooty mould fungus (*Capnodium* spp.) whose presence on strawberry plant surfaces interferes with physiological processes in the plant and impairs its aesthetic appearance. As other whitefly species, *A. lonicerae* has six developmental stages: egg, crawler (1st instar), two sessile nymphal instars (2nd and 3rd instars), the puparium (4th instar) and adult. During spring and summer females lay eggs one by one on the lower surfaces of leaves. The nymphs feed for several weeks, passing through three instars before pupating. In summer, adults emerge shortly afterwards, about 2 months after eggs were laid. There are several overlapping generations in a season and, since adults are long-lived, all stages of insect may occur together. This pest overwinters as adult or as pseudo-pupae (Alford, 2014). Honeysuckle whitefly adults have a light yellow body in varying degrees covered by white wax dust; wings are white with a faint grey curved line in the lower portion of the forewings (Stocks, 2012). Eggs are oblong, sized 0.27 × 0.1

mm, yellowish white in color and thickly covered with wax. Puparium is oval, varying in color from straw-yellow to yellow-greenish. Marginally it has very short waxy projections. Puparium is 900-1200 µm long and 600-900 µm wide. Puparium has a slightly wavy edge. Marginal setae on anterior and posterior margins are considerably long. Submarginal area of puparium is wrinkled. Dorsal disk is with long setae whose number and length depend on the host plant on which the species develop. A morphological feature of this species is rounded sculptures (median abdominal tubercles) situated on the central part of each second through seventh abdominal segment. Vasiform orifice is triangular in shape and distinctly toothed on the interior side of lateral fringe. Operculum is trapezoidal in shape and does not cover lingula, whose tip is clearly visible. Two long setae, projected from vasiform orifice, are situated on lingula tip. Caudal furrow is present, but underdeveloped. Caudal setae are very thick and long (Šimala, 2008).

Materials and methods

During the visual survey in August 2015 we collected samples of strawberry (*Fragaria x ananassa* Duchesne) leaves whose lower side hosted various whitefly instars. The sample was taken at Donja Papratnica site in Žepče Municipality. A magnifying lens of 20 times magnification was used for easier identification of sessile whitefly instars. Leaves with sessile stages of whitefly were collected in the middle of August. Adult stages of whitefly were sampled directly from surfaces of leaves using an aspirator. The leaf samples were placed and stored by dry method in an envelope (Martin, 1999). Examination of the samples and identification of the species were carried out at the Department of Plant Protection, Faculty of Agriculture and Food Technology University of Mostar. Genus and species identification was conducted based on morphological characteristics of puparium, using conventional identification methods and relevant morphological identification keys according to Martin *et al.* (2000). For an accurate identification a stereomicroscope (Leica EZ4D) and a compound microscope (Motic BA310) were employed. After identification of honeysuckle whitefly in Bosnia and Herzegovina we have done monitoring and sampling at locations with intensive strawberry production. Species monitoring included four sites in Žepče Municipality, (Zenica-Doboj Canton) during September 2015. During 2016 additional three cantons were included in monitoring (Central Bosnia Canton, Herzegovina-Neretva Canton and West Herzegovina Canton). At sites where we found honeysuckle whitefly, population density was also determined. We have taken randomly 100 plants per field. Number of adult insects per plant was visually surveyed early in the morning while they were idle. In order to survey sessile instars, we have also collected randomly 100 leaves per site, and stored them in paper bags with GPS coordinates and site data. We analyzed leaves under stereo microscope in laboratory. Sessile instars population density is shown as average number per trifoliolate leaf.

Results and discussion

Honeysuckle whitefly was found for the first time in Bosnia and Herzegovina in August 2015 at Donja Papratnica (44°25'47.93"; 17°59'17.78") Žepče Municipality (Zenica- Doboj Canton). By visual survey we identified adults and instars of honeysuckle whitefly on open field grown strawberries on black plastic mulch. After first finding, we included three additional sites (Donje Ravne, Matina and Lupoglav) in the Žepče Municipality during September 2015. Insects were recorded on all three of them. Highest average number of 2.88 sessile instars per trifoliolate leaf and 1.53 adults per plant were recorded at Donja Papratnica location. There were no significant differences among sites in number of pest, although lower number is recorded on three other sites. Sites, coordinates, population density in 2015 are given in Table 1.

First record of the honeysuckle whitefly *Aleyrodes loniceræ* (Hemiptera: Aleyrodidae) in Bosnia and Herzegovina and its incidence on cultivated strawberry

Table 1. Strawberry field locations monitored in 2015 with average numbers of honeysuckle whitefly (*Aleyrodes loniceræ*) sessile instars and adults

Canton	Municipality	Site	Coordinate	Average number per leaf/plant	
				Sessile instars	Adults
Zenica- Doboj Canton	Žepče	Donja Papratnica	44°25'47.93" 17°59'17.78"	2.88	1.53
		Donje Ravne	44°25'46.53" 18°00'52.84"	1.13	0.58
		Matina	44°28'55.92" 18°02'33.31"	0.86	0.32
		Lupoglav	44°26'18.77"	0.92	0.27
			18°03'52.73"		

Along with sites in Zenica – Doboj Canton we had widen research area in 2016 with three more cantons, Central Bosnia Canton, Herzegovina-Neretva Canton and West Herzegovina Canton. Research took part during August on 20 sites in open field strawberry production. We found pest in Central Bosnia Canton on Rauševac and Višnjica sites. Average number of pest was higher at Rauševac with 1.75 sessile instars per trifoliolate leaf and 1.28 adults per plant compared to Višnjica with 0.76 sessile instars and 0.47 adults. In Herzegovina – Neretva Canton we recorded honeysuckle whitefly at four sites in Mostar and Čitluk Municipalities. Highest average number was 0.35 sessile instars per leaf and 0.18 adults per strawberry plant at site Bašaga in Čitluk Municipality. Hodbina and Ševaš Njive sites were free of pest. We monitored in the West Herzegovina Canton eight sites in Ljubuški Municipality. Honeysuckle whitefly was found on six. We recorded highest average number in Veljaci with 0.27 sessile instars per trifoliolate leaf and 0.17 adults per plant. On two sites, Grab and Vašarovići, we did not record pest presence. We also noted minor damages due to honeydew secretion and sooty moulds on individual strawberry plants at Donja Papratnica (Zenica-Doboj Canton) and Rauševac (Central Bosnia Canton). Locations with honeysuckle whitefly and population density during 2016 were shown in Table 2.

Table 2. Monitored locations with average number of honeysuckle whitefly (*Aleyrodes loniceræ*) during 2016 season

Canton	Municipality	Site	Coordinate	Average number per leaf/plant	
				Sessile instars	Adults
Zenica-Doboj Canton	Žepče	Donja Papratnica	44°25'47.93" 17°59'17.78"	3.21	1.87
		Donje Ravne	44°25'46.53" 18°00'52.84"	1.87	0.79
		Matina	44°28'55.92" 18°02'33.31"	0.91	0.38
		Lupoglav	44°26'18.77"	1.12	0.78
			18°03'52.73"		
Central Bosnia	Kiseljak	Rauševac	43°59'55.39" 18°02'35.55"	1.75	1.28
		Višnjica	43°57'18.29"	0.76	0.47
			18°03'01.41"		

Herzegovina-Neretva Canton	Mostar	Blagaj I	43°15'14.94" 17°52'48.21"	0.20	0.10	
		Blagaj II	43°15'03.22" 17°52'14.41"	0.29	0.13	
	Čitluk	Hodbina	43°14'03.10" 17°50'56.44"	Pest presence not recorded		
		Blizanci	43°12'13.20" 17°44'50.04"	0.19	0.06	
		Bašaga	43°13'04.20" 17°40'42.64"	0.35	0.18	
	Čapljina	Ševaš Njive	43°09'11.80" 17°44'40.03"	Pest presence not recorded		
		Veljaci I	43°14'20.33" 17°25'47.41"	0.27	0.17	
	West Herzegovina Canton	Ljubuški	Veljaci II	43°14'31.90" 17°25'32.34"	0.21	0.12
			Šipovača I	43°14'53.64" 17°24'43.51"	0.10	0.06
		Ljubuški	Šipovača II	43°14'59.94" 17°24'42.04"	0.19	0.14
Vojnići			43°15'03.91" 17°26'53.04"	0.14	0.10	
Orahovlje		Orahovlje	43°13'11.91" 17°25'52.34"	0.13	0.05	
		Grab	43°12'18.01" 17°27'05.90"	Pest presence not recorded		
		Vašarovići	43°12'14.25" 17°28'52.74"	Pest presence not recorded		

Conclusions

Since we had recorded honeysuckle whitefly at more location in different parts of Bosnia and Herzegovina we concluded that this pest had been here for some years prior. Distribution of this species in other parts of the country along with different host plants should be surveyed in the following years. Although the recorded population of this pest is rather small, whitefly could become an important pest of strawberry in this area, since its increasing popularity among producers. This is also influenced by the fact that strawberry is still largely produced in an extensive way in the open field. The importance of honeysuckle whitefly is also supported by the fact that this is a highly polyphagous pest that can survive on a large number of host plants, which can be a constant source of infestation for strawberry production. Determination of new whitefly species in Bosnia and Herzegovina is of biological importance as well since its influence on entomofauna composition in the area. This also contributes to the knowledge of Aleyrodoidea superfamily diversity which has not been researched till today in Bosnia and Herzegovina.

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Prvi nalaz i intenzitet pojave jagodinog štitaštog moljca *Aleyrodes lonicerae* (Hemiptera: Aleyrodidae) na kultiviranoj jagodi u Bosni i Hercegovini

Sažetak

Jagodin štitašti moljac *Aleyrodes lonicerae* (Hemiptera: Aleyrodidae) novo je zabilježena vrsta u fauni štitaštih moljaca Bosne i Hercegovine. Vrsta je prvi puta utvrđena 2015. godine vizualnim pregledom listova jagode (*Fragaria x ananassa* Duchesne) na lokalitetu Donja Papratnica. Tijekom 2016. godine vrsta je utvrđena na 16 lokaliteta, a najveća brojnost populacije zabilježena je na lokalitetima Donja Papratnica (Zeničko-dobojska županija) i Rauševac (Srednjobosanska županija). Jagoda je komercijalno važna vrsta jagodastog voća u Bosni i Hercegovini te je pronalazak novog štetnika od velikog značaja za proizvodnju ove voćne kulture.

Ključne riječi: Aleyrodidae, Bosna i Hercegovina, *Aleyrodes lonicerae*, *Fragaria x ananassa*, prvi nalaz

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Utjecaj različitih polihidričnih alkohola na utekućenje trave *Miscanthus x giganteus*

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Sažetak

U radu su istražena svojstva utekućenja trave *Miscanthus x giganteus* (MxG) metodom utekućenja s polihidričnim alkoholima uz prisustvo sumporne kiseline (H₂SO₄) kao kiselog katalizatora pod točno određenim uvjetima utekućenja. Kao polihidrični alkoholi korišteni su polietilen glikol 400, dietilen glikol, etilen glikol i glicerol. Postotak netopljivog ostatka, postotak utekućenosti i hidroksilni broj određeni su kao vrijednosti koje označavaju svojstva utekućenja. Postotak netopljivog ostatka za različite polihidrične alkohole iznosio je od 1,18-17,45%, postotak utekućenosti od 82,25-98,82% te hidroksilni broj od 265-458 mg KOH/g. Kao najbolje otapalo utekućenja trave MxG pokazao se je glicerol.

Ključne riječi: *Miscanthus x giganteus*, polihidrični alkoholi, utekućena biomasa, netopivi ostatak, postotak utekućenosti, hidroksilni broj

Uvod

Trava *Miscanthus x giganteus* (MxG) je vrsta obnovljive agrokulturne biomase koja danas privlači sve više pažnje kao jedna od sirovina za široku lepezu iskorištavanja u proizvodnji različitih vrsta bioproizvoda te koja može djeomično zamjeniti fosilne izvore (Bilandžija, 2014). MxG je višegodišnja C₄ trava (zbog načina na koji skladišti ugljikov dioksid tijekom fotosinteze te pod kojim uvjetima raste) s odlikama koje ju svrstavaju u obećavajuću vrstu za proizvodnju bioproizvoda, posjeduje visok proizvodni potencijal i ekološki je vrlo prihvatljiva vrsta jer uspješno raste uz vrlo male količine pesticida i gnojiva (Lewandowski i sur., 2000). MxG, kao i sve ostale lignocelulozne biljke, građena je od kemijskih komponenata koji su po svojem sastavu visokomolekularni prirodni polimeri te tvore isprepletenu mrežu u staničnoj stijenci. U Tablici 1 je prikazan grupni kemijski sastav trave MxG u ovisnosti o različitim veličinama čestica uzoraka (Antonović i sur., 2019).

Tablica 1. Kemijski sastav trave MxG u ovisnosti o veličini čestica uzorka

Kemijska komponenta		Granulacija (mm)		
		1,19 – 2,00	0,71 – 1,19	0,50 – 0,71
Pepeo	%	1,67	1,99	2,40
Akcesorne tvari	%	0,98	0,96	1,26
Holoceluloza	%	66,27	66,57	67,56
Celuloza	%	51,34	51,31	50,69
Hemiceluloza	%	14,93	15,26	16,87
Lignin	%	31,09	30,48	28,77

Uobičajene metode koje se koriste u proizvodnji bioproizvoda (biogoriva, bioenergija, biokemikalije i biomaterijali) iz biomase su biokemijske i termokemijske pretvorbene metode (Krička i sur., 2017). Od termokemijskih metoda, kao

relativno nova metoda, imamo utekućenje biomase, koja pretvara iste u biorazgradive prirodne polimerne materijale i na taj način povećava njeno iskorištenje. Utekućenje drva možemo provesti s fenolom i ona rezultira produktima utekućenja koji su bogati fenolnim jedinicama te polihidričnim alkoholima čiji se produkti mogu koristiti kao polioli. Nadalje, postupak utekućenja je jedina metoda koja može u jednakim uvjetima utekućiti sve glavne kemijske komponente biomase, pri čemu se te komponente kemijski derivatiziraju i otapaju te pretvaraju u reaktivne molekule što ih čini pogodnim za daljnju sintezu u različite bioproizvode (Antonović i sur., 2012). Utekućena trava MxG pokazala je vrlo visoke vrijednosti hidroksilnog broja što je pretpostavka za moguću daljnju primjenu u različitim vrstama polimerizacija, na što se nadovezuje vrlo povoljan postotak netopivog ostatka i postotak utekućenosti bez obzira na veličinu čestica uzoraka. U usporedbi s prethodnim istraživanjima, utekućena trava MxG pokazala se je prihvatljivom za daljnju primjenu u različitim bioproizvodima na bazi utekućene biomase (Antonović i sur., 2019).

Kao otapalo utekućenja mogu se koristiti različite vrste polihidričnih alkohola koji različito utječu na svojstva utekućene različite biomase. Osim polihidričnih alkohola, na svojstva utječu različite vrste i količine katalizatora, omjer biomasa/otapalo, temperatura i vrijeme utekućenja. U nastavku, utekućena biomasa se može koristiti u proizvodnji različitih bioproizvoda. Najviše pozornosti pridano je primjeni utekućenog drva u pripremi i modifikaciji fenol-formaldehidnih smola rezolnog i novolačnog tipa, karbamid-formaldehidnih smola, poliuretanskih smola i pjena, izocijanatnih i epoksidnih smola, te njihovoj daljnjoj uporabi u novim vrstama materijala, koji imaju potencijalnu uporabu u različitim industrijama (Antonović i sur., 2011).

Kao nastavak istraživanja utekućenja i kemijske karakterizacije utekućene trave MxG, u ovom radu istraživano je utjecaj različitih polihidričnih alkohola (PEG400, DEG, EG i GLI) na svojstva utekućenja (postotak netopljivog ostatka, postotak utekućenosti i OH-broj) metodom kiselih katalizatora (H₂SO₄) pod točno definiranim uvjetima utekućenja.

Materijali i metode

Pokusno polje MxG postavljeno je na pokušalištu Agronomskog fakulteta Sveučilišta u Zagrebu u Šašinovcu (N 45° 51' 01.32"; E 16° 10' 35.85"). Istraživana kultura je posađena krajem travnja 2016. godine, a kao sadni materijal korištene su presadnice, dobivene mikropropagacijom. Uzorkovanje trave za potrebe daljnjeg laboratorijskog istraživanja provedena je krajem listopada 2018. godine. Uzorci MxG su usitnjeni u mlinu za usitnjavanje i prosijani na dimenzije čestice uzoraka 0,50-0,71 mm koje smo koristili dalje u postupku utekućenja.

Utekućena trava MxG pripremljena je na temelju prethodnih istraživanja (Antonović i sur., 2019). Kao reakcijske reagense utekućenja koristili smo polietilen glikol 400 (PEG400), dietilen glikol (DEG), etilen glikol (EG) i glicerol (GLI) kao podvrste polihidričnih alkohola, a sumporna kiselina rabljena je kao kiseli katalizator. Utekućenje se provodilo u reaktoru volumena 500 mL, opremljenim hladilom i mehaničkom miješalicom, s poklopcem na kojemu su bila četiri brušena grla. Postupak je tekao tako da je uzorak stavljen u reaktor i pomiješan s alkoholom u omjeru 1:5 te je dodana sumporna kiselina (3% na ukupnu masu alkohola). Reakcijska je smjesa utekućivana na 150°C kroz 120 min uz pomoć električnog grijača podržanoga termostatom.

Netopivi ostatak utekućene trave MxG nakon reakcije utekućenja određen je uz pomoć smjese dioksan/voda, koja se preporučuje kao univerzalno otapalo za utekućenu lignocelulozu ili biomasu (Antonović i sur., 2010). Utekućena je trava razrijeđena smjesom dioksan/voda u omjeru 8/2, te miješano magnetskom miješalicom 60 minuta. Nakon toga smjesa je filtrirana kroz B2 stakleni filtarski lončić na vakuumskoj boci. Ostatak je također više puta ispiran smjesom dioksan/voda, sve dok nije dobiven bezbojni filtrat. Potom je netopivi ostatak osušen u sušioniku na 103±2 °C do konstantne mase. Postotak netopljivog ostatka utekućenog drva određen je prema izrazu:

$$\text{Netopljivi ostatak} = \frac{\text{masa netopljivog ostatka}}{\text{masa utekućene trave MxG}} \quad (\%)$$

Postotak utekućenosti određen je prema izrazu:

$$\text{Utekućenost} = 100 - \text{Netopljivi ostatak} \quad (\%)$$

Hidroksilni broj (OH-broj) određen je prema ASTM normi D 4274-05. U dvije Erlenmeyerove tikvice od 250 mL odvađeno je 1,5-2,5 g utekućene trave MxG i dodano 10 mL reagensa. Reagens je bila smjesa piridina i anhidrida ftalne kiseline. U treću je tikvicu odmjeren samo reagens radi određivanja slijepe probe. Svaka je tikvica bila opremljena hladilom i magnetnom miješalicom s grijanjem. Na magnetnim su se miješalicama nalazile uljne kupelji uz pomoć kojih je održavana konstantna temperatura od 115 °C, a uz pomoć hladila kondenziran je reagens. Smjesa u tikvici zagrijavana je točno 60 minuta, mjereno od trenutka pojave prve kapi kondenzata reagensa. Nakon toga je ohlađenoj smjesi kroz hladilo dodano novih 50 mL piridina, te je obavljena titracija s 0,5 N natrijevim hidroksidom (NaOH), uz prisutnost fenolftaleina do ekvivalentne točke (nije se smjelo barem 30 sekundi pojaviti svjetlocrveno obojenje). Hidroksilni broj u mg KOH/g uzorka utekućenog drva određen je prema izrazu:

$$\text{OH-broj} = \frac{(B-A) \times c_{\text{NaOH}} \times 56,1}{m} \quad (\text{mg KOH/g})$$

gdje je: A – volumen NaOH upotrijebljenog za titraciju uzorka (mL); B – volumen NaOH upotrijebljenog za titraciju slijepe probe (mL); c_{NaOH} – molaritet NaOH (M); m – masa uzorka utekućene trave.

Rezultati i rasprava

U ovom radu istraživana je utjecaj različitih vrsta polihidričnih alkohola (PEG400, DEG, EG i GLI) na svojstva utekućenja kao što su postotak netopljivog ostatka, postotak utekućenosti i hidroksilni OH-broj a rezultati su prikazani u Tablici 1 i Slici 1. Svi rezultati su prikazani kao aritmetičke sredine i standardne devijacije pet mjerenja te je provedena analiza signifikantnosti (p-test i t-test za $p < .05$) u ANOVA programu.

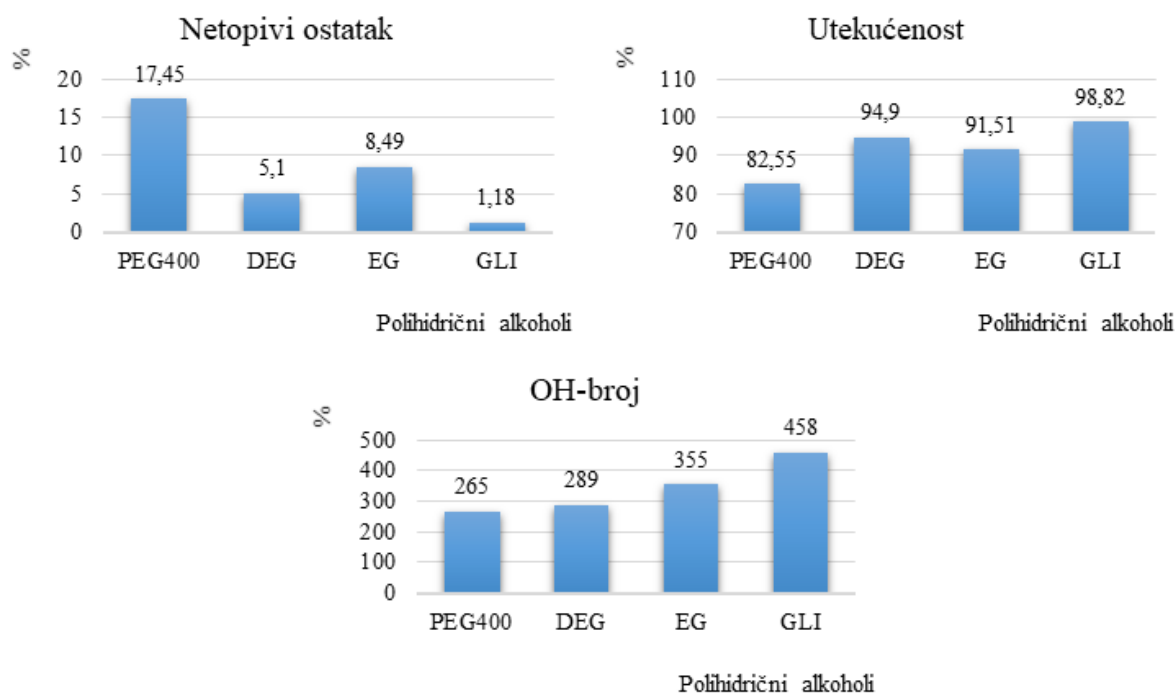
Tablica 2. Svojstva utekućene trave MxG u ovisnosti o različitim polihidričnim alkoholima

Polihidrični alkoholi	Svojstvo utekućenja					
	Netopivi ostatak		Utekućenost		OH-broj	
	\bar{x}	σ	\bar{x}	σ	\bar{x}	σ
PEG400	17,446	0,1139	82,554	0,1139	265	3,8079
DEG	5,100	0,0815	94,900	0,0815	289	5,4314
EG	8,494	0,1016	91,506	0,1016	355	6,8920
GLI	1,178	0,0476	98,822	0,0476	458	6,9642

\bar{x} – aritmetička sredina; σ – standardna devijacija

Hidroksilni broj je vrlo važna vrijednost utekućene biomase koja nam govori o njenoj sposobnosti u daljnjoj sintezi u različite biopolimere. Što je taj broj veći to je sposobnost vezivanja utekućene biomase uspješnija u daljnjim sintezama. Stoga vrijednost OH-broja ima prednost pred ostalim svojstvima utekućenja, kao što su postotak netopljivog ostatka i postotak utekućenosti. Postotak netopljivog ostatka za različite polihidrične alkohole iznosio je od 1,18 – 17,45%, postotak utekućenosti od 82,55 – 98,82% te hidroksilni broj od 265 – 458 mg KOH/g.

ANOVA analizom (p-test i t-test) utvrđeno je da nema signifikantne razlike unutar grupe rezultata pojedinih svojstava utekućenja za određeni alkohol. Međutim, signifikantnost je utvrđena kod svih svojstava utekućenja kada se usporede različite vrste alkohola. PEG400 pokazao je najlošija svojstva od svih istraživanih polihidričnih alkohola s najvećim postotkom netopivog ostatka (17,45), najmanjim postotkom utekućenosti (82,55) i OH-broja (265 mg KOH/g). Iako utekućeni MxG s PEG 400 od svih polihidričnih alkohola pokazuje povećani postotak netopivog ostatka i smanjeni postotak utekućenosti, bez obzira na to i dalje ima vrijednost OH-broja dovoljan za uspješnu polimerizaciju. Za njim slijede ostali polihidrični alkoholi (DEG, EG i GLI) s još boljim svojstvima. Na temelju prikazanih rezultata, kao najbolje otapalo utekućenja MxG pokazao se je glicerol (GLI) s najmanjim postotkom netopivog ostatka (1,18%), najvećim postotkom utekućenosti (98,82%) i najvećom vrijednošću OH-broja (458 mg KOH/g). Slične rezultate pokazala su i istraživanja na drugim vrstama biomase. Usporedbom s prijašnjim istraživanjima na drugim vrstama biomase (Antonović i sur., 2011), utekućena trava MxG pokazala je dovoljne vrijednosti svojstava utekućenja za daljnju primjenu.



Slika 1. Svojstva utekućene trave MxG u ovisnosti o različitim polihidričnim alkoholima

Nadalje, na temelju prijašnjih istraživanja, bolja svojstva utekućenja MxG mogu se dobiti mješanjem PEG400, DEG i EG s GLI u različitim omjerima te primjenom različitih katalizatora, temperature i vremena utekućenja pri čemu se ta svojstva najviše očituju u povećanju hidroksilnog broja što je i krajnji cilj. Stoga, u daljnjim istraživanjima utjecaja različitih polihidričnih alkohola na svojstva utekućenja trave MxG potrebno je provesti navedene radnje u postupku utekućenja.

Zaključak

Prema vrijednosti OH-broja kao funkcionalne skupine prirodnih komponenata trave MxG nastalih tijekom priključivanja različitih polihidričnih alkohola (PEG400, DEG, EG i GLI) koje smo aktivirali postupkom utekućenja prema istim i točno određenim uvjetima dobiveni su produkti utekućenja s povoljnim svojstvima za daljnje sinteze u različite bioproizvode. OH-broj kao najvažnije svojstvo utekućenja u daljnjoj primjeni ima prioritet ispred ostalih svojstava te prema tome glicerol (GLI) se je pokazao kao najbolje otapalo utekućenja. Postupak utekućenja s različitim polihidričnim alkoholima metodom kiselih katalizatora pokazao se je prigodnim za utekućenje trave MxG, uz mogućnost međusobnog mješanja u različitim omjerima s ciljem poboljšanja svojstava utekućenja.

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Influence of various polyhydric alcohols on the liquefaction of grass *Miscanthus x giganteus*

Abstract

The paper study the liquefaction properties of *Miscanthus x giganteus* (MxG) grass by the liquefaction method with different polyhydric alcohols in the presence of sulfuric acid (H₂SO₄) as an acid catalyst under specific liquefaction conditions. As polyhydric alcohols, polyethylene glycol 400, diethylene glycol, ethylene glycol and glycerol were used. The percentage of insoluble residue, the liquefaction percentage and the hydroxyl number were determined as values indicating the properties of the liquefaction. The percentage of insoluble residue for various polyhydric alcohols was 1.18 – 17.45%, the liquefaction percentage was 82.2 – 98.82% and the hydroxyl number was 265 – 458 mg KOH/g. Glycerol proved to be the best solvent for liquefying grass MxG.

Keywords: *Miscanthus x giganteus*, polyhydric alcohols, liquefied biomass, insoluble residue, liquefaction percentage, hydroxyl number

Arundo donax L. kao sirovina u biorafinerijskom procesu

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Sažetak

Cilj rada je istražiti energetska svojstva kulture *Arundo donax* L. te definirati mogućnost korištenja u biorafinerijskom procesu. Pepeo uz vlagu predstavlja temeljnu komponentu negorivih tvari biomase, a udio od 3% koji je nešto viši nego kod šumske biomase i dalje je niži od onoga kod većine poljoprivredne biomase. Od gorivih tvari najznačajniji su ugljik i sumpor, a utvrđene vrijednosti od 46% i 0,3% ukazuju na kvalitetu sirovine. Strukturni sastav upućuje na mogućnost korištenja u biorafinerijskom procesu, ali i u proizvodnji krutih biogoriva. Donja ogrjevna vrijednost kretala se oko 16 MJ kg⁻¹. Može se zaključiti da je *Arundo donax* L. kvalitetan alternativni izvor energije.

Ključne riječi: *Arundo donax* L., energetska iskoristivost, lignocelulozni sastav, gorive tvari, negorive tvari

Uvod

Zbog sve većih zabrinutosti koje proizlaze iz ovisnosti o fosilnim resursima, vrlo je poželjno proučavanje alternativnih izvora sirovina koji se temelje na biološkoj osnovi (Cherubini, 2010). Posljednjih godina biomasa druge generacije, odn. lignocelulozna biomasa pojavljuje se kao obnovljivi izvor zbog svoje godišnje obnovljivosti i velike godišnje količine biomase (Menon i Rao, 2012). Konkretno, višegodišnje rizomske trave dobivaju sve veći interes za održivu proizvodnju biomase zbog visokog potencijala prinosa, male potražnje i pozitivnog utjecaja u okoliš koji je povezan sa smanjenjem emisije CO₂ (Basso et al. 2006). Među raznim višegodišnjim travama identificiran je i *Arundo donax* L., uobičajenog naziva obična trska, koji ispunjava zahtjeve visokih iskorištenja biomase te posjeduje odgovarajuće karakteristike biomase za procese energetskog iskorištenja (Lewandowski i sur., 2003). Smatra se da je *Arundo donax* L. podrijetlom iz Azije, ali se smatra i autohtonom vrstom u regijama koje okružuju Sredozemno more. Ova biljka se širi širom svijeta, jer podnosi široku paletu ekoloških uvjeta, vrlo brzo raste i lako se razmnožava. Spada u C3 biljke, s visokom uspravnom stabljikom, koja pripada obitelji Gramineae (*Poaceae*) i jedna je od najvećih trava i vrlo je invazivna (Basso et al. 2006).

Ipak, kako bi se iskoristile različite komponente biomase i povećala dodana vrijednost, potrebno je provesti frakcioniranje lignoceluloze u tri glavne komponente, odnosno celulozu, hemiceluloze i lignin (Barana i sur., 2016). Navedeno frakcioniranje predstavlja temelj biorafinerijskog procesa i sastoji se od integriranog sustava pretvorbe za proizvodnju biogoriva, energije, topline i/ili kemikalija s dodanom vrijednošću iz biomase (Menon i Rao, 2012). Mnoga su istraživanja usmjerena na ekstrakciju potencijalno visoko vrijednim komponentama iz lignocelulozne biomase, poput lignina (Salanti i sur., 2010), nanokristalične celuloze (Johar i sur., 2012), silicijevog dioksida (Liou, 2004) i fermentiranog šećera za proizvodnju bioetanol (Lemons e Silva i sur., 2015).

Kako bi se prikazala kvaliteta biomase koriste se mnoga svojstva od kojih su najznačajnija negoriva i goriva svojstva, a najvažnije svojstvo za određivanje kvalitete je sadržaj kemijskih veza koje nose energiju, odnosno najzastupljeniji krajnji elementi (dušik, ugljik, sumpor, vodik i kisik) i sadržaj ukupnog pepela (Tao i sur. 2012). Korištenje biomase kao goriva prilikom proizvodnje toplinske i električne energije zahtijeva znanje o njezinoj ogrjevnoj vrijednosti (Krička i sur., 2017). Ogrjevna vrijednost je veličina za određivanje sadržaja energije u gorivu (Jenkins i sur., 1998). Često se izražava kao gornja i donja ogrjevna vrijednost. Biomasa je složena heterogena smjesa strukturnih organskih komponenti. Karakterizacija tih komponenti (kvalitativna i kvantitativna) u biomasi je bitna za određivanje daljnje prerade biomase (Antonović i sur., 2016) te razumjevanje mehanizma reakcije prilikom proizvodnje biogoriva.

Stoga je cilj ovog rada istražiti energetska svojstva kulture *Arundo donax* L. (goriva i negoriva svojstva, ogrjevnu vrijednost, kao i lignocelulozni sastav) te definirati mogućnost njenog korištenja u biorafinerijskom procesu.

Materijal i metode

Istraživanja su provedena na brzorastućoj energetskoj kulturi *Arundo donax* L. uzgojenoj na području grada Zagreba. Žetva je obavljena u veljači 2019. godine. Istraživanja energetske svojstava provedena su na Sveučilištu u Zagrebu Agronomskom fakultetu, na Zavodu za poljoprivrednu tehnologiju, skladištenje i transport, u Laboratoriju za istraživanje biomase i energetske iskoristivost u poljoprivredi. Istraživana su negoriva i goriva svojstva, ogrjevna vrijednost te lignocelulozni sastav. Kod toga su korištene metode za udio vlage, odnosno suhe tvari (HRN EN 18134-2:2015) u laboratorijskoj sušnici (INKO, Hrvatska), udio dušika (HRN EN ISO 16948:2015) na elementarnom analizatoru Vario MACRO (Elementar, Njemačka), udio pepela (HRN EN ISO 18122:2015) te udio koksa (EN 15148:2009) u mufolnoj peći (Nabertherm, SAD) i udio fiksiranog ugljika (EN 15148:2009) računski. Nadalje, udio ugljika i vodika (HRN EN ISO 16948:2015) te sumpora (HRN EN ISO 16994:2015) na na CHNS analizatoru (Elementar, Njemačka), dok se kisik izračunao računski kao ostatak C, H, N, S elemenata, kao i udio hlapljivih tvari sukladno EN 15148:2009. Gornja ogrjevna vrijednost određena je pomoću metode HRN EN 14918:2010 u adijabatskom kalorimetru (IKA, Njemačka), dok se donja ogrjevna vrijednost dobije računski. Određivanje udjela celuloze, hemiceluloze i lignina provedeno je modificiranom standardnom metodom (ISO 5351-1:2002).

Rezultati i rasprava

Energetska svojstva biomase definiraju se preko negorivih i gorivih tvari, lignoceluloznog sastava te ogrjevne vrijednosti.

U tablici 1 prikazane su negorive tvari energetske kulture *Arundo donax* L..

Tablica 1. Negorive tvari

PARAMETRI ANALIZE	<i>Arundo donax</i> L.
Vlaga (%)	10,23
Pepeo (%)	3,26
Fiksirani ugljik (%)	11,97
Koks (%)	14,65
Dušik (%)	0,69

Pepeo uvjetuje kvalitetu biogoriva te što je njegov sadržaj veći gorivo je lošije kvalitete. Sadržaj pepela kod kulture *Arundo donax* L. kretao se oko 3% što je znatno niži sadržaj od sadržaja pepela (5%) kojeg navodi Jegurimu i sur. (2010), ali ipak je viša od standarda šumske biomase koja iznosi maksimalno 2%. Ukoliko je sadržaj koksa i fiksiranog ugljika veći gorivo je kvalitetnije. Tako sadržaj fiksiranog ugljika kod *Arundo donax* L. iznosi 11,97% što je niža vrijednost od 18,4%, što navode Jegurim i sur. (2010).

U tablici 2 prikazane su gorive tvari energetske kulture *Arundo donax* L..

Tablica 2. Gorive tvari

PARAMETRI ANALIZE	<i>Arundo donax</i> L.
Ugljik (%)	46,38
Vodik (%)	6,19
Sumpor (%)	0,26
Kisik (%)	47,17
Hlapiva tvar (%)	75,12

Ugljik i vodik, kao elementi koji povećavaju ogrjevnu vrijednost biomase pojavljuju se u visokom postotku. Kod kulture *Arundo donax* L vrijednosti ugljika i vodika kod Licursi i sur. (2015) iznosile su za ugljik 44,8% te za vodik 6,0% što je u suglasju s dobivenim rezultatima. Sadržaj sumpora, štetnog elementa biomase u istraživanoj kulturi kretao se oko 0,26% što je u skladu s literaturnim navodima (Jegurim i sur., 2010). Kisik smanjuje ogrjevnu vrijednost biomase i pojavljuje se u velikom postotku što je također usporedivo s literaturnim navodima (Licursi i sur., 2015). *Arundo donax* L. pripada drugoj generaciji sirovina za proizvodnju biogoriva i ubrajaja se u tzv. lignoceluloznu biomasu. U tablici 3 prikazan je strukturalni, odnosno lignocelulozni sastav energetske kulture *Arundo donax* L.

Tablica 3. Lignocelulozni sastav

PARAMETRI ANALIZE	<i>Arundo donax</i> L.
Celuloza (%)	42,91
Hemiceluloza (%)	13,64
Lignin (%)	34,12

Dobiveni rezultati lignoceluloznog sastava, s obzirom na veći sadržaj celuloze i hemiceluloze, upućuju na mogućnost korištenja energetske kulture *Arundo donax* L. u biorafinerijskom procesu. Također, dobiven je i veći sadržaj lignina koji upućuje na mogućnost korištenja istraživane kulture u proizvodnji krutog biogoriva.

Svi prethodno navedeni parametri imaju utjecaj na ogrjevnu vrijednost biomase, stoga je u tablici 4 prikazana gornja i donja ogrjevna vrijednost istraživane energetske kulture *Arundo donax* L.

Tablica 4. Ogrjevna vrijednost

PARAMETRI ANALIZE	<i>Arundo donax</i> L.
Gornja ogrjevna vrijednost (MJ kg ⁻¹)	17,61
Donja ogrjevna vrijednost (MJ kg ⁻¹)	16,26

Iz Tablice 4 uočava se kako istraživana energetska kultura *Arundo donax* L. ima gornju ogrjevnu vrijednost oko 17,6 MJ kg⁻¹, što je vidljivo i kod Jegurim i sur. (2010) koji su dobili gornju ogrjevnu vrijednost 17,2 MJ kg⁻¹, dok Dahl i Obernberger (2004) navode višu gornju ogrjevnu vrijednost od 19,8 MJ kg⁻¹, ali s obzirom da agrotehnika i godina uzgoja neposredno utječu na ogrjevnu vrijednost biomase rezultati su u suglasju.

Zaključak

Temeljem vlastitih istraživanja mogućnosti korištenja biomase kulture *Arundo donax* L. u biorafinerijskom procesu, mogu se utvrditi kvalitetna energetska svojstva s obzirom na sadržaj negorivih tvari. Naime, pepeo koji najviše uvjetuje kvalitetu biogoriva kretao se relativno nisko u odnosu na ostalu poljoprivrednu biomasu. Gorive tvari takođe pokazuju dobra energetska svojstva, s obzirom na visok sadržaj ugljika i vodika koji povećavaju ogrjevnu vrijednost. Također, količina sumpora bila je relativno niska. Omjer celuloze, hemiceluloze i lignina ukazuje na povećani udio celuloze i hemiceluloze, što rezultira prijedlogom da se *Arundo donax* L. može koristiti u biorafinerijskom procesu, ali i za proizvodnju krutih biogoriva.

Napomena

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Arundo donax L. as raw material in the biorefinery process

Abstract

The aim of this study is to investigate the energy properties of *Arundo donax* L. and to define its use in the biorefinery process. Ash with moisture is a fundamental component of the non-combustible matter and a 3% share is slightly larger than in forest biomass but still better than in the most agricultural biomass. Of the combustible matter the most significant components are carbon and sulfur, and the established values of 46% and 0.3% indicate the quality of the raw material. The structural composition indicates the potential for use in the biorefinery process but also in the production of solid biofuels. The lower heating value was about 16 MJ kg⁻¹. It can be concluded that the *Arundo donax* L. is a quality alternative energy source..

Keywords: *Arundo donax* L., energy utilization, lignocellulosic composition, combustible matter, non-combustible matter

Biokompoziti kao proizvodi u biogospodarstvu

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Sažetak

Biogospodarstvo predstavlja zatvoreni sustav u kojem se otpad i nusproizvodi ponovno koriste kao izvor sirovine te se na taj način smanjuje utjecaj na okoliš. Poljoprivredna biomasa, kao sirovina za proizvodnju energije i prirodnih vlakana osobito je zanimljiva zbog svoje dostupnosti, obnovljivosti i prihvatljivosti. U posljednje vrijeme mogućnosti korištenja biomase kao novog izvora sirovine za proizvodnju ekološki prihvatljivih biokompozita, kemikalija i smola znatno su se povećale. U skladu s načelima biogospodarstva, poljoprivredni otpad ima velik potencijal postati važan izvor sirovine za proizvodnju biokompozita.

Ključne riječi: biogospodarstvo, biokompoziti, biomasa

Uvod

Poljoprivreda je kao gospodarska grana vrlo važna za ukupan razvoj pojedinih zemalja i regija, ali istovremeno može negativno utjecati na stanje okoliša. U poljoprivrednoj proizvodnji ostaju velike količine neiskorištene biomase, odnosno, više od 50% od ukupne biomase ne sakuplja se i ne koristi. Poljoprivredni otpad dostupan je u većim količinama kako u EU, tako i u RH te stoga postoji potencijal da postane izvor obnovljivih sirovina. Poljoprivredna je biomasa važan izvor obnovljive sirovine (Hodgson i sur., 2010) s velikim proizvodnim potencijalom, a korištenje biomase kao sirovine kontinuirano raste diljem Europe (Bilandžija i sur., 2018.; Krička i sur., 2017.). Velik dio poljoprivrednog otpada čini upravo lignocelulozni materijal, a najvažnija svojstva lignocelulozne biomase su vrlo dobra čvrstoća, zapaljivost, biorazgradivost i reaktivnost (Ashok, 2009.). Poljoprivrednu biomasu moguće je koristiti kao sirovinu za proizvodnju prirodnih vlakana te ako izvor sirovina neovisan o naftnim derivatima (Krička i sur., 2016.; Bhat Subrahmanya i sur., 2018.).

Globalno, lignocelulozna biomasa ima velik potencijal za industrijsku proizvodnju materijala i proizvoda, ali taj se resurs mora koristiti na okolišno povoljan, društveno prihvatljiv i održiv način (Samir i sur., 2005.). Najvažnija svojstva lignocelulozne biomase su vrlo dobra čvrstoća, zapaljivost, biorazgradivost i reaktivnost. Strukturni polimeri lignoceluloznih materijala su celuloza, hemiceluloza i lignin, a njihovi udjeli ovise o vrsti biljke (Fernandes i sur., 2009.). Općenito lignoceluloza sadrži 25-50 % celuloze, 20-40 % hemiceluloze i 10-35 % lignina (Ioelovich, 2014). Lignocelulozni materijali teško su razgradivi zbog kristalične strukture celuloze i kompleksne strukturne organizacije celuloze, hemiceluloze i lignina (Hatti-Kaul i sur., 2007.). Stoga je, prije samog procesa, npr. utekučenja biomase za dobivanje biokompozita (Tišler, 2002.), potrebno provesti odgovarajuće postupke predobrade (Fernandez i sur., 2009.; Misra i sur., 2015.). Predtretmanom je moguće poboljšati razgradnju sirovine, uklanjanje lignina, te tako omogućiti djelomičnu ili potpunu hidrolizu hemiceluloze te smanjenje količine kristalinične frakcije celuloze (Cardona i Sánchez, 2007.).

Biogospodarstvo i biomasa

Klimatske promjene, globalno zatopljenje i emisije stakleničkih plinova postali su prioritetno globalno pitanje razvoja. Glavni je izazov dugoročni razvoj gospodarstva sa smanjenom emisijom ugljikovog dioksida. Teži se učinkovitijem korištenju energije i resursa s ciljem smanjenja proizvodnje stakleničkih plinova. Sve se češće otvara polemika oko čovjekovog odnosa prema prirodi i okolišu (Dahl, A., 2008).

U 20. i 21. stoljeću došlo je do intenzivnog tehnološkog i društvenog napretka u svim sferama ljudskog života.

Industrijalizacija i globalizacija te potrošački mentalitet društva i linearno gospodarstvo doveli su do sve veće proizvodnje i ekspanzionalne potrošnja materijalnih dobara čime se crpe izvori iz prirode (Ragauskas i sur., 2006). Ograničenost prirodnih resursa te negativni utjecaj na okoliš uzrokovani potrošnjom zahtijevaju pronalazak novih modela za njihovo održivo korištenje. Budući da je prirodnih resursa sve manje, a s druge količine otpada rastu, pretvaranje poljoprivrednog otpada u novi resurs predstavlja mogućnost za razvoj biogospodarstva (Kalambura i sur., 2013). Europska strategija za pametan, održiv i uključiv rast kao jedan od osnovna 3 prioriteta razvoja predlaže održiv rast odnosno promicanje gospodarstva koje učinkovitije koristi resurse, koje je zelenije i konkurentnije, te omogućava prelazak s postojećeg, linearnog, na kružno gospodarstvo (Škrlec, 2016). Kružna ekonomija ne odbacuje sirovine koje se mogu još iskoristiti za proizvodnju novih proizvoda. Na taj se način manje iscrpljuju ograničene količine resursa, te se smanjuju količine otpada stoga se otvara nova fronta zelenih tehnologija i stvara novo ozračje zelene ekonomije (Bismarck i sur., 2006; Kalambura i sur., 2013).

S obzirom na svjetska ekonomska i ekološka pitanja povezana s velikom upotrebom petrokemijskih proizvoda, u proteklom desetljeću je sve veći interes za istraživanja mogućnosti korištenja otpadne biomase (Iqbal i sur., 2013.; Menon i Rao, 2012.). U skladu s principima održivog razvoja istražuju se metode i tehnologije alternativnog te prije svega efektivnog korištenja biomase za dobivanje „zelenih“ proizvoda i uporabe prirodnih polimernih materijala kao alternative petrokemijskim produktima (Lee, 2006.).

S rastućim trendovima proizvodnje koja se temelji na biološkoj osnovi proizvoda, koncept biorefinerije kontinuirano dobiva na značaju (Calvo-Flores i Dobado, 2010.). Iako je definicija samog pojma biorefinerije podvrgnuta raspravama, krajnji cilj proizvodnje u biorefineriji je stvaranje raznolikih proizvoda iz različite biomase kao sirovine (Lind i sur., 2009). Proces i tehnologije iz različitih područja, uključujući polimernu kemiju, bioinženjering i poljoprivredu (Ohara, 2003), korišteni u biorefinerijama mogu rezultirati različitim proizvodima kao što su gorivo, energija, kemikalije i različiti materijali iz biomase (FitzPatrick i sur., 2010).

Biokompoziti kao alternativa polimernim materijalima

Prirodni polimeri i biokompoziti poznati su i čovječanstvo ih je koristilo od davnina, ali upotreba prirodnih polimera i biokompozita u potpunosti je zanemarena u drugoj polovici 20. stoljeća ekspanzijom upotrebe sintetičkih polimera (Mihelcic i sur., 2003). Međutim kao rezultat zabrinutosti za stanje okoliša i iscrpljivanje fosilnih resursa, u 21. stoljeću ponovno raste zanimanje za istraživanje biopolimera i biokompozita što se dokazuje ekspanzionalno rastući broj patenata i publikacija o biokompozitnim materijalima (Satyanarayana i sur, 2019; Vilaplana i sur, 2010).

Biokompoziti su materijali dobiveni umjetnim spajanjem dvaju ili više materijala različitih svojstava s ciljem dobivanja materijala takvih svojstava kakve ne posjeduje niti jedna komponenta sama za sebe (Fowler i sur, 2006). Kompozitni se materijali sastoje od dvije faze, kontinuirane faze - matrice i diskontinuirane faze – punila ili ojačavala (Krajinović i sur., 2011.). Ako je matrica ili ojačalo prirodnoga podrijetla, riječ je o biokompozitima, a ako su i matrica i ojačalo prirodnog podrijetla, takav se materijal naziva zeleni kompozit (Ndazi, 2006). Kompozitni materijali ojačani prirodnim ojačalima u zadnjih nekoliko godina postali su zanimljivi kao alternativa polimernim materijalima (John i Thomas, 2008). Kompoziti ojačani česticama biomase imaju nisku cijenu i ekološki su prihvatljivi u usporedbi s tradicionalnim polimernim materijalima (Nadzi i sur, 2009). Iz tog razloga sve više zamjenjuju konvencionalna ojačanja (Kumar, 2017.; Yu i sur., 2006.).

Niz sektora suočava se s posebnim izazovima zbog ekološkog otiska ili ovisnosti o materijalima koji se nabavljaju izvan Europe (Satterfield i sur., 2009). Jedan od takvih sektora je proizvodnja polimernih materijala. Uporaba plastike u EU-u neprekidno raste, a velik problem za okoliš predstavljaju i velike količine polimera koji završavaju u oceanima, pa je ciljevima održivog razvoja do 2030. godine obuhvaćen cilj sprečavanja i znatnog smanjenja svake vrste onečišćenja mora plastikom. Osim toga, nafta je osnovna sirovina u proizvodnji plastike, a ona je neobnovljivi prirodni resurs. Imajući u vidu činjenicu da izvori nafte i prirodnog plina nisu neiscrpn, postoji potreba za razvojem obnovljivih sirovina (Kalambura i sur., 2014). Stoga korištenje biomase kao sirovine za proizvodnju vlakana kontinuirano raste diljem Svijeta. Složena kemijska raznolikost biomase kao sirovine pruža mogućnost stvaranja širokog spektra novih polimera. Jedno od mogućih rješenja leži u zamjeni plastike biokompozitima (Lee, 2006.).

Zaključak

Daljnji razvoj okolišno prihvatljivih materijala i širenje njihova tržišta ovisit će o istraživanjima i poboljšanju njihovih preradbenih i uporabnih svojstava. Posebno su aktualna istraživanja o upotrebi poljoprivrednog otpada, a posebice lignocelulozne biomase za proizvodnju biokompozita kao ekološki prihvatljivih materijala. Budući da upotreba polimernih materijala u Svijetu neprekidno raste, a velike količine polimernog otpada predstavljaju sve veći problem za okoliš, pretvaranje poljoprivrednog otpada u biokompozit kao alternativu polimernim materijalima predstavlja mogućnost za razvoj biogospodarstva. Razvoj proizvodnje biokompozita iz poljoprivrednog otpada svakako doprinosi očuvanju okoliša kroz izbjegavanje odlaganja otpada kao i efikasnijem trošenju resursa u skladu s principima održivog razvoja stoga biokompoziti imaju važnu ulogu u razvoju biogospodarstva.

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Biocomposites as products in the bioeconomy

Abstract

The bioeconomy is a closed system in which waste and by-products are reused as the primary source of raw materials. Agricultural biomass is interesting as a feedstock for energy and natural fiber production, especially because of its availability, renewability and acceptability. Therefore, the use of biomass as a raw material for fiber production is increasing worldwide. Recently, the possibility of using biomass as a new source of raw materials to produce environmentally friendly biocomposites, chemicals and resins have increased significantly. In accordance with the principles of bioeconomy, agricultural waste has great potential to become important source of raw material for bio composites production.

Keywords: bioeconomy, biocomposites, biomass

Usporedba empirijskih i teorijskih ogrjevnih vrijednosti miskantusa

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Sažetak

Eksperimentalne metode za procjenu gornje ogrjevne vrijednosti (HHV) su dugotrajne, skupe i imaju veću mogućnost pojave eksperimentalnih pogrešaka, stoga se u literaturi predlažu brojni izračuni s ciljem teorijskog utvrđivanja HHV. Stoga je cilj ovog istraživanja bio je analizirati 5 novih genotipova, križanaca *Miscanthus sacchariflorus* x *Miscanthus sinensis* te usporediti empirijske i teorijske vrijednosti HHV. Rezultati su pokazali da su se jednadžbe izvedene iz podataka dobivenih strukturalnom analizom biomase (lignin, fiksirani ugljik, fiksirani ugljik/hlapiva tvar) pokazale preciznijima za teorijski izračun HHV, odnosno nisu signifikantno različite od empirijski utvrđenih HHV.

Ključne riječi: novi genotipovi miskantusa, empirijska ogrjevna vrijednost, teorijska ogrjevna vrijednost

Uvod

Danas je miskantus vodeća višegodišnja poljoprivredna energetska kultura u Europi zbog svog potencijala za visoki prinos suhe tvari i sposobnosti da raste u širokom rasponu agroklimatskih uvjeta. Osim toga, miskantus je produktivan na tlima slabije kvalitete, uključujući slana tla i tla onečišćena teškim metalima. Budući da najviše istraženi genotip *Miscanthus x giganteus* pokazuje ograničenja s obzirom na abiotički stres, osobito sušu, intenzivno se razvijaju novi genotipovi, križanci *M. sacchariflorus* i *M. sinensis*, koji imaju veći potencijal uzgoja u stresnim uvjetima od samog *M. giganteus*.

Biomasa trave miskantus danas se još uvijek najčešće koristi kao sirovina u procesu neposrednog izgaranja, odnosno za proizvodnju toplinske i električne energije putem kogeneracijskih sustava ili u ložištima na čvrsta goriva za proizvodnju toplinske energije (Lewandowski i sur., 2018.). Jedno od najvažnijih svojstava biomase kao goriva je ogrjevna vrijednost, o kojoj ovisi energetska sadržaj i koja određuje učinkovitost upotrebe biomase (Acar i Ayanoglu, 2012.). Poznavanje ogrjevne vrijednosti biomase neophodno je za provedbu energetske analize i projektiranje bilo kojeg energetskeg sustava. Eksperimentalne metode za procjenu gornje ogrjevne vrijednosti (HHV) su dugotrajne, skupe i imaju veću mogućnost pojave eksperimentalnih pogrešaka (Nhuchhen i Abdul Salam, 2012.), stoga se u literaturi predlažu brojne jednadžbe za teorijski izračun HHV biomase, iz podataka dobivenih njenom osnovnom analizom (Acar i Ayanoglu, 2012.). Temeljem navedenog, cilj ovog istraživanja bio je analizirati 5 novih genotipova, križanaca *Miscanthus sacchariflorus* x *Miscanthus sinensis* te usporediti empirijske i teorijske ogrjevne vrijednosti.

Materijal i metode

U istraživanju je korištena biomasa 5 novih genotipova energetske kulture miskantus (u nastavku označeni od G1-G5), uzgojeni na fakultetskom pokušalištu Šašinovečki lug (45°50'59.1"N 16°11'25.8"E), na tlu lošije kvalitete (pseudoglej, pH 5,5-6,0), bez korištenja hranjiva. Uzorci su prikupljeni u kasnoj zimskoj žetvi prve vegetacijske sezone, u ožujku 2019. godine. Biomasa je osušena prirodnim putem, nakon čega su uzorci usitnjeni u laboratorijskom mlinu (IKA, Njemačka). Potom je standardnim metodama utvrđen sadržaj vode (HRN EN 18134-2:2015), pepela (HRN EN ISO 18122:2015), koks (EN 15148:2009), fiksiranog ugljika (računski) i hlapive tvari (EN 15148:2009). Elementarni sastav određen je standardnim metodama i to za sadržaj ugljika, dušika i vodika HRN EN ISO 16948:2015 te sumpora HRN EN ISO 16994:2016, dok je kisik dobiven računski. U IKA C200 kalorimetru (IKA, Njemačka) je utvrđena empirijska ogrjevna vrijednost u uzorcima (HRN EN 14918:2010). Nadalje, u uzorcima je utvrđen sadržaj lignina,

hemiceluloze i celuloze (modificirana metoda ISO 5351-1:2002). Jednadžbe za teorijski izračun gornje ogrjevne vrijednosti prikazane su u tablicama 1 i 2, a preuzete su iz dostupne literature (Saidur i sur., 2011.; Acar i Ayanoglu, 2012.; Nhuchen i Abdul Salam, 2012.).

Tablica 1. Jednadžbe za izračun HHV iz podataka elementarne analize

Ulazni parametri	Jednadžba izvedena iz podataka elementarne analize
CHNO (%)	$0,335C + 1,423H - 0,164O - 0,145N$
C (%)	$0,4373C - 1,6701$
CHONS (%)	$0,3516C + 1,16225H - 0,1109O + 0,0628N + 0,10465S$

Tablica 2. Jednadžbe za izračun HHV iz podataka strukturalne analize

Ulazni parametri	Jednadžba izvedena iz podataka strukturalne analize
Lignin (%)	$0,0979L + 16,292$
Fiksirani ugljik, FC (%)	$0,196FC + 14,119$
Fiksirani ugljik, FC; hlapive tvari, VM (%)	$0,312FC + 0,1708VM$

Usporedba teorijskih i empirijskih ogrjevnih vrijednosti hibrida miskantusa testirana je analizom varijance (ANOVA). Istraživanje je provedeno na 5 genotipova u tri ponavljanja (N=15). Utjecaj je označen kao signifikantan (statistički značajan) ukoliko je vjerojatnost prihvaćanja nulte hipoteze (H0) da su srednje vrijednosti svih vrsta jednake bila manja od 5 % ($p < 0,05$).

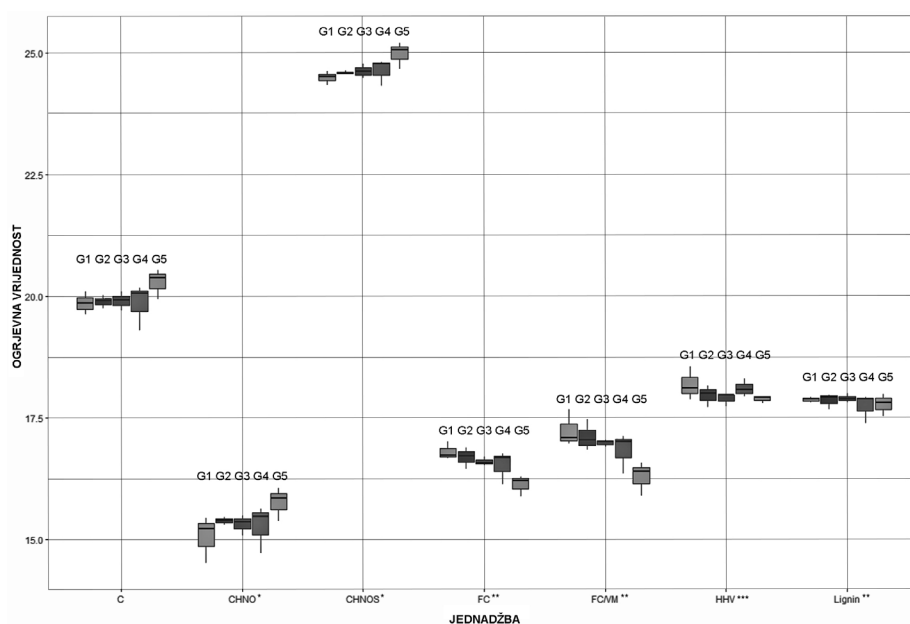
Rezultati i rasprava

Elementarna analiza nužna je za učinkovitu uporabu biomase, a njome se utvrđuje sadržaj ugljika (C), vodika (H), kisika (O), sumpora (S) i dušika (N) u gorivu. Vrijednosti ovih elemenata razlikuju se ovisno o vrsti biomase. Suprotno tome, strukturalna analiza je osnovna karakterizacija biomase s obzirom na sastav lignina, celuloze i hemiceluloze (Acar i Ayanoglu, 2012.). Osim toga, ona daje podatke o sadržaju vode, pepela i hlapivih tvari (Patel i Gami, 2012.). U Tablici 3 prikazan je sastav biomase, na temelju kojih parametara je utvrđena teorijska vrijednost HHV za pojedini genotip miskantusa.

Tablica 3. Sastav biomase miskantusa po pojedinom genotipu

Parametar \ Genotip	G1	G2	G3	G4	G5
Fiksirani ugljik (%)	13,28	13,20	12,83	12,38	10,39
Hlapive tvari (%)	75,54	75,70	76,64	76,49	76,91
Dušik (%)	1,54	1,30	1,03	1,40	0,74
Ugljik (%)	49,59	48,86	49,74	49,37	49,70
Sumpor (%)	0,13	0,12	0,12	0,12	0,10
Vodik (%)	3,90	3,91	3,91	3,93	3,91
Kisik (%)	44,84	45,81	45,19	45,18	45,56
HHV (MJ/kg)	18,12	18,12	18,01	17,75	18,01
Lignin (%)	16,51	16,62	15,57	17,35	12,79

Na Slici 1 grafički su prikazane prosječne teorijske vrijednosti HHV izračunate korištenjem jednadžbi prikazanih u tablicama 1 i 2 te podataka elementarne i strukturalne analize (Tablica 3), kao i vrijednosti HHV dobivene eksperimentalnim metodama, za svaki pojedini genotip (G1-G5).



Slika 1. Distribucija empirijskih i teorijskih vrijednosti HHV

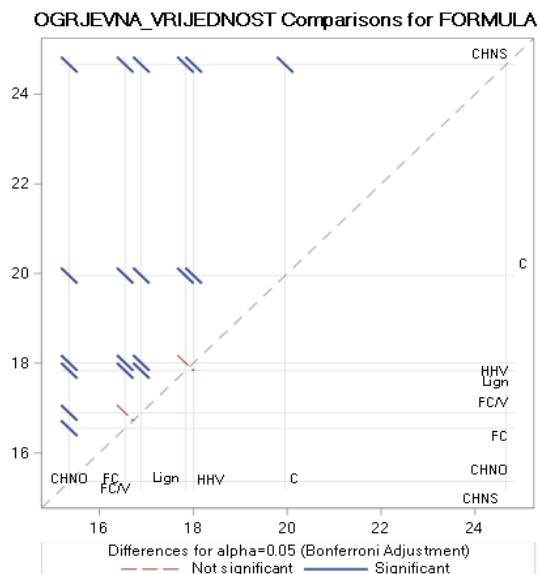
Iz Slike 1 je vidljivo da se jednadžbe koje u izračunu obuhvaćaju CHNO i CHNOS (*) najmanje podudaraju s eksperimentalno utvrđenom vrijednošću HHV (***). Jednadžba za izračun prema sadržaju ugljika (C) nije precizna, ali u usporedbi s jednadžbama CHNOS i CHNO je bliža izmjerenoj vrijednosti. Najpouzdanije su jednadžbe (**) za izračun prema sadržaju lignina, prema sadržaju fiksiranog ugljika (FC) i prema sadržaju fiksiranog ugljika i hlapivih tvari (FC/VM). Suprotno tome, u svom istraživanju, Nhuchhen i Abdul Salam (2012.) su utvrdili linearni negativni učinak odnosa FC/VM.

U Tablici 4 prikazana je srednja vrijednost svih genotipova za pojedinu jednadžbu, odnosno za teorijsku vrijednost HHV, kao i njihova standardna devijacija. Dobiveni rezultati testirani su analizom varijance (ANOVA) i uspoređeni s rezultatima gornjih ogrjevnih vrijednosti dobivenih eksperimentalnom analizom biomase miskantusa.

Tablica 4. Srednja vrijednost teorijski dobivenih HHV i njihova standardna devijacija

Ulazni parametri	N	HHV, MJ kg ⁻¹	
		Mean	Std Dev
C	15	19,9553	0,3014
CHNO	15	15,3606	0,3844
CHNOS	15	24,6593	0,2374
FC	15	16,5506	0,3030
FC/VM	15	16,8946	0,4431
HHV	15	18,0086	0,2172
Lignin	15	17,8346	0,1711

Na Slici 2 grafički su prikazane razlike između podataka empirijski i teorijski dobivenih vrijednosti HHV. Pune linije prikazuju vrijednosti koje se značajno razlikuju, a isprekidane linije prikazuju vrijednosti koje nisu značajno različite.



Slika 2. Razlike između podataka empirijski i teorijski dobivenih vrijednosti HHV

Iz Slike 2 je vidljivo da se teorijske vrijednosti HHV, dobivene jednadžbama koje u obzir uzimaju sastav CHNOS i CHNO, značajno razlikuju od empirijskih vrijednosti HHV. Teorijska vrijednost HHV, dobivena na temelju podataka o CHNOS, ima najveće odstupanje od prosjeka i zbog toga su ovi podaci najmanje relevantni za izračun HHV. Podaci teorijske vrijednosti HHV, dobiveni jednadžbama koje u obzir uzimaju sadržaj lignina i sadržaje fiksiranog ugljika i hlapivih tvari (FC, FC/VM), nisu značajno različiti od empirijski dobivenog HHV te su se u ovom istraživanju navedene jednadžbe pokazale najpouzdanijima u svrhu teorijskog izračuna HHV biomase novih genotipova miskantusa. Navedeno je u skladu s dosadašnjim istraživanjima, u kojima su Cordero i sur. (2001.), Parikh i sur. (2005.) te Sheng i sur. (2005.) utvrdili korelaciju između povećanja HHV i povećanja sadržaja hlapive tvari i fiksiranog ugljika.

Zaključci

U ovom istraživanju provedene su analize na 5 novih genotipova miskantusa, križanaca *Miscanthus sacchariflorus* x *Miscanthus sinensis* (tzv. sac x sin hibrida). Usporedbom empirijskih i teorijskih HHV biomase miskantusa, utvrđeno je da su se jednadžbe izvedene iz podataka elementarne analize (C, CHNO, CHNOS) pokazale manje točnima za izračun teorijske vrijednosti HHV, pri čemu se podaci teorijske HHV, dobiveni na temelju CHNOS i CHNO, značajno razlikuju od empirijski utvrđenih vrijednosti HHV. Nadalje, jednadžbe izvedene iz podataka dobivenih strukturalnom analizom (lignin, FC, FC/VM) biomase pokazale su se preciznijima za teorijski izračun HHV, odnosno nisu značajno različite od empirijski dobivenih vrijednosti HHV.

Napomena

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Comparison of empirical and theoretical heating values of miscanthus

Abstract

Experimental methods for estimating the higher heating value (HHV) are time-consuming, expensive, and have a greater chance of experiencing experimental errors, and therefore numerous calculations are proposed in the literature with the aim of theoretically determining the HHV. Hence, the aim of this study was to analyse 5 novel genotypes, crossbreeds of *Miscanthus sacchariflorus* x *Miscanthus sinensis* and to compare their empirical and theoretical HHVs. The results showed that the equations derived from the data obtained by structural analysis of biomass (lignin, carbon, fixed carbon/volatile matter) proved to be more accurate for the theoretical calculation of the HHV, i.e. they do not significantly differ from the empirically determined HHV.

Keywords: novel miscanthus genotypes, empirical heating value, theoretical heating value

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Proizvodnja energije iz sječke *Sida hermaphrodite* kao čvrstog biogoriva

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Sažetak

U radu su prikazana energetska svojstva sječke *Sida hermaphrodite* kako bi se utvrdila mogućnost korištenja iste kao čvrstog biogoriva. Dobiveni rezultati sadržaja ugljika i vodika kao osnovnih i najvažnijih elemenata svih vrsta goriva ukazuju na visoku kakvoću istraživane biomase. Lignocelulozni sastav upućuje na mogućnost korištenja kao čvrstog biogoriva. Sve navedeno je potvrđeno i visokom gornjom 18,07 MJ kg⁻¹ te donjom 16,75 MJ kg⁻¹ ogrjevnom vrijednosti.

Ključne riječi: *Sida hermaphrodita*, sječka, energetske vrijednosti

Uvod

Poljoprivreda je jedna od važnijih gospodarskih grana u Europskoj uniji i Hrvatskoj a dobro gospodarenje poljoprivredom, a time i poljoprivrednom biomasom od strateške je važnosti (Krička i sur., 2017a). Iako je poljoprivreda veliki potrošač energije, korištenjem ostataka proizvodnje može biti i veliki proizvođač s obzirom na to da svake godine ostanu velike količine neiskorištene biomase koja bi se mogla upotrijebiti u energetske svrhe. Postoje velike mogućnosti iskorištenja poljoprivredne biomase, kao što su proizvodnja humusa (zaoravanjem), stajnjaka, hrane za životinje, transportnih goriva te toplinske i/ili električne energije (Brkić, 2007). Poljoprivredna lignocelulozna biomasa ima znatan energetski potencijal jer predstavlja ostatke primarne poljoprivredne proizvodnje, odnosno nusproizvode nakon dorade/prerade poljoprivrednih sirovina u prehrambenoj industriji (Krička i sur., 2017b). Kao važan izvor lignocelulozne biomase koriste se i energetske kulture, a cilj njihovog uzgoja je proizvodnja, što je moguće veće, količine biomase po jedinici površine s ciljem njene pretvorbe u energiju. Energetske kulture mogu biti jednogodišnje ili višegodišnje. Energetske kulture karakterizira mogućnost konverzije u različite tipove i oblike biogoriva (kruta, plinovita, tekuća). Najčešće korištena kruta goriva su sječka, peleti i briketi, tekuća su biodizel i bioetanol, a plinovito je boplin.

Jedna od višegodišnjih energetskih kultura je *Sida hermaphrodita* koja je biljna vrsta iz porodice *Malvaceae* (sljezovi) (Krička i sur., 2017b). To je vrsta koja omogućava proizvodnju biomase u sustavima bez obrade tla, čuvajući tako strukturu tla pa je značajna za uzgoj na marginalnim tlima (Beare i sur., 1994). *Sida hermaphrodita* postaje prodorna u drugoj godini uzgoja jer pohranjuje fotosintezom stvorene hranjive tvari u gustu mrežu podzemnih stabljika (rizoma) i time uspješno suzbija korove (Borkowska i Molas, 2012). Naraste do isine od 1-4,5 metra (obično do oko 3), a prinosi iznose do 25 t ha⁻¹ (Denisiuk, 2006). Dugotrajnost nasada, jednostavnost uzgoja i velike sposobnosti prilagodbe na različite klimatske uvjete i uvjete tla ukazuju na velike potencijalne mogućnosti korištenja ove vrste (Borkowska i Styk 2006).

Iz svega navedenog cilj ovog rada istražiti proizvodnju energije iz sječke *Sida hermaphrodite* kao čvrstog biogoriva preko analiza gorivih i negorivih svojstva, ogrjevne vrijednost i lignoceluloznog sastava.

Materijal i metode

Istraživanja su provedena na sječki energetske kulture *Sida hermaphrodita* uzgojene na području grada Zagreba. Žetva je obavljena u veljači 2019. godine. Istraživanje je provedeno u Laboratoriju Zavoda za poljoprivrednu tehnologiju, skladištenje i transport, Sveučilišta u Zagrebu Agronomskog fakulteta.

Istraživana su negoriva i goriva svojstva, lignocelulozni sastav te ogrjevna vrijednost. Analiziran je sadržaj vode

(HRN EN 18134-2:2015) u laboratorijskoj sušnici, pepela (HRN EN ISO 18122:2015) i koksa (CEN/TS 15148:2009) u mufolnoj pećnici, fiksnog ugljika i hlapljive tvari (EN 15148:2009) računski. Gornja ogrjevna vrijednost (HRN EN 14918:2010) određena je u adijabatskom kalorimetru (IKA, Njemačka), dok se donja ogrjevna vrijednost dobila računski. Sadržaj dušika (HRN EN ISO 16948:2015), ugljika i vodika (HRN EN ISO 16948:2015) te sumpora (HRN EN ISO 16994:2015) određen je CHNS analizatorom, dok se kisik izračunao računski kao ostatak C, H, N, S elemenata. Određivanje udjela celuloze, hemiceluloze i lignina provedeno je modificiranom standardnom metodom ISO 5351-1:2002 u laboratoriju.

Rezultati i rasprava

Biomasa, kao kruto gorivo, sastoji se od gorivih i negorivih svojstava. Rezultati istraživanja negorivih svojstva prikazani su u tablici 1, dok su u tablici 2 prikazana goriva svojstva sječke *Side hermaphrodite*.

Tablica 1. Rezultati negorivih svojstava

Istraživani parametri	Sida hermaphrodita
Vlaga (%)	8,03
Pepeo (%)	2,68
Fiksirani ugljik (%)	5,45
Koks (%)	8,13
Dušik (%)	0,58

Temeljem dobivenih vrijednosti negorivih svojstava sječke *Side hermaphrodite* sadržaj pepela koji određuje dobru ili lošu kvalitetu goriva i čim je manji gorivo je kvalitetnije bio je 2,68%, što je u suglasju s količinom pepela od 2,56% koju navode Stolarki i sur. (2014). Sadržaj koksa i fiksnog ugljika u biomasi smatra se pozitivnim svojstvima biomase jer predstavljaju količinu energije izgaranjem određene količine biomase (Garcia i sur., 2012; Jurišić i sur., 2017). Sadržaj koksa kod *Side hermaphrodite* iznosio je 8,13%, dok je sadržaj fiksnog ugljika iznosio je 5,45% što je u suglasju sa vrijednosti od 5,09% dobivenoj u istraživanju Krička i sur. (2017a). Sadržaj dušika bio je 0,58% dok Šiaudinis i sur. (2015) utvrđuju sadržaj dušika od 0,75%.

Tablica 2. Rezultati gorivih svojstava

Istraživani parametri	Sida hermaphrodita
Ugljik (%)	48,97
Vodik (%)	6,03
Sumpor (%)	0,21
Kisik (%)	44,21
Hlapiva tvar (%)	83,84

Ugljik je osnovni i najvažniji element svih vrsta goriva i određuje njegovu kvalitetu te što ga je više gorivo je kvalitetnije. Vodik uz ugljik čini osnovni sastav svakog goriva i povećani udio vodika poboljšava kvalitetu goriva (Vassilev i sur., 2010). Sadržaj ugljika bio je 48,97%, a vodika 6,03% što su neznatno više vrijednosti od dobivenih iz literature. Naime, Howaniec i Smolinski (2011) navode sadržaj ugljika 47,18% i vodika 5,68%.

Sumpor utječe na povećanje stakleničkih plinova te se uz dušik smatra kritičnim elementom u biomasi u smislu emisije stakleničkih plinova. Šiaudinis i sur. (2015) utvrđuju sadržaj sumpora 0,17%, dok je u ovome istraživanju sadržaj sumpora bio viši i iznosio je 0,21%. Sadržaj kisika bio je 44,21%, dok su prema navodima literature Howaniec i Smolinski (2011) i Šiaudinis i sur. (2015) i dobili vrijednosti od 35,73% do 41,94%, što su nešto niže vrijednosti. Sadržaj hlapive tvari bio je 83,84% što je u skladu sa navodima Krička i sur. (2017a).

U tablici 3 prikazani su rezultati lignoceluloznog sastava, a u tablici 4 rezultati ogrjevne vrijednosti sječke *Side hermaphrodite*.

Tablica 3. Rezultati lignoceluloznog sastava

Istraživani parametri	<i>Sida hermaphrodita</i>
Celuloza (%)	39,91
Hemiceluloza (%)	26,84
Lignin (%)	28,08

Kako bi se definiralo za koju vrstu goriva je najbolje koristiti određenu energetska kulturu mora se definirati čvrstoća, biorazgradljivost te zapaljivost biomase što se definira pomoću lignoceluloze (Olesen i Plackett, 1999). U koliko lignoceluloza sadrži više lignina pogodnija je za izravno izgaranje (Hodgson i sur., 2010), a u koliko ima više celuloze i hemiceluloze za proizvodnju tekućih goriva (Lewandowski i sur., 2003). Sadržaj celuloze bio je 39,91%, hemiceluloze 26,84%, a lignina 28,08%, što su vrijednosti koje su djelomično u suglasju s navodima literature prema kojem Wróblewska i sur. (2009) utvrđuju udio celuloze od 41,02%, hemiceluloze od 17,05% i lignina od 26,04%.

Tablica 4. Rezultati ogrjevne vrijednosti

Istraživani parametri	<i>Sida hermaphrodita</i>
Gornja ogjevna vrijednost (MJ kg ⁻¹)	18,07
Donja ogjevna vrijednost (MJ kg ⁻¹)	16,75

Gornja (HHV) i donja (LHV) ogrjevna vrijednost *Side hermaphrodite* iznosila je za gornju ogrjevnu vrijednost 18,07 MJ kg⁻¹ te za donju 16,75 MJ kg⁻¹. Stolarski i sur. (2013) istražili su gornju ogrjevnu vrijednost *Sida hermaphrodita* i dobili su vrijednosti 18,90 MJ kg⁻¹, što je nešto viša vrijednost nego dobivena u ovome istraživanju.

Zaključak

Temeljem vlastitih istraživanja sječke *Side hermaphrodite* može se zaključiti da se može koristiti za proizvodnju energije procesom izravnog izgaranja. Sadržaj negorivih svojstava (vlaga, pepeo, koks, fiksirani ugljik i dušik), a posebice koks, fiksiranog ugljika i dušika u granicama je očekivanih vrijednosti. Sadržaj gorivih svojstva (ugljik, sumpor, vodik, kisik i hlapljive tvari) posebice ugljika i vodika kao osnovnih i najvažnijih elemenata svih vrsta goriva ukazuju na visoku kakvoću istraživane biomase. Dobiveni rezultati lignoceluloznog sastava biomase ukazuje na mogućnost korištenja istraživane biomase za različite tipove biogoriva, a posebice čvrstog biogoriva. Gornja ogrjevna vrijednost od 18,07 MJ kg⁻¹ te donja ogrjevna vrijednost iznosila od 16,75 MJ kg⁻¹ ukazuju na visoki energetski potencijal istraživane kulture.

Napomena

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Energy production from *Sida hermaphrodita* chips as solid biofuel

Abstract

The paper presents the energy properties of *Sida hermaphrodite* chips with aim to determine the potential for its use as a solid biofuel. Obtained results indicate that carbon and hydrogen content as the basic and most important elements for all fuel types indicate on the high quality of the investigated biomass. The lignocellulosic compositions of the chips indicated on the possibility of its use as solid biofuel. All this was confirmed by the high upper heating value of 18.07 MJ kg⁻¹ and lower heating value of 16,75 MJ kg⁻¹

Keywords: *Sida hermaphrodita*, chips, energy value

Upotreba biomase invazivne biljne vrste amorfe (*Amorpha fruticosa* L.) u proizvodnji energije

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Sažetak

Cilj ovog rada bio je analizama biomase invazivne vrste amorfe (*Amorpha fruticosa* L.) prikupljene sa različitih lokacija na području Sisačko-moslavačke županije utvrditi njena energetska svojstva i potencijal za neposredno izgaranje i proizvodnju toplinske energije, te proces pirolize (proizvodnja bioulja kao energenta i biougljena kao proizvoda dodane vrijednosti). Obradom dobivenih podataka i interpretacijom rezultata, amorfa je opravdala pretpostavke velikog energetskog potencijala u proizvodnji energije i time otvorila vrata upotrebi invazivnih vrsta u proizvodnji energije nakon uklanjanja iz prirode.

Ključne riječi: neposredno izgaranje, piroliza, bioulje, biougljen, energetska iskoristivost

Uvod

Količina obnovljive energije proizvedene u EU, godišnje se povećava za 5,3 % (Eurostat, 2018). Biomasa jedan je od obnovljivih izvora energije. Razvoj tehnologije u energetskom sektoru (rasplinjavanje, izgaranje, piroliza, anaerobna fermentacija), pruža mogućnosti šireg korištenja biomase i doprinosi ideji zamjene fosilnih goriva kao primarne energije biomasom, slijedom čega različite sastavnice biomase postaju zanimljive.

Flora Hrvatske izrazito je bogata i raznolika, te broji oko 5600 vrsta i podvrsta unutar kojih je zabilježeno i registrirano više od **600 alohtonih vrsta**. Njih 74, uključujući i amorf, smatra se invazivnim za područje Hrvatske. Nagli porast broja takvih vrsta prate mnogobrojne izmjene nacionalnih zakonodavstava, nadzor prometa roba i ljudi, te konkretne mjere uklanjanja vrsta (Mitić, 2014). Iskorištavanje njihove biomase moglo bi pridonijeti povećanju udjela proizvodnje obnovljive energije, smanjenje emisije stakleničkih plinova te socio-ekonomskih šteta u poljoprivredi, šumarstvu, turizmu, zdravstvu i drugo. Amorfa je sjevernoamerička listopadna, drvenasta invazivna vrsta, koja je u Hrvatsku unesena početkom 20. stoljeća. Zbog svog agresivnog širenja, predstavlja problem u prirodnoj obnovi nizinskih poplavnih šuma gdje vrlo često čini dominantnu biljnu vrstu. S druge strane, predstavlja bioenergetski potencijal Hrvatske gdje svojim morfološkim značajkama u kratkom vremenu stvara značajnu količinu biomase po jedinici površine (Krpan i sur., 2014). Biomasa amorfe u hrvatskim šumama raste u prirodi bez ikakvih agrotehničkih mjera i troškova, što ju čini konkurentnom u proizvodnji biomase te bi uključivanjem u obnovljive izvore energije pridonijela smanjenju troškova obnove šuma i otvaranju novih mogućnosti rada energetskih postrojenja (Vuletić i sur., 2015). Stoga je u ovom radu, analizama energetskih svojstava prikupljenih uzoraka ispitana mogućnost korištenja spomenute sirovine u proizvodnji toplinske i električne energije te u proizvodnji biougljena i bioulja.

Materijal i metode

Biomasa invazivne biljne vrste amorfe, za ovo istraživanje, prikupljena je krajem mjeseca travnja 2018. godine na pet lokacija na području Lonjskog polja, Sisačko-moslavačke županije. Lokacije prikupljanja biomase su: Šumski predio Gaj (L1), Područje Lonjsko polje „Plavi most“ (L²), Područje Gredica (L3) Područje Josura (L4) i Čeprlin (L5). Na svakoj od lokacija prikupljeni su izdanci amorfe s listovima bez korijena, koji su zatim pipremljeni za daljnje analize održivanjem sadržaja vode u svježem uzorku, sušenjem i usitnjavanjem u laboratorijskom mlinu (IKA, Njemačka), a sve analize rađene su u tri ponavljanja. Standardnim metodama utvrđeni su sadržaj vode (HRN EN 18134-2:2015), pepela (HRN EN ISO 18122:2015), koks (EN 15148:2009), fiksiranog ugljika (računski) i hlapivih tvari (EN 15148:2009). Udio celuloze, hemiceluloze i lignina odredilo se modificiranom standardnom metodom (ISO

5351-1:2002). Sadržaj dušika (N), ugljika (C) i vodika (H) (HRN EN ISO 16948:2015) te sumpora (S) (HRN EN ISO 16994:2015) određen je Macro CHNS analizatorom (Analysensysteme GmbH, Njemačka), u skladu s protokolima za određivanje, dok se sadržaj kisika izračunao iz razlike. Ogrjevna vrijednost utvrđena je standardnom metodom (EN 14918:2010) IKA C200 kalorimetrom (IKA Analysentechnik, Njemačka). Udio proizvedenog biougljena i bioulja odredio se računski, nakon procesa pirolize u laboratorijskim uvjetima pri temperaturi od približno 400 °C.

Rezultati i rasprava

Uzorci amorfe koji su prikupljeni na području Lonjskog polja, podrvrgnuti su analizama energetske svojstava kako bi se odredili parametri koji utječu na proces izgaranja i proces pirolize. Osnovne značajke šumske ili drvene biomase kao energenta jesu kemijski sastav, ogrjevna vrijednost, temperatura samozapaljenja, temperatura izgaranja te fizikalna svojstva koja utječu na izgaranje (Alakangas, 2005).

Udio vode, jedan je od važnijih parametara za određivanje kvalitete biomase, iz razloga što smanjuje njenu ogrjevnju vrijednost. Dio energije koji se otpušta tijekom procesa izgaranja troši se na isparavanje vode te se iz tog razloga smatra gubitkom topline (Francescato i sur., 2008). Obzirom da je povoljne uvjete za širenje našla u slivnim područjima nizinskih vodotokova, kao i poplavnim šumama hrasta lužnjaka te preferira vlažna staništa uz obale rijeka i potoka; za očekivati je da amorfa ima veći postotak vode u biomasi. U tablici 1 prikazani su rezultati analize sadržaja vode u uzorcima za pojedinu lokaciju koja se kreće oko 12,13 % u suhoj tvari biomase. Usporedno sa ostalim vrstama biomase koje se koriste u proizvodnji energije, McKendry (2002.) navodi kako je sadržaj vode kod biomase drva u vrijednosti od 20 %, kod stabljike ječma 30 % i pšenice 16 %, dok su Kumabe i sur. (2007) za drvenaste vrste dobili vrijednosti od 5,2 do 32,3%.

Pepeo je anorganski ostatak te je nepoželjan u gorivu jer smanjuje njegovu kvalitetu, kao i toplinsku vrijednost (Šilić i sur., 2012). Sadržaj pepela u biomasi amorfe po lokacijama, prikazan je u tablici 1. Prema Francescato i sur. (2008) sadržaj pepela u biomasi kreće se od 0,2 do 6,2 %. Rezultatima dobivenim u istraživanju Xiong i sur. (2008), utvrđen je sadržaj pepela kod amorfe od 2,82 %. Kumabe i sur. (2007) za drvenaste vrste dobili su vrijednosti pepela koje su se kretale od 0,6 do 1,1%. U analiziranim uzorcima ovog istraživanja utvrđene su slične vrijednosti te je prosječna vrijednost pepela sa svih lokacija iznosila 3,03 %.

Sadržaj koksa predstavlja ostatak suhe destilacije te što ga ima više, gorivo je kvalitetnije (Francescato i sur., 2008). U tablici 1, prikazani su rezultati analize sadržaja koksa u suhom uzorku koji prikazuju da se udio koksa kretao od 10,73 % do 12,52 %. Jurišić i sur. (2017) navode da važnije energetske kulture poput miskantusa imaju udio koksa oko 14,97 %, dok se srednja vrijednost koksa kod amorfe kretala oko 11,9 %.

Sadržaj fiksiranog ugljika, uz pepeo predstavlja kruti ostatak nakon gorenja i isparavanja hlapivih komponenti. Udio fiksiranog ugljika u uzorcima amorfe kreće se od 8,16 % do 9,40 % što su nešto niže vrijednosti od onih dobivenih analizom biomase važnijih energetske kulture u RH sa 11,40 % (Jurišić i sur., 2017). Također, McKendry (2002) je za drvenu biomasu dobio vrijednosti fiksiranog ugljika oko 17 %, dok su Kumabe i sur. (2007) za drvenaste vrste dobili vrijednosti od 11,8 % do 30,9 %.

Tablica 1. Karakteristike uzoraka biomase amorfe (s.t.)

Amorfa (<i>Amorpha fruticosa</i> L.)					
	Lokacija 1	Lokacija 2	Lokacija 3	Lokacija 4	Lokacija 5
Voda [%]	10,35	12,82	12,69	13,35	11,46
Pepeo [%]	4,00	3,12	2,58	2,05	3,38
Koks [%]	12,42	12,52	10,73	11,04	11,91
Cfix [%]	8,42	9,40	8,16	9,00	8,54
Hlapive tvari [%]	77,23	74,67	76,58	75,60	76,63
LHV [MJ/kg]	15,92	15,97	15,43	15,32	16,27
HHV [MJ/kg]	17,31	17,34	16,80	16,66	17,63
Produkti pirolize					
Biougljen	46,90	45,63	42,36	47,68	33,20

Bioulje	35,79	34,08	35,68	35,38	45,20
*HHV [MJ/kg]	29,56	24,58	29,51	28,34	38,22

*Gornja ogrjevna vrijednost (HHV) biougljena dobivenog procesom pirolize

Tijekom procesa izgaranja, biomasa se razgrađuje na hlapive plinove i kruti ostatak, te su u tablici 1, prikazani rezultati analiza gdje se sadržaj hlapivih tvari u amorfi kreće oko 76,11 %. Prema Vassilev i sur. (2010), sadržaj hlapivih tvari u drvnoj biomasi iznosi otprilike 71,9 %, dok su Kumabe i sur. (2007) za drvenaste vrste dobili vrijednosti od 35,7 do 82,4 %.

Gornja ogrjevna vrijednost (HHV) predstavlja toplinu koja se oslabada pri potpunom izgaranju količine nekog goriva u uvjetima kada se nastala vodena para iz dimnih plinova kondenzira te kada se dimni plinovi ohlade na temperaturu od 0 °C (Šilić i sur., 2012). U tablici 1, prikazani su rezultati kalorimetrije. Sukladno dobivenim rezultatima koji se kreću oko 17,15 MJ/kg za gornju i 15,78 MJ/kg za donju ogrjevnu vrijednost, postoji statistički značajna razlika ogrjevnih vrijednosti između uzoraka s različitih lokacija. Garcia i sur. (2012) navode gornje ogrjevne vrijednosti za energetske kulture *Mischanthus x giganteus* od 17,7 MJ/kg, dok Xiong i sur (2008) navode za amorfu ogrjevnu vrijednost od 17,85 MJ/kg. Krpan i sur. (2014), za suhu tvar amorfe navode ogrjevnu vrijednost od 20,2 MJ/kg, dok su Kumabe i sur. (2007) za druge drvenaste vrste dobili ogrjevne vrijednosti od 19,7 do 30,9 MJ/kg. Jurišić i sur. (2017), za važnije energetske kulture u RH dobili su oko 17 MJ/kg. Navedene literaturne vrijednosti pokazuju da biomasa amorfe zadovoljava pretpostavke potencijala u dobivanju energije.

Piroliza je postupak termičke razgradnje organske tvari u anaerobnim uvjetima, pri visokim temperaturama (> 400 °C). Proces pirolize organske tvari je vrlo kompleksan te se sastoji od različitih simultanih i sukcesivnih reakcija koje se odvijaju tijekom zagrijavanja organske tvari u reaktivno neutralnom okruženju pri čemu nastaju biougljen, bioulje te hlapivi plinovi (Jurišić i sur., 2017). Iz rezultata u tablici 1, vidljivo je da se udio dobivenog bioulja iz biomase amorfe kreće oko 43 %, dok se udio dobivenog biougljena kreće oko 37 %. Najviše bioulja dobiveno je pirolizom biomase lokacije 4, dok je najviše biougljena dobiveno pirolizom biomase lokacije 5.

U tablici 1, navedena je i gornja ogrjevna vrijednost za biougljen dobiven pirolizom biomase amorfe, čije se vrijednosti kreću od 24,58 - 38,22 MJ/kg što je znatno više od ogrjevnih vrijednosti biomase amorfe te možemo zaključiti da biougljen amorfe predstavlja kvalitetan energent.

U tablici 2, prikazan je lignocelulozni sastav biomase amorfe, odnosno udio celuloze, hemiceluloze i lignina. Prema dobivenim rezultatima, biomasa amorfe sadrži oko 41,00 % celuloze, 20,72 % hemiceluloze i oko 29,60 % lignina. Gledajući udio lignina, najviši udio ima lokacija 4 (30,87 %) što ju čini najpogodnijom za proizvodnju energije izravnim izgaranjem, dok najniži udio celuloze ima lokacija 5 (35,35%).

Tablica 2. Prikaz aritmetičkih sredina mjerenih parametara biomase amorfe (s.t.)

Amorfa (<i>Amorpha fruticosa</i> L.)					
	Lokacija 1	Lokacija 2	Lokacija 3	Lokacija 4	Lokacija 5
Sadržaj celuloze, hemiceluloze i lignina					
Celuloza [%]	44,02	41,48	40,96	43,23	35,35
Hemiceluloza [%]	20,45	19,87	19,83	18,23	25,21
Lignin [%]	26,60	32,00	30,06	30,87	28,49
Sadržaj CHNS i O					
C [%]	45,79	45,46	45,54	44,73	45,20
H [%]	6,35	6,26	6,26	6,14	6,25
N [%]	2,61	2,32	1,78	1,98	2,58
S [%]	0,44	0,28	0,23	0,25	0,29
O [%]	44,81	45,68	46,18	46,90	45,69

U tablici 2, prikazan je i elementarni sastav analizirane biomase. Prikazan je sadržaj dušika, ugljika, sumpora, vodika

i kisika u biomasi amorfe. Prema istraživanju koje su proveli Krpan i sur. (2009), u drvu amorfe na pokusnim poljima utvrđeno je 46,2 % ugljika, dok je u kori amorfe utvrđeno 49,6 % ugljika. Xiong i sur (2008) su u suhoj tvari biomase amorfe utvrdili 48,06 % ugljika, 0,74 % dušika, 6,41 % vodika i manje od 0,01% sumpora. Budući da se dušik i sumpor tijekom izgaranja gotovo u potpunosti pretvaraju u NO_x i SO₂ spojeve, koji imaju negativan utjecaj na okoliš (García i sur., 2012), poželjan je njihov što manji udio u biomasi. Analizom istraživane biomase amorfe sa pet različitih lokacija ovog istraživanja dobiveni su rezultati za ugljik koji se kreću oko 45 %, dušika oko 2 %, vodika oko 6 %, sumpora oko 0,30 % te kisika oko 46 %. Sadržaj ugljika je nešto niži, no ostali rezultati su vrlo slični rezultatima iz drugih istraživanja.

Zaključak

Na temelju provedenih analiza biomase invazivne alohtone biljne vrste amorfe (*Amorpha fruticosa* L.) i produkata pirolize (bioulja i biougljena) može se zaključiti:

1. Biomasa amorfe pokazala je zadovoljavajuće vrijednosti istraživanih svojstava (sadržaj vlage, pepela, koksa, fiksiranog ugljika, hlapivih tvari, gornje ogrjevne vrijednosti, kao i lignocelulozni i elementarni sastav) te se isti nalaze unutar literaturnih vrijednosti za ovu vrstu biomasu, ali i energetske kulture poput miskantusa.
2. Slijedom rezultata, opravdana je pretpostavka da je amorfa kvalitetna sirovina za procese izravnog izgaranja i proces pirolize iz biomase. Tome u prilog idu u usporedbi s ostalim literaturnim podacima niži udio vode, pepela, hlapivih tvari, viši sadržaj koksa i fiksiranog ugljika, visoka ogrjevna vrijednost te sadržaj ugljika, celuloze, lignina i hemiceluloze.
3. Dobiveni produkti pirolize, upućuju da ova vrsta ima veliki potencijal u procesu pirolize, odnosno u proizvodnji bioulja kao energenta i biougljena kao proizvoda dodane vrijednosti (dok ga visoka gornja ogrjevna vrijednost čini još boljim energentom po jedinici mase).

Obzirom na rezultate, zaključeno je da je invazivna alohtona biljna vrsta amorfa zadovoljavajuće kvalitetna sirovina za proces neposrednog izgaranja, kao i proces pirolize. Provedenim analizama, amorfa je opravdala pretpostavke velikog energetskog potencijala te ukazala na mogućnost upotrebe invazivnih vrsta u odnosu na dosadašnju upotrebu komercijalnih vrsta drveća ili energetskih nasada u proizvodnji energije.

Napomena

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Use of invasive plant species Indigobush (*Amorpha fruticosa* L.) biomass in energy production

Abstract

This study aimed to analyze biomass of the invasive species Indigobush collected from five different locations in the Sisak-Moslavina County to determine its energy properties and potential for direct combustion and heat production, as well as the pyrolysis process (producing bio-oil as an energy source and biochar as a value-added product).

With the analyzes carried out, Indigobush justified the assumptions of great energy potential in energy production and opened the door to the use of invasive species after their removal from nature for these purposes in comparison to the previous use of commercial tree species.

Keywords: direct combustion, pyrolysis, biofuels, biochar, energy utilization

