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PRODUKTIVNE KARAKTERISTIKE NOVE SORTE LJUBIČASTOG KROMPIRA (*Solanum tuberosum* cv. Bergerac)*

Amer Bošnjaković[1], Drena Gadžo[1], Mirha Đikić[1], Teofil Gavrić[1], Lejla Čengić[1]

Prethodno saopštenje - *Previous statements*

Rezime

Krompir je u Bosni i Hercegovini (BiH) jedan od važnijih usjeva i sadi se na oko 35 000 ha, a prosječan prinos je do 12 t ha^{-1}. Tradicionalno se godinama sade iste sorte, sličnih kvalitativnih karakteristika, iako je ponuda novih sorata bogata. Fenolska jedinjenja, fitohemikalije koje nastaju kao sekundarni metaboliti, pokazuju antioksidativna svojstva te pozitivan fiziološki efekat.
Jedna od novijih sorti u BiH je holandska sorta Bergerac koju odlikuje ljubičasta boja pokožice i mesa.
Tokom 2021. godine su obavljena uporedna ispitivanja nove sorte Bergerac i sorte Dezire koja je godinama jedna od raširenijih sorti u BiH.
Sadnja je obavljena u dvije varijante: sa i bez primjene mikrobiološkog đubriva Slavol. Kod obje sorte Slavol je uticao na visinu prinosa, broj i krupnoću gomolja po biljci. Sorta Dezire je u varijanti sa primjenom Slavola ostvarila prosječan prinos od 48,4 t ha^{-1}, a Bergerac 37,1 t ha^{-1}.
Prosječan prinos u kontrolnoj varijanti (bez primjene mikrobiološkog đubriva) kod sorte Dezire iznosio je 31,2 a kod sorte Bergerac 33,1 t ha^{-1}.
Prosječan broj gomolja po biljci je bio veći kod sorte Bergerac, dok je Dezire imao u prosjeku krupnije gomolje (promjer ≥40 mm).
Istraživanje je pokazalo da između ispitivanih sorti postoji razlika u antioksidativnoj aktivnosti. Sorte sa ljubičastim mesom ispoljavaju veću antioksidativnu aktivnost, pa je tako kod sorte Bergerac ona bila veća za 53,7% i iznosila je 267,23 mg GAE/kg FW, dok je kod sorte Dezire ona bila prosjeku 123,68 mg GAE/kg FW.

Ključne riječi: *ljubičasti krompir, sorta, mikrobiološko đubrivo, prinos, antioksidativna aktivnost*

UVOD

Danas je krompir po ukupnoj proizvodnji četvrti, najvažniji usjev u svijetu, nakon kukuruza, pšenice i riže. U 2020. godini je proizvedeno preko 359 miliona tona a

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uzgajao se na oko 16,5 miliona hektara u oko 160 svjetskih zemalja (FAOStat, 2022). Bogatog je hemijskog sastava u kojem dominiraju skrob (više od 14%), bjelančevine (preko 2,5%), šećeri (preko 1,3%) mineralne materije (preko 1%) i ostale materije. Prisustvo različitih materija kao što su fenoli, antocijani, lipidi, vitamin C, riboflavin, tiamin i niacin čine ga potpunom hranom (Changan *et al.*, 2020). Redovna konzumacija krompira ima i zdravstvene dobrobiti koje uključuju poboljšanje probave, smanjenje nivoa holesterola, zaštita crijeva, smanjenje nastanka karcinoma, jačanje imunološkog sistema, usporavanje procesa starenja, održavanje nivoa natrija i kalija u krvi (Kumar *et al.*, 2020).

Južna Amerika, odnosno, područje Anda je pradomovina krompira gdje se on uzgajao još prije 8.000 godina i to je centar njegove biološke i genetičke raznolikosti. Krompir žute, narandžaste ili ljubičaste boje mesa se uzgaja i konzumira od davnina u područjima Perua, Ekvadora, Bolivije i Kolumbije, a sadašnje poboljšane sorte obojenog mesa nastale su ukrštanjem takvih starih autohtonih populacija i modernih evropskih sorti. Različita boja mesa potiče od vrste i sadržaja prisutnih pigmenata, pa tako lutein i zeaksantin dovode do razvoja žute, a antocijan do ljubičaste i nijansi crvene boje mesa krompira (Burgos *et al.*, 2013). Potražnja za krompirom obojenog mesa se u posljednje vrijeme sve više povećava jer potrošačima je sve važniji nutritivni aspekt hrane, a pigmenti, posebno antocijan su povezani sa povećanim antioksidativnim kapacitetom (Oertel *et al.*, 2017).

U Bosni i Hercegovini se krompir najčešće koristi za ishranu ljudi termički obrađen (pržen, pečen ili kuhan), a manje je zastupljena prerada u oplemenjene proizvode za ljudsku ishranu ili kao sirovina za proizvodnju skroba, alkohola, za ishranu stoke i sl.

Danas u svijetu postoji preko 4.000 sorata od čega se na Sortnoj listi BiH nalazi oko 360, ali nažalost u široj proizvodnji prisutno je svega nekoliko desetina sorti. Od 2020. godine na našem tržištu se prvi put pojavila i sorta ljubičaste boje mesa Bergerac, koja je selekcionisana u Holandiji. Zahvaljujući prisustvu antocijana, ljubičasti krompir je jak antioksidans, pa je iz tih razloga cilj ovog rada bio da se u poređenju sa krompirom, uobičajenim za naša područja, utvrdi njegova mogućnost proizvodnje, prinos ali i antioksidativna vrijednost i sadržaj fenola.

MATERIJAL I METOD RADA

Zemljišni uslovi

Zemljište na kojem je postavljen ogled je odgovarajuće za proizvodnju krompira. Neutralne je do blago kisele reakcije, visokog sadržaja humusa i dobro opskrbljeno pristupačnim fosforom i kalijem (tab. 1).

Tabela 1. Analiza plodnosti zemljišta
Table 1. Soil fertility analysis

Parametri *Parameters*	Vrijednosti *Values*	Karakteristike zemljišta *Soil characteristics*
pH (H_2O)	7,4	Neutralno/*Neutrally*
pH (KCl)	6,6	Blago kiselo/*Mildly acid*
% humusa	8,2	Jako humozno tlo/*Very humorous soil*
mg P_2O_5/100 g tla	39	Veoma dobro opskrbljeno/*Very well stocked*
mg K_2O/100 g tla	61	Veoma dobro opskrbljeno/*Very well stocked*

Vremenski uslovi

Prosječne temperature i prosječne količine padavina za period od januara do avgusta 2021. godine prikazani su u tabeli 2.

Tabela 2. Srednja mjesečna temperature i suma oborina u 2021. godini
Table 2. Average monthly air temperature and amount of precipitation

Mjesec / *Month*								
I	II	III	IV	V	VI	VII	VIII	
Srednja mjesečna temperatura zraka (°C) / *Average monthly air temperature*								
1,2	5,8	5,	8,8	15,9	20,9	23,6	22,3	
Suma oborina (mm) / *Amount of precipitation*								Ukupno/*Total*
21,8	43,9	36,2	70,6	67,2	73,6	69,2	103,8	486,3

Tokom 2021. godine nije bilo ekstremnih odstupanja u pogledu oborina i ukupna suma za prvih osam mjeseci godine (do vađenja krompira) iznosila je 486,3 mm. Okvirne potrebe krompira za vodom u vegetacionom periodu iznose 460-480 mm (Pejić *et al.*, 2014). Temperature se bile visoke, posebno u junu i julu i znatno više od višegodišnjeg prosjeka. Prema Muminoviću i sar. (2014) najbolji uslovi za rast krompira su u područjima gdje srednja temperatura najtoplijeg mjeseca vegetacije ne prelazi 18,5°C.

Ogled je postavljen kao dvofaktorijalni (sorta, đubrenje) po šemi slučajnog blok rasporeda u četiri ponavljanja. Razmak sadnje je bio 60 x 30 cm, dubina oko 10 cm. Sadnja je obavljena 24. aprila 2021. godine, u četiri reda dužine 10 m.
Za sadnju su korištene dvije sorte krompira: Bergerac i Dezire.
Bergerac je srednje rana sorta selekcionisana u Holandiji, boja mesa i klice joj se kreće od svijetlo do tamnoljubičaste. Stabljika je nešto tamnija u odnosu na uobičajene sorte a gomolji sitniji i izduženog oblika.
Desire je srednje kasna, prinosna sorta sa ujednačenim krupnim krtolama, ovalnog do izduženog oblika crvene pokožice i svijetložute boje mesa.
U varijanti sa đubrenjem korišten je Slavol tečno mikrobiološko đubrivo odnosno stimulator rasta, koji je namijenjen za prihranu ratarskih i povrtlarskih kultura.

Certifikovan je za primjenu u organskoj i tradicionalnoj poljoprivrednoj proizvodnji jer ne sadrži hemijske dodatke i pozitivno djeluje na biljke, zemljište i životnu sredinu.
Priprema zemljišta je obavljena po sistemu obrade za jare usjeve, uz unošenje oko 40 t ha^{-1} stajnjaka.
Prije sadnje, gomolji na varijanti na kojoj se primjenjivao Slavol su bili potopljeni u 10% rastvor Slavola u trajanju od 30 minuta, što je u skladu sa preporukama proizvođača đubriva. Drugi tretman Slavolom je bio folijarni tretman 2% rastvorom, neposredno pred cvjetanje i treći 15 dana kasnije. Varijanta bez primjene Slavola poslužila je kao kontrolna varijanta.
Uzorci za određivanje proizvodnih osobina su uzimani iz svakog ponavljanja i to po deset biljaka iz dva središnja reda slučajnim odabirom. Evidentiran je prinos, krupnoća gomolja, sadržaj fenola i antioksidacijska aktivnost. Vađenje krompira je obavljena 20. avgusta 2021. godine.
Određivanje ukupnih fenola u uzorcima krompira rađeno je UV/VIS spektrofotometrisjkom metodom, zasnovanoj na obojenoj reakciji fenola sa Folin-Ciocalteau reagensom, mjerenjem apsorbanse na talasnoj dužini od 600 nm (Ough i Amerine, 1988), uz galnu kiselinu kao standard.
Antioksidacijska aktivnost uzoraka je određivana spektrofotometrijskom pFRAP metodom (Meng *et al.*, 2011), zasnovanoj na reakciji fenolskih jedinjenja sa $FeCl_3$ i $K_3[Fe(CN)_6]$, pri čemu nastaje plavi kompleks sa maksimumom aposrpcije na 700 nm. Kao standard je korištena galna kiselina.
Statističke analize rađene su pomoću softverskog programa SPSS 22.

REZULTATI I DISKUSIJA

U proizvodnji krompira broj gomolja po biljci i prinos je sortna karakteristika ali je i pod uticajem ekoloških faktora i agrotehnike (Gadžo i sar., 2011). Na brojnost gomolja i njihovu masu osim sortimenta značajno utiče i gustina sadnje i oblik vegetacionog prostora (Jovović, 2011; Shun-Lin Zheng *et al.*, 2016).

Tabela 3. Prosječan broj i masa gomolja po biljci (g)
Table 3. The average number and mass of tubers per plant (g)

Sorta /*Cultivar*	Tretman / *Treatments*	Broj gomolja po biljci / *Tubers number/plant*	Masa gomolja po biljci / *Tubers mass/plant*	Prosječan prinos / *Average yield*
			-g-	-t ha^{-1}-
Bergerac	Slavol	22,8^{ns}	991,0^{a}	37,1^{a}
	Kontrola/ *Control*	23,4^{ns}	883,5^{b}	33,1^{b}
	Prosjek/ *Average*	23,1**a**	937,3**ns**	35,1**b**
Dezire	Slavol	14,5^{ns}	1293,7^{a}	48,4^{a}

Kontrola/ *Control*	11,3[ns]	848,7[b]	31,8[b]
Prosjek/ *Average*	12,9**b**	1071,2**ns**	40,1**a**

[a] i [b]indeksirana slova označavaju statistički značajnu razliku između tretmana / superscript letters indicate significant differences between treatments
a i **b** boldirana slova označavaju statistički značajnu razliku izemeđu sorti / bold letters indicate significant differences between cultivars

Iz tabele 3 se vidi da je Bergerac ostvario statistički značajno veći prosječan broj gomolja po biljci (23,05) u poređenju sa sortom Dezire koja je formirala oko 13 gomolja, što je i očekivano s obzirom da je veliki broj, uglavnom sitnijih gomolja, njena sortna osobina. Razlog za formiranje velikog broja gomolja može biti visoka plodnost zemljišta, dobra struktura i visok sadržaj humusa, a pored toga i činjenica da je tokom godine bilo i dovoljno vlage što je pospješilo rast i razvoj krompira.

Rivelli & De Maria (2018) su utvrdili da se između osam lokalnih populacija i tri komercijalne sorte krompira koje su ispitivali u Italiji, Dezire ističe brojem formiranih gomolja po biljci (9), ujednačenom krupnoćom ali i dobrom prilagodljivošću pedoklimatskim uslovima proizvodnje.

Prema Akass *et al.* (2014) broj gomolja po biljci se povećava sa smanjenjem vegetacionog prostora dok se prinos istovremeno smanjuje. Oni su utvrdili da je najveći broj gomolja bio pri sadnji na razmak 60 x 20 cm, dok je najmanji bio pri razmaku 70 x 40 cm.

Zheng *et al.* (2016) navode da na brojnost gomolja, njihovu masu i prinos utiču veličina ali i oblik hranidbenog prostora, tj. odnos razmaka između redova i u redu.

Tretman mikrobiološkim đubrivom nije uticao na broj formiranih gomolja niti kod jedne sorte.

Sorta Dezire je imala za oko 12,5% veću prosječnu masu gomolja po biljci (1071,2 g) od sorte Bergerac čiji je prosjek iznosio 937,3 grama ali statistički značajne razlike između njih nisu postojale. Tretman mikrobiološkim đubrivom je kod obje sorte statistički značajno uticao na povećanje prosječne mase gomolja po biljci (Tab. 3)

Miskoska - Milevska *et al*. (2020) su tokom jednogodišnjih ispitivanja Slavola u krompiru utvrdili da Slavol značajno utiče na povećanje površine lista, gustine stominih otvora, što je značajno za uspješnije odvijanje procesa fotosinteze, rasta i produktivnosti biljke.

Obje sorte su realizovale visoke prinose a tretman mikrobiološkim đubrivom značajno ga je i dodatno povećao. Tretman ljubičastog krompira Slavolom ostvario je prosječan prinos od 37,1 t ha^{-1} a bez Slavola prinos je bio niži za 4 t ha^{-1} (33,1). Dezire je u tretmanu sa Slavolom postigao prosjek od 48,4 t ha^{-1}što je značajno veći prinos od kontrolne varijante. Ukoliko bi se u ponovljenim istraživanjim potvrdilo pozitivno

djelovanje Slavola to bi bio značajan doprinos povećanju prinosa krompira. Posmatrajući prinose sorti, očekivano, ljubičasti krompir je imao niži prinos koji je ova sorta nadomjestila svojim kvalitativnim karakteristikama. U svakom slučaju prinos iznad 35 t ha^{-1}je zavidan uspjeh ove sorte. Dezire svojim prosječnim prinosom od oko 40 t ha^{-1} opravdava višedecenijsku zastupljenost i široku upotrebu u većem području Bosne i Hercegovine.

Za visok prinos, osim sortimenta neophodno je obezbijediti i odgovarajuću kombinaciju đubriva. Petropoulos *et al.* (2020) koristeći pet različitih kombinacija đubriva u krompiru utvrdili su da je najefikasnije đubrenje azotnim đubrivom koje se sporo otapa u zemljištu.

Stockem *et al.* (2021) su koristeći različite matematičke jednadžbe izračunali da se može prognozirati broj, masa, oblik gomolja i prinos, a sve u zavisnosti od veličine i oblika vegetacionog prostora. Tačnost prognoze je u korelaciji sa veličinom parcele za koju se određuju navedeni parametri.

Krupnoća gomolja je sortna osobina ali značajno zavisi i od agrotehnike i ekoloških uslova proizvodnje. S obzirom da su ekološki uslovi i agrotehnika bili isti razlike u krupnoći kod ispitivanih sorti su posljedica sortnih odlika (Gavrić i sar., 2012).

Tabela 4. Broj i masa gomolja različite krupnoće
Table 4 Number and mass of tubers of different sizes

Sorta/ *Cultivar*	Tretman / *Treatments*	Broj gomolja po biljci / *Number of tubers per plant*			Masa gomolja po biljci / *Mass of tubers per plant*		
		≤25 mm	25-40 mm	≥40 mm	≤25 mm	25-40 mm	≥40 mm
Bergerac	Slavol	6,7^{a}	12,6^{ns}	3,5^{ns}	100^{a}	584^{ns}	307^{b}
	Kontrola/ *Control*	6,3^{b}	13,3^{ns}	3,7^{ns}	94^{b}	519^{ns}	311^{a}
	Prosjek /*Average*	6,5**a**	12,9**a**	3,6**b**	97**a**	552**a**	309**b**
Desire	Slavol	4,3^{a}	3,8^{ns}	5,5^{ns}	90^{a}	266^{ns}	945^{a}
	Kontrola/ *Control*	2,5^{b}	3,7^{ns}	5^{ns}	51^{b}	241^{ns}	589^{b}
	Prosjek	3,4**b**	3,7**b**	5,3**a**	71**b**	254**b**	767**a**

^{a} i ^{b}indeksirana slova označavaju statistički značajnu razliku između tretmana / superscript letters indicate significant differences between treatments
a i **b** boldirana slova označavaju statistički značajnu razliku izemeđu sorti / bold letters indicate significant differences between cultivars

U tabeli 4. su prikazani rezultati nakon klasiranja gomolja u tri frakcije krupnoće: sa promjerom ispod 25 mm, 25-40 mm i promjerom iznad 40 mm. Bergerac je sorta koja je genetski predodređena da formira sitnije gomolje, zbog čega su i frakcije krupnoće netipične za merkantilni krompir koji je uobičajen na našim prostorima, što je i potvrđeno ovim istraživanjem. Najmanje zastupljena frakcija kod ove sorte je promjera iznad 40 mm (3,6) koja je kod sorte Dezire bila dominantna (5,3). U ukupnom broju

gomolja, kod sorte Bergerac najviše ih je bilo u rasponu prečnika 25-40 mm, što je za oko 71% više nego kod Dezirea.

Tretman mikrobiološkim đubrivom je najveći uticaj pokazao na broj najsitnijih gomolja (≤25 mm), dok na prosječnu brojnost formiranih gomolja preostale dvije frakcije pozitivnog uticaja nije bilo niti kod jedne sorte.

Sadržaj fenola i antioksidativna aktivnost

Krompir ima visoku antioksidativnu vrijednost i čuva organizam od slobodnih radikala koji nastaju u procesu oksidacije i mogu oštetiti važne strukture ćelije, ubrzati starenje ili oštetiti nasljednu DNK. Te činjenice čine krompir zanimljivijim proizvodom, posebno forme sa obojenim mesom (Buturac, 2008).

Tabela 5. Sadržaj fenola GAE/100g svježe mase i antioksidativna aktivnost (mg GAE/kg FM)
Table 5. Total phenolic content (mg GAE/100 g FM) and antioxidant activity (mg GAE/kg FM)

Sorta /*Cultivar*	Tretman / *Treatments*	Sadržaj ukupnih fenola / *Total phenolic content*	Antioksidativna aktivnost / *Antioxidant activity*
		mg GAE/100g FM	mg GAE/kg FM
Bergerac	Slavol	410,72[a]	283,32[a]
	Kontrola/ *Control*	320,56[b]	251,12[b]
	Prosjek/ *Average*	365,64**a**	267,23**a**
Dezire	Slavol	305,55[a]	138,68[a]
	Kontrola/ *Control*	244,30[b]	108,68[b]
	Prosjek/ *Average*	279,92**b**	123,68**b**

[a] i [b]indeksirana slova označavaju statistički značajnu razliku između tretmana / superscript letters indicate significant differences between treatments
a i **b** boldirana slova označavaju statistički značajnu razliku izemeđu sorti / bold letters indicate significant differences between cultivars

Rezultati sadržaja ukupnih fenola i antioksidativna aktivnost prikazani su u tabeli 5. Sadržaj ukupnih fenola je statistički značajno zavisio od sortimenta. Sorta ljubičaste boje mesa je imala 365,64 mg 100 g^{-1} FM što je za preko 23% više od sadržaja fenola u sorti Dezire (279,92).
Tretman Slavolom je kod obje sorte statistički značajno povećao antioksidacijsku aktivnost. Između sadržaja fenola i antioksidativne aktivnosti utvrđena je pozitivna korelacija što potvrđuju i istraživanja Valcarcel *et al.* (2015) i Reyes *et al.* (2005). Ovi rezultati su u saglasnosti sa istraživanjima Mystkowska *et al.* (2020). Oni su proučavajući tri sorte krompira u dvogodišnjem eksperimentu utvrdili da ukupan saržaj fenola zavisi od sortimenta a tretman biostimulatorima je povećao sadržaj fenola bez obzira na sortu. Sorte obojenog mesa imaju dva do tri puta veći antioksidativni potencijal u poređenju sa sortama žute boje mesa (Lachman & Hamouz, 2005). Veći

sadržaj fenola i veću antioksidatvnu vrijednost kod sorti ljubičaste i crvene boje mesa potvrđuju i Brown (2005) kao i Bellumori *et al.* (2017).
Na sadržaj fenola u krompiru najviše utiče genotip, pa tako najviše fenola ima kod sorti krompira sa ljubičastom bojom mesa ali njihov sadržaj zavisi i od stepena zrelosti, odnosno momenta vađenja (Frankova *et al.*, 2022).
Tokom industrijske prerade krompira u procesima guljenja, sječenja ili bilo kojim drugim postupkom dezintegracije tkiva dolazi veoma brzo do promjene boje, koja se može sačuvati primjenom nekih inhibitora posmeđenja koji neminovno vode degradaciji fenolnih jedinjenja. Prema rezultatima Begić-Akagić i Piližota (2010) sorta Dezire se ističe brzom degradacijom fenolnih materija, stoga iako jedna od raširenijih sorti u BiH, ne preporučuje se za industrijsku preradu.

ZAKLJUČAK

Bergerac, nova sorta ljubičaste boje mesa, je u jednogodišnjem ogledu postigla prosječan prinos gomolja od oko 35 t ha^{-1}. Formirala je veći broj, ali znatno sitnijih gomolja od sorte Dezire. Prosječan sadržaj ukupnih fenola iznosio je 365,64 mg GAE/100 g FM ili 23% više od ukupnih fenola utvrđenih u gomoljima sorte Dezire. Antioksidativni potencijal je također značajno veći nego kod sorte Dezire.
Zbog svojih kvalitativnih karakteristika Bergerac predstavlja sortu vrijednu pažnje, pa stoga ova istraživanja treba nastaviti i promovirati njeno uvođenje u širu proizvodnju.

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PRODUCTIVE CHARACTERISTICS OF A NEW VARIETY OF PURPLE POTATO (*Solanum tuberosum* cv. Bergerac)

Summary

Potato, as one of the most important crops in Bosnia and Herzegovina, covers an area of 35,000 ha with average yield of up to 12 t ha^{-1}. New potato variety - Bergerac, with purple flash and skin color, has been used for comparison with traditionally used Desire variety. Research was carried out during 2021. Phenolics, as secondary metabolites, have great physiological impact, due to their antioxidative activity.

Two ways of planting were used: with and without (control variant) microbiological fertilizer Slavol application. In both varieties, Slavol affected yield, height, and the number and size of tubers per plant.

The average yield in the control variant (without the application of microbiological fertilizer) for the variety Dezire was 31.2 t ha^{-1} and 33.1 t ha^{-1} for the variety Bergerac. With Slavol, Desire variety achieved an average yield of 48.4 t ha^{-1}, while Bergerac produced a yield of 37.1 t ha^{-1}. The average number of tubers per plant was higher in Bergerac variety, while Desire had larger tubers (average diameter ≥40 mm).

Study indicates that potatoes with purple flesh have a higher antioxidant activity. The antioxidant activity for the Bergerac variety was 53.7% higher then Desire with 267.23 mg GAE/kg FW, while Desire variety had average amount of 123. 68 mg GAE/kg FW.

Key words: *purple potato, variety, microbiological fertilizer, yield, antioxidant activit*

SJEVERNO-AMERIČKA TREŠNJINA MUHA *Rhagoletis cingulata* Loew (Diptera: Tephritidae), NOVI KARANTENSKI ŠTETNIK U BOSNI I HERCEGOVINI

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Originalni znanstveni rad - *Original scientific paper*

Sažetak

Sjeverno-američka trešnjina muha (*Rhagoletis cingulata* Loew) (Diptera: Tephritidae) podrijetlom je iz Sjeverne Amerike. U Europi je prvi put utvrđena 1983. godine u Švicarskoj. Vrsta *R. cingulata* je karantenski štetnik za Bosnu i Hercegovinu i trenutno se nalazi na Listi I, Dio A, Odjeljak I, Pravilnika o listama štetnih organizama, listama bilja, biljnih proizvoda i reguliranih objekata. Najvažnije biljke domaćini na području Europe su trešnja (*Prunus avium* L.) i višnja (*Prunus cerasus* L.). Ličinke vrste *R. cingulata* se razvijaju u plodovima ovih voćnih kultura te napadnuti plodovi postaju neprikladni za konzumaciju i prodaju. Istraživanje prisutnosti ovog štetnika u nasadima višnje i trešnje provodi se od 2015. godine na sedam lokaliteta na području Hercegovačko-neretvanske županije. Za praćenje prisutnosti vrste *R. cingulata* korištene su fluorescentno žute Csalomon® PALz i Rebell® amarillo ljepljive lovke. Svim postavljenim lovkama dodan je Csalomon® hranidbeni atraktant za *Rhagoletis* spp. Prvi nalaz vrste *R. cingulata* na području BiH zabilježen je sredinom srpnja 2022. godine na lokalitetu Rodoč (Mostar). Na lovkama postavljenim u nasad višnje ukupno je ulovljeno pet odraslih jedinki. Osim na lokalitetu Rodoč, štetnik je krajem srpnja 2022. godine utvrđen i na području općine Čitluk, na lokalitetima Krehin Gradac i Blizanci. Na oba lokaliteta na postavljenim lovkama ulovljena su po dva imaga sjeverno-američke trešnjine muhe. Identifikacija vrste rađena je na osnovu morfoloških karakteristika odraslih jedinki.

Ključne riječi*: sjeverno-američka trešnjina muha, Rhagoletis cingulata, karantenski štetnik, Bosna i Hercegovina*

UVOD

Vrsta *Rhagoletis cingulata* Loew je nearktičkog podrijetla, s područja Sjeverne Amerike, a prvi put je pronađena u državi Massachusetts (Loew, 1862). U Europi je

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nazočnost ove vrste prvi put utvrđena 1983. godine u Švicarskoj (Merz, 1991). Na području Njemačke vrsta je utvrđena 1993. godine (Lampe i sur., 2005), a na području Nizozemske, 2001. godine. Naknadnim istraživanjem je utvrđeno da je vrsta raširena u priobalnom i središnjem dijelu države (EPPO, 2004). Prvi nalaz vrste na području Belgije zabilježen je 2004. godine (Baugnée, 2006), dok je na području Mađarske vrsta prvi put zabilježena 2006. godine (Szeöke, 2006). U Hrvatskoj je *R. cingulata* zabilježena tijekom 2005. godine u Splitu (Bjeliš, 2007), a u Sloveniji tijekom 2007. godine na više lokacija u okolici mjesta Ormož (Seljak i Bjeliš, 2008). Iste godine vrsta je zabilježena i u Austriji (Egartner i sur., 2010). Prisustvo ove vrste je utvrđeno 2009. godine u Poljskoj (EFSA, 2014), Francuskoj, 2010. godine (EPPO, 2013), Crnoj Gori, 2013. godine (Radonjić, 2013), te Republici Češkoj 2014. godine (EPPO, 2014). Vrsta *R. cingulata* spada u *Rhagoletis cingulata* (Loew) kompleks, zajedno s vrstom *Rhagoletis indifferens* Curran i još dvije vrste koje nisu štetnici kultiviranog bilja. *R. cingulata* i *R. indifferens* su vrlo srodne i slične vrste, vrlo rasprostranjene na području istočne i zapadne obale Sjeverne Amerike. Do 1966. godine (Bush, 1966. cit. Bjeliš, 2007), obje vrste su nazivane *R. cingulata*, a sva tadašnja literatura nije pravila razliku između ove dvije vrste. Kao posljedica toga, svi podatci o vrsti *R. cingulata* s područja Sjeverne Amerike odnose se na vrstu *R. indifferens* (Benjamin, 1934; Phillips, 1946; Foote i Blanco, 1963. cit. Bjeliš, 2007). *R. cingulata* kao i trešnjina muha *Rhagoletis cerasi* L., uzrokuje "crvljivost" plodova višnje *Prunus cerasus* L., i trešnje *Prunus avium* L., a simptomi napada na plodovima domaćina su jednaki (Bjeliš, 2007). *R. cingulata* napada rašeljku *Prunus mahaleb* L. i crnu trešnju *Prunus serotina* Ehrhart, te ukrasne vrste *Prunus virginiana* L. i *Prunus pensylvanica* (Bush, 1966. cit. Bjeliš, 2007). Prema Weems (1972), može se razviti i u plodovima vrsta *Chionanthus virginica* L. i *Osmanthus americanus* (L.) Gray. Odrasli oblik sjajno je crne boje, veličine 4-5 mm, štitić (*scutellum*) je bijele boje, goljenjače i stopala su žućkaste boje. Na krilima se uočavaju poprečne i kose crnkaste oznake, a na zatku četiri bijele poprečne pruge (Slika 1). Odrasli oblici u prirodnim uvjetima mogu živjeti oko 40 dana (Christenson i Foote, 1960). Ženke polažu jaja ispod pokožice plodova biljaka domaćina. Ženka u prosjeku odloži od 300 do 400 jaja. Iako u jedan plod može biti odložen veći broj jaja, samo jedna ličinka se razvije u plodu. Nakon 3 - 8 dana, iz jaja izlaze ličinke. Ličinka je žućkasto bijele boje, dužine oko 5 mm, zašiljena u predjelu glave. Ličinka tijekom razvoja prolazi kroz tri stupnja, a razvoj u prosjeku traje 2 - 5 tjedana. Prema Weems (1972) razvoj ličinki pri temperaturi od 25 °C traje 11 dana. Kukuljenje se odvija u tlu na dubini od 3 do 8 cm, ispod krošnji stabla biljka domaćina. Prema Weems (1972), mali postotak muha javi se tijekom kolovoza i rujna kao druga generacija. Međutim, većina jedinki treba cijelu godinu da završi ciklus razvoja (Christenson i Foote, 1960; Weems, 1972). U početku, napadnuti plodovi ne otpadaju nego vise u krošnji. Na njima su vidljiva mjesta uboda (mjesto ovipozicije), oko kojih je tkivo obično blijede boje i blago uleglo (Bjeliš, 2007). Let odraslih muha

i transport plodova koji sadrže žive ličinke je najčešći način širenja vrste *R. cingulata* u nova nenapadnuta područja. Postoji mogućnost unošenja štetnika u nova područja i putem tla, u stadiju kukuljice (Bjeliš, 2007). Mjere suzbijanja koje se provode na području EPPO regije za suzbijanje europske trešnjine muhe (*R. cerasi*) jednake su onima koje se provode u Sjevernoj Americi za suzbijanje *R. cingulata*, i mogu se primijeniti u slučaju pojave navedenih vrsta bilo gdje u EPPO regiji (Bjeliš, 2007). Nakon što se utvrdi prisutnost štetnika na nekom području, neophodno je skupljati i uništavati otpale napadnute plodove biljaka domaćina. Ukoliko je moguće, potrebno je ukloniti stabla divljih domaćina u okolici detektiranog žarišta. Visoku učinkovitost za suzbijanje vrsta iz roda *Rhagoletis* pokazali su pripravci na osnovi dimetoata (Boller i Prokopy, 1976), te pripravci iz skupine sintetskih piretroida (Belangeri sur., 1985). U Sjevernoj Americi primjena gotovog mamca Success Bait u dozi 1 l/ha pokazala je visoku učinkovitost suzbijanja vrste *R. cingulata* (Pelz-Stelinski i sur., 2006. cit. Bjeliš, 2007).

MATERIJAL I METODE

Istraživanje prisutnosti sjeverno-američke trešnjine muhe na području Hercegovine provodi se od 2015. godine do danas, na nekoliko lokaliteta u nasadima trešnje i višnje. Osim u nasade ovih voćnih kultura, lovke su postavljane i na pojedinačna stabla u okućnicama. Na području općine Čitluk istraživanje je provedeno na četiri lokaliteta: Blizanci (43º12'13"S; 17º44'50"I), Krehin Gradac (43º13'04"S; 17º42'52"I), Bašaga (43º13'24"S; 17º40'35"I) i Potpolje (43º12'57"S; 17º41'06"I). Na području grada Mostara istraživanje je provedeno na tri lokaliteta: Rodoč (43º18'10" S; 17º48'34" I), Buna (43º14'34" S; 17º50'44" I) i Jasenica (43º16'46" S; 17º48'12" I). Za praćenje prisutnosti sjeverno-američke trešnjine muhe korištene su žute ljepljive Rebell® amarillo lovke švicarskog proizvođača Andermatt Biogarten AG, i fluorescentno žute PALz lovke mađarskog proizvođača Csalomon®. Svim lovkama je dodan i hranidbeni atraktant za vrste *R. cerasi*/*R. cingulata* mađarskog proizvođača Csalomon®. Prema navodima proizvođača, dodatak atraktanta lovkama može povećati ulov muha za 50 - 70%, što potvrđuju i istraživanja (Tóth i sur., 2014), na osnovu kojih dodatak hranidbenog atraktanta lovkama značajno utječe na broj ulovljenih muha. Lovke su postavljane u krošnje stabala na visinu od 2 do 3 m u vrijeme početka mijenjanja boje ploda iz zelene u žutu boju. Lovke su, radi lakšeg pregleda i determinacije ulovljenih muha, mijenjane svakih 7 dana. Prikupljene lovke su pregledane uz pomoć binokularne lupe (Leica EZ4D) u laboratoriju Zavoda za zaštitu bilja na Agronomskom i prehrambeno-tehnološkom fakultetu Sveučilišta u Mostaru. Determinacija vrste je rađena sukladno identifikacijskim ključevima (White i Elson - Harris, 1992).

Sl. 1. Odrasli oblik vrste *R. cingulata* (foto: Zovko, M.)
Fig. 1. Adult form of species R. cingulata (photo: Zovko, M.)

REZULTATI I RASPRAVA

Prvi nalaz sjeverno-američke trešnjine muhe (*R. cingulata*) u Bosni i Hercegovini zabilježen je 13. srpnja 2022. godine na području grada Mostara (lokalitet Rodoč), kada je na Csalomon® PALz lovci uhvaćen jedan imago. Na istom lokalitetu, tjedan dana kasnije, zabilježen je ulov tri imaga na PALz i jednog imaga na Rebell® amarillo lovci. Osim na lokalitetu Rodoč, štetnik je 26. srpnja 2022. godine utvrđen i na lokalitetima Blizanci i Krehin Gradac (Čitluk). Na oba lokaliteta ulovljena su po dva imaga *R. cingulata*. Na lokalitetima Buna i Jasenica (Mostar), te Potpolje i Bašaga (Čitluk), na postavljenim lovkama nije zabilježen ulov sjeverno-američke trešnjine muhe.

Lokaliteti na kojima je provedeno istraživanje prisutnosti sjeverno-američke trešnjine muhe, te broj ulovljenih imaga po lokalitetima, prikazan je u tabeli broj 1.

Tabela 1. Lokaliteti na kojima su postavljene lovke i broj uhvaćenih imaga sjeverno-američke trešnjine muhe (*R. cingulata*) u 2022. godini
Table 1.Localities where traps were set and the number of captured the eastern cherry fruit fly (R. cingulata) adults in 2022.

Tip lovke/*Trap type*	Rebell® amarillo	Csalomon® PALz
Lokaliteti/*Sites*	Broj ulovljenih muha/*Number of flies caught*	
Blizanci – Čitluk	0	2
Potpolje – Čitluk	0	0
Krehin Gradac – Čitluk	1	1
Bašaga - Čitluk	0	0
Rodoč - Mostar	1	4
Jasenica – Mostar	0	0
Buna - Mostar	0	0

Lokaliteti na kojima je potvrđena prisutnost sjeverno-američke trešnjine muhe (*R. cingulata*) prikazani su na karti (Slika 2).

Sl. 2. Raširenost sjeverno-američke trešnjine muhe *Rhagoletis cingulata* na području Bosne i Hercegovine
Fig. 2 Current distribution of the eastern cherry fruit fly Rhagoletis cingulata in Bosnia and Herzegovina

Iako vrste *R. cerasi* i *R. cingulata* imaju veoma sličnu biologiju, *R. cingulata* zahtijeva više temperature kako bi završila razvoj stadija kukuljice, te se odrasle jedinke javljaju kasnije u odnosu na vrstu *R. cerasi* (Lampe i sur., 2005). Prema istraživanju Lampe i sur. (2006), let sjeverno-američke trešnjine muhe započinje sredinom lipnja i traje sve do kraja kolovoza. Prema našim istraživanjima, prvi ulov *R. cingulata* zabilježen je sredinom srpnja, desetak dana nakon što je na postavljenim lovkama prestao ulov trešnjine muhe *R. cerasi*. Prema Bjeliš (2007), *R. cingulata* se u Hrvatskoj javlja dvadesetak dana nakon vrste *R. cerasi*, pa je za očekivati značajniji napad plodova kasnijih sorata trešnje, te poglavito višnje maraske. Prema Lampe i sur. (2005), prisutnost *R. cingulata* u Europi, odnosno kasniji let vrste, dovodi do napada kasnih sorti trešnje i višnje koje inače "izbjegnu" napad od *R. cerasi*. U Njemačkoj je zabilježen značajniji napad plodova kasne sorte višnje "Schattenmorellen" (EFSA, 2014). Iako je u pojedinim državama, poput Nizozemske, Njemačke i Mađarske, *R. cingulata* široko raširena i prisutna u velikoj brojnosti, u Austriji, Belgiji i Poljskoj, vrsta je ostala prisutna na relativno ograničenom području (EFSA, 2014). Istraživanja provedena u Hrvatskoj (Bjeliš i sur., 2016), pokazala su veoma sporo širenje vrste u nova područja, mali ulov muha na postavljenim lovkama. Isti autori navode da ekonomske štete nisu zabilježene, iako je *R. cingulata* na području Hrvatske prisutna duži niz godina. Radonjić i sur. (2019), navode da nakon prvog nalaza vrste u Crnoj Gori 2013. godine na lokalitetu Lastva Grbaljska, naknadnim dvogodišnjim monitoringom na lokalitetima Ulcinj, Bar, Baošići i Bigova, nije utvrđena njena prisutnost, te autori navode da se vrsta nije udomaćila na području Crne Gore.

ZAKLJUČCI

Sjeverno-američka trešnjina muha je karantenski štetnik za Bosnu i Hercegovinu, i trenutno se nalazi na Listi I, Dio A, Odjeljak I, Pravilnika o listama štetnih organizama, listama bilja, biljnih proizvoda i reguliranih objekata. Na području Bosne i Hercegovine, vrsta je prvi put zabilježena sredinom srpnja 2022. godine na lokalitetu Rodoč (Mostar). Krajem srpnja prisutnost vrste zabilježena je i na području općine Čitluk, na lokalitetima Krehin Gradac i Blizanci. Do sada je na području BiH, na lovkama postavljenim u krošnje stabala višnje i trešnje, ulovljeno samo devet odraslih jedinki muhe. Uz raširenu vrstu *R. cerasi*, prisutnost nove štetne vrste *R. cingulata* predstavlja dodatnu prijetnju proizvodnji trešnje i višnje, važnih voćnih kultura na području Hercegovine. S obzirom na to da vrsta napada više vrsta iz roda *Prunus*, postoji velika mogućnost njenog širenja na cijelom području Bosne i Hercegovine. Budući da se radi o prvom nalazu ove vrste na području Bosne i Hercegovine, neophodno je provesti dodatna istraživanja, postavljanjem vizualnih lovki na širem području, kako bi se utvrdila njena raširenost, vrijeme pojave, te dinamika populacije u našim agroekološkim uvjetima.

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THE EASTERN CHERRY FRUIT FLY *Rhagoletis cingulata* Loew (Diptera: Tephritidae), A NEW QUARANTINE PEST IN BOSNIA AND HERZEGOVINA

Summary

The eastern cherry fruit fly (*Rhagoletis cingulata* Loew) (Diptera: Tephritidae), originates from North America and appeared in Europe in the eighties. Species *R. cingulata* is a quarantine pest for Bosnia and Herzegovina and it is currently on List I,

Part A, Section I, of the Rulebook on the list of harmful organisms. Major cultivated host plants of *R. cingulata* in Europe are sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.). The larvae of this species damage the internal tissues of fruit, making the infested fruit unsuitable for consumption and sale. A survey of eastern cherry fruit fly was carried out from the growing season of 2015 until 2022 at seven sampling sites in Herzegovina-Neretva Canton. Fluorescent yellow Csalomon® PALz and Rebell® amarillo sticky traps with synthetic feeding attractant (*Rhagoletis* spp. lure) were used for monitoring of the fly presence. The five flies of *R. cingulata* were caught in mid September 2022 in a sour cherry orchard at site Rodoč (municipality of Mostar). At the end of September 2022, the presence of the pest was confirmed at two more locations: Krehin Gradac and Blizanci (municipality of Čitluk). In both locations two flies were caught. Identification of the caught eastern cherry fruit flies was based on morphological characteristics.

Key words: *eastern cherry fruit fly, Rhagoletis cingulata, Quarantine pest, Bosnia and Herzegovina*

BANKA SJEMENA KOROVSKIH BILJAKA U OBRADIVOM ZEMLJIŠTU*

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Originalni naučni rad - *Original scientific paper*

Rezime

Banka sjemena korovskih biljaka u obradivom zemljištu predstavlja važan izvor zakorovljavanja usjeva. Poznavanje banke sjemena je važno zbog prognoze nicanja te pravilnog odabira herbicida. Cilj ovog istraživanja bio je utvrditi banku sjemena korova u obradivom zemljištu nakon dva predusjeva (krompir i pšenica) i u usjevu lucerke, sa tri različite dubine: 0-10 cm, 10-20 cm i 20-30 cm. Primijenjene su dvije metode: fizička ekstrakcija sjemena i metoda naklijavanja. Veći broj korovskih vrsta je identifikovan pomoću metode naklijavanja (23), dok je metodom fizičke ekstrakcije zabilježeno 19 vrsta. Najveći broj jedinki je evidentiran u uzorku gdje je predusjev bila pšenica, potom slijedi lucerka, a najmanje nakon krompira.

Ključne riječi: *banka sjemena korova, pšenica, lucerka, krompir, dubina tla*

UVOD

Banka sjemena korova predstavlja rezervu održivog sjemena prisutnog na površini i rasutog u cijelom profilu zemljišta. Akumulirano sjeme korova predstavlja izvor zakorovljavanja u narednim godinama (Barić i sar., 2014). U biljnoj proizvodnji rezerve sjemena u zemljištu predstavljaju primarni izvor novog zakorovljavanja jednogodišnjim korovskim vrstama (Kovačević i Momirović, 2000). Poznavanje banke sjemena unutar proizvodnog zemljišta je važno zbog prognoze nicanja te pravilnog odabira herbicida (Forcella i sar., 1992). Sjeme nekih korovskih biljaka može da klija odmah nakon plodonošenja. To su sjemena koja obično imaju kratku životnu sposobnost i zadržavaju se u zemljištu veoma kratko u zavisnosti od mogućnosti širenja. Druga grupa sjemena korovskih biljaka zadržava duži vremenski period svoju životnu sposobnost te svake godine klija jedan dio od ukupne količine sjemena u zemljištu (Janjić i sar., 2003). Razvojem ekološki prihvatljivih mjera suzbijanja, analize prisutnih sjemenki korova u banci sjemena tla dobile su na važnosti kao metode rane mogućnosti prognoze buduće zakorovljenosti. Planiranje prihvatljive strategije suzbijanja korova teško je obaviti samo na osnovu poniklih korova u usjevu. Poznavanjem sastava i gustoće sjemenki korova u banci sjemena tla može se predvidjeti

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buduća zakorovljenost, ali se može i procijeniti efikasnost svih prethodno provedenih mjera suzbijanja korova na nekom polju (Barić i sar., 2014). Osnovne dvije metode koje se koriste za identifikaciju sjemena korovskih biljaka u zemljištu su: metod ekstrakcije u kojoj se sjeme sakuplja iz uzoraka zemljišta i na različite načine izdvaja (ispiranje, flotacija) i vegetacioni metod u kojoj se sjeme, prisutno u uzorku zemljišta, ostavlja da naklijava, a identifikacija vrsta se vrši na osnovu morfoloških karakteristika klijanaca.

Cilj ovog istraživanja je procijeniti brojnost i sastav korovske zajednice iz banke sjemena oraničnog sloja zemljišta (0-30 cm) koje je u prethodnoj vegetacionoj sezoni bilo pod različitim usjevima.

MATERIJAL I METODE

Uzorkovanje zemljišta je obavljeno početkom aprila, sa tri parcele, od kojih je na prvoj parceli pretkultura bila pšenica, na drugoj krompir, a treća je pod usjevom lucerke. Osnovna obrada tla nakon krompira i pšenice je obavljena u jesen, a lucerka je u trećoj godini vegetacije. Uzorci su uzeti sa tri dubine oraničnog sloja (0-10, 10-20, 20-30 cm). Za svaku navedenu dubinu urađeno je uzorkovanje u 8 tačaka (W model). Spajanjem pojedinačnih uzoraka nastao je zbirni. Od svakog zbirnog uzorka odvagano je po 400 g tla, što je predstavljalo jedan prosječan uzorak. Za procjenu rezervi sjemena primijenjene su dvije metode: fizička ekstrakcija sjemena i metod naklijavanja, a po 200 g (0,2 kg) zemljišta je korišteno za svaku metodu. Metoda fizičke ekstrakcije je sprovedena na način da je u plastičnu posudu stavljen uzorak zemljišta zajedno sa 300 ml vode u cilju lakšeg ispiranja uzoraka zemljišta kroz sita promjera 2, 1 i 0,5 mm pod mlazom tekuće vode. Ostatak uzorka na situ prenesen je na papir i ostavljen da se suši na sobnoj temperaturi. Nakon sušenja, izdvojeno je sjemena korova, njegova determinacija i brojanje. Determinacija sjemena korova urađena je pomoću Skender i sar. (1998), Parkinson i sar. (2013), Zollinger i Harstad (2017), kao i kolekcije sjemena korova na Poljoprivredno-prehrambenom fakultetu u Sarajevu. Na osnovu determinisanog sjemena, po dubinama i usjevima, izračunat je ukupan broj sjemenki korova po m^2 prema sljedećoj formuli:

$$Broj\ sjemenki\ po\ m^2 = \frac{Broj\ sjemenki\ \times 130}{0{,}2}$$

0,2 - masa jednog uzorka (kg); 130 - masa zemljišta u kg do dubine od 0 do 10 cm na 1 m^2. Metoda naklijavanja se odnosi na praćenje nicanja korova. Na dno plastičnih posuda, promjera 226×176×36 mm stavljen je tanki drenažni sloj kvarcnog pijeska, zatim je 2/3 zapremine posude napunjeno komercijalnim humusom. Nakon toga, stavljen je uzorak zemljišta dubine do 1 cm, koji se prekrio kvarcnim pijeskom radi sprječavanja pojave pokorice. Ovako napunjene posude su se držale na toplom i sunčanom mjestu. Ponikli korovi su determinisani u fazi razvijenih kotiledona do prvih pravih listova nakon čega su uklonjeni iz posude. Determinacija je urađena prema

Šariću (1991) i priručniku Weed communities of Europe (1971). Vrste koje je bilo teže identificirati su presađene u druge posudice i nastavile da rastu sve do determininacije. Prikupljeni podaci su statistički obrađeni pomoću softvera za statističku analizu podataka IBM SPSS Statistics. S obzirom da su podaci kategorijskog tipa primijenjen je neparametarski Hi kvadrat test. Rezultati ovog testa pokazuju procentualnu zastupljenost kategorijskih varijabli kroz različite nivoe ispitivanih faktora. Vrijednost Pearsonovog Hi kvadrata ukazuje na zavisnost ispitivanih faktora. Zaključci su doneseni na osnovu signifikantnosti Pearsonovog Hi kvadrat testa.

REZULTATI ISTRAŽIVANJA I DISKUSIJA

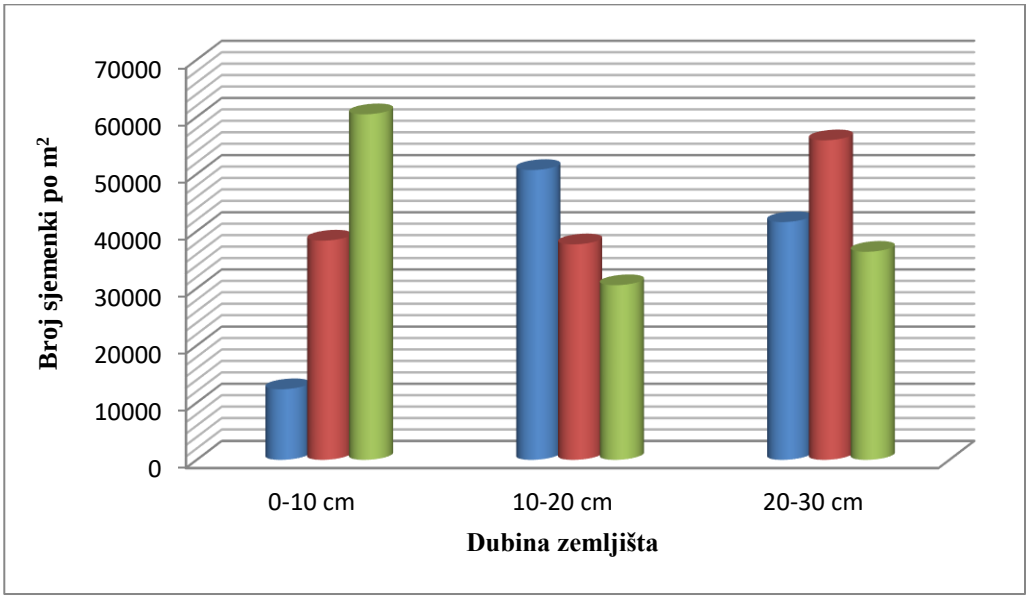
Metoda fizičke ekstrakcije sjemena

Vrste i brojnost sjemena korova dobijene metodom fizičke ektrakcije predstavljeni su u tabeli 1.

Tab. br. 1. Brojnost korovskih vrsta u banci sjemena dobijenih fizičkom ekstrakcijom sjemena
Table 1. The number of the recorded species in the weed seed bank, obtained by the extraction method

Korov Weed	**Krompir Potato**				**Lucerka Alfalfa**				**Pšenica Wheat**			
	0-10 cm	10-20 cm	20-30 cm	**Suma**	0-10 cm	10-20 cm	20-30 cm	**Suma**	0-10 cm	10-20 cm	20-30 cm	**Suma**
Polygonum aviculare	8	0	0	**8**	11	9	5	**25**	3	2	1	**6**
Echinochloa crus-galli	0	0	0	**0**	2	0	0	**2**	2	7	20	**29**
Chenopodium album	0	8	8	**16**	15	6	4	**25**	19	14	15	**48**
Amaranthus retroflexus	0	0	0	**0**	12	9	9	**30**	13	16	14	**43**
Digitaria sanguinalis	0	0	0	**0**	0	0	1	**1**	5	0	20	**25**
Lamium purpureum	0	1	0	**1**	2	0	0	**2**	2	0	1	**3**
Capsella bursa-pastoris	2	44	43	**89**	23	10	35	**68**	15	14	10	**39**
Convolvulus arvensis	3	0	1	**4**	0	0	0	**0**	0	1	0	**1**
Abutilon theophrasti	2	1	0	**3**	0	0	0	**0**	0	0	0	**0**
Sinapis arvensis	4	0	0	**4**	0	0	0	**0**	0	0	0	**0**

Polygonum persicaria	0	23	11	**34**	0	0	0	**0**	0	0	0	**0**
Polygonum lapathifolium	0	1	1	**2**	21	12	1	**34**	0	2	3	**5**
Hibiscus trionum	0	0	0	**0**	4	0	1	**5**	0	0	0	**0**
Rumex crispus	0	0	0	**0**	2	0	0	**2**	0	0	0	**0**
Cerastium glomeratum	0	0	0	**0**	1	1	0	**2**	0	0	0	**0**
Glechoma hederacea	0	0	0	**0**	1	1	0	**2**	0	0	0	**0**
Senecio vulgaris	0	0	0	**0**	1	0	1	**2**	0	0	0	**0**
Portulaca oleracea	0	0	0	**0**	0	1	0	**1**	0	0	0	**0**
Raphanus raphanistrum	0	0	0	**0**	0	0	0	**0**	0	2	2	**4**
Ukupno sjemenki	**19**	**78**	**64**	**161**	**95**	**49**	**57**	**201**	**59**	**58**	**86**	**203**
Procenat	11,8	48,4	39,8	100	47,3	24,4	28,3	100	29,1	28,6	42,3	100

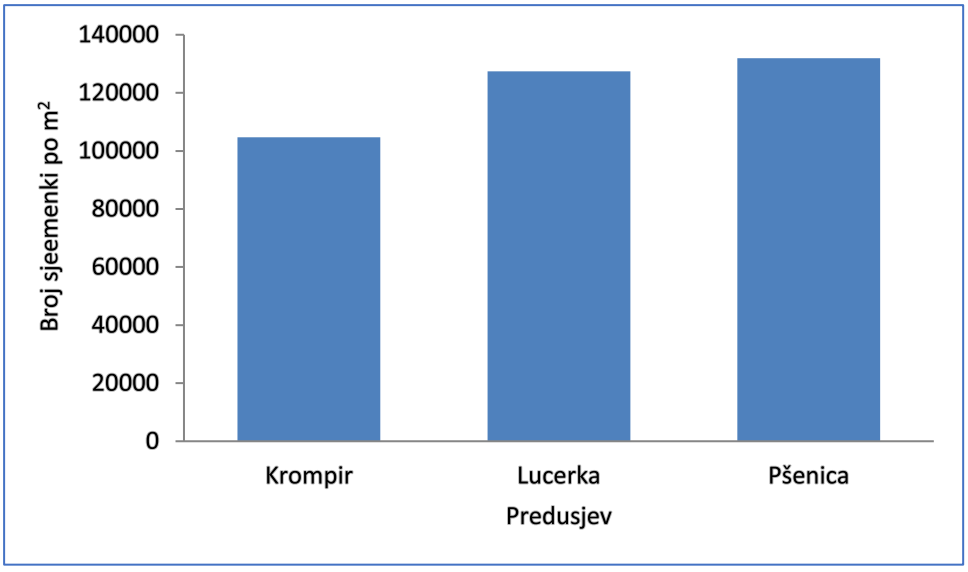


Graf. br. 1. Ukupan broja sjemenki korovskih vrsta u zavisnosti od dubine zemljišta i usjeva

Fig. 1. The total number of weed seeds depending on the depth of the soil and crop

Primjenom metode fizičke ekstrakcije sjemena ukupno je determinisano devetnaest korovskih vrsta (tabela 1). U tlu gdje je predusjev bio krompir evidentirano je 9 vrsta,

ispod pšenice 10 i u lucerki 11. Dominiraju jednogodišnje vrste. *Capsella bursa-pastoris* je identifikovana na svim dubinama i kod sva tri usjeva. Zemljište na kojem je gajena pšenica je sadržavalo najveći broj sjemenki po uzorku - 203, a ono pod krompirom najmanji broj - 161. Preračunato na m^2 u zemljištu (korištenjem već navedene formule) pod pšenicom na dubini od 0 do 30 cm nalazi se 131.950 sjemenki, pod lucerkom 130.650, a pod krompirom 104.650 (graf. 2). Janjić i sar. (2005) navode da se u oraničnom sloju do 30 cm može naći od 200 do 300.000 m^{-2} sjemena korovskih biljaka. U istraživanju Saulić i sar. (2017a) u monokulturi soje registrovano je 50.625 sjemenki korova na m^2.



Graf. br. 2. Ukupan broj sjemenki korova po kulturama
Fig. 2. Total number of weed seeds per crop

Na osnovu statističke analize (x^2=60,55 i sig=0,00<0,05) zaključuje se da postoji povezanost između usjeva i dubine zemljišta u zastupljenosti sjemena korova. Statističke značajne razlike se mogu uočiti između lucerke i dvije preostale kulture. Kod lucerke je zabilježen najveći broj sjemenki u površinskom sloju zemljišta (47,4%), kod krompira identifikovan je najveći broj sjemenki u srednjem sloju zemljišta (48,4%), dok je kod pšenice taj broj bio najveći u najdubljem sloju zemljišta (42,4%). Najmanji broj sjemenki identifikovan je u površinskom sloju zemljišta čija je pretkultura krompir (11,8%).

Banku sjemena korova istraživali su Konstantinović i sar. (2010; 2012; 2016) sa tri dubine (0-10 cm, 10-20 cm i 20-30 cm) i tri usjeva (pšenica, šećerna repa i djetelina). Najveći broj korovskih vrsta na sve tri parcele je determinisan u površinskom sloju zemljišta odnosno od 0 do 10 cm što predstavlja posljedicu neadekvatne primjene agrotehnike, prije svega, nedostatka dubokog oranja za uzgoj poljoprivrednih kultura.

Blagojević i sar. (2014) su u usjevu kukuruza istraživali banku sjemena sa tri dubine 0-10 cm, 10-20 cm i 20-30 cm, a ukupno je determinisano 19 korovskih vrsta, a najveći broj sjemena po m^2 korovskih biljaka determinisan je u sloju zemljišta od 0-10 cm.
U našem istraživanju najveći broj sjemenki u površinskom sloju zabilježen je kod lucerke što je i očekivano jer zemljište nije orano, dok je kod pšenice i krompira primijenjena uobičajena agrotehnička mjera oranje u jesen, te je i zabilježen veći broj sjemenki u dubljim slojevima.

Metoda naklijavanja

Banka sjemena korova dobijena metodom naklijavanja je predstavljena u tabeli 2.

Tab. 2. Brojnost korovskih vrsta u banci sjemena dobijenih metodom naklijavanja sjemena
Table 2. The number of the recorded species in the weed seed bank, obtained by the germination method

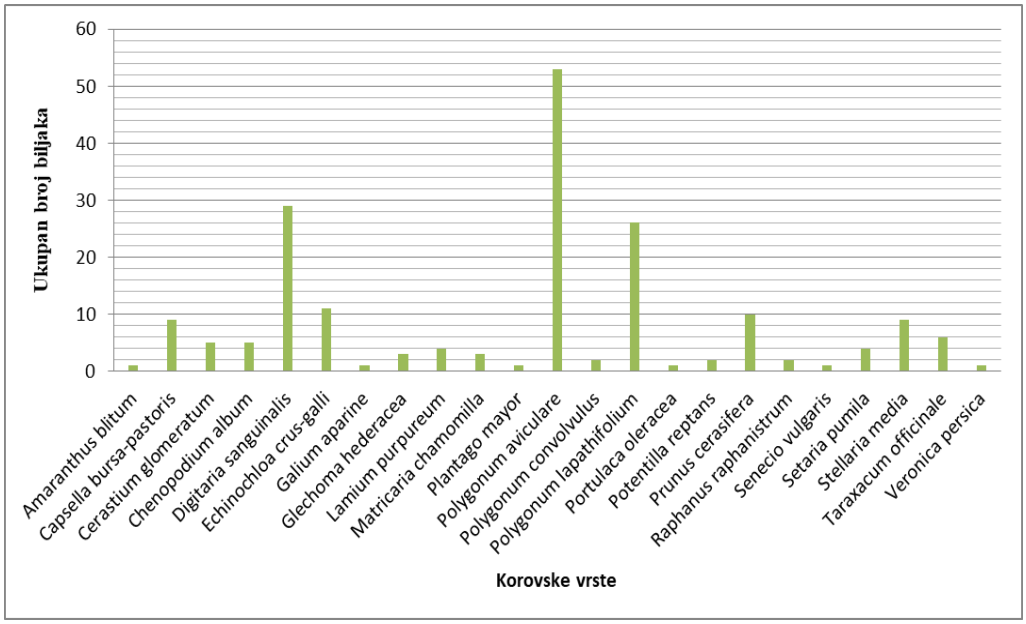
Korov Weed	**Krompir Potato**				**Lucerka Alfalfa**				**Pšenica Wheat**			
	0-10 cm	10-20 cm	20-30 cm	**Suma**	0-10 cm	10-20 cm	20-30 cm	**Suma**	0-10 cm	10-20 cm	20-30 cm	**Suma**
Polygonum aviculare	13	3	7	**23**	9	4	5	**18**	5	1	6	**12**
Echinochloa crus-galli									2	4	5	**11**
Chenopodium album					1	2	2	**5**				
Amaranthus retroflexus									0	0	1	**1**
Digitaria sanguinalis	1	1	0	**2**	1	0	0	**1**	4	5	17	**26**
Lamium purpureum									1	1	2	**4**
Capsella bursa-pastoris	1	2	1	**4**	3	0	2	**5**				
Polygonum lapathifolium	2	0	3	**5**	9	3	4	**16**	0	0	5	**5**
Potentilla reptans									0	0	2	**2**
Setaria glauca									0	3	1	**4**
Cerastium glomeratum	1	0	0	**1**	3	1	0	**4**				

Glechoma hederacea					1	0	2	**3**				
Senecio vulgaris					0	0	1	**1**				
Portulaca oleracea	0	0	1	**1**								
Raphanus raphanistrum					0	0	1	**1**	0	0	1	**1**
Polygonum convolvulus	0	0	1	**1**	1	0	0	**1**				
Plantago mayor	0	0	1	**1**								
Prunus cerasifera	2	0	2	**4**	1	0	4	**5**	1	0	0	**1**
Matricaria chamimolla	1	0	1	**2**	1	0	0	**1**				
Stelaria media	3	0	0	**3**	1	0	0	**1**	2	3	0	**5**
Veronica persica	0	0	1	**1**								
Taraxacum officinale					0	0	1	**1**	3	1	1	**5**
Gallium aparine									0	0	1	**1**
Ukupan broj klijanaca	**24**	**6**	**18**	**48**	**31**	**10**	**22**	**63**	**18**	**18**	**42**	**78**
Procenat	50,0	12,5	37,5	100	49,2	15,9	34,9	100	23,1	23,1	53,8	100

Metodom naklijavanja ukupno su determinisane 23 korovske vrste. Ovom metodom je zabilježen veći broj korova u odnosu na metodu fizičke ekstrakcije sjemena (19 korovskih vrsta). Vučković i sar. (2011) su determinisali veći broj korovskih biljaka metodom fizičke ekstrakcije sjemena. Shah i sar. (2017) su poredili metod fizičke ekstrakcije sjemena sa metodom naklijavanja. Više korovskih vrsta determinisano je metodom fizičke ekstrakcije u poređenju sa metodom naklijavanja. U vlastitim analizama banke sjemena korova, korištenjem pomenutih metoda dobili smo različite rezultate. Metoda fizičke ekstrakcije sjemena pokazala je veću brojnost sjemena/m² i raznolikost korovskih vrsta u poređenju sa drugom metodom. Metoda naklijavanja može znatno podcijeniti gustoću sjemena m^{-2} korovskih vrsta zbog grešaka povezanih sa dormantnošću sjemena i ekološkim zahtjevima za klijanje.

Najveći broj poniklih biljaka (82 biljke) zabilježen je na dubini 20-30 cm. Potom slijedi površinski sloj zemljišta sa 73 ponikle biljke, a najmanji broj poniklih biljaka je determinisan u srednjem sloju zemljišta odnosno na dubini 10-20 cm. *Polygonum aviculare* (53) se izdvaja kao vrsta sa najvećim brojem poniklih jedinki (graf. 3). Potom slijedi *Digitaria sanguinalis* sa 29 poniklih biljaka. I u radu Vučković i sar. (2011) ove dvije korovske biljke su imale najveći broj poniklih jedinki. Po brojnosti se dalje ističu *Polygonum lapathifolium* (26), *Echinochloa crus-galli* (11 biljaka) i *Prunus cerasifera* (10 biljaka). Korovska vrsta *Chenopodium album* je u metodi fizičke ekstrakcije identifikovana sa 89 sjemenki, ali su njene ponikle biljke bile rijetke (pet

biljaka). Slični podaci su dobijeni u istraživanju Tracy i Sanderson (2000) koji zaključuju da sjemenki ove korovske vrste ima dosta u banci, ali da je rijetka u vegetaciji.



Graf. br. 3. Ukupan broj klijanaca korovskih biljaka
Fig. 3. Total number of weed seedlings

Prema rezultatima ove metode ne postoji statistički značajna povezanost između različitih vrsta korova i dubine zemljišta u odnosu na broj poniklih biljaka na zemljištu kod sva tri predusjeva.
Kako navode Saulić i sar. (2017b) na rezultate ovakvih istraživanja utiče i odabir adekvatne strategije i metode uzorkovanja, kao i metode procjene rezerve sjemena korovskih biljaka u zemljištu. Ogledi ovakvog tipa svrstaju se u dugoročna ekološka istraživanja a dobijeni podaci bi mogli da posluže za formiranje baze i izradu modela za prognozu pojave korova pri određenim agroekološkim uslovima.

ZAKLJUČCI

Banka sjemena korova u zemljištu nakon uzgoja pšenice, krompira i u lucerki je vrlo bogata vrstama ali i brojnošću jedinki. Metodom fizičke ekstrakcije sjemena determinisano je 19 korovskih vrsta. Sjemena korovskih vrsta *Polygonum aviculare, Chenopodium album, Lamium purpureum, Capsella-bursa pastoris* i *Polygonum lapathifolium* su identifikovana na svim dubinama kod sva tri predusjeva, pri čemu se *Capsella-bursa pastoris* i *Chenopodium album* izdvajaju po brojnosti (196 i 89). Metodom naklijavanja ukupno su determinisane 23 korovske vrste. Najveći broj jedinki

je evidentiran na uzorku gdje je predusjev bila pšenica (79), potom slijedi lucerka (63), najmanje nakon krompira (48). Najveći broj poniklih biljaka zabilježen je kod korovske vrste *Polygonum aviculare* (53), a potom slijedi uskolisni korov *Digitaria sanguinalis* sa 29 biljaka.

Obje korištene metode imaju i prednosti i nedostatke. Za metodu fizičke ekstrakcije sjemena potreban je kraći vremenski period u odnosu na metodu naklijavanja, dok je za drugi metod potreban duži vremenski period za identifikaciju. Svakako da istovremeno provođenje obje metode daju bolju sliku banke sjemena korova u tlu.

Poznavanje banke sjemena unutar proizvodnog zemljišta je važno jer njenim ispitivanjem dobijamo informacije koje korovske vrste i sa kolikom brojnošću sjemena se nalaze u oraničnom sloju zemljišta, odnosno u sloju dubine od 0 do 30 cm. Sjeme koje se nalazi u zemljištu predstavlja izvor zakorovljavanja narednih usjeva. Na osnovu analize banke sjemena korova u zemljištu, može se prognozirati nicanja korovskih biljaka, ali i pravilan odabir herbicida za njihovo suzbijanje.

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WEED SEED BANK IN ARBLE SOIL

Summary

The weed seed bank is an important source of crop weeding. Knowledge of the weed seed bank is very important for the prediction of weed germination and the proper selection of herbicides. The aim of this research was to determine the weed seed bank in arable soils that were previously used for potato and wheat, and in the plot where alfalfa growing. Soil samples were taken at three different depths: 0 - 10 cm, 10 - 20 cm and 20 - 30 cm. Two methods were applied: physical seed extraction and germination method. A greater number of weed species were identified using the germination method (23), while 19 species were recorded using the physical extraction method. The largest number of weed was recorded in the sample where the previous crop was wheat, followed by alfalfa, and the least after potato.

Key words*: weed seed bank, wheat, alfalfa, potato, soil depth*

BIOGEOGRAPHIC DIFFERENTIATION OF EPIGEAN FRESHWATER AMPHIPODS (AMPHIPODA: CRUSTACEA) IN BOSNIA AND HERZEGOVINA

Mahir Gajević[1], Sadbera Trožić-Borovac[1], Aldijana Mušović[1], Adi Vesnić[1], Samir Đug[1]

Original scientific paper

Summary

The inland fauna of epigean freshwater gammarids in Bosnia and Herzegovina is diverse and abundant. This region has a favorable geographical position on the Balkan Peninsula. Excluding Ponto-Caspian species originating in brackish waters and freshwater subterranean taxa, there are 6 formally recognized epigean freshwater species recorded from this country. They belong to 2 genera, from the family Gammaridae, *Gammarus* 4 species and *Echinogammarus* 2 species. We provide new data with high-resolution distribution maps, thus improving the knowledge of the ranges of these taxa. Studied species display substantial altitudinal variability and, fragmented distribution. They occur abundantly, particularly in springs and streams, from lowlands to sub-mountainous and mountainous regions. The mosaic distribution of epigean freshwater amphipod species in B&H shows that this region is particularly suitable for biogeographical analyses of this group. Their large-scale distribution patterns remain obscure due to insufficient data, consequently limiting biogeographical interpretations.

Key words: *Bosnia and Herzegovina, biogeography, distribution, Gammarus, Echinogammarus*

INTRODUCTION

Understanding past influences that have affected current species distributions can be gained via studying distribution patterns (Brown *et al*., 1996). Because of their limited ability to disperse and the fragmented nature of freshwater environments, freshwater amphipod crustaceans are particularly well-suited for biogeographical investigations (Väinölä *et al*., 2008; Hou *et al*., 2011). Amphipods are predominantly aquatic benthic animals that do not possess free-swimming larval stages or resistant propagules, and thus are prone to genetic differentiation and isolation (Barnard & Barnard, 1983). Additionally, a lot of freshwater taxa exhibit allopatric or discontinuous distributions, which are frequently thought to be the result of geologically-related secondary events like island separation, sea level changes, and continental breakup, or that adhere to prehistoric drainage patterns (Hogg *et al*., 2006; Finston *et al*., 2007; Bauzà-Ribot *et al*., 2011, 2012).

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Freshwater amphipod species are rather numerous on the European continent, and diversity rises in the southeast (Väinölä *et al*., 2008). Due to Bosnia and Herzegovina's advantageous geographic location, which places it in one of the significant biodiverse regions in Europe - the Balkan Peninsula - the country has a relatively rich diversity of amphipod species. In terms of biodiversity and endemism, Dinarid Mountains in Bosnia and Herzegovina are likewise regarded as a hotspot location (Ivković & Plant, 2015). Furthermore, the area was a significant glacier refuge throughout the Pleistocene glaciations (Brus, 2009) and underwent active geological events in the Late Tertiary (Argnani, 2018). Because of its diverse terrain, Bosnia and Herzegovina offers a suitable environment for studying amphipod biogeographical trends at a more focused, finer scale. As a result, it can shed additional light on the mechanisms shaping the geographic distributions of freshwater benthic crustaceans.

The biogeographical differentiation of Bosnia and Herzegovina is primarily conditioned by its geographical position, climate, and altitude. The vegetation in the northern, eastern, western, and central parts of Bosnia and Herzegovina has similarities with the vegetation in Central Europe, while the vegetation in Herzegovina and the western and southwestern parts of Bosnia shows similarities with the vegetation in Mediterranean areas. Three biogeographical regions can be differentiated in the area of the Dinarides: the Mediterranean, the Continental, and the Alpine region (Đug & Škrijelj, 2009).

The Ponto-Caspian brackish/freshwater taxa and generally stygobiotic (subterranean) species are excluded from this study since it primarily focuses on epigean freshwater amphipod species, which complete their life cycle in surface freshwaters and occasionally appear in subterranean waters. There are six formally recognized native amphipod taxa that fit the aforementioned requirements and are found in Bosnia and Herzegovina's inland surface freshwaters. Those species belong to the genera *Gammarus* Fabricius, 1775 and *Echinogammarus* Stebbing, 1899 from the Gammaridae family.

Although the epigean amphipod fauna of Bosnia and Herzegovina is abundant and diversified, little is known about the distribution patterns of these species. Studies addressing these issues typically concentrated on national level (Trožić-Borovac, 2014).

We analyze pertinent literature and add new, in-depth data to update distribution maps of Bosnia and Herzegovina-inhabited epigean freshwater amphipod species.

MATERIALS AND METHODS

The material for this research was collected from 90 sample locations between 2007 and 2015 across the entire country of Bosnia and Herzegovina. A benthic hand-net with a mesh size of 250 µm was used to collect the samples, which were then preserved in either 70% or 96% ethanol or 4% formaldehyde solution. We examined every microhabitat that was accessible at each sampling location. A review of the literature was done from the pertinent studies, including the most current ones, and distribution data were collected. The distribution maps were created using data from the literature

and this study. Taxa were identified using the morphological delimitation criteria of the following authors: G. Karaman & Pinkster (1977a; 1977b; 1987), Pinkster (1993) and Stock (1968). For each investigated locality, geographic coordinates were determined using a GPS device during sampling or subsequently. Distribution maps with mapped localities were created in the ArcMap program from the ArcGIS 9.2 software package.

RESULTS AND DISCUSSION

During the research for this study, a total of four species from the genus *Gammarus* and two species from the genus *Echinogammarus* were found in watercourses in Bosnia and Herzegovina (tab. 1). Within the genus *Gammarus*, the presence of the species *Gammarus balcanicus* Schaferna, 1922, *Gammarus bosniacus* Schaferna, 1922, *Gammarus fossarum* Koch, 1836 and *Gammarus roeselii* Gervais, 1835 was recorded. Within the genus *Echinogammarus*, two species *Echinogammarus acarinatus* (S. Karaman, 1931) and *Echinogammarus thoni* (Schaferna, 1922) were recorded.

Table 1. List of epigean freshwater amphipod taxa found in Bosnia and Herzegovina, their type localities, distribution ranges and habitats.
Habitat abbreviations: Sp-springs, St-streams, R-rivers, L-lakes, Rl-reservoir lakes.

Taxa	**Type locality**	**Range**	**Habitats in B&H**
Gammarus balcanicus Schaferna, 1922	Kolašin, Monte Negro	SE Europe, Asia Minor	Sp, St, R
Gammarus bosniacus Schaferna, 1922	Sarajevo, B&H	B&H, the basin of the upper course of the Bosna River	Sp, St, R
Gammarus fossarum Koch, 1836	Regensburg, Germany	Western, Central and SE Europe, Asia Minor	Sp, St, R
Gammarus roeselii Gervais, 1835	Coulanges-sur-Yonne, France	Central and SE Europe, Asia Minor	R
Echinogammarus acarinatus (S. Karaman, 1931)	Mostar, B&H	SE Europe	St, R
Echinogammarus thoni (Schaferna, 1922)	Lake Deransko, BiH	SE Europe	L, Rl

The species *G. bosniacus* is an endemic species of crayfish for Bosnia and Herzegovina, described and up to this research found only on a narrow stretch from Vrelo Bosna to Rimski Most. Until now, it was considered that this species inhabits only the source of the Bosna River, where it was described by Schaferna in 1922, and again described by Karaman G. S. in 1975. For this species, there are data that it was present in the Bosna River as far as the mouth of the Miljacka River (Šenk, 1956). In addition to the Bosna River in its headwaters, this species was also found in the rivers that are on the Bjelašnica mountain or drain water from that terrain (Bjelašnica and Bijela). Given this distribution, it is possible to assume that the center of origin of this species is on the

mentioned mountain, and that it spread to the headwaters of the Bosna River via underground waters.

The most widespread species of gammarids in Bosnia and Herzegovina is *G. balcanicus* recorded at 70 sites. It is spread through all biogeographical regions in Bosnia and Herzegovina. This species in B&H has distribution in the Glina River basin, Una River basin, Bosna River basin, Drina River basin, Cetina River basin and in the Neretva River basin. *G. balcanicus* is a species that has a wide distribution in Southeast Europe and Asia Minor (Barnard & Barnard, 1983; G. Karaman & Pinkster, 1987; Özbek & Ustaoğlu, 2006; Özbek *et al*., 2009). This species is the most widespread gammarid in Romania as well (Petrescu, 1994). According to Copilas-Cocian et al. (2014), this species has the widest habitat altitude range among freshwater amphipods in Romania, which ranged between 16 and 1530 m, and most localities are between 300 and 600 m. It occurs most often in springs and streams, and occasionally in caves and rivers. In certain localities, it was found in coexistence with the species *G. fossarum* and *G. roeselii* (Copilas-Cocianu *et al*., 2014).

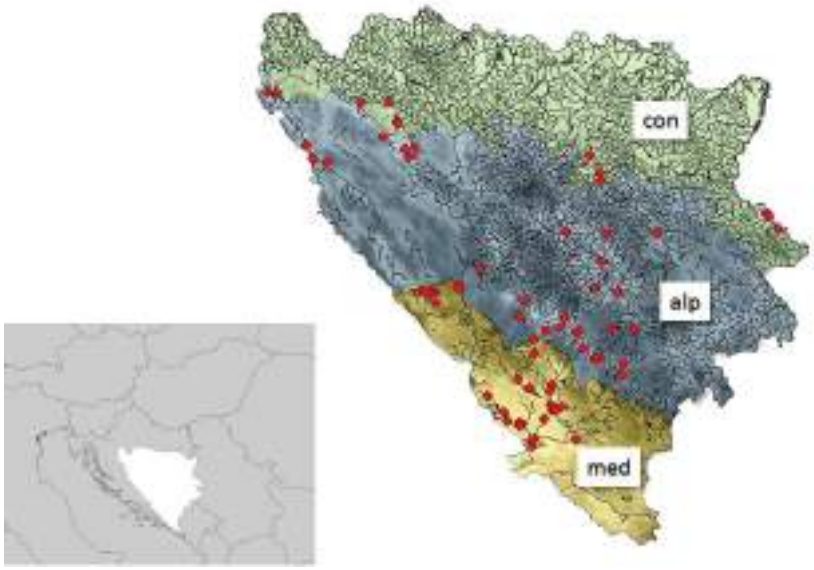

Figure 1. Distribution of *G. balcanicus*. con – continental, alp – alpine, med – mediterranean.

The species *G. fossarum* in Bosnia and Herzegovina was found only in the Black Sea basin, in the Una, Vrbas, Bosna, and Drina River basins, and in the immediate Sava River basin. *G. fossarum* has a wide range that includes western, central, and southeastern Europe and reaches northern Anatolia (G. Karaman & Pinkster, 1977a; Barnard & Barnard, 1983; Özbek & Ustaoğlu, 2006). In Romania, it occurs in the western part of the Carpathians in two isolated regions, one in the northwest and the other in the southwest. The altitude range of localities where this species has been recorded ranges from 47 to 860 m, and most often between 300 and

550 m. Populations from southwestern Romania also occur in lowland rivers, while northwestern populations are restricted to springs and streams in sub-mountainous areas. In some localities, *G. fossarum* coexists with *G. balcanicus* and *G. roeselii* (Copilas-Cocianu *et al*., 2014).

During this research, the species *G. roeselii* was found at four localities in the Una River basin. Three localities were recorded on the Una River itself (Bosanska Krupa and two localities near Bosanska Otoka), and one locality on the Sana River (Sanski Most). In Bosnia and Herzegovina, this species is found predominantly in the watercourses of the Continental region. The species *G. roeselii* is distributed in Western, Central, and South-Eastern Europe as well as South-Eastern Europe, and in the western part of Turkey (G. Karaman and Pinkster, 1977a; Barnard & Barnard, 1983; Jazdzewski & Roux, 1988; Özbek & Ustaoğlu, 2006). In Romania, it is present in several regions (Motaş *et al*., 1962; Pârvulescu 2009). It is a typical lowland taxon that occurs mainly at altitudes below 200 m. This species is the most ecologically plastic gammarid, which can be found in springs, streams, rivers, and occasionally in lakes and swamps (Motaş *et al*., 1962). It can co-occur with *G. balcanicus* and *G. fossarum*.

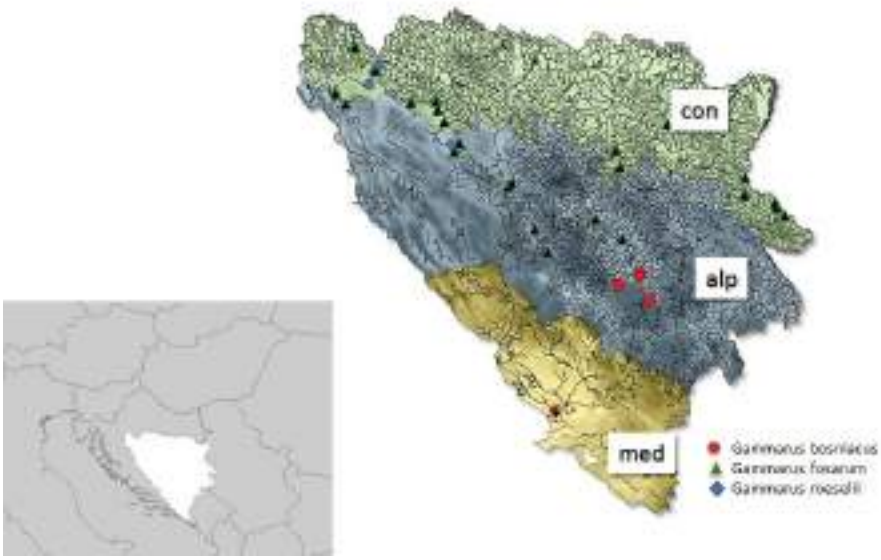

Figure 2. Distribution of *G. bosniacus*, *G. fossarum* and *G. roeselii*. con – continental, alp – alpine, med – mediterranean.

Analyzing the distribution of individual species from the genus *Gammarus*, it was determined that there are overlapping zones of certain habitats for some species. The most noticeable mixing between populations of different species was recorded between *G. balcanicus* and *G. fossarum*. For these two species, habitats overlap in the Una River basin, in the locality downstream from Bihać, and in the rivers Sana and Sanica. Common habitats for these two species were also observed in the Bosna River basin, in

its upper and middle reaches. Overlapping habitats were also noted in the area of the Drina River basin.

The genus *Echinogammarus* is the second most numerous genus within the family Gammaridae, in which 53 species have been described. Most of them inhabit freshwater and brackish waters, and only a small number are exclusively marine species. More than most of the species of this genus known so far (35 or 66%) are distributed in Southern Europe with the center of endemism in the Iberian Peninsula, where 20 endemic species have been described. The distribution and ecology of endemic species are poorly researched, and many species are known only from a few sites or from the type locality (Pinkster, 1993).

The species *E. acarinatus* was found in both major sea basins in Bosnia and Herzegovina and the Continental and Mediterranean regions. In the Black Sea basin, it was found in the Vrbas River basin in the Duboka river near Bugojno at an altitude of 715 m. In the Bosna River basin, this species was found in Plava Voda, a tributary of Lašva near Travnik, near the watershed between the Vrbas and Bosna basins. In the Adriatic Sea basin, this species is present in the Neretva River basin in two localities, the Radobolja River and the mouth of the Buna in the Neretva.

The species *E. acarinatus* was first described as a form of *G. pungens f. acarinata* (Schaferna, 1922). After ten years, Karaman, S. (1931) raised this form to species level and called it *Ostigammarus acarinatus*, while Karaman, G. (1970) included this species in the genus *Echinogammarus*. The type locality of this species is the Buna River near the mouth of the Neretva. The area of this species includes the source of the river Krka, Butižnica (a tributary of the Krka), Jadro, Lake Vrana near Biograd, salt springs near Trogir, the river Jadrtovac near Šibenik, Neretva near Metković, a small stream near Dubrovnik, a stream near Lake Vrana and the stream Stobreč in Croatia (Karaman, 1931; Karaman, 1970; Pinkster, 1993), and in the Buna and Radobolja rivers near Mostar (Karaman, 1970). The species *E.s bosnensis* S. Karaman, 1934 is synonymized with the species *E. acarinatus*. The type locality for the mentioned synonymized species is Šumeće Vrelo near Travnik. As this species is widespread in various watercourses in the areas of Croatia and Bosnia and Herzegovina, where certain abiotic factors vary greatly, it can be said that it is a eurivalent species that occur in waters where temperatures range from 10-25ºC, with electrical conductivity of 365-1007 μScm^{-1}, and inhabits saline springs and freshwater ecosystems (Karaman, 1931; Žganec, 2009). This species reaches a significantly higher number of individuals on plant substrate (Žganec, 2009). Further research is necessary to determine the range of tolerance of this species for certain abiotic factors in the aquatic environment.

The species *E. thoni* in Bosnia and Herzegovina was found only in the Neretva River basin in four localities. Earlier findings of this species in Lake Deran, Bregava, and Buna rivers were confirmed. During the research, this gammarid was also recorded in the Salakovac reservoir on the Neretva River and represents the northernmost point of distribution of this species. After the description of this species, it was found in five more localities in the Neretva delta in Croatia and Bosnia and Herzegovina, as well as in the upper course of the Jadro river near Split (Karaman, 1929, 1934; Karaman,

1969). This gammarid was also recorded in the Buna and Bregava rivers, near the confluence with the Neretva. More recently, this species has also been found in the Canj stream near Bar, and in the Orahovica river near Skadar lake in Montenegro (Grabowski & Pešić, 2005; Žganec *et al*., 2010). In more recent research, *E. thoni* was found in several new localities in Croatia and watercourses in Albania (Žganec *et al*., 2010). So far, only the species *G. balcanicus* has been found in the middle part of the Neretva River from the Amphipoda order (Škrijelj, 2002). The physical and chemical conditions recorded in this hydro accumulation show that this population of the mentioned species is well adapted to life in depths of up to 20 m, and with a relatively constant and low temperature, which is up to 10°C, which confirms its good adaptation to different living conditions in different types aquatic habitats. This species is well adapted to the conditions of high salt concentration in the water, and tolerates concentrations up to 6.9‰, and it reaches its highest abundance in summer in warm watercourses, where the temperature during this season is between 20 and 25°C. The distribution center is located in the southern part of the Neretva River delta, where it inhabits most of the lower reaches, and probably also most of its tributaries in that part (Žganec *et al.*, 2010).

Figure 3. Distribution of *E. acarinatus* and *E. thoni*.

Biogeographical distribution of freshwater epigean amphipod species in B&H expresses different patterns. *G. bosniacus* was found only in the Alpine region and *E. thoni* only in the Mediterranean. *G. balcanicus* inhabits watercourses in all three biogeographical regions in B&H, but 60% of recorded sites are in the Alpine region, and 27% are in the Mediterranean. *G. roeselii* and G. *fossarum* were recorded in the Alpine and Continental regions. According to our data, these two species are mostly distributed in the

Continental region (fig. 4). *E. acarinatus* has equal distribution in the Mediterranean and alpine regions.

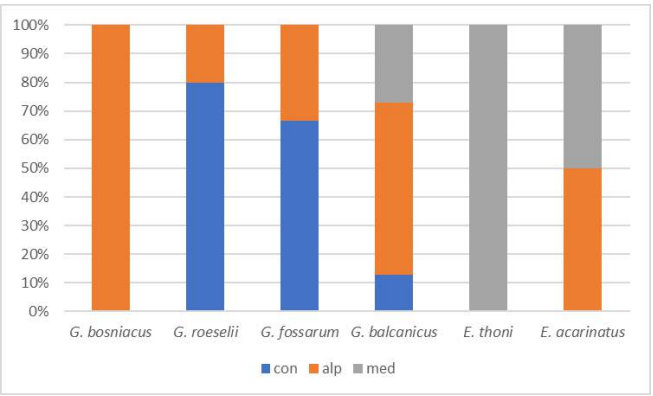

Figure 4. Biogeographical range of epigean freshwater gammarids in B&H. con – continental, alp – alpine, med – Mediterranean.

The habitat analysis of species from the genus *Gammarus* covers locations from 0 to 1200 meters above sea level. The species *G. balcanicus*, which was found in sites ranging from sea level to 1200 meters, shows the widest amplitude of variation in altitude (fig. 5).

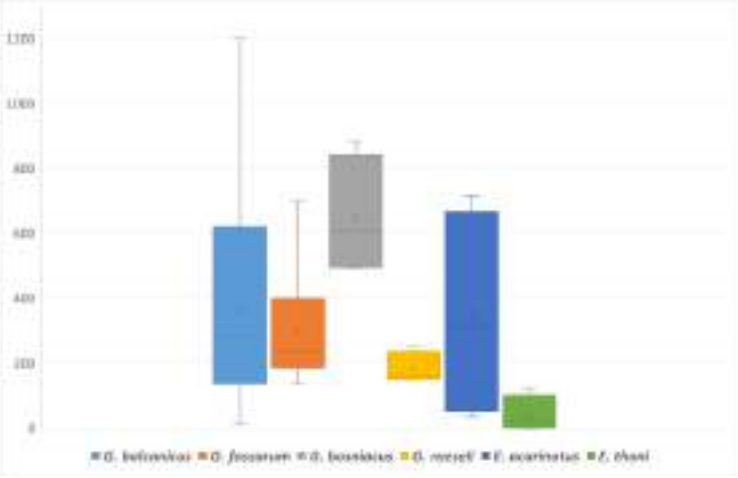

Figure 5. Boxplots representing the altitudinal ranges of studied epigean amphipod taxa in Bosnia and Herzegovina.

However, the largest number of localities where this species has been recorded ranges from 200 to 600 meters above sea level. The species *G. fossarum* was found in sites from 150 to 750 meters above sea level with most sites in the range of 400 to 600 meters. The altitude range of the habitat for the *G. bosniacus* ranged from 450 to 950 meters above sea level. The species *G. roeselii*, which was observed in sites whose altitude

ranged from 180 to 250 meters above sea level, shows the smallest range of variation in habitat height (Fig. 5). Habitats of two *Echinommarus* species ranges from 0 to 750 meters above sea level. The wider altitudinal range has *E. acarinatus*, and *E. thoni* was found only in habitats between 0 and 123 meters above sea level (fig. 5).

CONCLUSION

To fully appreciate Bosnia and Herzegovina's unique fauna of epigean freshwater amphipods, additional taxonomic research is required. The patchy and altitudinal variability of the epigean freshwater amphipod distributions in B&H is distinctive features. These make up a suitable model system for studying biogeography and phylogeography at a fine scale, with implications for further research in ecology, adaptation, and speciation of freshwater amphipods. This is due to their limited capacity for dispersal as well as the heterogeneous topography and geology of this area.

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BIOGEOGRAFIJA NADZEMNIH SLATKOVODNIH RAKUŠACA (AMPHIPODA: CRUSTACEA) U BOSNI I HERCEGOVINI

Rezime

Kopnena fauna nadzemnih slatkovodnih rakušaca u Bosni i Hercegovini je raznolika i bogata. Dinaridi imaju povoljan geografski položaj na Balkanskom poluotoku i predstavljaju jedan od centara biodiverziteta. Isključujući ponto-kaspijske vrste koje potječu iz bočatih voda i slatkovodne podzemne vrste, postoji 6 službeno priznatih nadzemnih slatkovodnih vrsta zabilježenih u Bosni i Hercegovini. Sve vrste spadaju u 2 roda, iz porodice Gammaridae, *Gammarus* 4 vrste i *Echinogammarus* 2 vrste. U ovom radu su izneseni novi podaci s kartama distribucije visoke rezolucije, čime se poboljšava poznavanje rasprostranjenosti ovih vrsta. Proučavane vrste pokazuju znatnu visinsku varijabilnost i fragmentirane obrasce distribucije. Javljaju se u većim brojevima, osobito u izvorima i potocima, od nizinskih do pretplaninskih i planinskih krajeva. Mozaička distribucija ovih vrsta u BiH pokazuje da je ovo područje posebno pogodno za biogeografske analize ove skupine. Njihovi obrasci distribucije ostaju nejasni zbog nedovoljno podataka, što posljedično ograničava biogeografska tumačenja.

Key words: *Bosna i Hercegovina, biogeografija, distribucija, Gammarus, Echinogammarus*

EFFICIENCY OF ORGANIC WASTE RECYCLING THROUGH VERMICOMPOSTING PROCESS

Adrijana Filipović[1,2], Ana Mandić[1], Alisa Hadžiabulić[3], Antonio Stipanović[1], Helena Brekalo[1]

Original scientific paper

SUMMARY

The agricultural sector and growing food production needs relies on chemical fertilizers. This initially had positive effects on production, but over the last decade the negative impacts of overuse have resulted in low crop productivity, increased pest and disease infestation, soil degradation and consequent adverse effects on environmental parameters. As one of the possibilities in organic farming we found vermicomposting as the more sustainable practice. For the assembly of vermicomposting, mature cattle manure was utilized in three different combinations with organic material and earthworms inoculations (the household waste, the grass clippings and mixture). The experiment was set up by random block design in three replications. Analyzes of qualitative parameters of final product showed a pH reaction neutral to slightly alkaline. The nitrogen compound varied between 2.7-2.9 %, while organic carbon varied between 45.59-47.41% showing a C/N ratio of 16.7-16.67. The potassium content varied between 1.1-1.2% K_2O and phosphorus from 0.3-0.5%. The experiment indicates quite good results of vermicomposting of household waste with manure, showing a neutral pH reaction optimal content of ash and carbon with almost similar and good NPK contents and increased C/N ratio of 16.7 and with satisfying trace elements content and heavy metal contents under the limits. These results indicate that vermicomposting can increase quality of final product.

Key words: *vermicomposting, earthworms, recycling, organic waste*

INTRODUCTION

Vermicomposting may be a mesophilic process (Edwards and Burrows, 1988) that involves a joint action of earthworms (active at 10-32 °C) and mesophilic microbes (Benitez *et al*., 1999) for the conversion of organic wastes into a valuable conclusion called vermicompost. Sinha *et al*. (2010) stated that earthworms restore and improve

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soil fertility and significantly boost crop productivity. It's been found that over the last 20 years, 75% of the agricultural sector relies on chemical fertilizers in agricultural production. This initially had positive effects on production, but over the last decade the negative impacts of overuse have resulted in low crop productivity, increased pest and disease infestation, soil degradation and consequent adverse effects on environmental parameters. This has led to the emergence of the employment of organic agriculture through the varied organic amendments, bio pesticides and bio control measures by researchers and farmers in numerous countries, which has created selective markets for organic products. This can be also supported by the increased use of solid organic waste produced at different levels which mainly includes 46% of organic waste globally, incorporated into soil and water causing various pollutants. Such organic waste is recycled by processes like vermicomposting which may produce organic fertilizers rich in nutrients that are an enriched source of beneficial microbes. Increased public interest and environmental problems associated with the utilization of manure are directing farmers towards alternative solutions, and composting enables a big reduction of environmental problems associated with the employment of assorted manure fertilizers. One of the simplest explanations for vermicomposting is a process that uses earthworms to transform organic residues into a secondary product named vermicompost that can be used as a fertilizer for crop production (Dominguez, 2004). Vermicompost improves the physical, chemical and biological properties of the soil as well contributes to organic enrichment (Chauhan and Singh, 2013). Vermicomposting is the biotechnological method of composting by using different species of earthworms to boost waste conversion mechanism and achieve better product (Devi and Prakash, 2015). The aim of this study is to provide a vermicompost enriched by nutrient and microorganism utilized from mixed bio waste using earthworms.

MATERIALS AND METHODS

For the assembly of vermicompost, partly mature manure was utilized in three different combinations with organic material and earthworms. Mature manure was used as an initial substrate for composting with California earthworms (*Eisenia fetida,* Figure 1).

Figure 1. California earthworms (*Eisenia fetida*)

The experiment was organized in line with a random block design in three replications. The initial mature manure was placed in three combinations with household waste, the grass clippings, while the controls were a combination of the previous two combinations. Moistening (if required) was performed in order to maintain the moisture level at 40- 50 %. The composting material was prepared in plastic airtight boxes with a volume of 40 L dimension 36 x 35 x31 cm (L x W x H), at the underside and sidelined wrapped with dark waterproof canvas with small holes. Inoculation composting of epigeic California earthworms was added to every separate box (10 worms per kg material) on an initial mass of 5 kg of manure per treatment. In each box, 1 kg of the mentioned organic materials were added to the surface at weekly intervals. Vermicompost was mixed, finely sieved, sampled, and analyzed after 6 months. The mixtures of used treatments were shown in Figure 2.

Figure 2. Organic materials used for vermicomposting (Household waste, grass clips, and a mixture of the two)

For qualitative analysis, a median mass sample was taken, and also the standard chemical parameters, substitution pH reaction (in 1 mol dm^{-3} KCl) potentiometrically in step with BAS ISO 10390:2009 v/v 1:5) and active pH reaction (in H_2O) was analyzed. Nitrogen contents in vermicomposting mass were determined by the Kjeldahl method (BAS ISO 11261: 2010). Determination of potassium and phosphorus was performed by wet incineration and sample analysis on a phosphorus spectrophotometer and a potassium flame photometer. Values are expressed as P_2O_5 and K_2O in percentage. Total carbon was calculated from the results obtained by the determination of the organic matter. The C/N ratio was calculated using the information of total carbon in dry matter and total nitrogen. The dry matter content was obtained by drying the sample at 105° C to constant mass. Electrical conductivity (EC, mS cm^{-1}, TDS g dm^{-3} and salinity ‰) was analyzed in the ratio of 1:2 (w/v) by a digital portable conductivity meter (HACH semsyion5). Volatile solid content was determined by loss ignition method (dry mass basis) at 550°C for 1 h. The total organic carbon content was calculated from volatile solids (Mohee *et al.*, 2008). Extraction of elements soluble

in aqua regia Ca, Mg, Zn, Cu, Cd, Fe, Pb and Na were done according to ISO 11466:2000 and determination was done by the method of flame and graphite atomic absorption spectrometry: ISO 11047:2000 on the device Perkin Elmer FIAS 800.
Before the inoculation of worms on composting mixture, the manure utilized in the trial was analyzed.
The results were reported as the mean of three replicates with standard error (SE). ANOVA and Tukey's test was used to test the effect of different chemical properties of the vermicompost products due to the earthworm activities. The probability level used for statistical significance was $p < 0.05$ for all measured parameters. For statistical processing the data's GenStat 7 software (Laws Agricultural Trust, Rothamsted Experimental Station) was used.

RESULTS AND DISCUSSION

Results of initial cattle manure used in the trial indicated alkaline pH reaction (8.3) and lower nitrogen and higher carbon content comparing to vermicompost treatment resulting in a C/N ratio of 25.57. Potassium content was low 0.5 % K_2O similar to phosphorus content of 0.8% P_2O_5. Results of this trial with vermicomposting for basic parameters are shown in the table 1 below:

Table 1. The treatment used in the vermicomposting process presented some chemical characteristics

Parameters	Initial cattle manure	Cattle manure and domestic waste	Cattle manure and grass clippings	Mixture (control)
pH H_2O	8.3±0,2	7.2 ± 0.14^{ns}	8.3 ± 0.44^{ns}	7.1 ±0.47^{ns}
pH KCl	7.5±0,1	6.8 ± 0.9^{ns}	7.0 ± 0.37^{ns}	6.7 ± 0.34^{ns}
%N	1.9±0,11	2.7 ± 0.01^{ns}	2.8 ± 0.10^{ns}	2.9 ±0.12^{ns}
%P_2O_5	0.5±0,21	0.3 ± 0.11^{ns}	0.3 ± 0.31^{ns}	0.5 ± 0.15^{ns}
%K_2O	0.8±0,14	1.2 ± 0.08^{ns}	1.1 ± 0.09^{ns}	1.2 ± 0.19^{ns}
% ash	17,4±0,85	21.58 ± 0.99^{ns}	20.20 ± 1.4^{ns}	18.46 ±1.05^{ns}
%C	48.6±3,6	45.59 ± 2.9^{ns}	46.39 ± 5.3^{ns}	47.41 ± 3.8^{ns}
C/N	25.57±2,2	16.7 ± 1.1^{ns}	16.29 ± 2.1^{ns}	16.67 ± 1.3^{ns}
g dm^{-3} TDS	2,01±0,63	2.46 ± 0.32^{ns}	2.81 ± 0.83^{ns}	2.56 ± 0.61^{ns}
‰ Salinity	0,5±0,55	2.53 ± 0.32^{ns}	2.90 ± 0.86^{ns}	2.67 ± 0.61^{ns}
mS/cm EC	1,01±0,3	4.70 ± 0.18^{ns}	5.33 ± 0.45^{ns}	4.92 ± 0.79^{ns}
% Ca	1,98±0,15	2.27 ± 0.50^{ns}	2.45 ± 0.14^{ns}	2.26 ± 0.11^{ns}
% Mg	0,50±0,18	0.67 ± 0.11^{ab}	0.9 ± 0.1^{a}	0.53 ± 0.06^{b}
mg kg^{-1} Cd	0,52±0,03	0.60 ± 0.02^{a}	0.53 ± 0.02^{b}	0.53 ± 0.02^{b}
mg kg^{-1} Pb	4,46±0,52	11.85 ± 0.81^{ns}	10.47 ± 0.79^{ns}	8.13 ± 2.01^{ns}
mg kg^{-1} Cu	38,4±2,34	40.7±4.41^{ns}	43.2±0.51^{ns}	42.8±0.98^{ns}
mg kg^{-1} Zn	119,7±6,32	183.0±10.7^{ns}	161.9±4.6^{ns}	171.6±2.99^{ns}

mg kg^{-1} Fe	3,19±1,2	5.96±0.88^{a}	4.29±0.49^{ab}	3.39±0.79^{b}
mg kg^{-1} Na	100,7±11,2	586±16.46^{a}	338±17.95^{b}	514±47.66^{a}

Values of three different vermicompost are mean ± standard error (n = 3). ns- non significant Means in a row followed by different letters are significantly different at p < 0.05 (Tukey test).

The collected data indicate that using organic material together with cattle manure shows a pH reaction neutral to slightly alkaline. In this trial cow manure was used as basis for vermicompost treatments showing lower pH compared to initial one, probably due to presence of various vegetables and fruit home waste and of course earthworms activity. This is in align with Gandhi *et al*. (1997) that stated earthworm activity reduced not only pH but also C/N ratio in manure. According to Dickerson (2001) recommended pH for vermicomposting is around 6-7 as in lower pH the bacterial activity decrease, and worms that do not like it, will migrate or probably die. Brinton (2000) recommended a threshold for vermicomposting A-class range, pH 6.5-8.4. According to organic amendments for pH range it is suggested 6.0 to 8.5 of several legislations (e.g. Italy, Belgium, Spain) to ensure compatibility with most plants (Hogg *et al*., 2002). We can confirm that in our research all three-vermicomposting mixtures have active and substitution pH neutral, accept alkaline active pH in cattle gun and grass clippings probably due to the grass component, but anyway all fits in suggested range. A larger population of earthworms can significantly contribute to the aeration of the compost mass, which prevents a drastic drop in the pH value, while aerobic condition affects ammonium consumption and prevent pH increment (Rostami, 2011). The nitrogen compound in presented three treatments varied between 2.7%, 2.8% and 2.9 % N while total carbon varied between 45.59%, 46.39%, 47.41% showing a C/N ratio of 16.7, 16.29 and 16.67. C/N compounds in vermicomposting mixtures have decreased up to 34% to 36% from initial cattle gung. Significant decrease of C/N ratio comparing to initial cattle manure was in vermicompost with grass clippings, probably due to uniform mass and earthworms who can easily digest it. A C/N ratio less than 20 confirms organic waste mineralization which indicates compost maturity; however, a C/N ratio less than 12 is also preferred for agricultural purpose (Shak *et al*., 2014). Rostami (2011) stated that vermicompost process will progress properly by starting the process with a C/N ratio around 25-30 and it will decrease during the process. We have decresment in C/N which is in ailing with previous statement. According to Tchobanoglous *et al*. (1993) carbon reduces because heterotrophic bacteria use organic material as source of electron and carbon is oxidized to CO_2 and releases to atmosphere. Content of carbon (C%) was higher in treatment with the domestic waste and grass clipping probably regarding the complex mixture and it is possible that the complexity of mixtures required longer period for mineralization process. According to Boruah *et al*.(2019) the reduction of organic carbon during vermicomposting is mainly attributed to the respiratory activity of microorganisms and earthworms with a synchronized increase in nitrogen added through the mucus and nitrogenous excretion by the worms. The potassium content varied between 1.1-1.2% K_2O and phosphorus varied within 0.3-0.5% P_2O_5. Slightly different results were obtained by Pukan et al.,

(2013) using slurry method (non-enriched and enriched) and conventional method that found total K_2O content varied from 0.69, 0.73 and 0.65% while for P_2O_5 content varied 1.1, 1.51 and 0.82%. Results of Bhat *et al.* (2013) indicated a range of potassium and phosphorus 2.47% P_2O_5 and 2.37% K_2O for final vermicompost. But diversity in nutrient compound probably could be due to the domestic waste and its nutritional value and of course initial material and favorable condition that can speed up by earthworm activity. Higher potassium content is expected in organic compounds, and we recorded 37 to 50% higher content of potassium comparing to initial cattle manure. Suhane (2007) asserts that vermicompost has a minimum of 4 times more nutritive than cattle manure compost. Based on the presented results, the experiment indicates quite good results of vermicomposting of household waste with cattle manure, showing a neutral pH reaction and showing the best content of ash and carbon with good NPK contents and with slightly increased C/N ratio of 16.7. It's to be noted that a decline within the C/N ratio to < 20 indicates a sophisticated degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes (Senesi, 1989). It's believed that a C/N ratio below 20 is indicative of acceptable maturity, while a ratio of 15 or lower is preferable (Morais and Queda 2003). As we can see (Table 1) ash content was increased in vermicomposting materials compering to initial cattle manure as Gupta and Garg (2008) confirm that the increase in the ash content illustrates faster rate consuming the wastes by the earthworms and the microbial assimilation is also performing the degradation process rapidly.

In our trial regarding a different organic waste EC as most important factor of vermicompost phytotoxicity varied between 4.7-5.33 mS cm^{-1} and these results are in accordance with Majlessi *et al.* (2012) who reached value of 4.9 mS cm^{-1} in the final product. Increase in EC caused by ions discharge some researchers explained that happened due to the breakdown of organic substrates, ammonium, phosphate, potassium, nitrate, and calcium (Gong *et al.*, 2019; Yuvaraj *et al.*, 2019; Paul 2020). Mitchell (1997) states that electrical conductivity of vermicompost from cattle manure in his research was 2.5-3.1 mS cm^{-1}, while Tiquia and Tam (1998) found that compost with an EC of 3.5-4.7 mS cm^{-1} did not reduce germination in their research on seeds and root elongation of cabbage and spinach. Some biological test with vermicompost on excessive salinity could be good confirmation of phytotoxicity. Salinity can be also developed from nitrogen mineralization and production of organic acids, even these factors indicate the compost stability and not its maturity. Iannotti *et al.*, (1994) found 32% of analyzed samples shown EC values greater than 4 dS m^{-1} (6.1 to 15 dS m^{-1}), probably due to the raw material used (i.e. food waste). Heavy metals, like Pb, Cd, Cu, Zn contents in vermicompost regardless of different materials of vermicomposting were found to be below permissible limit. Different materials used in all three treatment in trial result in calcium content that varied between 2.53% - 2.9%, and that was in align with findings of Suthar (2009) in vermicompost (2.0–2.57% Ca). It was reported by Srimathi *et al.* (2019) that significant increment of magnesium in vermicompost elevates initial Mg content from 0.37-0.41% to 0.35-0.65% wich is in aligmant with our results. From our trial it is important to emphasize the significant

higher content of magnesium in vermicompost with grass clippings probably regarding the rich chlorophyll content in grass. The magnesium is the central atom of the chlorophyll molecule, with each molecule containing 6.7% magnesium. For the total concentrations of Cd, Fe and Na it was found significant difference among the treatments. All three values (Cd, Fe and Na) were significantly higher in first treatment with cattle manure and home waste which probably refers to some residues applied with waste and these values were lower in initial material. Measured heavy metal contents in the final vermicompost were under the US EPA standard limits (US EPA, 1993). In accordance with applied organic material within the treatments, these results show that vermicompost could rises the speed of organic matter and increase the capacity of carbon sequestration.

CONCLUSION

An appropriate pH environment, N and C contents, which are reflected in a favorable C/N ratio, create an environment in which earthworms can reproduce, recycle and efficiently process organic substances and ultimately result in quality vermicompost. Through vermicomposting, the nutrient content could be elevated resulting in good and adequate fertilization manure that could be widely applied. But when it comes to pH and EC regarding the obtained vermicompost's the most suitable soil for application would be the one with lower pH reaction, especially the combination of cattle manure and grass clippings. The recycle of organic material trough the vermicomposting could be definitely positive ecofriendly way of reducing of waste and producing organic fertilizer amendments.

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UČINKOVITOST RECIKLIRANJA ORGANSKOG OTPADA KROZ POSTUPAK VERMIKOMPOSTIRANJA

Rezime

Poljoprivredni sektor i rastuće potrebe za proizvodnjom hrane uvelike se oslanjaju na kemijska gnojiva. To je u početku imalo pozitivne učinke na proizvodnju, no tijekom posljednjeg desetljeća negativni učinci pretjerane uporabe rezultirali su niskom produktivnošću usjeva, povećanom zarazom štetočinama i bolestima, degradacijom tla i posljedičnim štetnim učincima na parametre okoliša. Kao jednu od mogućnosti u organskom uzgoju pronašli smo vermikompostiranje kao održiviju praksu. Za pripravljanje vermikompostne smjese korišten je zreli stajski gnoj u tri različite kombinacije s organskim otpadom i uz inokulaciju glista (kućni otpad, pokošena trava i mješavina-kontrola). Eksperiment je postavljen slučajnim bloknim rasporedom u tri ponavljanja. Analize kvalitativnih parametara konačnog proizvoda pokazale su neutralnu do blago alkalnu pH reakciju. Dušik je varirao između 2,7-2,9 %, dok je organski ugljik varirao između 45,59-47,41 % pokazujući omjer C/N od 16,7-16,67. Sadržaj kalija varirao je između 1,1-1,2% K_2O i fosfora od 0,3-0,5%. Eksperiment pokazuje dosta dobre rezultate vermikompostiranja kućnog otpada sa stajskim gnojem, pokazujući neutralnu pH reakciju, optimalan sadržaj pepela i ugljika s gotovo sličnim i dobrim sadržajem NPK i povećanim C/N omjerom od 16,7 te sa zadovoljavajućim sadržajem elemenata u tragovima i sadržajem teških metala ispod granica. Ovi rezultati pokazuju da vermikompostiranje može povećati kvalitetu konačnog proizvoda.

Ključne riječi: *vermikompostiranje, gliste, recikliranje, organski otpad*

PROIZVODNJA BIOPLINA IZ ORGANSKOG OTPADA OD PROČIŠĆAVANJA SOJE*

Matko Bošnjaković[1], Vesna Gantner[2], Sanja Antunović[2], Davor Kralik[2]

Orginalni naučni rad - *Orginal scientific paper*

Rezime

Soja se uglavnom koristi kao osnova za proizvodnju hrane za ljude i životinje. U procesu obrade, prvi korak tehnološke prerade soje je mehaničko pročišćavanje od nečistoća. Pročišćavanjem soje nastaje otpad velikog energetskog potencijala. U Republici Hrvatskoj se u 2021. godini proizvelo 228.000 tona soje, a prema podacima za 2022. godinu jednog od otkupljivača soje u Republici Hrvatskoj, prosječni udio nečistoće iznosio je 6,79%. Uzevši u obzir navedeni postotak nečistoća, dolazimo do podatka da je u 2021. godini nastalo oko 15.481 tonu otpada od pročišćavanja soje. Korištenjem navedenog otpada kao sirovine u procesu anaerobne fermentacije može se proizvesti značajna količina bioplina, koji se zatim može iskoristiti kao pogonsko gorivo za proizvodnju energije. Provedenim laboratorijskim ispitivanjima utvrđeno je da se postupkom anaerobne fermentacije od jedne tone otpada pročišćavanja soje može proizvesti oko 513,81 m^3 bioplina s udjelom metana od oko 57,2%. Kalkulacijom dolazimo do podatka da je navedena količina otpada dovoljna za proizvodnju oko 19.000 MWh električne energije godišnje. S prosječnom potrošnjom električne energije u kućanstvima u 2021 godini u Republici Hrvatskoj od 2.728 kWh, ukupna proizvedena električna energija od otpada od pročišćavanja soje dovoljna je da podmiri godišnje potrebe za električnom energijom za 6.980 kućanstava.

Ključne riječi: *soja, anaerobna fermentacija, organski otpad, energija*

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UVOD

Soja (*Glycine max* (L.) Merrill) pripada starim ratarskim kulturama. Potječe iz Azije, s područja Dalekog istoka (Kine, Japana, Indije i dr.) gdje se uzgaja već više od 4.000 godina. Biljka se može koristiti u razne svrhe, te je njena iskoristivost maksimalna, jer se koristi njeno zrno i stabljika. Zrno soje jedan je od glavnih izvora jestivih ulja i bjelančevina za prehranu ljudi i hranidbu domaćih životinja. Ono može biti korišteno u razne industrijske svrhe (Lakić, 2016.).

U Hrvatskoj se soja prvi puta pojavljuje između 1876. i 1878. godine, kada je austrijski biokemičar Friedrich Haberlandt provodio pokuse na cijelom području Austrougarske te u Dubrovniku. Na samom području Hrvatske, točnije na imanju Korija kraj Virovitice, Stjepan Čmelik nabavio je novu kolekciju sorata soje izravno iz Kine i Mandžurije. Između 1931. i 1934. godine ta soja se proširuje na istočno područje Slavonije na imanju u Ernestinovu gdje ostvaruje urod između 1.600 i 2.200 kg ha^{-1} pod imenom Osječanka ili Čmelikova (Lakić, 2016.).

Čisto sjeme je sjeme koje pripada deklariranoj vrsti, gdje spada zrelo i neoštećeno sjeme vrste, normalne veličine.
Primjese su sve ono što nije čisto sjeme deklarirane vrste.
Dijele se na:
- organske korisne,
- organske nekorisne i
- neorganske (anorganske) primjese

Organske korisne primjese: spadaju oštećena zrna, sjeme stranih kultura i lom zrna. Organske primjese su korisne jer služe kao stočna hrana. Organske nekorisne primjese čine sjemenke korova, ostaci biljke, pljeva, slama, dijelovi špage, mrtvi i živi kukci i grinje, te njihovi fragmenti i ekskrementi i mikroorganizmi. Najopasniji su živi nametnici, te sjeme korova koje prenosi vlagu na zrno. Neorganske primjese su sve ono što je anorganskog podrijetla (prašina, kamenčići, pijesak, komadiće žice i sl.) (Rozman i Liška, 2011).
Prisutnost primjese znatno utječe na kvalitetu skladištenja i učinkovitost sušenja, te ukoliko se ne izdvoje imati će negativan utjecaj na kvalitetu gotovog proizvoda.

Prema istraživanju iz 1991. godine (William, 1996) sadržaj primjesa u soji je bio slijedeći: 37,5% biljni ostaci stabljike soje, 24,2% lom zrna, 23,5% korov, 9,6% prašina, 2,4% cijelo zrno soje, 2,3% mahune, 0,6% insekti.

Prema posljednjem Izvješću o poljoprivrednoj proizvodnji u 2021., a kojega je objavio Državni zavod za statistiku (DZS), te je godine proizvedeno 228.000 tona soje požnjevenih na 87.000 hektara, odnosno, prinos po hektaru je bio 2,6 tona, dok je u

2020. godini proizvedeno 266.000 tona soje požnjevenih na 86.000 hektara, odnosno, prinos po hektaru te je godine bio 3,1 tonu.

Tablica 1. Površina i proizvodnja žitarica i ostalih usjeva u 2021. – privremeni podaci, DZS
Table 2. Area and production of cereals and other crops in 2021 - provisional data, DZS

	Ostvarena proizvodnja					
	površina, u 000 ha	prirod t ha^{-1}	ukupno, tona u 000	površina, tis. ha	prirod t ha^{-1}	ukupno, tona, u 000
	2020			**2021**		
Kukuruz - ukupno	288	8,4	2.431	287	7,8	2.231
Krumpir (kasni i sjem.)	7	20,2	140	7	15,3	101
Soja	86	3,1	266	87	2,6	228
Suncokret	39	3,1	120	41	3,0	125
Šećerna repa	10	74,0	774	10	70,3	717
Lucerna, sijeno	26	7,6	201	28	6,9	191
Silažni kukuruz	30	41,9	1.261	25	35,2	883

Prema podacima za 2022. godinu jedne od većih poljoprivrednih tvrtki u Republici Hrvatskoj, a koja otkupljuje i soju, udio primjesa, tj. otpada nakon pročišćavanja se penje i do 20,4%, dok je prosječni udio primjesa iznosio 6,79%.
Kada bi navedeni prosječni udio primjesa primijenili na ukupnu količinu proizvedene soje u 2021. godini, a koja je iznosila 228.000 tona, dolazi se do ukupne količine primjese za 2021. godinu od 15.481,2 tona.

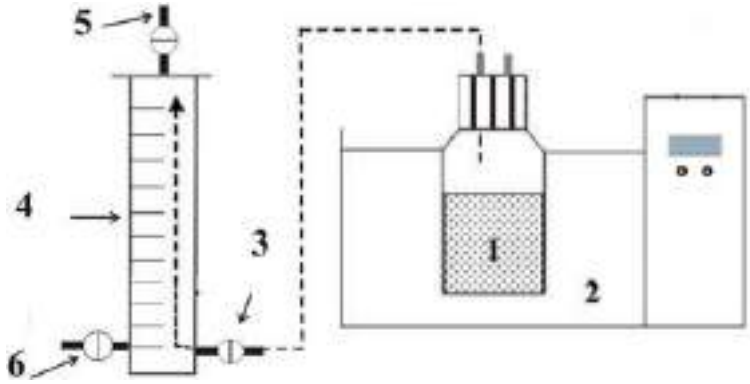
Jedna od primjena i način kako korisno iskoristiti primjesu soje jest korištenje kao sirovine za proizvodnju bioplina, odnosno električne energije, na bioplinskom postrojenjima procesom anaerobne fermentacije (AF).

MATERIJALI I METODE

Pravilnikom „Kontrola kakvoće soje, suncokreta i uljane repice u otkupu“ (NN 88/2010) definirano je:
(1) Soja koja se otkupljuje mora sadržavati najmanje 13% vlage i najviše 2% nečistoće.
(2) Ukoliko kvaliteta soje odstupa od minimuma iz stavka 1. ovoga članka pri obračunu troškova otkupa cijena se za svaki dodatni postotak proporcionalno smanjuje odnosno povećava za 1%.

Istraživanje je provedeno u Laboratoriju za biomasu i obnovljive izvore energije pri Fakultetu agrobiotehničkih znanosti Osijek. Za potrebe istraživanja korištena je goveđa

gnojovka s farme muznih krava i organski otpad nakon prosijavanja soje. Anaerobna fermentacija (AF) je provedena u diskontinuiranim reaktorima zapremine 1,0 l pri termofilnim uvjetima (55°C) i vremenu hidraulične retencije od 28 dana. Kontrolnu grupu (K) činila je goveđa gnojovka (500 g), a eksperimentalnu grupu činila je 475 g goveđe gnojovke i 25 g organski otpad nakon prosijavanja soje (PS). Sve grupe postavljene su u tri ponavljanja. Anaerobna fermentacija je svakodnevno praćena, te je prikupljan proizvedeni bioplin u baždarene mjerne posude koje su gibljivim prozirnim crijevom povezane sa reaktorom.



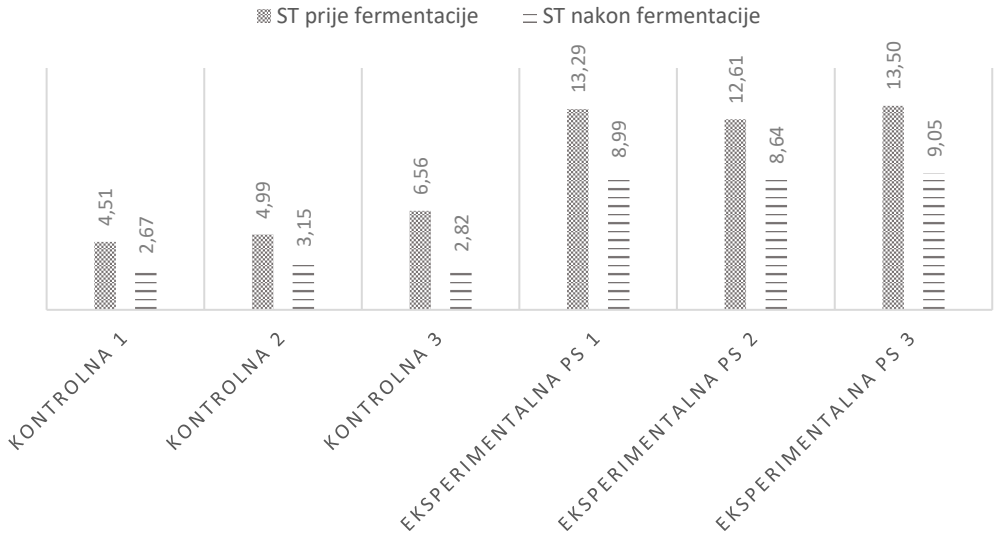
Slika 1. Shematski prikaz aparature za anaerobnu fermentaciju (D. Kralik)
Picture 1. Schematic representation of apparatus for anaerobic fermentation (D. Kralik)

1 - reaktor sa supstratom za kodigestiju; 2 - vodena kupelj; 3-ulazni ventil; 4 - graduirani cilindar sa zasićena otopina natrijevog klorida; 5 – izlazni ventil za uzorkovanje plina; 6 - izlazni ventil za zasićenu otopinu

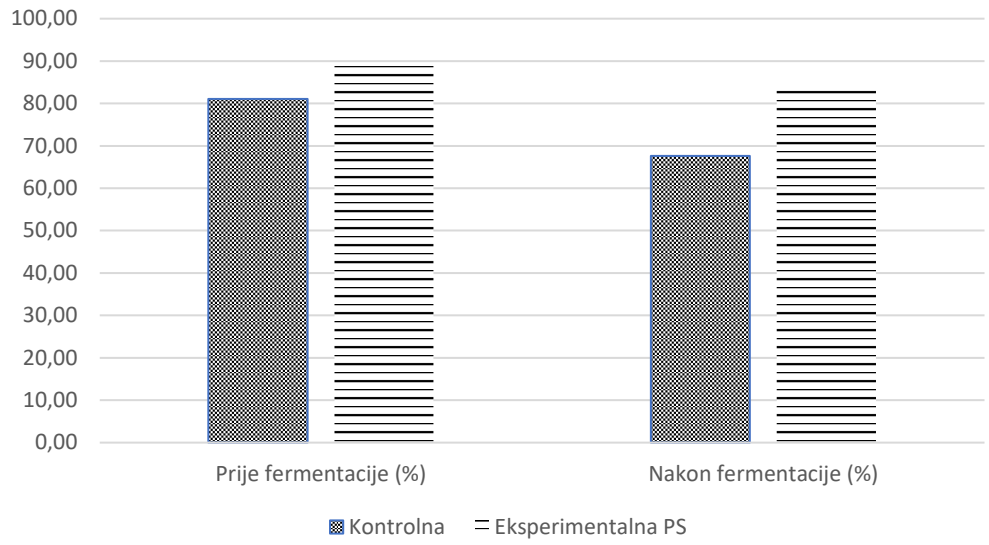
Prema Thompsonu (2001) određen je udio suhe i organske tvari u uzorcima. Suha tvar određena je nakon sušenja u sušioniku na 105 °C do konstantne mase, a organska tvar žarenjem 4 sata na 550 °C u mufolnoj peći. Elektrokemijskim mjerenjem utvrđena je pH vrijednost.
Prikupljeni plin analiziran je pomoću plinskog detektora Optima 7 biogas i određen je udio CH_4 (%), H_2S (ppm), CO_2 (%), O_2 (%), kalorična vrijednost i ogrijevn vrijednost.
Normalnost raspodjele numeričkih podatak provjerena je Shapiro - Wilkovim testom, te zbog toga što podaci ne slijede normalnu raspodjelu, prikazni su medijanom i granicama interkvartilnog raspona. Razlike između kontrolne (K) i ekspirementalne (PS) skupine, testirane su Mann Whitney. U testu sve P vrijednosti su dvostrane. Razina značajnosti je postavljena ALPHA = 0,05. Za satitičku analizu korišten je statistički program MedCalc ver. 20.115.

REZULTATI ISTRAŽIVANJA

Prosječni udio suhe tvari (ST) u goveđoj gnojovci (kontrolnoj skupini (K)) iznosio je 5,35%, a organskog otpada nakon pročišćavanja soje 69,09%. Prosječni udio ST u eksperimentalnoj skupini (PS) nakon dodavanja 25 g otpada nakon pročišćavanja soje u 475g goveđe gnojovke na početku fermentacije iznosio je 13,13%.
Po završetku AF udio ST smanjio se u odnosu na početni udio ST kod K skupine za 44,82%, a kod PS skupine za 32,26%. Grafikon 1.

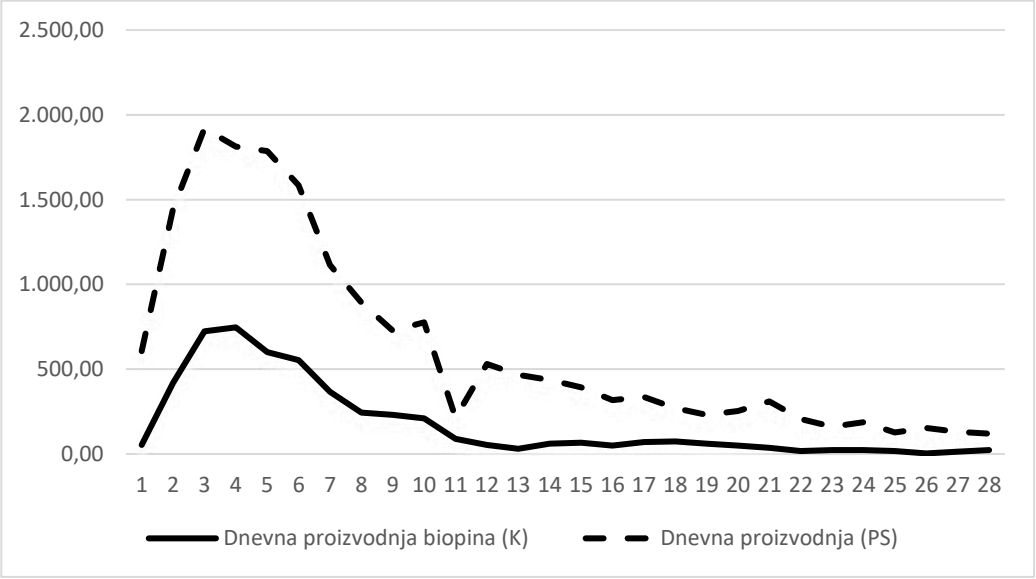


Grafikon 1. Udio ST prije i nakon anaerobne fermentacije
Graph 1. Dry matter before and after anaerobic fermentation



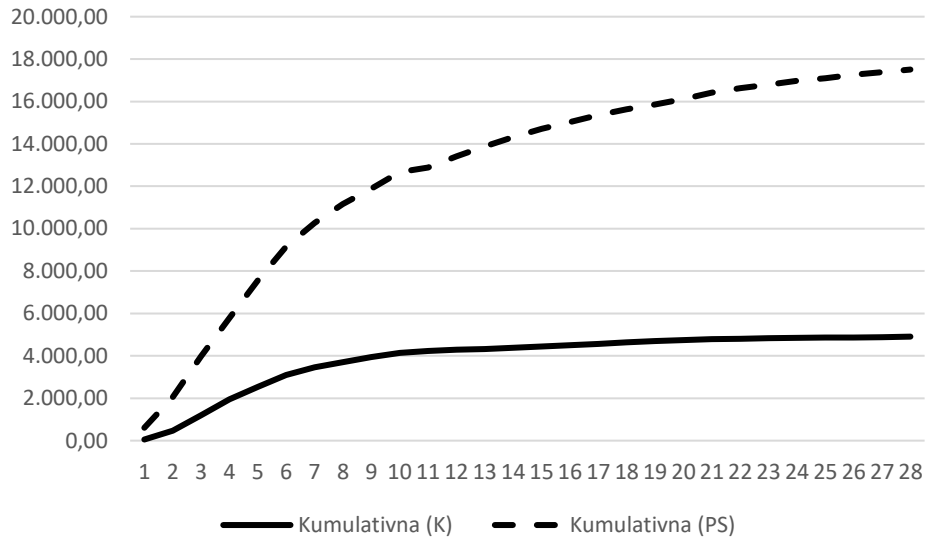
Grafikon 2. Prosječni udio organske tvari prije i nakon procesa AF
Graph 2. The average proportion of organic matter before and after the AF process

Dnevna proizvodnja bioplina bila je najintezivnija u prvih trinaest dana, ali maksimum je postignut od drugog do petog dana anaerobne fermentacije. Kod eksperimentalne grupe maksimum je postignut treći dan s proivodnjom od 1.920 ml bioplin/dan, a kod kontrolne grupe četvrti dan od 746 ml bioplina/dan (Grafikon 3).



Grafikon 3. Dinamika dnevne proizvodnje bioplina
Graph 3. Daily production of biogas

U grafikonu 4 prikazana je razlika u ukupnoj proizvodnji bioplina tijekom 28 dana anaerobne fermentacije (AF).



Grafikon 4. Kumulativna proizvodnja bioplina
Graph 4. Total production of biogas

Kod eksperimentalne grupe proizvodnja je iznosila 17.330 ml tijekom AF od 28 dana, dok je kod kontrolne proizvodnja za isto vrijeme iznosila 4.420 ml bioplina. Značajno je veća dnevna (Mann Whitney U test, $P < 0,001$) i ukupna količina proizvedenog plina (Mann Whitney U test, $P = 0,008$) kod EP u odnosu na kontrolnu skupinu (Tablica 2).

Tablica 2. Razlike u CH_4 i količini plina između kontrolne i EP skupine
Table 3. Differences in CH4 and gas volume between control and EP groups

	Median (IQR)		Difference	95% CI	*P**
	Control Group (K)	EP (PS)			
CH_4 (%)	60,2 (58,2–67,8)	57,2 (53,5–62,9)	-4,8	-9,8 to 1,2	0,09
Dnevna količina proizvedenog plina [ml]	85 (42,5–340)	365 (210–835)	235	125-430	< **0,001**
Ukupna količina plina [ml]	4.420 (4.390–5.678)	17.330 (16.776–18.558)	125.331	11.010 – 14.850	**0,008**

IQR – interquartile range; EP – eksperimentalna primjesa soje; CI – confidence interval
*Mann Whitney U test

Medijan koncentracije metana tijekom 28 dana anaerobne fermentacije kod kontrolne grupe iznosila je 60,2% (interkvartilnog raspona od 58,4 do 67,5%), a kod eksperimentalne grupe iznosila je 57,2% (interkvartilnog raspona od 53,6 do 62,9 %). Istraživanjem je utvrđeno da je iz jedne tone svježeg organskog otpada nakon pročišćavanja soje moguće proizvesti 513,81 m^3 (BPP) bioplina s udjelom metana od 57,2 %. Slične rezultate u svojim istraživanjima su dobili (Kuratorium für Technik und Bauwesen in der Landwirtschaft) u Njemačkoj, gdje su iz jedne tone svježe mase otpada od pročišćivanja proizveli 547,7m^3 bioplina s udjelom metana od 54%.

Tablica 3. Sirovinski prinos bioplina, KTBL
Table 4. Biogas yield

Naziv sirovine	Suha tvar	Organska suha tvar	Prinos bioplina		Kvaliteta bioplina
	% ST	% oST	l_n/kg oST	m_n^3/t SM	% CH4
Otpad od pročišćavanja	89,2	83,5	656,0	547,7	54,0

RASPRAVA

Sukladno svemu navedenom možemo izračunati da je iz jedne tone svježe mase otpada od pročišćavanja soje moguće proizvesti oko 1.230 kWh električne energije.

BPP – bioplinski potencijal sirovine [m^3/t_{SM}]
BMP – biometanski potencijal sirovine [m^3/t_{SM}]
$\%CH_4$ – udio metana u bioplinu proizvedenog iz određene sirovine

$$BMP_{t.SM} = \frac{BPP_{t.SM} \times \%CH_4}{100}$$

$$BMP_{t.SM} = \frac{513{,}81 \times 57{,}2}{100} = 293{,}90\ m^3_{CH_4}/t_{SM}$$

$LHV_{CH4} = 9{,}972$ kWh/m^3 - donja ogrjevna vrijednost metana

$\eta_{el} = 0{,}42$[3] - stupanj korisnog djelovanja pretvorbe u električnu struju

$$EP_{t.SM} = BMP_{t.SM} \times LHV \times \eta_{el}$$

$$EP_{t.SM} = 293{,}90 \times 9{,}972 \times 0{,}42 = 1.230{,}92\ kWh_{el}$$

Prema godišnjem izvješću za 2020. godinu Hrvatske energetske regulatorne agencije (HERA) u Republici Hrvatskoj broj priključnih mjesta na „niski napon – kućanstva" je bio 2.227.106, koja su navedene godine potrošila 6.075.185 MWh električne energije. Sukladno navedenom prosječna godišnja potrošnja električne energije jednog kućanstva iznosi 2,728 MWh.

ZAKLJUČAK

Bioplinska postrojenja su postrojenja za proizvodnju energije iz obnovljivih izvora. Ideja izgradnje navedenih postrojenja je prvenstveno stvaranje nove vrijednosti iz organskih nusproizvoda i otpada, tj. biti dodatna karika u lancu proizvodnje i iskoristiti neiskorišteno. Trenutno, gotovo svim bioplinskim postrojenima u Republici Hrvatskoj osnova za proizvodnju bioplina je kukuruzna silaža.

Situacija na tržištu poljoprivrednih proizvoda, točnije kontinuirani rast cijena energetskih usjeva, pa tako i kukuruzne silaže, nameće pitanje isplativosti proizvodnje energije na bioplinskim postrojenjima. Iz tog razloga, vlasnici bioplinskih postrojenja sve su više u potrazi za novim sirovinama, tj. nastoje što više smanjiti utjecaj energetskih usjeva u proizvodnji zamjenom sa organskim nusproizvodima i otpadom.

Jedna od takvih sirovina zasigurno može biti i otpad od pročišćavanja soje. Ako u obzir uzmemo da u Republici Hrvatskoj godišnje nastaje oko 15.481,2 tona otpada od pročišćavanje soje, a sukladno laboratorijskom istraživanju i izračunu možemo reći da od jedne tone svježe mase otpada od pročišćavanja soje se može proizvesti oko 1.230 kWh električne energije, dolazi se do podatka da je navedena količina otpada dovoljna za proizvodnju oko 19.000 MWh električne energije, tj. da zadovolji potrebe za električnom energijom oko 6.980 kućanstava u Republici Hrvatskoj, odnosno 0,3%.

Nažalost, ili na sreću, trenutna situacija s cijenama nabave energetskih usjeva, vlasnike bioplinskih postrojenja vraćaju na „osnovne postavke", a to je proizvodnja energije iz trenutno već dostupnih sirovina. Otpad od pročišćavanja soje zasigurno je jedna od takvih sirovina, ali dodatnim ulaganjem u poljoprivrednu proizvodnju, kao i u bioplinska postrojenja, mogu se još više iskoristiti razni poljoprivredni i industrijski nusproizvodi i otpadi.

[3] Podatak za bioplinski motor proizvođača Jenbacher, model JMS 416 GS-B.L

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PRODUCTION OF BIOGAS FROM ORGANIC WASTE FROM SOYBEAN CLEANING

Summary

Soy is mainly used as a base for the production of food. The first step in the technological processing of soybeans is mechanical cleaning from impurities. Cleaning process creates waste with great energy potential. In 2021 228.000 tons of soybeans were produced in the Croatia, and according to data for 2022 from one of the buyers of soybeans in the Croatia, the average amount of impurities was 6,79%. Taking into account the stated percentage of impurities, we can conclude that in 2021 about 15.481 tons of waste from soybean were generated. By using the mentioned waste as a raw material in the anaerobic digestion process, a significant amount of biogas can be produced, which can be used as fuel for energy production. Laboratory tests have shown that one ton of soybean cleaning waste can produce about 513,81 $m^3$of biogas, of which about 57,2% is CH_4. We calculate that the specified amount of waste is sufficient for the production of 19.000 MWh of electrical energy per year. With the average consumption of electrical energy in households in 2021 in Croatia of 2.728 kWh, the total electrical energy produced from soybean waste is sufficient to meet the annual electrical energy needs of 6,980 households.

Key words: *soybean, anaerobic digestion, organic waste, energy*

MODERN CATTLE BREEDING AND CLIMATE CHANGE - CHALLENGES AND RESPONSES*

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Review paper

Summary

According to the reports of the UN Food and Agriculture Organization (FAO), in anthropogenic emissions of greenhouse gases, animal husbandry participates with 14.5% (translated into CO2 equivalent, about 7.1 Gt), which puts it in third place, behind the energy sector and industry. and in front of the traffic. On the other hand, climate changes significantly affect livestock production in terms of increasing the frequency of animal diseases (heat stress), changes in environmental factors (microclimatic conditions) and changes in nutritional conditions (impact on grassland composition). Reducing greenhouse gas emissions will only be possible by adopting good agricultural practices. That is why the researches carried out for this purpose are more numerous and more current in a large number of countries.
Key words*: animal husbandry, climate change, heat stress, greenhouse gases*

INTRODUCTION

Climate changes and their consequences emerged as one of the biggest global problems on the planet Earth from the beginning of the industrial revolution until today, and they arise as a direct consequence of human activities.

The average temperature increases due to the so-called greenhouse effect, which causes an increase in the concentration of certain gases in the air. The most significant impact is the concentration of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). The concentration of CO_2 (57%) has the greatest impact on climate change, while the percentage share of CH_4 and N_2O is 27% and 16%, respectively (Johnson *et al*., 2007). Global warming leads to an increase in the average temperature of the air, land, sea and ocean, and thus to a change in the precipitation regime, resulting in extreme droughts which are becoming more frequent; short-term heavy rains that lead to flash floods, landslides, erosion; occurrences of atmospheric phenomena uncharacteristic for certain areas (storms and tornadoes).

The change in the rainfall regime has a negative impact on the entire agricultural activity. There is a decrease in arable land, changes in the composition and quality of

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the soil. Also, the yield (amount of plant products used in food) and the quality of plant products (incidence of mycotoxins, change in chemical composition and nutritional value). Also, its has direct and indirect negative effects on livestock production.
Paradoxically, one of the strongest negative contributors to greenhouse gas emissions and global climate change is agriculture. Cultivation of land, conversion of land (deforestation, destruction of plant cover, drying of water bodies), use of artificial fertilizers, production of animal feed, processing of agricultural products and transport increase CO_2 emissions. Enteric processes in ruminants and manure production are a significant source of methane (Rojas-Downin *et al.*, 2017). Livestock production affects soil degradation, air and water pollution and the destruction of biodiversity. (Bellarbi *et al.*, 2013; Reinolds *et al.*, 2010; Steinfeld *et al.*, 2006; Thornton and Gerber, 2010).
Given that agricultural activity has a significant contribution to the disruption of climate (third major impact, after the energy and industrial sectors), the attention given to this situation must be proportional to it. It is important to work on raising global awareness and education about the seriousness of the situation in all segments. The beginning of the process of adaptation and adjustment of cattle production to climate change is the training of agricultural experts who will be the bearers of modernization and new research in this area. This is necessary in order to meet the needs for skilled and prepared workforce in the agricultural sector in the conditions of climate change.

CATTLE PRODUCTION IN CONDITIONS OF CLIMATE CHANGE

Climate change affects cattle production in two ways - indirectly and directly.
The indirect impacts are primarily reflected in the difficult supply of water and food. Agriculture uses 70% of freshwater resources, making it the world's largest consumer (Thornton *et al.*, 2009). The frequent occurrence of long-term droughts leads to difficulties in supplying animals with water. At the same time, drought leads to a decrease in the yield of forage crops. In addition to yield reduction, in the regime of disrupted water supply, there is a more frequent occurrence of plant diseases and pests and the occurrence of mycotoxins in plant-based nutrients. This significantly reduces the quality and nutritional value of plants. One of the indirect effects of climate change on cattle production is the change of agro-ecological zones. Climate changes at low latitudes generally go in a negative direction, leading to arid climate conditions. In areas on higher latitudes, climate change leading to an increase in temperature could be beneficial in terms of allowing a longer growing season for plants (Yohannes, 2016). A change in climatic conditions often leads to the appearance of various diseases uncharacteristic for certain areas. Pathogenic agents, as well as the vectors that transmit them, are also changing. Changes in climate lead to changes in pathogens or parasites, the spread of vector-borne diseases, food-borne diseases, and reduce host resistance to food and water scarcity (Nardone *et al.*, 2010; Thornton *et al.*, 2009; Tubiello *et al.*, 2008).
The direct influence of climatic and microclimatic conditions on animals is reflected in

the incidence of the so-called heat stress, which has multiple negative effects. Cattle are able of withstanding large fluctuations in air temperature (their thermal comfort is between 10 and 20^0C) and easily adapt to temperatures up to -40^0C with exercise and adequate nutrition. A serious problem is adaptation to high air temperature (above 22^0C), especially if it is accompanied by high air humidity (Samolovac, 2016). The most common indicator used to determine and evaluate heat stress, as well as to evaluate the impact of heat stress on production and reproductive characteristics of cows, is the temperature-humidity index (THI), which is determined based on temperature and air humidity. The negative influence of the TH index, whose value is higher than 68, is multiple.

- Growth and development of animals in conditions of heat stress are slowed down, primarily due to reduced consumption and conversion of food, which results in a decrease in body weight and condition of animals. The mode of food intake changes, so that animals prefer to consume food at night when the temperature is lower and closer to the thermal optimum. Heat stress causes hormonal and metabolic changes in the body. Hormones that initiate catabolic reactions in the body are activated, liver function decreases, food intake decreases, and the immune response to pathogenic agents decreases (Nardone *et al.*, 2010).
- Heat stress has a negative effect on milk production in terms of quantity and composition, i.e. quality of milk. In conditions of heat stress, the content of milk fat and fat free dry matter in milk decreases. The decrease in protein content is less pronounced. The quality of milk decreases because the number of bacteria and somatic cells in milk increases, which reduces the nutritional value of milk and milk products (Samolovac *et al.*, 2012., Mićić *et al.*, 2022).
- Due to the negative impact of high temperatures and air humidity on the hormonal status, in female animals there is an irregular ovulation cycle and a weaker expression of estrus. In male animals, poorer spermatogenesis occurs. All this impairs the conception rate and affects poor reproductive results.
- With the increase in external temperature, heat release increases through the mechanism of vasodilation in the skin, but up to a certain limit. If the temperature of the environment exceeds 31^0C, vasodilatation cannot ensure the efficient release of heat, so there is an increase in the body temperature of animals (increase in rectal temperature). Thermoregulation is also carried out by evaporation of water through the skin (sweating) or through the respiratory organs (fast breathing with the mouth open). This causes increased water consumption. Cortisol - the stress hormone - is also secreted more in the body, which impairs the body's ability to adapt and resist. Consequently, morbidity and mortality in herds increases.

CLIMATE CHANGE UNDER THE INFLUENCE OF CATTLE PRODUCTION

Cattle production has a negative impact on the climate in several ways, mainly through the emission of greenhouse gases (GHG) - carbon dioxide (CO_2), methane (CH_4) and

nitrous oxide (N_2O). According to some research, the livestock sector contributes with 10-12% (Crossona *et al.*, 2007), and even up to 14.5% to global greenhouse gas emissions (Rojas-Downin *et al.*, 2017). At the same time, the emission of CH_4 contributes the most (44%). According to FAO reports, the agricultural sector, taking into account direct and indirect emissions, is responsible for about 30% of global warming. Fossil fuels used on farms; deforestation; change of land cultivation method, change of land use, etc., are the most significant sources of CO_2 emissions. Fermentation in the digestive tract of ruminants, organic waste (primarily manure), biomass burning, cultivation of rice, etc., lead to an increase in methane concentration. The increase in the concentration of nitrogen oxides occurs due to the irrational use of artificial fertilizers.

Cattle production is considered to be a direct or indirect cause of soil degradation, air and water pollution, and destruction of biodiversity. Direct GHG emissions from animal sources include enteric fermentation, respiration, and excretion (Jungbluth *et al.*, 2001). The two main sources of CH_4 on cow farms with high-yielding dairy are enteric fermentation and CH_4 release from manure. Enteric fermentation involves biochemical processes in the foreguts of ruminants during which CH_4 is produced. At the same time, methane produced by enteric fermentation has a share of 25% in anthropogenic CH_4 emissions (Wuebbles and Hayhoe, 2002), and cattle, for the most part (95%), eliminate it by regurgitation and exhaling, while only a small part is removed by flatulence (Kirovski *et al.*, 2022). Manure is also a significant source of CH_4, especially when stored in anaerobic conditions and inadequately "processed".

Indirect gas emissions refer to gases obtained as a result of the cultivation of fodder crops, the use of manure and inorganic fertilizers, the use of machinery for agricultural activities, the processing of livestock products, transportation and distribution, the conversion of land necessary for livestock production, etc.

When looking at cattle production, research shows that the biggest negative contribution to GHG emissions is milk production and cattle fattening for meat production, which accounts for 65% of the total greenhouse gas emissions. If the assessment is made on the basis of the obtained products, meat production participates the most with 41%, followed by milk production with 20% (Gerber *et al.*, 2013).

The conversion of forests into pastures and arable land is a continuous process as a result of the constant pressure of livestock production to meet the nutritional needs of animals. Land use change affects the natural carbon cycle. Releasing a large amount of carbon into the atmosphere increases greenhouse gas emissions. Forests accumulate more carbon in soil and vegetation compared to crops and pastures, thereby reducing CO_2 emissions into the atmosphere. By clearing forests and increasing the area under pasture and arable land, CO_2 emissions into the atmosphere also increase.

Animal feed production accounts for 45% of global anthropogenic GHG emissions related to animal husbandry (Gerber *et al.*, 2013). For the most part, due to the use of manure and artificial fertilizers, especially nitrogen (Steinfeld *et al.*, 2006), and a significant amount of these gases are also released during the processing and transportation of nutrients.

POTENTIAL SOLUTIONS

Adaptation of cattle production to increasingly pronounced climate changes and the ever-present phenomenon of heat stress can be achieved in two ways, biological and technological adaptation.

Biological adaptation implies: intensive selection of dairy cattle, in addition to selection for production and functional traits, use of those bulls for AI whose offspring are more resistant to heat stress, use of genomic selection; introduction of new breeds for milk production.

Technological solutions are, among others: changing the way and time of feeding (increasing the quality of nutrients, increasing the share of energy in the meal, using additives in the diet, feeding during the part of the day with lower air temperatures), providing sufficient amounts of water, controlling the temperature and humidity of the air in buildings (using various applications), thermal insulation, ventilation and cooling of facilities for housing animals, enabling the movement of animals by keeping them on free ranges, controlling insects, etc.

Some of the mentioned solutions have a double positive effect, in a way that they also have a positive effect on climate change. By changing the technology of breeding and feeding, introducing new breeds and using genomic selection, the productivity of animals increases. This results in a larger amount of product per number of farmed animals per unit area, which reduces the emission of harmful gases.

Changing the diet and cultivation method reduces the emission of enteric methane. However, the method of storing manure is more important for reducing the concentration of methane. Manure management and adequate use (storage in aerobic conditions, use of solid manure, etc.) significantly reduces the spread of this gas.

The use of pastures, afforestation, reduction of intensively cultivated areas, reduces the emission of carbon dioxide into the atmosphere.

In addition to all of the above, the use of nitrogen fertilizers in limited amounts and for a limited time (only when and where they are necessary, in the vegetation phase when they are necessary and in the smallest amount that meets the needs of the plants) reduces the harmful impact of nitrogen oxides.

If we look at all of the above, it can be concluded that the organic method of production has a significant potential to mitigate the consequences of harmful anthropogenic effects on the climate, which is also confirmed by some studies (Johnson *et al.*, 2007.).

CONCLUSION

Agricultural activity contributes to global climate change through the emission of greenhouse gases (GHG) such as carbon dioxide, methane and nitrous oxide. At the same time, agriculture could be a solution to climate change through the widespread adoption of climate change mitigation and adaptation actions. Global action must be focused on reducing GHG emissions, preserving the climate, but also adequate

development of agricultural production in order to ensure safe food supply, eradication of hunger, malnutrition and poverty, especially in underdeveloped regions of the world.

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SAVREMENO GOVEDARSTVO I KLIMATSKE PROMENE – IZAZOVI I ODGOVORI

Rezime

Prema izveštajima Organizacije UN za hranu i poljoprivredu (FAO) u antropogenim emisijama gasova staklene bašte stočarstvo učestvuje sa 14,5% (prevedeno u CO_2 ekvivalent, oko 7,1 Gt), što ga stavlja na treće mesto, iza energetskog sektora i industrije, a ispred saobraćaja.

S druge strane, klimatske promene značajno utiču na stočarsku proizvodnju u smislu povećanja učestalosti pojave bolesti životinja (toplotni stres), promene faktora životne sredine (mikroklimatski uslovi) i promene u uslovima ishrane (uticaj na sastav travnjaka). Smanjenje emisije gasova sa efektom staklene bašte biće moguće samo usvajanjem dobrih poljoprivrednih praksi. Zbog toga su istraživanja koja se vrše u tom cilju, sve brojnija i aktuelnija u velikom broju zemalja.

Ključne reči: *stočarstvo, klimatske promene, toplotni stres, gasovi staklene bašte*

THE VARIATIONS IN BIOCHEMICAL PARAMETERS IN BLOOD AND MILK DUE TO MASTITIS INDICATION AND COWS PARITY*

Ivana Jožef[1], Marcela Šperanda[2], Mislav Đidara[2], Ranko Gantner[2], Muhamed Brka[3], Vesna Gantner[2]

Original scientific papers

Summary

With the aim of determination of the variability of biochemical parameters in blood plasma and milk samples depending on mastitis incidence and parity, blood and milk samples were taken from 75 high-yielding Holstein cows. The mastitis incidence was defined accordingly to daily lactose content and daily somatic cell count (test-day records). The obtained results indicate that the differences between the analysed biochemical parameters in blood plasma, due to mastitis score classes (accordingly to daily somatic cell count, SCC) were present and statistically significant ($P < 0.05$) in some parameters (AST and GGT). Also, different patterns regarding mastitis scoring (DCL of SCC) were determined in some parameters. It was established that there was an increase in the levels of most of the analysed biochemical parameters in blood plasma and milk compared due to animal parity, except for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity. Therefore, when using test-day records as an indicator for the mastitis risk and health status of an animal both scoring ways should be used, but in the case of mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for explicit detection.

Key words: *biochemical parameters, test-day records, mastitis, Holstein*

INTRODUCTION

Inefficiency in milk production, such as the occurrence of animal disorders/diseases, can have great economic consequences. Although epidemic infectious diseases can result in large economic losses and generate much greater public attention, production disorders/diseases are economically more important for the overall efficiency of livestock production. Selection to increase milk yield imposes stress on the overall animal and especially on the udder, so high-yielding cows have an increased risk of mastitis. The disproportion between the genetically determined ability for milk

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production and the limitations in improving the energy value of the ration may be the cause of metabolic disorders (Puppel & Kuczyńska, 2016). Metabolic burden in the liver function is often associated with abomasal displacement, ketosis, mastitis, parturient paresis, placenta retention and endometritis, which usually occur in postpartum cows. Therefore the priority for intensive milk production is the prevention of metabolic disorders/diseases. The identification of new and less expensive biomarkers can enable earlier detection of animals at risk and consequently improve treatment strategies and milk yield and reduce the use of antibiotics. Many studies conducted in various countries have represented the incidence rate of clinical mastitis (CM) and its estimated effects and risk factors. Jamali *et al*., (2018) reported that parity was found to be a risk factor for new intramammary infection (IMI) in general, suggesting that the cow's intramammary and anatomical (e.g. teat sphincter patency) defense mechanisms may deteriorate with age. These non-standard defense mechanisms may explain the higher incidence of CM and CM recurrence in older cows. Furthermore, Gonçalves *et al*. (2018) noticed that second- and third-lactation cows had greater milk loss associated with somatic cell counts, than primiparous cows. Antanaitis *et al*. (2021) observed that the decrease in lactose concentration and the increase in the number of somatic cells in milk were directly related to the presence of the causative agent of subclinical mastitis in dairy cattle. Since the percentage of lactose in milk decreased with parity, while milk yield and number of somatic cells increased, it was hypothesized that changes in lactose percentage reflect the history of mammary gland infection and can be considered a mammary memory indicator (Costa *et al.*, 2020).
Metabolites can reflect the environment and the nutritional status of cells, the role of drugs and environmental pollutants, and the influence of other external factors (Hu *et al*., 2021). Furthermore, metabolites in the blood can completely reflect the physiological and biochemical state of dairy cows, so it is frequently used to detect the occurrence of various disorders/diseases in cows. Different metabolites in milk can also indirectly reflect whether cows have mastitis or not. The knowledge and analysis of blood and milk biochemical parameters could be useful to assess and prevent udder health disorders/diseases. The metabolic changes are closely related to clinical and subclinical disease after calving, lactation, and to reproductive performance - factors that significantly affect the profitability of dairy production (Kuczyńska *et al*., 2021). The aim of this work was to determine the values of biochemical indicators in blood plasma and milk of Holstein cows regarding the classes of daily lactose content (which indicates the risk of mastitis) and the number of somatic cells (which indicates the animal's health condition) separately for each parity (second and third).

MATERIALS AND METHODS

The research was performed on a commercial dairy cattle farm situated in East Croatia. During the research, blood and milk samples were taken from 75 cows of the Holstein breed with average daily milk production of 39.30±9.02 kg (Table 1). Blood samples of cows were taken from the coccygeal vein into tubes with lithium heparin anticoagulant

(Becton Dickinson, Plymouth, England, UK). Samples were centrifuged (1.500 g/10 min at 4°C) and plasma was separated and frozen at -80°C until analyses. Samples of milk were taken into clean tubes, centrifuged (12.000 g/30 min at 4°C) and milk plasma was separated and stored at -80°C until analyses. Biochemical parameters in blood and milk plasma were determined using the automatic clinical chemistry analyser Beckman Coulter AU400 (Beckman Coulter, Germany). The concentration of β-hydroxybutyrate (BHB) was determined using commercial kits (Randox Laboratories Ltd, Crumlin, UK) by the enzymatic colourimetric method.

Also, test-day records (from regular milk recording accordingly to the AT4 method) of selected cows were taken from the central database of HAPIH (Croatian Agency for Agriculture and Food). Test-day records were corrected accordingly to the ICAR guidelines (ICAR, 2017). In regard to daily lactose content (DLC), cows were divided into two classes: mastitis risk (DLC < 4.5%) and normal cows (DLC ≥ 4.5%). Furthermore, in accordance with the daily somatic cell count (SCC), animals were divided into three classes: normal healthy animals (SCC < 200,000/ml); cows in mastitis risk (SCC = 200,000 - 400,000/ml) and cows with mastitis (SCC > 400,000/ml). Tab. 1 presents the basic statistical parameters of daily production traits (daily milk yield, daily lactose content and somatic cell count).

Tab. 1. Variability of daily milk production traits of selected animals (n = 75)

Trait	**Mean**	**SD**	**CV**	**Min**	**Max**
Daily milk yield, kg	39.30	9.02	22.95	18.60	59.80
Daily lactose content, %	4.46	0.22	4.95	3.57	4.85
Somatic cell count	1,420,673	2,205,156	155.22	31,818	10,844,296

The basic variability of analyzed biochemical parameters in blood plasma is shown in table 2, while table 3 shows the basic variability of analyzed biochemical parameters in milk.

Tab. 2. Variability of analyzed biochemical parameters in blood plasma of selected animals

Trait	**Mean**	**SD**	**CV**	**Min**	**Max**
Aspartate amino transferase (u/l)	139.319	68.090	48.873	56.290	396.100
γ-glutamil transferase (u/l)	33.561	16.391	48.840	8.700	106.600
Glucose (mmol/l)	3.036	0.466	15.334	1.600	3.940
Urea (mmol/l)	4.496	0.825	18.340	2.280	6.770
Protein (g/l)	84.374	5.562	6.592	73.200	103.400
Albumin (g/l)	32.043	2.547	7.948	23.100	36.000
Triglyceride (mmol/l)	0.115	0.022	19.422	0.070	0.190
β-hydroxybutyrate (mmol/l)	0.495	0.173	34.988	0.230	1.110
Fe (μmol/l)	23.722	7.018	29.587	6.000	42.600

Ca (mmol/l)	2.163	0.234	10,814	0.610	2.550

Tab. 3. Variability of analyzed biochemical parameters in milk of selected animals

Trait	**Mean**	**SD**	**CV**	**Min**	**Max**
Aspartate amino transferase (u/l)	15.095	11.379	75.387	3.755	53.955
γ-glutamil transferase (u/l)	335.803	83.286	24.801	110.125	632.900
Glucose (mmol/l)	0.519	0.174	33.450	0.163	0.945
Urea (mmol/l)	5.426	1.203	22.182	1.428	8.403
Protein (g/l)	35.689	5.186	14.530	14.825	47.350
Albumin (g/l)	22.373	2.390	10.685	9.900	26.625
Fe (μmol/l)	23.109	14.757	63.857	2.550	58.475
Ca (mmol/l)	3.179	0.551	17.343	1.303	4.523

The variability of biochemical parameters in blood plasma and milk due to different lactose content and somatic cell count classes were tested using least square means in GLM procedure in SAS (SAS Institute Inc., 2019) separately for each parity. Following statistical model was used:

$$y_{ijkl} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\, ln(305/d_i) + b_4\, ln^2(305/d_i) + M_j + D_k + e_{ijkl}$$

Where:

y_{ijkl} = estimated biochemical parameters;

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficients (lactation curve by Ali and Schaeffer, 1987);

d_i = days in milk i (i = 11 to 537 day);

M_j = fixed effect of month of measurement j (j = V., VI., VII.),

D_k = fixed effect of lactose content classes k (k = normal / mastitis risk) or somatic cell count class k (k = normal / mastitis risk / mastitis),

e_{ijkl} = residual.

In order to test the significance ($p < 0.05$) of the differences in biochemical parameters due to different lactose content or somatic cell count classes the Tukey-Kramer's studentized range test in GLM procedure in SAS (SAS Institute Inc., 2019) were used.

RESULTS AND DISCUSSION

The statistical analysis showed that the effects of lactation stage, measurement month, and lactose content or somatic cell count class included in the used statistical model were statistically significant ($P < 0.01$). The values of the biochemical parameters in blood plasma regarding the mastitis score classes (accordingly to daily lactose content in milk and somatic cell count) in second and third lactation are presented in Table 4.

The value of aspartate aminotransferase (AST) in blood plasma showed a significant decrease in cows in the second lactation associated with an increase in the number of somatic cells, but also a slight decrease in relation to the increased lactose content. In

cows in the third lactation, lower values of AST were determined in animals at risk (DLC < 4.5%) and significantly (P < 0.05) lowest value was observed in cows with mastitis (scored by somatic cell count, SCC > 400,000). The highest value of γ-glutamyl transferase (GGT) in plasma was determined in animals at risk of mastitis (SCC = 200,000 to 400,000) and in normal animals (DLC > 4.5%) in the III. parity. Although LSMeans values of other analysed biochemical parameters were not statistically significantly different (p > 0.05), some patterns were observed. For biochemical parameters in blood plasma, depending on the mastitis classes from healthy to animals at risk and/or in mastitis, an increase, according to the daily lactose content (DLC), was observed in the levels of AST, glucose, albumin, BHB and Fe in cows of the II. parity; as well as protein and triglycerides in cows in the III. lactation, while Fe increased in all animals regardless of the parity. A consistent decrease in blood plasma concentration, according to an increased daily somatic cell count (SCC), was observed for urea, protein and Fe in cows in the II. parity and an increase in glucose concentration in cows in the III. lactation. The concentrations of other parameters varied, noting that the highest values of albumin and Ca, and the lowest values of TGC were found in animals at risk, in both parities.

Tab. 4. LSmeans of the biochemical parameters in blood plasma regarding lactose and SCC classes separately by each parity (II.; III.)

Trait	**Parity**	**DLC, %**		**SCC**		
		Mastitis risk	**Normal status**	**Mastitis**	**Mastitis risk**	**Normal status**
Aspartate amino transferase (u/l)	2	170.451^{A}	140.667^{A}	136.226^{A}	120.522^{A}	210.759^{B}
	3	120.160^{A}	148.158^{A}	107.912^{A}	196.589^{B}	162.151^{B}
γ-glutamil transferase (u/l)	2	30.987^{A}	33.561^{A}	35.310^{A}	27.065^{A}	31.109^{A}
	3	32.954^{A}	36.627^{A}	29.290^{A}	56.760^{B}	38.071^{A}
Glucose (mmol/l)	2	3.060^{A}	3.025^{A}	3.086^{A}	2.909^{A}	2.991^{A}
	3	2.989^{A}	3.157^{A}	3.118^{A}	3.069^{A}	2.856^{A}
Urea (mmol/l)	2	4.052^{A}	4.244^{A}	4.093^{A}	4.329^{A}	4.370^{A}
	3	4.590^{A}	4.788^{A}	4.732^{A}	4.485^{A}	4.550^{A}
Protein (g/l)	2	81.889^{A}	85.251^{A}	85.865^{A}	80.325^{A}	83.709^{A}
	3	85.210^{A}	83.471^{A}	83.807^{A}	84.014^{A}	86.905^{A}
Albumin (g/l)	2	32.277^{A}	31.456^{A}	31.624^{A}	32.010^{A}	31.487^{A}
	3	31.620^{A}	33.156^{A}	32.299^{A}	33.688^{A}	31.194^{A}
Triglyceride (mmol/l)	2	0.110^{A}	0.116^{A}	0.115^{A}	0.110^{A}	0.117^{A}
	3	0.119^{A}	0.105^{A}	0.115^{A}	0.104^{A}	0.116^{A}
β-hydroxybutyrate (mmol/l)	2	0.483^{A}	0.420^{A}	0.432^{A}	0.474^{A}	0.415^{A}
	3	0.503^{A}	0.556^{A}	0.555^{A}	0.453^{A}	0.467^{A}
Fe (µmol/l)	2	24.834^{A}	23.855^{A}	23.086^{A}	24.882^{A}	26.441^{A}
	3	23.004^{A}	23.781^{A}	21.705^{A}	30.402^{A}	24.481^{A}
Ca (mmol/l)	2	2.239^{A}	2.082^{A}	2.098^{A}	2.213^{A}	2.111^{A}
	3	2.208^{A}	2.131^{A}	2.162^{A}	2.291^{A}	2.181^{A}

** DLC – daily lactose content, %; SCC – somatic cell count; Values within the same row marked with different letter differ statistically highly significant (P<0.05)*

The values of the biochemical parameters in cows' milk regarding the mastitis score classes (accordingly to daily lactose content and somatic cell count) in II. and III. lactation is presented in Table 5. Regarding the biochemical parameter in milk, depending on the mastitis classes from healthy to risky animals and/or in mastitis, a decrease was observed, according to the daily lactose content (DLC), in the levels of AST, GGT, urea, protein and Fe in cows of the II. parity; albumin in cows in the III. lactation. Furthermore, the concentration of glucose and Ca in milk were similar in cows in the II. lactation, but slightly decreased in the milk of cows in the next lactation. A consistent increase in the level of biochemical parameters in milk, according to the increased daily number of somatic cells (SCC), was observed for AST, GGT and urea, in both parities, and for the protein concentration in cows of the III. parity and a continuous decrease in the glucose concentration in cows in the III. lactation. The highest values of other biochemical parameters in milk were found in animals at risk regardless of the parity.

Tab. 5. LSmeans of the biochemical parameters in milk regarding lactose and SCC classes separately by each parity (II.; III.)

Trait	Parity	DLC, %		SCC		
		Mastitis risk	Normal status	Mastitis	Mastitis risk	Normal status
Aspartate amino transferase (u/l)	2	12.807^{A}	15.415^{A}	16.043^{A}	13.245^{A}	12.230^{A}
	3	17.129^{A}	15.402^{A}	18.621^{A}	14.802^{A}	10.557^{A}
γ-glutamil transferase (u/l)	2	316.493^{A}	337.885^{A}	349.199^{A}	344.042^{A}	271.057^{A}
	3	355.392^{A}	345.538^{A}	356.714^{A}	383.929^{A}	318.110^{A}
Glucose (mmol/l)	2	0.568^{A}	0.568^{A}	0.534^{A}	0.650^{A}	0.594^{A}
	3	0.464^{A}	0.538^{A}	0.437^{A}	0.594^{A}	0.622^{A}
Urea (mmol/l)	2	4.801^{A}	5.192^{A}	4.952^{A}	5.329^{A}	5.282^{A}
	3	5.563^{A}	5.480^{A}	5.601^{A}	5.386^{A}	5.373^{A}
Protein (g/l)	2	34.857^{A}	35.491^{A}	35.006^{A}	36.400^{A}	35.280^{A}
	3	36.760^{A}	36.005^{A}	37.366^{A}	37.014^{A}	33.234^{A}
Albumin (g/l)	2	22.791^{A}	22.198^{A}	21.761^{A}	23.319^{A}	21.935^{A}
	3	22.704^{A}	22.959^{A}	22.904^{A}	24.193^{A}	21.744^{A}
Fe (µmol/l)	2	16.685^{A}	23.865^{A}	20.146^{A}	26.779^{A}	23.060^{A}
	3	26.327^{A}	25.059^{A}	24.452^{A}	31.355^{A}	27.585^{A}
Ca (mmol/l)	2	3.190^{A}	3.190^{A}	3.192^{A}	3.491^{A}	2.903^{A}
	3	3.238^{A}	3.309^{A}	3.297^{A}	3.680^{A}	2.944^{A}

** DLC – daily lactose content, %; SCC – somatic cell count; Values within the same row marked with different letter differ statistically highly significant (P<0.05)*

Comparing the concentrations of biochemical parameters in blood plasma to milk, a considerable decrease in AST, glucose and protein, and a slighter decrease in the concentration of albumin in milk compared to blood were found in all cows. A large increase in concentration in milk compared to plasma was found for GGT, and a slightly lower increase in urea and Ca concentrations.

The increase in the concentrations of most of the examined biochemical parameters in blood plasma and milk compared due to parity was determined. The exception of the determined trend was observed for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity.

The increased levels of various enzymes in the milk occur mainly due to the increased permeability of microcirculatory vessels in inflamed areas along with the leakage from degenerated/necrotic parenchymal cells and leukocytes. Aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) are important catabolic enzymes that play an important role in the function of animal liver. Also, variations in AST and GGT are frequently associated with abomasal displacement, ketosis, mastitis, parturient paresis; retain placenta and endometritis, which often occur in postpartum cows. Kuczyńska *et al.* (2021) stated that multiparous cows were characterised by a higher deterioration of the blood plasma parameters, particularly in the AST, GGT and BHB levels compared to primiparous. The same authors noticed the value of AST in the blood serum in the range from 67.00 U/L (the oldest cows at the end of lactation) to 100.39 U/l (cows in the second lactation in the first 100 days of lactation). Furthermore, the same authors emphasised that the influence of the parity on blood plasma GGT was significant with the lowest GGT activity found in the blood of primiparous cows at the early lactation. Djokovic *et al*. (2017) determined statistically significant ($P<0.01$) higher activity of AST in blood serum in early lactation cows compared to the mid-lactation cows, while there was no significant difference ($P>0.05$) in milk serum AST due to the stage of lactation. Liu et al. (2012) determined that AST activities in milk were significantly lower ($P<0.001$), and GGT activity was significantly higher ($P<0.001$) compared to blood plasma. Also, the same authors reported significant positive correlations ($P<0.001$) between the activities of these enzymes in milk and blood plasma. Batavani *et al.* (2003) found a similar distribution of AST in the milk of normal and subclinical ewes as well as a higher concentration of AST than in blood serum. Similarly, Babaei *et al*. (2007) reported no significant differences in milk AST in relation to subclinical mastitis, with a higher concentration in milk in relation to blood serum. However, a considerable amount of AST in homogenates from healthy mammary gland tissue suggests that a major source of AST in normal and mastitic milk is the mammary gland secretory cells (Batavani *et al*., 2003).

Increases in plasma glucose are primarily attributed to changes in feed intake and reduced demand for lactose synthesis in the mammary gland in response to intramammary infection (IMI) challenge, while lower plasma β-hydroxybutyrate (BHB) may be associated with the increased transfer into milk. Nyman *et al*., (2008) noticed that the greater serum concentrations of BHB and glucose before calving were associated with lesser SCC levels at first test milking. Primiparous cows have a lower incidence of mastitis than older cows, but in early lactation, they have as great or greater, the prevalence of mastitis than older cows, possibly indicating a lesser ability to cope with stressors occurring during the periparturient period. Guan *et al*., (2020) emphasised that although the results of BHB analysis showed an association with the risk of clinical or subclinical mastitis, the elevated BHB levels are mainly related to

ketosis in dairy cows and cause relatively minimal changes in immune function. Therefore, BHB could not be directly related to the inflammatory process.

Saleh *et al*. (2022) pointed out that the considerable rise in blood glucose in the subclinical mastitis cows could be attributed to higher cortisol levels. Furthermore, the biochemical examination of blood samples in some studies demonstrated a substantial decrease in serum total protein, albumin, and globulin (Saleh *et al*., 2022). Because vascular permeability is enhanced, this decrease may be linked to the leaking of albumin and other serum proteins into milk. Albumin is a negative acute-phase systemic protein that migrates to inflamed tissues via increased vascular permeability and performs a variety of physiological activities, including antioxidants, and is regarded as an immune-inflammatory biomarker (Kaneko *et al*., 2008). During the acute phase response, albumin production is suppressed and amino acids in the liver are diverted to the synthesis of acute phase proteins (Sadek *et al*., 2017). Furthermore, albumin breakdown is accelerated in the presence of elevated glucocorticoids; hence, illness stress and increased cortisol may result in a drop in blood albumin levels. Tripathy *et al*. (2018) indicated a significant ($p<0.05$) increase in various serum biochemical parameters such as protein and AST and a significant decrease in glucose and Ca concentration in cows with mastitis compared to apparently healthy ones (mastitis negative control cows). They also found a significant ($p<0.05$) decrease in protein value in mastitis cows' milk.

Iron, after adsorption from the digestive, passes into the bloodstream and is transported therein, attached to the glycoprotein transferrin, iron-binding protein. This protein is never fully saturated with iron and in that way ensures that no free iron remains in circulation (Ratledge and Dover, 2000). Ganz (2018) explained that the pathogenic microbes have evolved specialized mechanisms for obtaining iron from the host during infections, but the mammalian host has evolved multiple mechanisms of innate immunity that limit the availability of essential nutrient iron to infecting microbes. Antibacterial systems are helpless when iron becomes freely available and this result in rapid extracellular bacterial growth and greatly increased bacterial virulence. Furthermore, Tsukano and Suzuki, (2020) reported that the changes in the blood Fe concentration could be useful as biomarkers of inflammatory disease, including in cows with mastitis. Finally, El Zubeir *et al*. (2005) reported a significant negative effect of mastitis on the variation in Ca concentration in the blood serum that is they determined a significant decrease ($P < 0.01$) in blood serum Ca in samples from clinically infected cows.

CONCLUSIONS

This work aimed to determine the values of biochemical parameters in blood and milk samples, depending on the classes of daily lactose content (which indicates the risk of mastitis) and classes of the number of somatic cells (which indicates the animal's health condition) in cows of the II. and III. parity. The differences between the analysed biochemical parameters in blood plasma, due to mastitis score classes (accordingly to

daily somatic cell count, SCC) were present and statistically significant ($P < 0.05$) in some parameters (AST and GGT). Also, different patterns regarding mastitis scoring (DCL of SCC) were determined in some parameters. It was established that there was an increase in the levels of most of the analysed biochemical parameters in blood plasma and milk compared due to animal parity, except for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity. Therefore, when using test-day records as an indicator for the mastitis risk and health status of an animal both scoring ways should be used, but in the case of mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for explicit detection.

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VARIJACIJE BIOKEMIJSKIH PARAMETARA U KRVI I MLIJEKU USLIJED INCIDENCIJE MASTITISA I PARITETA KRAVE

Rezime

U cilju utvrđivanja varijabilnosti biokemijskih parametara u uzorcima krvne plazme i mlijeka ovisno o incidenciji mastitisa i paritetu krave, uzorkovani su uzorci krvi i mlijeka od 75 visokoproizvodnih krava Holstein pasmine. Incidencija mastitisa definirana je sukladno dnevnom sadržaju laktoze, DCL te dnevnom broju somatskih stanica, SCC (test-day record). Dobiveni rezultati pokazuju da su razlike između analiziranih biokemijskih parametara u krvnoj plazmi, ovisno o razredima mastitisa (prema dnevnom broju somatskih stanica), prisutne i statistički značajne ($P < 0,05$) u nekim parametrima (AST i GGT). Također, u nekih su parametara utvrđeni različiti obrasci varijablnosti ovisno o načinu incidencije mastitisa (DCL of SCC). Utvrđeno je povećanje razine većine analiziranih biokemijskih parametara u krvnoj plazmi i mlijeku u odnosu na viši paritet životinja, osim za TGC i Fe u krvnoj plazmi, te glukozu u mlijeku gdje su koncentracije uglavnom niže u sljedećim paritet. Stoga, kada se zapisi na kontrolni dan (test-day record) koriste kao pokazatelj rizika od mastitisa i zdravstvenog statusa životinje, trebaju se koristiti oba načina bodovanja, ali u slučaju rizika od mastitisa ili pojave mastitisa, druge dijagnostičke metode (kao što su različiti testovi za mastitis) trebaju biti korištene za nedvojbeno otkrivanje.

Ključne riječi: *biokemijski parametri, test-day records, mastitis, Holstein*

THE EFFECT OF METABOLIC DISORDER RISK ON THE VARIABILITY OF BIOCHEMICAL PARAMETERS IN COWS' BLOOD AND MILK REGARDING THE SAMPLING MONTH*

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Original scientific paper

Summary

Aiming the determination the variability of biochemical parameters in blood plasma and milk of Holstein cows regarding the metabolic disorder risk (based on F/P ratio classes) separately for each sampling month (May, June, July), blood and milk were sampled from 75 high-yielding Holsteins reared on a dairy cattle farm in East Croatia. The metabolic disorder risk was defined concerning the daily fat/protein ratio (F/P) obtained from test-day records (F/P < 1.1 indicating acidosis risk; F/P in [1.1, 1.5], the normal status of an animal; F/P > 1.5, indicating ketosis risk).
The conducted analysis showed variability in the values of the biochemical parameters in both blood plasma and milk due to metabolic disorder risk and month of sampling. Accordingly, when different metabolites are used as an indicator of the metabolic disorder risk, correction for the systematic effects (stage of lactation and parity, sampling month) should be applied.

Key words: *biochemical parameters, blood, milk, metabolic disorders, Holstein*

INTRODUCTION

Reduced profitability of dairy cattle farms is frequently caused by the occurrence of animal disorders/diseases. The best way to realize the economically and ecologically sustainable functioning of a dairy farm is to prevent the occurrence of certain disorders/diseases and solve problems in a subclinical form. The above implies constant monitoring of the state of animals in production and the use of various indicators to assess the state of the animal from the health, production, reproductive and welfare aspects. Since farmers have to monitor and analyse a big quantity of data on daily basis as well as react in time to prevent potential problems in production, dairy cattle farming represents one of the most demanding animal productions (Gantner, 2020; Gantner

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et al., 2021). Furthermore, to optimize all segments of dairy cattle farm functioning and to enable the expression of the genetic potential of an animal, the farmer must be aware of animals' needs and the characteristics of the production environment. The intensive selection for high milk production reduced the resistance of the animal to negative external influences and increased the stress level of the organism, which results in an increased frequency of occurrence of various disorders/diseases in dairy cows. Puppel and Kuczyńska (2016) stated that the disproportion between the genetic production potential and the limitations in the digestive system could cause the occurrence of metabolic disorders. The disproportion of the level of production and the digestive system, especially at the beginning of lactation, significantly burdens the functioning of the liver and consequently creates prerequisites for the occurrence of abomasal displacement, mastitis, parturient paresis, placenta retention and endometritis, acidosis and ketosis. Ametaj (2017) emphasised that different factors, such as changes in the ration, reduced food intake, weight loss, negative energy balance and hypocalcemia can cause sets of different disorders during this period postpartum period. The same autor continued that in the period after calving and in early lactation, the most common metabolic disorders are sub-acute and acute ruminal acidosis, laminitis, ketosis, fatty liver, displaced abomasum, milk fever, downer cow, retained placenta, liver abscesses, metritis, mastitis and bloat. furthermore, the occurrence of one metabolic disorder is highly correlated with the occurrence of another one (Suthar *et al*., 2013; Ametaj, 2017). Ametaj (2017) noticed that ruminal acidosis in subacute and acute forms often occurs in high-producing dairy cows in the first month of lactation and cows with a high intake of dry matter, while ketosis is generally a result of negative energy balance (usually due to increased daily milk production). Since the occurrence of subclinical disorders frequently results in high economic losses caused by decreased production, decreased reproduction and increased involuntary culling as well as therapy costs (Suthar *et al*., 2013), it is essential to notice and prevent disorders in the subclinical phase before the development of clinical signs and possible occurrence of some other disorder. Hu *et al.* (2021) stated that the various metabolites can indicate the status of living cells. For instance, metabolites in the blood can indicate the physiological and biochemical state of the organism of a dairy cow, so it is often used to detect the occurrence of some disorders/diseases in the animal. Furthermore, different metabolites in milk can also indicate the metabolic and healthy status of an animal. Therefore, these metabolites could be used for the prevention of disorders/diseases occurrence.

This research aimed to determine the variability of biochemical parameters in blood plasma and milk of Holstein cows regarding the risk of metabolic disorder occurrence (based on F/P ratio classes) separately for each sampling month (May, June, July).

MATERIALS AND METHODS

During the research, blood and milk were sampled from 75 high-yielding Holsteins reared on a dairy cattle farm in East Croatia. Blood was taken from the animals' coccygeal veins into tubes with lithium heparin anticoagulant (Becton Dickinson,

Plymouth, England, UK). In the laboratory, samples were centrifuged (1.500 g/10 min at 4°C) and plasma was separated and frozen at -80°C until analyses. Furthermore, milk was taken into clean tubes, centrifuged (12.000 g/30 min at 4°C) and milk plasma was separated and stored at -80°C until analyses. Using the automatic clinical chemistry analyser Beckman Coulter AU400 (Beckman Coulter, Germany) biochemical parameters were determined in prepared samples. The concentration of β-hydroxybutyrate (BHB) was determined by the enzymatic colourimetric method (using commercial kits, Randox Laboratories Ltd, Crumlin, UK).

Furthermore, test-day records (obtained in regular milk recording that performs accordingly to the AT4 method) of selected Holsteins were taken from the central database of HAPIH (Croatian Agency for Agriculture and Food). During the preparation of data, test-day records were logically controlled accordingly to the ICAR guidelines (ICAR, 2017). Concerning fat/protein ratio (F/P), cows were divided into three classes: F/P < 1.1, acidosis risk; F/P in [1.1, 1.5], normal; F/P > 1.5, ketosis risk. Tab. 1 presents the basic statics of daily milk yield and content of selected Holstein cows regarding the sampling month.

Tab. 1. Basic statistics of daily milk yield and content of selected Holstein cows regarding the sampling month

Trait	N	Mean	SD	CV	Minimum	Maximum
May						
DMY, kg	30	39.36	9.48	24.10	18.60	59.80
DFC, %	30	3.60	1.09	29.68	1.99	6.94
DPC, %	30	3.34	0.34	10.28	2.70	4.11
F/P ratio	30	1.10	0.31	27.89	0.56	1.94
June						
DMY, kg	26	38.36	8.94	23.31	24.20	54.80
DFC, %	26	3.92	1.10	27.96	2.12	7.56
DPC, %	26	3.42	0.25	7.38	3.02	4.07
F/P ratio	26	1.15	0.34	29.45	0.65	2.48
July						
DMY, kg	19	40.50	8.70	21.48	19.90	55.70
DFC, %	19	3.93	1.42	36.26	2.15	8.61
DPC, %	19	3.41	0.37	10.91	2.66	4.26
F/P ratio	19	1.18	0.55	46.64	0.58	3.24

*DMY – daily milk yield; DFC – daily fat content; DPC – daily protein content; F/P – fat/protein

The variability of biochemical parameters in blood plasma and milk due to fat/protein classes were tested using least square means in GLM procedure in SAS (SAS Institute Inc., 2019) separately for each sampling month (May, June, July). Following statistical model was used:

$$y_{ijkl} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\, ln(305/d_i) + b_4\, ln^2(305/d_i) + P_j + D_k + e_{ijkl}$$

Where:
y_{ijkl} = estimated biochemical parameter in blood serum or milk;
μ = intercept;
b_1, b_2, b_3, b_4 = regression coefficients (lactation curve by Ali and Schaeffer, 1987);
d_i = days in milk i (i = 11 to 537 day);
P_j = fixed effect of parity i (i = II., III+),
D_k = fixed effect of fat/protein ratio classes k (k = acidosis risk / normal / ketosis risk),
e_{ijkl} = residual.
Tukey-Kramer's studentized range test in GLM procedure in SAS (SAS Institute Inc., 2019) were applied in order to test the significance ($p < 0.05$) of the differences in biochemical parameters in blood plasma and milk due to fat/protein ratio classes separately for each sampling month (May, June, July).

RESULTS AND DISCUSSION

The effects included in the used statistical model (lactation stage, parity, and F/P ratio class statistically significantly ($P < 0.01$) affected values of biochemical parameters in blood plasma and milk of selected Holsteins. The variability of the biochemical parameters in blood plasma regarding the metabolic disorder risk (F/P ratio classes) separately for each sampling month (May, June, July) is presented in Table 2. Differences in the values of the biochemical parameters in blood plasma regarding the risk of metabolic disorder separately for each month of sampling (May, June, July) were found, although they were not statistically significant ($p > 0.05$).
The value of aspartate aminotransferase (AST) in blood plasma was highest in cows with acidosis risk. Also, the decreasing trend of AST was observed in cows in normal status and in acidosis risk regarding the sampling month. Similarly, the highest value of γ-glutamyl transferase (GGT) in blood plasma was determined in animals at risk of acidosis with an increasing trend regarding the month of sampling. The concentration of glucose in blood plasma also varied regarding the sampling month with the lowest values observed in June while the highest concentration was measured in the blood plasma of cows at ketosis risk in May. Furthermore, the highest concentration of urea in May and June was in healthy cows, while in July the highest urea concentration was determined in the blood plasma of cows at acidosis risk. The highest concentration of protein was observed in the blood plasma of cows at risk of ketosis in May and June, while in July highest protein concentration was in normal cows. Regarding the albumin concentration, the lowest value was observed in June in cows at ketosis risk. The lowest concentration of triglyceride was observed in acidosis-risk animals, while the lowest β-hydroxybutyrate was in June in ketosis-risk cows. The highest concentration of Fe in blood plasma was determined in acidosis-risk animals, while the highest value of Ca was observed in ketosis-risk cows in May.

Tab. 2. LSmeans of the biochemical parameters in blood plasma regarding fat/protein ratio classes separately by sample month (May, June, July)

Trait	Month	Fat/protein ratio		
		< 1.1 Acidosis risk	**1.1 – 1.5 Normal status**	**> 1.5 Ketosis risk**
Aspartate amino transferase (u/l)	May	144.195 ^{A}	132.912 ^{A}	88.996 ^{A}
	June	174.313 ^{A}	157.299 ^{A}	80.525 ^{A}
	July	129.657 ^{A}	107.023 ^{A}	137.727 ^{A}
γ-glutamil transferase (u/l)	May	32.489 ^{A}	24.715 ^{A}	26.628 ^{A}
	June	36.573 ^{A}	34.665 ^{A}	24.934 ^{A}
	July	41.789 ^{A}	34.335 ^{A}	37.226 ^{A}
Glucose (mmol/l)	May	3.195 ^{A}	3.381 ^{A}	3.621 ^{A}
	June	2.533 ^{A}	2.734 ^{A}	2.447 ^{A}
	July	3.292 ^{A}	3.206 ^{A}	3.208 ^{A}
Urea (mmol/l)	May	4.354 ^{A}	4.689 ^{A}	3.478 ^{A}
	June	4.614 ^{A}	4.767 ^{A}	4.297 ^{A}
	July	4.599 ^{A}	4.340 ^{A}	2.902 ^{A}
Protein (g/l)	May	85.717 ^{A}	83.626 ^{A}	86.235 ^{A}
	June	82.799 ^{A}	80.230 ^{A}	83.341 ^{A}
	July	86.700 ^{A}	88.751 ^{A}	79.283 ^{A}
Albumin (g/l)	May	32.170 ^{A}	32.499 ^{A}	32.593 ^{A}
	June	31.889 ^{A}	33.129 ^{A}	29.385 ^{A}
	July	32.229 ^{A}	32.409 ^{A}	25.964 ^{A}
Triglyceride (mmol/l)	May	0.112 ^{A}	0.122 ^{A}	0.130 ^{A}
	June	0.113 ^{A}	0.115 ^{A}	0.131 ^{A}
	July	0.107 ^{A}	0.117 ^{A}	0.107 ^{A}
β-hydroxybutyrate (mmol/l)	May	0.584 ^{A}	0.574 ^{A}	0.464 ^{A}
	June	0.451 ^{A}	0.445 ^{A}	0.338 ^{A}
	July	0.366 ^{A}	0.515 ^{A}	0.455 ^{A}
Fe (µmol/l)	May	26.892 ^{A}	24.105 ^{A}	17.551 ^{A}
	June	24.390 ^{A}	23.976 ^{A}	11.911 ^{A}
	July	23.647 ^{A}	23.224 ^{A}	21.325 ^{A}
Ca (mmol/l)	May	2.303 ^{A}	2.240 ^{A}	2.332 ^{A}
	June	1.983 ^{A}	2.055 ^{A}	1.949 ^{A}
	July	2.174 ^{A}	2.174 ^{A}	2.172 ^{A}

** Values within the same row marked with different letter differ statistically significant (P<0.05)*

The values of the biochemical parameters in milk regarding the risk of metabolic disorder separately for each month of sampling (May, June, July) are presented in Table 2. Differences in the values of the biochemical parameters in milk regarding the metabolic disorder risk were found, and were statistically significant ($p < 0.05$) only for AST and albumin concentration. The highest concentration of AST was determined in the milk of ketotic-risk cows in June, while the highest value of GGT was observed in ketotic-risk cows in July. The concentration of glucose was highest in the milk of acidosis-risk and lowest in ketosis-risk cows. A similar trend regarding the risk of

metabolic disorders was also observed for the value of urea in milk with the highest values determined in June. The lowest determined concentration of protein and albumin was in the milk of ketotic-risk cows in May. Furthermore, in the same animals, the lowest concentration of Fe and Ca in milk were observed.

Tab. 3. LSmeans of the biochemical parameters in blood plasma regarding fat/protein ratio classes separately by sample month (May, June, July)

Trait	**Month**	**Fat/protein ratio**		
		< 1.1 Acidosis risk	**1.1 – 1.5 Normal status**	**> 1.5 Ketosis risk**
Aspartate amino transferase (u/l)	May	7.024 ^{A}	9.486 ^{A}	10.829 ^{A}
	June	19.153 ^{A}	20.786 ^{A}	43.134 ^{A}
	July	15.399 ^{A}	19.771 ^{A}	15.376 ^{A}
γ-glutamil transferase (u/l)	May	279.661 ^{A}	315.916 ^{A}	332.696 ^{A}
	June	308.205 ^{A}	394.518 ^{B}	375.193 ^{AB}
	July	342.663 ^{A}	442.823 ^{A}	468.324 ^{A}
Glucose (mmol/l)	May	0.562 ^{A}	0.457 ^{A}	0.411 ^{A}
	June	0.560 ^{A}	0.537 ^{A}	0.288 ^{A}
	July	0.621 ^{A}	0.434 ^{A}	0.407 ^{A}
Urea (mmol/l)	May	5.168 ^{A}	5.730 ^{A}	3.934 ^{A}
	June	6.402 ^{A}	5.989 ^{A}	5.556 ^{A}
	July	4.752 ^{A}	4.546 ^{A}	3.467 ^{A}
Protein (g/l)	May	30.676 ^{A}	34.662 ^{A}	28.382 ^{A}
	June	40.354 ^{A}	39.059 ^{A}	38.418 ^{A}
	July	35.507 ^{A}	35.315 ^{A}	40.450 ^{A}
Albumin (g/l)	May	20.686 ^{A}	23.129 ^{A}	18.494 ^{B}
	June	23.129 ^{A}	23.242 ^{A}	22.255 ^{A}
	July	23.020 ^{A}	22.182 ^{A}	23.818 ^{A}
Fe (µmol/l)	May	11.895 ^{A}	17.751 ^{A}	7.518 ^{A}
	June	30.939 ^{A}	27.002 ^{A}	16.412 ^{A}
	July	28.036 ^{A}	24.139 ^{A}	45.251 ^{A}
Ca (mmol/l)	May	2.768 ^{A}	3.067 ^{A}	2.658 ^{A}
	June	3.086 ^{A}	3.699 ^{A}	3.851 ^{A}
	July	3.460 ^{A}	3.546 ^{A}	3.425 ^{A}

** Values within the same row marked with different letter differ statistically significant (P<0.05)*

Dieho *et al*. (2016) pointed out that inadequate feeding management and imbalanced ration significantly raise the possibility of metabolic disorders occurrence. Furthermore, the occurrence of some metabolic disorders could result in variability of the concentration of biochemical parameters in cows' blood and milk. Determining the differences in the biochemical parameters in blood and milk concerning animal health could enable the detection and prevention of disorders/diseases development.

The results of the conducted analysis showed that the differences in the values of the biochemical parameters in blood plasma and milk regarding the metabolic disorder risk were present, but were statistically significant ($p < 0.05$) only for Aspartate amino

transferase (AST) and albumin concentration in milk. Also, there were notable differences in all analysed parameters due to sampling months (May, June, and July). The determined effect of sampling month could be the consequence of variations in feeding management and microclimatic conditions on the farm. Similarly, fluctuations of catabolic liver enzymes in blood plasma vs milk as in this research were determined by Liu *et al*. (2012). The same authors reported higher GGT and lower AST concentrations in milk than in blood plasma. Furthermore, the higher urea concentration in the blood serum, determined mainly in acidosis-risk cows, could indicate inefficient nitrogen utilization. For instance, Stefanska *et al*. (2020) reported that in cows with low rumen pH, milk urea nitrogen rises significantly. Although β-hydroxybutyrate (BHB) did not vary significantly in this research (variability was determined regarding the indication of metabolic disorder and the month of sampling, and the highest values did not undoubtedly indicate only subclinical ketosis), Guan *et al*. (2020) emphasised that the elevated BHB levels are mainly related to ketosis in dairy cows. The obtained results pointed out the lowest concentrations of protein, albumin, Fe and Ca in the milk, as well as the lowest concentrations of Fe in the blood plasma of ketotic-risk cows. Tsukano and Suzuki (2020) noted that the differences in the blood Fe concentration could be an indicator of the same inflammatory disease.

CONCLUSIONS

This work aimed to determine the variability of biochemical parameters in blood and milk samples due to the classes of fat/protein ratio (which indicates the risk of metabolic disorders) regarding the sampling month (May, June, and July). The effects included in the used statistical model (lactation stage, parity, and F/P ratio class statistically significantly ($P < 0.05$) affected values of biochemical parameters in blood plasma and milk of selected Holsteins. Also, the conducted analysis showed variability in the values of the biochemical parameters in both blood plasma and milk due to metabolic disorder risk and month of sampling. Accordingly, when different metabolites are used as an indicator of the metabolic disorder risk, correction for the systematic effects (stage of lactation and parity, sampling month) should be performed.

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UTJECAJ RIZIKA OD METABOLIČKIH POREMEĆAJA NA VARIJABILNOST BIOKEMIJSKIH PARAMETARA U KRVI I MLIJEKU KRAVA S OBZIROM NA MJESEC UZORKOVANJA

Rezime

U cilju utvrđivanja varijabilnosti biokemijskih parametara u krvnoj plazmi i mlijeku Holstein krava s obzirom na rizik od pojave metaboličkih poremećaja (na temelju razreda omjera F/P) zasebno za svaki mjesec uzorkovanja (svibanj, lipanj, srpanj), krv i mlijeko uzorkovani su iz 75 visokoproizvodnih krava Holstein pasmine uzgojanih na farmi mliječnih krava u istočnoj Hrvatskoj. Rizik od metaboličkog poremećaja definiran je u temeljem dnevnog omjer masti/protein (F/P) dobiven iz zapisa na kontrolni dan – test-day records (F/P < 1,1 što ukazuje na rizik od acidoze; F/P u [1.1, 1.5], normalan status životinje ; F/P > 1,5, što ukazuje na rizik od ketoze).
Provedena analiza pokazala je varijabilnost vrijednosti biokemijskih parametara u krvnoj plazmi i mlijeku uslijed razreda rizika od metaboličkih poremećaja i mjeseca uzorkovanja. U skladu s tim, kada se različiti metaboliti koriste kao pokazatelj rizika od metaboličkog poremećaja, potrebno je primijeniti korekciju za fiksne utjecaje (stadij laktacije i pariteta, mjesec uzorkovanja).

Ključne riječi: *biokemijski parametri, krv, mlijeko, metabolički poremećaji, Holstein*

CHANGES IN THE COMPOSITION OF MARE'S MILK DUE TO THE EFFECT OF ANIMAL'S AGE (PARITY)*

Maja Gregić[1], Mirjana Baban[1], Pero Mijić[1], Vesna Gantner[1], Tina Bobić[1]

Original scientific paper

Summary

Mare's milk is traditionally used in the regions of Central Asia by nomadic people. Also, there is a long history of utilisation and processing of mare's milk. Furthermore, in recent decades, mare's milk has been researched as a functional food throughout Europe. The production of mare's milk is highly demanding. Since the composition of the mare's milk depends on various factors, the aim of the work was to determine the variability of the composition of the Lipizzaner mare's milk regarding parity. Mare's milk was collected from the 16 Lipizzaner mares bred at a stud farm in eastern Croatia. Regarding parity, the mares were classified into two groups: (A) younger (first to third lactation, n = 8) and (B) older (over third lactation, n = 8). It was found that the amount of milk fat and lactose is higher in the milk of older mares, while the amount of protein was lower in the same animals. Concerning the aspect of functional food in the human diet, protein represents the most important component of mare's milk. Therefore, it can be concluded that the milk of the younger mares is richer in protein and more recommended as a functional food.

Keywords: *mare's milk, functional food, composition*

INTRODUCTION

Mare's milk is traditionally used in the regions of Central Asia by nomadic people. Also, there is a long history of utilisation of mare's milk. Furthermore, in recent decades, mare's milk has been researched as a functional food throughout Europe. The production technology of mare's milk is highly demanding (Alatrović *et al*., 2017). Mare's milk represents a highly valued and sought-after raw material for the processing of innovative dairy products. Also, mare's milk is rich in nutrients. Furthermore, the changes in the composition of mare's milk have been poorly investigated in the production of the raw material. Components in mare's milk depend on various factors such as the stage of lactation and different environmental factors (Sonntag *et al*., 1996). The fatty acid composition of mare's milk is especially high in UFAs (unsaturated fatty acids) which

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is considered to be beneficial for consumers (Gregić *et al*., 2022). Regarding the true whey N and non-protein nitrogen (NPN) mare's milk has a similar content as human and goat's milk (Potočnik *et al*., 2011). Research done so far indicates that mare's milk could be beneficial in treating atopic dermatitis as well as in improving skin appearance (Pieszka *et al*., 2016). Furthermore, mare's milk has been proven to play a role in curing Crohn's disease, ulcerative colitis, as well as hepatitis and chronic gastric ulcers. Mare milk, along with qymyz, has been reported to have a wide range of therapeutic effects (Sabrina *et al*., 2019; Teichert *et al*., 2021; Kondybayev, 2021). The utilization of mare's milk for direct consumption, which is today in Europe sold mainly as fresh or frozen raw milk, requires the adoption of adequate facilities, equipment, and management of animals and milk to achieve products with high hygienic standards (Salimei & Park 2017). Accordingly, to Fišera *et al.* (2018) the composition of a mare's milk varies in regard to the physiological state of an animal, while the effects such as the mare's age, the number of foaling, sex of foal etc. did not affect milk nutrient composition. According to Schryver *et al*. (1986), the total solids and ash content of mare's milk varied regarding the stage of lactation, with values of 12 and 0.61% respectively, at the end of the first week of lactation, 10.5 and 0.45% at 4 weeks of lactation, 10 and 0.38% at 8 weeks of lactation and 10.2 and 0.32% at 16 weeks of lactation. Furthermore, the basic composition of the mare's milk was as follows: solids-not-fat in the amount of 84.4 g/kg, fat 15.1 g/kg, lactose 65.3 g/kg, and total protein 24.2 g/kg (combined of casein in the amount of 14.6 g/kg, and whey protein, 9.5 g/kg). Accordingly, to Teichert *et al*. (2021), the basic composition of Polish Coldblood mare's milk was as follows: solids-not-fat, 84.4 g/kg, fat 15.1 g/kg, lactose 65.3 g/kg, and total protein 24.2 g/kg (made up of casein, 14.6 g/kg, and whey protein, 9.5 g/kg). Čagalj *et al*. (2014) reported that mare's milk of Croatian Coldblood horses on average contained: 10.2 % of total solids, 1.23 % of milk fat, 1.76 % of proteins, 0.71 % of casein and 6.26 % lactose (Marchis *et al*., 2019). Furthermore, the fat content of mare's milk varies between 1.88 g/100 g (in the first lactation) and 2.17 g/100 g (in the third lactation) while the content of protein in milk varies between 1.74 g/100g (in the first lactation) and 1.92 g/100 g (in the third lactation) (Marchis *et al*., 2019). Furthermore, Pikul & Wójtowski (2008) recommended the collection of milk from mares in the period from the 4th and 5th month of lactation. The aim of the work was to determine the variability of the composition of the Lipizzaner mare's milk regarding parity.

MATERIALS AND METHODS

During the research milk was collected from 16 Lipizzaner mares reared on a state stud farm located in Slavonia. The body weight of selected mares was in the interval between 450 and 535 kg while wither height was between 156 and 162 cm. Furthermore, during the research mares were fed the same ration. During the research, all animals were grazing on meadow grass pasture. Additionally, 4.5 kg/day of oats and hay ad libitum was given to the selected mares. During the research, all mares were clinically healthy. The milking and sampling were performed once a day in the morning after 3 hours of

physical separation from their foals by hand from both teats of the udder. Sampled milk was analysed using a MilkoScan™ FT120 (Foss Tecator AB Hilleroed, Denmark) in the laboratory. Furthermore, the solids-not-fat, fat, lactose, casein, whey protein, and ash contents were determined in the milk samples. Selected mares were in the fourth and sixth months of lactation. Finally, regarding age, mares were classified into two groups: (A) younger (from first to third lactation, n = 8) and (B) older (over third lactation, n = 8).

Logical control of data and statistical analysis was carried out in the statistical program SAS. MS Excell was used for the graphic presentation of the data.

RESULTS AND DISCUSSION

Differences in the composition of mare's milk depending on the age of Lipizzan mares are shown in Figure 1. It can be seen that age affects the variability of individual components of a mare's milk and that the composition of a mare's milk differs in younger and older mares. Furthermore, changes in composition depend on the individual substance. The amount of fat and lactose (g/100 g) in the mare's milk is higher in older mares, while the amount of protein (g/100 g) is higher in younger animals. Furthermore, the milk of younger mares had a higher protein and ash composition. Milk fat in mare's milk is extremely interesting from a nutritional point of view in the diet and processing of mare's milk.

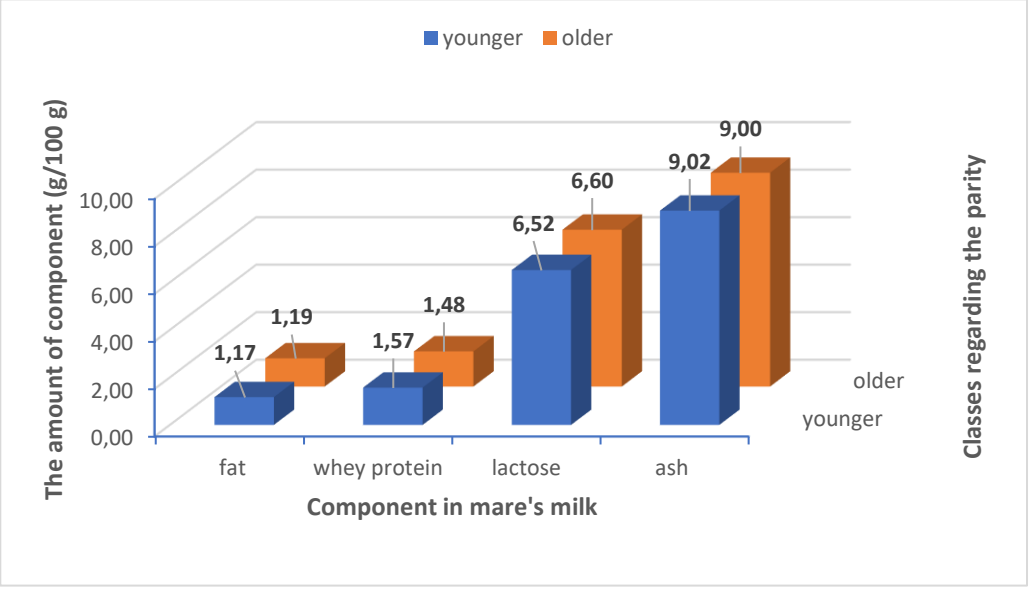

Figure 1. The amount of fat, whey protein, lactose and ash (g/100 g) in milk of Lipizzaner mares regarding the parity class (younger (n=8) and older (n=8))

Changes in the composition of mare's milk during lactation were determined by Schryver *et al*. (1986). In addition, the variability in the composition of proteins and fat in mare's milk due to the influence of the stage of lactation was determined by Marchis *et al*. (2019). Teichert *et al*. (2021) determined changes in the composition of mare's milk regarding the order of lactation both in Lipizzaner breeds and in cold-blooded heavy horse breeds. In addition, the same authors state that the milk of the Lipizzaner breed of mares is nutritionally more suitable for younger mares due to its higher protein content. Kondybayev (2021) states that the milk of older Lipizzan mares is suitable for processing into fermented dairy products due to the higher content of milk fat than in the milk of younger mares. Taking into account the overall composition and nutritional value of the milk of both young and old mares, it is recommended for consumption regardless of the number of lactations.

CONCLUSIONS

Based on the performed research it was determined that the animals' age (parity) affects the composition of milk. The amount of fat and lactose in the mare's milk was higher in older mares, while the amount of protein was higher in younger ones. The amount of ash did not vary due to the animal's age.

Concerning the aspect of functional food in the human diet, protein represents the most important component of mare's milk. Therefore, it can be concluded that the milk of the younger mares is richer in protein and more recommended as a functional food. Finally, further research on the protein composition of mare's milk should be performed.

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PROMJENE U SASTAVU KOBILJEG MLIJEKA USLIJED UTJECAJA DOBI ŽIVOTINJE (PARITETA)

Rezime

Kobilje mlijeko tradicionalno koriste nomadski narodi u središnjoj Aziji. Također, duga je povijest korištenja i prerade kobiljeg mlijeka. Nadalje, posljednjih se desetljeća kobilje mlijeko istražuje kao funkcionalna hrana diljem Europe. Proizvodnja kobiljeg mlijeka je vrlo zahtjevna. Budući da sastav kobiljeg mlijeka ovisi o različitim čimbenicima, cilj ovoga rada bio je utvrditi varijabilnost sastava mlijeka lipicanskih kobila s obzirom na paritet. Kobilje mlijeko prikupljeno je od 16 kobila uzgojanih na ergeli u istočnoj Hrvatskoj. S obzirom na paritet, kobile su razvrstane u dvije skupine: (A) mlađe (od prve do treće laktacije, n = 8) i (B) starije (iznad treće laktacije, n = 8).

Utvrđeno je da je količina mliječne masti i laktoze veća u mlijeku starijih kobila, dok je količina proteina manja kod istih životinja. S aspekta funkcionalne hrane u ljudskoj prehrani, proteini predstavljaju najvažniji sastojak kobiljeg mlijeka. Stoga se može zaključiti da je mlijeko mlađih kobila bogatije bjelančevinama i preporučljivije kao funkcionalna hrana.

Ključne riječi: *kobilje mlijeko, funkcionalna hrana, sastav*

A STUDY OF SOME PRODUCTION CHARACTERISTICS OF DUPSKA PRAMENKA LAMBS WITH THE AIM OF GENETIC CHARACTERIZATION OF THE BREED*

Božo Važić[1], Biljana Rogić[1], Ivan Pihler[2], Vesna Gantner[3]

Summary

Characterization of animal genetic resources includes all activities related to morphometric, production and genetic characterization. The aim of these work was production characterization of Dupska pramenka, as a valuable genetic resource of Bosnia and Herzegovina. In these paper, parameters of birth weight and development of lambs up to the age of 180 days were monitored. The lambs were raised in extensive feeding conditions. Birth weight was measured for a total of 116 lambs, 47 female and 69 male. The average birth weight of lambs is 3.81 kg, females 3.78 and males 3.83. The weight of the lambs was also recorded at the age of 30, 90 and 180 days old. A total of 30 lambs were monitored, 15 female and 15 male. The average weight of lambs was 10.43 kg (30 days), 20.95 kg (90 days) and 32.75 kg (180 days). Average daily gain of lambs was 221.00 g (0-30 days), 175.67 g (30-90 days) and 130.33 g (90-180 days). The total gain of lambs was 6.62 kg (0-30 days), 10.51 kg (30-90 days) and 11.70 kg (90-180 days). The results showed that there is a statistically significant correlation between some analyzed parameters.

Key words*: autochthonous breed, characterization, weigh of lambs, daily and total gain, correlation*

INTRODUCTION

Sheep production in Bosnian and Herzegovina (BiH) is mostly based on the production of autochtonous sheep breed with modest production characteristics. Dupska pramenka belongs to the autochthonous strain of pramenka breed, with a triple direction of production: milk, meat and wool. The original habitat of these strains is in the central part of Bosnia and Herzegovina, especially on the Vlašić mountain and the surrounding municipalities. Most of the sheep production in BiH is based on the breeding of Dupska pramenka sheep.

The characterization of animal genetic resources must consider an appropriate system of identification of individuals, a quantitative and qualitative description of the breed,

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habitat and production systems in which the animals are raised and which they are adapted (Caput *et al*., 2010). The goal of genetic characterization is to gain more knowledge about resources, their current and potential future use in food production under defined environmental conditions, and their current status regarding endangerment (FAO, 1984).

The characterization of animal genetic resource is based in three directions: morphologic, production and genetic characterizazion. Morphological characterization of Dupska pramenka sheep breed is reported (Važić *et al*., 2017a, Važić *et al*., 2017b). Genetic characterization was performed through hemoglobin polymorphism (Važić et al., 2015; and Važić *et al*., 2017c), and DNA characterization (Ćinkulov *et al*., 2008; Ćurković *et al*., 2016). According to production characterization, some results of milk production can be found (Važić *et al*., 2003). Momić (2014) reported the classification, linear measurements, texture, and meat colour, and Drzaic *et al.* (2017) report the effect of sex on slaughter traits and measurements of Dupska pramenka sheep lamb. Analysis of Pramenka lamb meat was part of the research by Grabež *et al.* (2019) and the results showed that the meat of the Dupska Pramenka has certain advantages compared to the meat of the lambs of the Norwegian White and the Pivska Pramenka sheep.

The aim of these staudy was to improve results of production characterization of Dupska pramenka breed, through analysis parameters of growth and development of lambs aged up to 180 days.

MATERIAL AND METHODS

The birth weight of the lambs was measured at a total of 116 lambs from 109 ewes (7 ewes had twins). 30 lambs, 15 male and 15 female, were randomly selected to measure growth parameters. The body weight of the lambs was measured four times: at birth, at 30, 60 and 180 days of age. Based on the measured weights, average daily growth and total achieved growth of lambs were recalculated. The lambs were always with their mothers from birth to the age of 180 days. In the first 15 days of life, the lambs suckled, and from the third week they had available meadow hay and concentrate for lambs in limited amount.

Descriptive statistics were performed for birth weight, body weight, average daily growth and total achieved growth of lambs until the age of 180 days. Phenotypic correlations for lambs aged 0-90 days and lambs aged 90-180 days were calculated. For lambs aged up to 90 days, correlations were made for the following traits: birth weight (X1), body weight at 90 days (X2), total achieved growth (X3) and average daily gain (X4). For lambs aged 90-180 days, correlations were made for the following traits: body weight at 90 days (X5), body weight at 180 days (X6), total achived growth at 90-180 days (X7) and average daily growth at 90-180 days (X8). The statistical program SPSS version 22.0 was used for data processing.

RESULTS AND DISCUSSION

The average birth weight of lambs was 3.81 kg, recpectively 3.78 female, 3.83 male lambs (table 1). The small difference in the birth weight of lambs between male and female lambs can be explained by the fact that there were more twins in the total number of male lambs, which have a lower birth weight compared to singles.

Table 1. The birth weight of lambs, kg

sex	n	mean	SD	SEM	CV	Min	Max
F	47	3.78	0.50	0.07	13.23	2.55	4.80
M	69	3.83	0.69	0.08	17.75	1.50	5.15
F+M	116	3.81	0.62	0.06	16.27	1.50	5.15

F-female; M-male; n-number; SD-standard deviation; SEM-standard error of mean; CV-coefficient of variation; Min-minimal value; Max-maximale value

The fertility of Dupska Pramenka sheep is very low. Based on 109 monitored births, 116 lambs were obtained, that means that only 7 sheep had twins. Comparing with other Pramenka strains, it can be concluded that the birth weight of Dupka Pramenka lambs is higher than Pirotska Pramenka lambs, 2.61 kg (Antić, 1954), similar to Svrljiska and Sjenicka (Milosavljević, 1955; Trajić, 1978), but smaller compared to Kupreška lambs (Palina, 1957).

In order to monitor the dynamics of growth and development of lambs, the weight of lambs aged 30, 60 and 180 days was measured. The results are shown in table 2. The body weight of lambs 30 days aged ranged from 9.99 kg (female) to 10.87 kg (male).

Table 2. The body weight of lambs, aged 30, 60 and 180 days

age (days)	sex	n	mean	SD	SEM	CV	Min	Max
30	M	15	10.87	1.47	0.39	13.52	8.70	13.30
	F	15	9.99	1.44	0.39	14.41	7.90	12.30
	M+F	30	10.43	1.52	0.28	14.57	7.90	13.30
90	M	15	21.61	1.91	0.51	8.84	17.10	24.50
	F	15	20.29	1.59	0.43	7.84	17.90	22.90
	M+F	30	20.95	1.88	0.35	8.97	17.10	24.50
180	M	15	33.87	3.21	0.86	9.48	24.10	37.20
	F	15	31.62	1.70	0.45	5.38	28.20	34.40
	M+F	30	32.75	2.80	0.52	8.55	24.10	37.20

F-female; M-male; n-number; SD-standard deviation; SEM-standard error of mean; CV-coefficient of variation; Min-minimal value; Max-maximale value

In the first month of growth, the lambs consumed mother's milk, and from the fifteenth day they were given concentrate for lambs in limited amounts. With the development of the pre-gastric, the lambs could also take hay, which was given to their mothers.

Comparing with other Pramenka strains, it can be concluded that the lambs weight aged 30 days of Dupka Pramenka is higher than Sjenicka (Mastilović and Slijepčević, 1957), but smaller compared to Ovčepoljska (Tokovski *et al*., 1987). The body weight of Dupska Pramenka lambs aged 90 days ranged from 20.29 (female) to 21.61 kg (male). The obtained results are similar with Lička (Mikulec *et al*., 1979), higher than Sjenička (Milosavljević, 1955) and Šarplaninska (Bičanin, 1958), but smaller than Ovčepoljska Pramenka lambs (Tokovski *et al*., 1987). The body weight of Dupska Pramenka lambs aged 180 days ranged from 31.62 (female) to 33.87 kg (male), similar to other Pramenka strain, except Pivska Pramenka lambs which was significantly higher (Marković, 1994).
The movement of average daily growth of lambs is followed, and results are shown in table 3. The average daily growth of Dupska pramenka lambs in the first 30 days was 221 g, respectively 232 g on male and 210 g on female lambs.

Table 3. The movement of average daily growth of lambs (g)

age (days)	sex	n	mean	SD	SEM	CV	Min	Max
0-30	M	15	232.00	50.23	13.43	21.65	160	310
	F	15	210.00	48.99	13.10	23.33	140	280
	M+F	30	221.00	50.82	9.43	23.00	160	310
30-90	M	15	178.67	23.91	3.34	13.38	140	250
	F	15	173.33	14.47	3.54	7.19	150	190
	M+F	30	175.67	19.09	3.54	10.87	140	250
90-180	M	15	134.67	26.30	7.03	19.53	80	170
	F	15	126.00	20.26	5.42	16.08	70	150
	M+F	30	130.33	23.87	4.43	18.32	70	170

F-female; M-male; n-number; SD-standard deviation; SEM-standard error of mean; CV-coefficient of variation; Min-minimal value; Max-maximale value

Obtained results are similar with other results reported in literature. The average daily growth on Svrljiska pramenka lambs was 123 g (Živković and Kostić, 1953), which is lower in compare with obtained results. Spiridović and Jašović (1989) reported 283 g daily growth on male and 256 g on female Baljuša Pramenka lambs, which is higher in compare whith obtained data.
The average daily growth of Dupska pramenka lambs from 30 to 90 days was 175.67 g, respectively 178.67 g on male and 173.67 g on female lambs. Trajić (1978) reported daily growth for Sjenička pramenka male lambs 140 g, respectively female lambs 125 g, which is lower than obtained results. The average daily growth of Dupska pramenka lambs aged from 90 to 180 days was 130.33 g. In order to monitor the dynamics of growth and development of lambs, the total achieved growth until the age of 180 days was measured. The results are shown in table 4.

Table 4. The total achieved growth of lambs until the age of 180 days (kg)

age (days)	sex	n	mean	SD	SEM	CV	Min	Max
0-30	M	15	6.94	1.48	0.40	21.33	4.85	9.15
	F	15	6.29	1.48	0.40	23.05	4.30	8.50
	M+F	30	6.62	1.51	0.28	22.81	4.30	9.15
30-90	M	15	10.65	1.41	0.37	13.24	8.20	14.70
	F	15	10.36	0.71	0.19	6.85	8.70	11.20
	M+F	30	10.51	1.12	0.21	10.66	8.20	14.70
90-180	M	15	12.07	2.33	0.62	19.30	7.00	14.90
	F	15	11.33	1.81	0.49	15.98	6.50	13.80
	M+F	30	11.70	2.12	0.39	18.12	6.50	14.90

F-female; M-male; n-number; SD-standard deviation; SEM-standard error of mean; CV-coefficient of variation; Min-minimal value; Max-maximale value

The male lambs had a higher average total growth in all three monitored intervals. The difference between the sexes increased with age, which is expected because male lambs develops faster than female lambs.
Phenotypic correlations for growth traits are calculated. According the obtained results it can be concluded that there is correlation between some growth traits (table 5).

Table 5. The values of coefficients of correlation for growth characteristics of lambs

age	0-90 days					
relations	X1:X2	X1:X3	X1:X4	X2:X3	X2:X4	X3:X4
coefficient of correlation	0.17	-0.03	-0.04	0.98*	0.97*	0.99*
age	90-180 days					
relations	X5:X6	X5:X7	X5:X8	X6:X7	X6:X8	X7:X8
coefficient of correlation	0.63*	-0.07	-0.04	0.75*	0.75*	1.00*

Coefficient of correlation between body weight at 90 days (X2) and total achived growth (X3) and average daily growth (X4) at lambs 90 days old are statisticaly significant. Also, statisticaly significant correlation between total achived growth (X3) and average daily growth (X4) was confirmed. For lambs aged 90-180 days, a statistically significant correlation between body weight at 90 (X5) and body weight at 180 days (X6), and this indicates that the lambs that had a higher body weight at 90 days retained this advantage until the age of 180 days. Also, the statisticaly significant correlation between body weight at 180 days (X6) and total achived growth (X7) and average daily growth (X8) was confirmed.

CONCLUSION

Dupska pramenka sheep is a valuable genetic resource of Bosnia and Herzegovina and the most of sheep production in BiH is based on the breeding of these sheep. The average birth weight of lambs was 3.81 kg. The average weight of lambs aged 30, 90 and 180 days was 10.43 kg, 20.95 kg and 32.75 kg. According obtained results we can conclude that there is a statistically significant correlation between some analyzed traits. Based on the literature data, there are no more recent works that investigated the growth and development of lambs not only of Dupka Pramenka, but also of other strains from Balkan Peninsula. The obtained results are valuable from the aspect of characterization of Dupska pramenka sheep as an animal genetic resources.

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ISTRAŽIVANJE NEKIH PROIZVODNIH KARAKTERISTIKA JANJADI DUPSKE PRAMENKE S CILJEM GENETIČKE KARAKTERIZACIJE RASE

Rezime

Karakterizacija animalnih genetičkih resursa podrazumijeva sve aktivnosti vezane za morfološke, proizvodne i genetičke karakteristike. Cilj rada je bila proizvodna karakterizacija dupske pramenke, kao vrijednog genetičkog resursa Bosne i Hercegovine. U radu su praćeni parametri mase pri rođenju i razvoja jagnjadi uzrasta do 180 dana. Porođajna masa je mjerena kod 116 jagnjadi, 47 ženskih i 69 muških. Prosječna porođajna masa jagnjadi je bila 3,81 kg, odnosno 3,78 kg ženskih i 3,83 kg muških jagnjadi. Težina jagnjadi je također mjerena pri uzrastu od 30, 90 i 180 dana. Ukupno je praćeno 30 jagnjadi, 15 ženskih i 15 muških. Prosječna masa jagnjadi je bila 10,43 kg (30. dana), 20,95 kg (90. dana) i 32,75 kg (180. dana). Prosječni dnevni prirast jagnjadi je bio 221,00 g (0-30 dana), 175,67 g (30-90 dana) i 130,33 g (90-180 dana). Ukupni prirast jagnjadi je bio 6,62 kg (0-30 dana), 10,51 kg (30-90 dana) i 11,70 kg (90-180 dana). Rezultati su pokazali statistički značajnu korelaciju između nekih praćenih parametara.

Ključne riječi: *autohtona rasa, karakterizacija, masa jagnjadi, dnevni i ukupni prirast, korelacije*

INTERNATIONAL MARKETS IMPACT ON WHEAT PRICES IN THE REPUBLIC OF NORTH MACEDONIA*

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Abstract

The production of wheat, as one of the elementary and strategic nutrition crops, in North Macedonia is insufficient and cannot meet the national demands, which results with negative trade balance with import of 74,937 tons of wheat valued at 17.9 million EUR. The main goal of this paper is to analyse the influence of international markets on wheat prices in the Republic of North Macedonia. Data on the purchase price of wheat have been analysed using statistical methods and models from December 2009 to December 2021. A total of 145 monthly time series with data on the purchase price of wheat for Macedonia, the EU, Bulgaria, Romania and Serbia have been analysed and compared. The Pearson correlation coefficient shows a relatively high relationship and mutual dependence of purchase prices in Macedonia, compared to prices in the EU, Bulgaria, Romania and Serbia (over 65%). The purchase prices of wheat in Macedonia have the highest relationship and mutual dependence with the price movement in Serbia (0.78), and the lowest with the price movement in the EU (0.66). The estimated lag length of the autoregressive process (AIC, BIC, FPE and HQIC tests), on average shows 2 lags (months) later time reaction of the wheat prices in Macedonia with the comparison countries countries. The forecasting model (Granger causality test) shows that time series of wheat prices can be convenient for forecasting wheat prices in Macedonia. As of October 2021, the model clearly shows the impact of food economic crisis and unexpected, immediate rise of wheat prices as result of the post Covid-19 and Ukraine war crisis.
This research and analysis model can provide significant information for the wheat price trends, forecasting and markets shock, as management and decision-making tools for producers, traders and processors, but also for the policy makers.

Keywords: *international markets, agri-food prices, wheat prices, forecasting.*

INTRODUCTION

The agricultural and food (agri-food) sector is important for the economy of the Republic of North Macedonia, which has a great contribution in the formation of the gross domestic product (7.60% of the GDP) and participation in the trade exchange of

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the country. The Republic of North Macedonia is an import dependent country. According to the data of the State Statistics Office, the total value of the export of goods from the Republic of North Macedonia in 2021 is 6,922,573 thousand EUR, and the import is 9,638,290 thousand EUR. The coverage of imports with exports is 71.82%, and the trade deficit amounts to 2,715,717 thousand EUR. The EU member states (77.33%) and the countries of the Western Balkans (11.81%) have the largest share in the export of goods, and the EU member states (46.24%) in the import. The export of agricultural and food products in 2021 has share of 9.05% of the total export, while the import has share of 9.79% of the total import. According to the Annual Agricultural Report of the Ministry of Agriculture, Forestry and Water Management (MAFWE, 2021), the most significant trade partner for agri-food and fish products of the Republic of North Macedonia are the EU countries, with a total value of trade in 2020 of 679.7 million EUR. As a result of the crisis with the Covid-19 pandemic and the exit of the United Kingdom from the European Union, the export of agri-food and fish products has a decrease of 5.2% of exports and 5.9% of imports compared to 2019.

In addition to the economic dimension, the agri-food sector is also significant from a social aspect, considering its importance for the rural population. According the Structural research of agriculture holdings (SSO, 2016), 178,125 agriculture holdings are involved in agriculture production, out of which only small number (280) are business entities.

Wheat, as one of the elementary and strategic nutrition crops, is the most common crop sown on 70,515 ha or about 44.39% of the sown areas under cereal crops (158,836 ha) in 2021 (SSO, 2022). The production of wheat in 2021 was about 243,676 tons with average yields of 3,463 kg/ha. The production is insufficient and cannot meet the national demands and needs, which results with negative trade balance with import of 74,937 tons of wheat, valued at 17.9 million EUR. The export is only 21,395 tons with value of 4.6 million EUR (SSO, MakStat database).

The analysis of the integration of the markets, as well as the impact of this connection on the prices of the products, are of special interest for understanding how certain changes or shocks in a certain market affect and are reflected on other markets. One of the key principles of economics is that markets allow prices to be transmitted horizontally (spatially) and vertically (Conforti, 2004). Scientific researchers and economists in general have always shown great interest in researching the connection and influence between prices, although it is generally known in theory that other factors, especially the product and its characteristics, are also of great importance in the way of connecting markets and explaining of the trade balance (Asche, Jaffry, & Hartmann, 2007).

Towards the end of the twentieth century, most scientific research focused on the vertical integration of markets (Goodwin & Holt, 1999); (Balcombe & Morrison, 2002), with a primary focus on price and its movement in the food supply chain. Scientific and empirical research of integration and interdependence level of markets is

mainly based on time series analysis of price movements and margin distribution at the different levels (participants) in the food supply chain.

In the last period, the interest in horizontal integration and the influence that exists between spatially (regionally) separate markets, is especially increasing, as result of increasing globalization and liberalization of markets and trade. The interest of scientific society in the movement of prices of agri-food products and the level of horizontal integration and mutual influence of markets has increased as a result of the emergence of the so-called food crisis in the period 2007-2008, when the prices of agri-food products on international markets begin to vary significantly with the occurrence of large falls and increases in sales prices (European Commission, 2008); (Irwin, Sanders, & Merrin, 2009).

The importance of the horizontal integration of the markets is further increased as a result of the application of an increasingly restrictive policy of intervention by the states in the direction of protecting their own production and markets. Through research on horizontal integration, information can also be obtained about the level of freedom or protectionism in a certain market. The period of crisis and major shocks in the markets of agricultural and food products forced the states to apply a more restrictive policy and stricter measures to protect their markets (Tangermann, 2011). Such increased protection and intervention of the states contributes to a greater disparity between supply and demand, greater isolation of the markets, followed by non-objective price formation and inefficient movement of products. This situation also contributes to increased regional imbalance and the occurrence of surplus and deficit of food. In that direction, information on the horizontal integration of markets is of great importance for defining intervention measures in order to reduce the imbalance and prevent insecurity in food supply (Goletti & Babu, 1994). The critical review of Fackler and Goodwin (Fackler & Goodwin, 2001), emphasizes that economic movements and markets are increasingly influenced by social and political situations in different countries, which has a special impact on the changes in the approach of scientific research on the integration of markets.

The basic theory of market integration and price formation is based on the spatial arbitrage rule. This theory is based on the assumption that the difference in the price of the product between related markets that trade and mediate among themselves will not exceed the costs of transferring it. If the price difference is greater than the transfer costs, the profit-making opportunities will be used by various intermediaries (arbitrageurs) who will be involved in the trade process. The result of this theory is the law of one price (Marshall, 1920); (Fackler & Goodwin, 2001), according to which the product will have the same price expressed in the same currency in different markets, when the costs incurred for the transfer of that product from one market to another are included. According to (Tomek & Robinson, 2003), in perfectly integrated markets that trade with each other, the price difference is equal to the transfer costs, and in markets that they do not trade with each other, the price difference is less than the transfer costs.

The whole system of market integration, as well as the degree of influence of such integration on product prices, is influenced by a number of factors. Still the main factors (Piero, 2004) that influence the movement and formation of prices between different markets can come from transportation and transportation costs, including costs of providing information, negotiation, as well as the costs of monitoring the entire process of transport are included (Williamson, 1986) or costs of searching for a partner, checking the seriousness of the partner, negotiating with the partner, transferring the product, monitoring the realization, as well as court costs in certain cases when the contract will not be fulfilled (Staal, Delgado, & Nicholson, 1997).

The market positioning, competitiveness and market power influence price formation. In addition, he role of intermediaries and their position and market power can increase the final price for consumers (Wohlgenant, 1999); (Azzam, 1999); (Goodwin & Holt, 1999); (Dhar & Cotterill, 1998); (McCorriston, Morgan, & Rayner, 2001).

Productivity and profitability of production contribute to increasing market competitiveness and strength and as such have an impact on horizontal integration and formation on prices. However, when it comes to vertical integration and its impact on price formation in the food supply chain, productivity and profitability of production have a different effect compared to horizontal integration and their impact on market competitiveness and strength (McCorriston, Morgan, & Rayner, 2001).

The problem of price dependence in scientific research is also addressed from the perspective of the impact that occurs as a result of the relationships and interaction that exists between different agricultural products (Esposti & Listorti, 2013). Prices are influenced by the complementarity or complementarity of a certain agricultural-food product with another similar or different product (Saadi, 2011). The level of trade and price dependence is always lower in models that assume product heterogeneity, compared to models that assume homogeneity (Armington, 1969).

Different currencies and exchange rates, through their ratio and their stability, affect the level of market integration, the volume of trade and the formation of prices. Prices are additionally influenced by the ability of firms to adjust their marketing strategy and pricing strategy in relation to exchange rate changes and different markets (Dornbusch, 1987); (Froot & Klemperer, 1989); (Knetter, 1993).

Last, but not least, the import and export policy have a direct impact on horizontal integration, the level of trade and the formation of prices. From the point of view of import and export policy, non-tariff (non-tariff) barriers such as: variable tariffs (customs and subsidies), tariff quotas, protective tariffs and technical barriers have a great impact on market integration and price formation. Compared to tariffs which are measurable, non-tariff barriers are difficult to quantify, measure and assess. They may result in additional costs for exporters. Such costs are almost always borne by the exporters and usually represent a bigger problem for small exporters and companies and they are much more affected compared to larger and export-strong companies (OECD, 2017).

The main working hypothesis of this paper is that the agricultural and food sector in the Republic of North Macedonia has a modest competitive power and is significantly influenced by regional and world trends, while international market movements have a strong impact on domestic production and prices of agricultural and food products.
The main goal of this paper is to analyse the influence of international markets on wheat prices in the Republic of North Macedonia.
Considering that most of the market information systems and generally provide information only on agri-food prices, this paper is focused only on the analysis of the impact of international markets in terms of prices and price movements, not taking into consideration other factors that influence the formation of prices of wheat and agri-food products.

MATERIAL AND METHODS

Data on the purchase price of wheat have been analysed using statistical methods and models for the period from December 2009 to December 2021. A total of 145 months times series with data on the purchase price of wheat for Macedonia, the EU, Bulgaria, Romania and Serbia have been analysed and compared.
For the domestic markets, the data for the monthly wheat price indices of agricultural and food products from the State Statistical Office were used. The price indexes are translated into prices by using the index and the price of purchased products in the baseline year 2015, calculated based on value and quantity of purchase wheat (SSO, 2015). Data for the EU countries are taken from the official agricultural data site of the European Commission (EC, Agridata, 2022). For Serbia, data from the official Agricultural Market Information System of Serbia (STIPS, 2022) were used.
Exponential smoothing as a method is especially effective and needed when the time series has its own trend, but also a seasonal component that changes over time. The method was used for the alignment (weighting) of the series through the use of alignment constants and assigning a weight to each value, which at the same time allows for the purification of the series from certain seasonal atypical variations. At the same time, depending on the model used, the degree of importance of newer or older data can be determined and accordingly the model can give more importance to one or the other in the alignment.
Since in our case the data time series have a pronounced trend with multiplicative seasonal variation where the mean, growth rate and seasonal variation change over the years, we used triple exponential smoothing and the multiplicative Holt-Winters model with multiple degrees of seasonal smoothing. character.
Correlation and correlation coefficient in statistics is an indicator that should show the relationship (linear or proportional) between two quantitative variables and the mutual dependence of one variable on the other. There are several statistical models for determining correlation, such as Pearson correlation coefficient (PCC), Pearson product-moment correlation coefficient (PPMCC), Bivariate correlation. In any case, all models basically measure the degree of linear correlation between two sets of data

and determine the degree of covariance of two variables and the product of their standard deviations. The correlation coefficient can have a minimum value of -1 and a maximum value of +1. At the minimum value of -1, the coefficient shows that there is a perfect inversely proportional dependence between the variables and that as one variable increases, the other decreases in the same proportion. The opposite situation is at a maximum value of +1, which indicates that by increasing one variable, the other variable increases in the same proportion. If the correlation coefficient approaches 0, then the dependence between the observed variables is very small or does not exist.

In our case, we used the Pearson correlation coefficient to compare and determine the correlation and dependence of the purchase prices of agricultural and food products in the Republic of North Macedonia compared to other countries. In addition to the correlation test and the correlation coefficient, a test was also made for the statistical significance of the correlation, expressed through the t test (Student's t-test), and for the strength of the correlation, expressed through the coefficient of determination.

Augmented Dickey Fuller test, or ADF test for short, was used to analysed the character of the time series, which is one of the most common statistical tests used to determine whether a certain time series is stationary or non-stationary. ADF is a unit root test that tests the null hypothesis against the first lag of an unknown (compared) variable. The basic assumption of the null hypothesis is that there is the presence of a single common root and $\alpha=1$ and if a lower p-value level of significance is obtained (lower than 0.05) it is a basis for rejecting the null hypothesis which shows essentially that the time series is stationary and the trend of the series itself will not affect the trends of other series.

Estimating the number of lags of a regression is one of the key tasks in econometrics and model analysis. Determining the number of lags is essentially determining the number of time lags that will be included in the model. Several criteria are used to select the order of the time delay and there are several models.

In this paper, determining the optimal number of lags is done using Akaike's information criterion (AIC), Bayesian information criterion (BIC), final prediction error (FPE) and Hannan–Quinn information criterion (HQIC). The results of the model are obtained through a process of their minimization and the model with the lowest value is selected as the relevant lag or time delay.

At the end of the statistical analysis, the Granger causality test was used, which essentially determines whether and to what extent one time series can be used to predict another. The test was first proposed in 1969 as a simple regression, but was improved by Clive Granger who argued that dependence in the economy can be tested by measuring the future predicted value of the time series using previous data from another time series. Granger's test essentially finds predicted causality, the use of only the term causality is a relative misnomer for the model that Granger himself named as temporally related later in 1977. Essentially the model instead of testing whether a variable value causes certain changes in other parameters, essentially analyses the dependency and tests whether the variable predicts the other parameters. The time series shows whether there is such a relationship, and through certain tests (t-test and F-test) on the lags of the

variable value, it shows the statistical significance of its prediction of the future values of the other parameters.

RESULTS AND DISCUSSION

The graphic analysis of the data for the purchase prices of wheat in Macedonia and the EU shows that the prices in Macedonia are higher than the prices in the EU, with the exception of the middle of 2019 and the last months of 2021 (Figure 1).

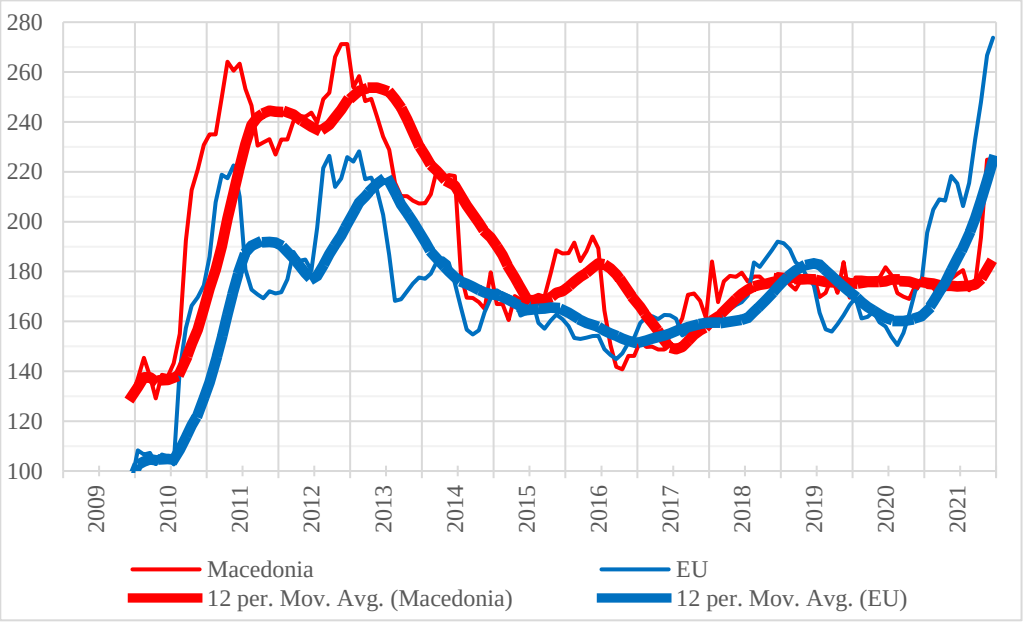

Figure 3. Purchase prices of wheat in Macedonia and the EU, in EUR per 1,000 tons, December 2009 – December 2021 and price trends (12-month moving average)

From the same graphic, it can be seen that the price trend (expressed through a 12-month moving average) of purchase prices in Macedonia and the EU has a relatively high and almost the same trend and mutual dependence in the period until the middle of 2015. After this period, the trend significantly decreased.

The graphic analysis of wheat purchase prices compared to prices in Serbia as non-EU country, shows the similar trend (Figure 2). Although prices in Macedonia are higher compared to Serbia, this difference is relatively smaller compared with the EU and in certain periods (especially from 2009 to mid in 2011), they are almost at the same level. On the other hand, the price trend has a much higher relationship and mutual dependence compared with the EU, almost throughout the whole analysed period with the exception of the small deviation that occurs in 2021.

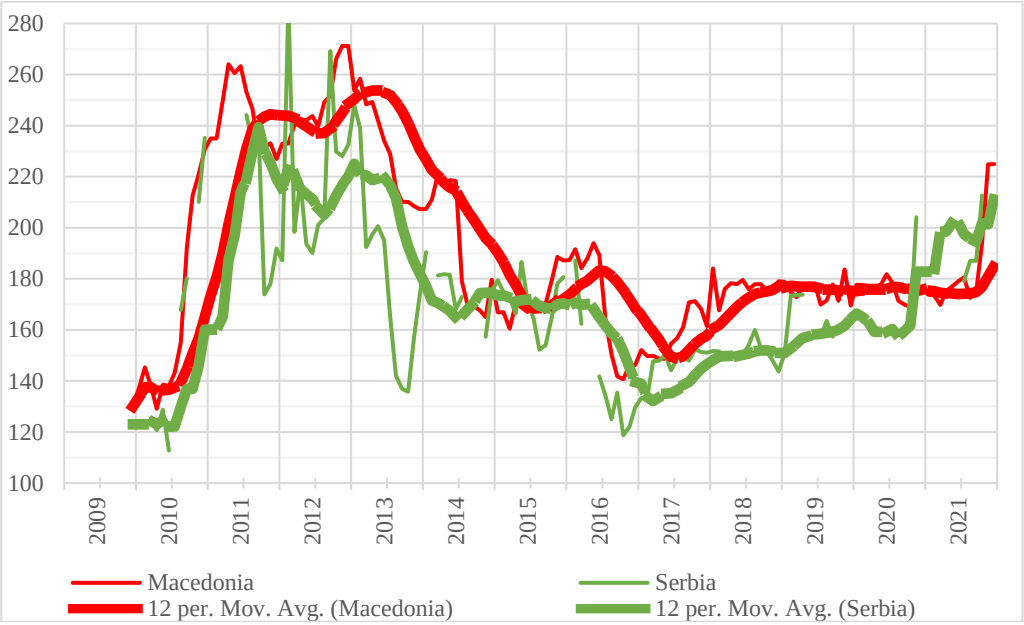

Figure 4. Purchase prices of wheat in Macedonia and Serbia, in euro per 1,000 tons, December 2009 – December 2021 and price trends (12-month moving average)

From all the analysed data for 145 months, in the process of the exponential smoothing for 37 monthly data for which there was no information on the purchase prices of wheat, the values were calculated and replaced with the mean value of the previous months. The Goodness of fit statistics and degree of suitability and deviation of the model from the real data shows a high level of fitness of the model for Macedonia ($R^2 = 0.94$) and relatively for other countries.

Table 1 present the minimum, maximum and average purchase prices of wheat, as well as the standard deviation for Macedonia, EU, Bulgaria, Romania and Serbia, for the period from December 2009 to December 2021.

Table 5. Purchase prices of wheat in EUR per 1,000 tons, December 2009 – December 2021

Country	**Minimum**	**Maximum**	**Average**	**Standard deviation**
Macedonia	129.12	271.24	190.73	35.18
EU	96.63	273.78	174.00	29.83
Bulgaria	96.97	259.48	169.45	34.75
Romania	106.93	255.33	166.19	28.28
Serbia	112.71	288.30	177.07	37.92

The table shows that in the period from December 2009 to December 2021, Macedonia has the highest average monthly purchase price of wheat of 190.73 EUR/t or 0.19 EUR/kg of purchased wheat. At the same time, Romania has the lowest average monthly price of 166.19 EUR/t. The lowest monthly purchase price was recorded in the EU at 96.63 EUR/t, while Serbia at the same time has data for a month with the highest purchase price of 288.39 EUR/t. The highest monthly standard deviation of prices is in Serbia (37.92 EUR/t) and the lowest in Romania (28.28 EUR/t).
The correlation coefficient shows a relatively high relationship and mutual dependence of purchase wheat prices in Macedonia, compared to prices in the EU, Bulgaria, Romania and Serbia (over 65%). The purchase prices of wheat in Macedonia in terms of the compared countries have the highest relationship and mutual dependence with the price movement in Serbia (0.78), and the lowest with the price movement in the EU (0.66).

Table 6. Correlation coefficient of wheat purchase prices

Countries	**Macedonia**	**EU**	**Bulgaria**	**Romania**	**Serbia**
Macedonia	**1.00**	**0.66**	**0.74**	**0.69**	**0.78**
EU	**0.66**	**1.00**	**0.92**	**0.91**	**0.73**
Bulgaria	**0.74**	**0.92**	**1.00**	**0.92**	**0.81**
Romania	**0.69**	**0.91**	**0.92**	**1.00**	**0.79**
Serbia	**0.78**	**0.73**	**0.81**	**0.79**	**1.00**

*The values in bold have significant statistical importance with α=0.05 and different from 0

After the ADF test for stationary character of the time series, the obtained p-value significance level is lower than 0.05 and determined that the time series is stationary.
Table 3 presents that the number of lags and months of time delay of wheat purchase prices in Macedonia compared to the other countries. The values are ranging from no delay (BIC test) to a delay of two months (AIC and FPE test). According to the Hanna-Quinn test (HQIC) the delay is only one month.

Table 7. Number of lags and months of time delay of purchase prices of wheat in Macedonia

	AIC	**BIC**	**FPE**	**HQIC**
0	24.15	24.27*	3.08E+10	24.2
1	23.65	24.33	1.86E+10	23.93*
2	23.61*	24.87	1.801e+10*	24.12
3	23.75	25.58	2.08E+10	24.49
4	23.77	26.17	2.14E+10	24.74
5	24.01	26.98	2.77E+10	25.22
6	23.98	27.53	2.75E+10	25.42

*Values marked with an asterisk is the lag number or monthly lag

In our case, we took 2 (two) lags as a basis as input for forecasting model purchase prices of wheat in Macedonia and performing Granger causality test.

Table 8. Real, forecast and deviation of the forecast to real purchase prices of wheat in Macedonia, in EUR per 1,000 tons

Month	Actual prices	Forecast prices	Difference (forecast-actual)	Monthly difference
11/2020	174.45	178.63	4.18	4.18
12/2020	176.83	179.49	2.66	-1.52
1/2021	173.82	181.79	7.97	5.31
2/2021	173.53	183.42	9.89	1.93
3/2021	169.80	184.63	14.83	4.93
4/2021	175.11	185.55	10.44	-4.38
5/2021	177.10	186.22	9.12	-1.32
6/2021	179.02	186.77	7.76	-1.37
7/2021	180.52	187.26	6.73	-1.02
8/2021	172.54	187.72	15.18	8.44
9/2021	175.03	188.17	13.14	-2.04
10/2021	193.70	188.62	-5.08	-18.22
11/2021	224.87	189.06	-35.81	-30.73
12/2021	224.96	189.48	-35.48	0.33
1/2022	225.70	189.86	-35.83	-0.36
2/2022	225.68	190.20	-35.48	0.35
3/2022	225.68	190.48	-35.20	0.28
4/2022	236.02	190.70	-45.32	-10.12
5/2022	244.44	190.87	-53.57	-8.26
6/2022	249.36	190.98	-58.39	-4.82
Average	**198.91**	**187.00**	**-11.91**	**-2.92**

From Table 4, it can be concluded that forecasted purchase prices of wheat with the Granger causality test and model until September 2021 is suitable and has a tendency for modest overestimate of the prices trend, which results with a symbolically higher value than the real ones. On average for this period, the model has positive difference of 9.26 euro/t or insignificant 0.01 EUR per kilogram of wheat. In average, monthly difference for this period is 1.19 euro/t.

The negative trends start from October 2021, when the predicted purchase prices of wheat start to be significantly lower than the actual ones. The highest negative monthly difference can be noticed in October (-18.22 EUR/t) and November (-30.73 EUR/t) in 2021 and April (-10.12 EUR/t) in 2022, Still, this is insignificant 0.01 EUR per kilogram of wheat. This results to have in total average negative difference of the model of -11.91 EUR/t or -2.92 EUR/t per month, for the whole forecasted period. Still, even this difference is insignificant and is less than -0.01 EUR per kilogram of wheat.

CONCLUSIONS

The correlation coefficient (Pearson correlation coefficient) shows a relatively high relationship and mutual dependence of purchase prices in Macedonia, compared to prices in the EU, Bulgaria, Romania and Serbia (over 65%), which clearly confirms that Macedonia wheat production has a modest competitive power and is significantly influenced by regional and world trends, while international market movements have a strong impact on domestic production and prices of agricultural and food products.

The purchase prices of wheat in Macedonia in terms of the compared countries have the highest relationship and mutual dependence with the price movement in Serbia (0.78) with which Macedonia has a long-lasting connection and importing dependence when it comes to the cereals import and especially wheat.

Estimating the lag length of autoregressive process for a time series (AIC, BIC, FPE and HQIC tests), on average shows 2 lags (months) later time reaction of the wheat prices in Macedonia with compared countries.

The forecasting model (Granger causality test) shows that wheat prices time series can be convenient in forecasting wheat prices in Macedonia. The model starting from October 2021, clearly shows the impact of food economic crisis and unexpected, immediate rise of wheat prices as result of the post Covid-19 and Ukraine war crisis.

This research and proposed methodology can provide significant and timely information for the wheat price trends and forecasting future trends and markets shock, which can be used as substantial management and decision making tools for producers, traders and processors, but also for the policy makers, especially to quickly react to markets shock and respond to the emerging situation with adequate public policies.

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UTICAJ MEĐUNARODNOG TRŽIŠTA NA CIJENE PŠENICE U REPUBLICI SJEVERNOJ MAKEDONIJI

Rezime

Proizvodnja pšenice, kao jedne od osnovnih i strateških prehrambenih kultura u Sjevernoj Makedoniji je nedovoljna i ne može da zadovolji nacionalne potrebe, što rezultira negativnim trgovinskim bilansom sa uvozom od 74.937 tona pšenice u vrijednosti od 17,9 miliona evra. Osnovni cilj ovog rada je analizirati uticaj međunarodnih tržišta na cijene pšenice u Republici Sjevernoj Makedoniji. Podaci o otkupnoj cijeni pšenice analizirani su statističkim metodama i modelima od decembra 2009. do decembra 2021. Ukupno je 145 mjesečnih vremenskih serija sa podacima o otkupnoj cijeni pšenice za Makedoniju, EU, Bugarsku, Rumuniju i Srbiju. analizirali i uporedili. Pirsonov koeficijent korelacije pokazuje relativno visoku povezanost i međusobnu zavisnost nabavnih cena u Makedoniji, u poređenju sa cijenama u EU, Bugarskoj, Rumuniji i Srbiji (preko 65%). Otkupne cijene pšenice u Makedoniji imaju najveći odnos i međusobnu zavisnost sa kretanjem cijena u Srbiji (0,78), a najnižu sa

kretanjem cijena u EU (0,66). Procijenjena dužina kašnjenja autoregresivnog procesa (AIC, BIC, FPE i HQIC testovi), u prosjeku pokazuje 2 laga (mjeseca) kasnije vremensku reakciju cijena pšenice u Makedoniji sa zemljama za poređenje. Model predviđanja (Grangerov test uzročnosti) pokazuje da vremenske serije cijena pšenice mogu biti pogodne za predviđanje cijena pšenice u Makedoniji. Od oktobra 2021, model jasno pokazuje uticaj ekonomske krize hrane i neočekivanog, trenutnog porasta cijena pšenice kao rezultat ratne krize poslije Covid-19 i rata u Ukrajini.
Ovaj model istraživanja i analize može pružiti značajne informacije za trendove cijena pšenice, prognoze i tržišni šok, kao alate za upravljanje i donošenje odluka za proizvođače, trgovce i prerađivače, ali i za kreatore politike.

Ključne riječi: *međunarodna tržišta, poljoprivredno-prehrambene cijene, cijene pšenice, prognoze*

DECENTRALIZATION OF AUTHORITY, POSSIBILITIES AND OBSTACLES TO ATTRACTING INVESTMENTS IN AGRICULTURAL PRODUCTION IN THE EXAMPLE OF SERBIA, BOSNIA AND HERZEGOVINA AND CROATIA*

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Original scientific papers

Summary

The goal of this research was, based on the actual jurisdiction of local governments, assigned after the decentralization of the central government, to analyze the possible assumptions that affect the attraction of investments in agricultural production and to point out the current weaknesses of individual segments of economic policy.
The paper analyzes individual indicators that, according to the Doing business list, make it easier to start a business using an analytical method. The research was conducted for Serbia, Bosnia and Herzegovina and Croatia for the period 2019 and 2020. Furthermore, the research focuses on indicators that are the responsibility of local politics, and which can contribute to a better competitive position in the market.
The general presented data concludes that there is room for reforms and that at all levels of government, and especially at the local level, additional measures must be taken to improve and facilitate business conditions. The analyzed indicators are very significant because they show the willingness of local governments to adapt to market trends. Furthermore, local plans, studies and strategies must facilitate procedures that would prioritize the developing and improving agricultural production.

Key words: *decentralization, spatial plan, local policy, investment policy, investments*

INTRODUCTION

Decentralization implies transferring duties, jurisdiction and finances from the central level to local authorities. The purpose of decentralization in transitional countries is to prepare all levels of government for better functioning in the global and national environment and to strengthen local and regional authorities and their capacities in order to realize more successfully solving political, economic and social problems. This

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process affects the transformation in the authority structure in society, essentially changing the role of the state because it loses a considerable part of its jurisdiction.
Decentralization can be political, administrative, economic and financial (Đorđević, 2009). Political decentralization strengthens the autonomy of local administration, but also more actively involves citizens in decision-making processes (Cheema and Rondinelli, 2007). Administrative decentralization includes the administrative component of management and implies that the local administration takes over a considerable part of the tasks from the state. Therefore, through decentralization, jurisdictions are divided between state and local administrative bodies, and the previously hierarchical relations between these levels of government are replaced by partnership relations and cooperation. Fiscal (tax) decentralization is an essential component of all forms of decentralization, especially devolution. Decentralized units of local government units cannot carry out their work independently, unless they have sufficient and constant financial resources, as well as the right to autonomously decide on the type of local taxes, on the rate, independently collect taxes and spend the budget. Ferrara (2010) emphasizes type of decentralization implies the strengthening of the political and financial autonomy of local authorities, and it also opens up space for competition between local authorities in the direction of creating the best environment for investments, which as a rule greatly contributes to the development of society (fiscal federalism). Finally, economic decentralization is the process of transition from managing the economy from one political centre to a system in which the influence of the market on economic flows is emphasised (Horwath, 2000).
Spatial planning as activity results from the decentralization of authority that has been transferred to the local level. In the practice of urbanism, spatial plans are internationally recognized documents on the basis of which spatial development is managed and taken care of. Ehler and Douvere (2007) stated that spatial plans focus on public infrastructure: roads, water, electricity, waste removal, transportation, and construction facilities. The increase in capital investment in facilities, equipment, infrastructure, and similar raises business potential (Dunning, 1972). The global experience of using spatial planning should enable more efficient use of public infrastructure. Given the lack of resources, spatial planning provisions should direct the utilization of natural resources toward those directional to enhance the production of new values. Furthermore, spatial plans should be the basis for creating a vision of the future on the one hand, but at the same time, they cannot delimit the existing material and institutional structure. Adopting a spatial plan represents the process of adopting selective decisions about future activities based on defined existing geographic areas of a certain population, often under the jurisdiction of the government (Portman, 2011). It is essentially a planning concept that has been extensively researched, and involves defining priorities and subsequent outcomes; that is, creating, implementing and monitoring the outcome of the plan itself. Spatial plans, respecting socio-economic, natural, cultural-historical and landscape values, elaborate the principles of spatial planning, determine the goals of spatial development, and the organization, protection, use and purpose of space (Holland 2010).

Spatial planning, as a set of activities, guarantees the management of the area of the organized society as a valuable and limited resource. Key components, or aspects, of spatial planning that have to be ensured, are coordination between the plan and infrastructure provision. These components include the following (Hostovsky, 2000):
1. the current state and projections of the future number of inhabitants that should be foreseen in the plan in order to model the development of the appropriate infrastructure,
2. the locations of future infrastructure development must be precisely defined,
3. existing infrastructure capacities must be realistically presented,
4. adaptation of the spatial plan according to the outcomes (state infrastructure construction plans, quality standards, industrial requirements, environmental protection requirements, etc.) that are expected in the future,
5. the responsibility of persons according to the prepared plan,
6. the existence of coordination in the planning of spatial conditions between all interested parties.

Using geomarketing information, economic development projects align population growth with increasing economic power (Hirt and Block, 2005), thereby enabling states to implement the necessary procedures and actions to realize the future's planned vision achieving the goals of regulating the development of agricultural production should protect settlements, infrastructure and landscape at all decision-making levels. At the same time, the polycentric development of the network of municipalities should be planned and carefully directed, and further spontaneous and unplanned construction should be prevented in order to care for the protection of natural values. Since space is becoming increasingly limited by non-renewable resources, in contrast to the increasing dynamics and expansion of the economy and social needs, space is becoming more and more of a limiting element.

Investments represent a very significant generator of the development of business activities, and therefore it is necessary to create prerequisites for their attraction. Considering the powers that local self-governments received through the process of decentralization, creating of an encouraging environment requires a series of actions that must be approached studiously. Precisely because of this, it is necessary to make decisions at the local level that will minimize the effect of negative externalities. The local government must maintain continuous cooperation with expert teams of various profiles with the aim of creating social well-being.

For Serbia, Bosnia and Herzegovina and Croatia, agricultural production constitutes a significant segment of the gross social product. Agricultural production from raw materials to processing has significant opportunities for engaging all production factors. That is why it is necessary to strive for allocative efficiency, that is, the state when no reorganization of production improves the current state of some actors, nor worsens the state of other actors (Samuelson, 1992).

The goal of this research was, based on the actual jurisdiction of local governments, assigned after the decentralization of the central government, to analyze the possible assumptions that affect the attraction of investments in agricultural production, and to point out the current weaknesses of individual segments of economic policy.

MATERIALS AND METHODS

The research in this paper covers the territory of Serbia, Bosnia and Herzegovina and Croatia. Based on the data of the Doing business list, for the years 2019 and 2020, a comparison of the conditions of business complexity was made for all three countries, i.e., the current situation was shown, and then compared with the situation from the previous period. The analyzed factors are grouped into spatial, procedural and fiscal.

Tab. 1. Parameters of ease of doing business according to the Doing business list Rank 190 (WBG, 2022)

Indicator	A score of the implemented reform	DB 2020 Rank	DB 2020 Score	DB 2019 Score	Change in score point
Serbia					
Average		44	75.7	73.9	1.8
Starting a business	Negative	73	89.3	92.6	3.3
Work with building permits	Positive	9	85.3	84.4	0.9
Obtaining electricity	Positive	94	75.2	70.0	3.2
Ownership registration	-	58	71.8	71.2	0.6
Getting a loan	-	67	65.0	65.0	-
Protection of minority investors	Positive	37	70.0	62.0	8
Paying taxes	Positive	85	75.3	74.8	0.5
Trading across borders	-	23	96.6	96.6	-
Implementation of the contract	Positive	65	63.1	61.9	1.2
Insolvency resolution	Positive	41	67.0	60.8	6.2
Bosnia and Hercegovina					
Average		90	65.4	65.4	-
Starting a business	-	184	60.0	59.8	0.2
Work with building permits	-	173	48.6	48.6	-
Obtaining electricity	-	74	79.0	78.9	0.1
Ownership registration	-	96	63.6	63.6	-
Getting a loan	-	67	65.0	65.0	-
Protection of minority investors	-	88	56.0	56.0	-
Paying taxes	-	141	60.4	60.4	-
Trading across borders	-	27	95.7	95.7	-
Implementation of the contract	-	93	57.8	57.8	-
Insolvency resolution	-	37	68.2	67.8	-
Croatia					
Average		51	73.6	73.0	0.6
Starting a business	Positive	114	85.3	82.6	2.7
Work with building permits	Positive	150	57.8	55.7	2.1
Obtaining electricity	-	37	86.8	83.6	3.2

Ownership registration	Positive	38	77.4	73.9	3.5
Getting a loan	Negative	104	50.0	55.0	5
Protection of minority investors	-	37	70.0	70.0	-
Paying taxes	-	49	81.8	81.8	-
Trading across borders	-	1	100	100	-
Implementation of the contract	-	27	70.6	70.6	-
Insolvency resolution	-	63	56.5	56.2	0.3

Furthermore, the accumulation of investments in the year 2020 will be presented and compared with the year 2019 based on data from statistical reports (BHAS, 2022; DZS, 2022; RZS, 2022).

RESULTS AND DISCUSSION

The indicators from Table 1 represent the results of local policy management. The presented indicators show that it is easiest to do business in Serbia; that is, Serbia is the best-ranked country compared to the other two. Also, Serbia has the most positively assessed reforms. Bosnia and Herzegovina has the worst results, followed by Croatia. However, looking at the overall position of both countries, they are above half of the ranking, given that the Doing business list analyzes the business of 190 countries. Furthermore, from the above results show that the most active investment policy is conducted in Serbia. Serbia is ranked 44th on the Donig business list (WBG, 2022). Croatia is in the 51st position, while Bosnia and Herzegovina is in the 90th.

The general conclusion from the presented data is that there is space for reforms and that at all levels of government, and especially at the local level, additional measures must be taken in order to improve and facilitate business conditions. The analyzed indicators are very significant because they show the willingness of local governments to adapt to market trends. Furthermore, local plans, studies and strategies must facilitate procedures that prioritize developing and improving agricultural production.

If the presented indicators are analyzed individually (Figure 1), it could be observed that it is easiest to trade across the border in Croatia (1st position), while Bosnia and Herzegovina is in the 27th position. Compared to other countries, it is easiest to obtain a building permit in Serbia, and it is in the 9th position. In Croatia (150th and 114th position) and Bosnia and Herzegovina (173rd and 184th position), it is the most difficult to obtain building permits and start a business, which is a direct consequence of bad local politics. Serbia has the worst result in connecting to the electricity grid (94th position), which may be a consequence of poorly developed electrical energy infrastructure or a consequence of the cumbersome and sluggish administrative apparatus in the Electric Power Industry of Serbia. However, if one looks at the evaluation of the implemented reforms, it can be seen that there is progress in reforming the economic policy.

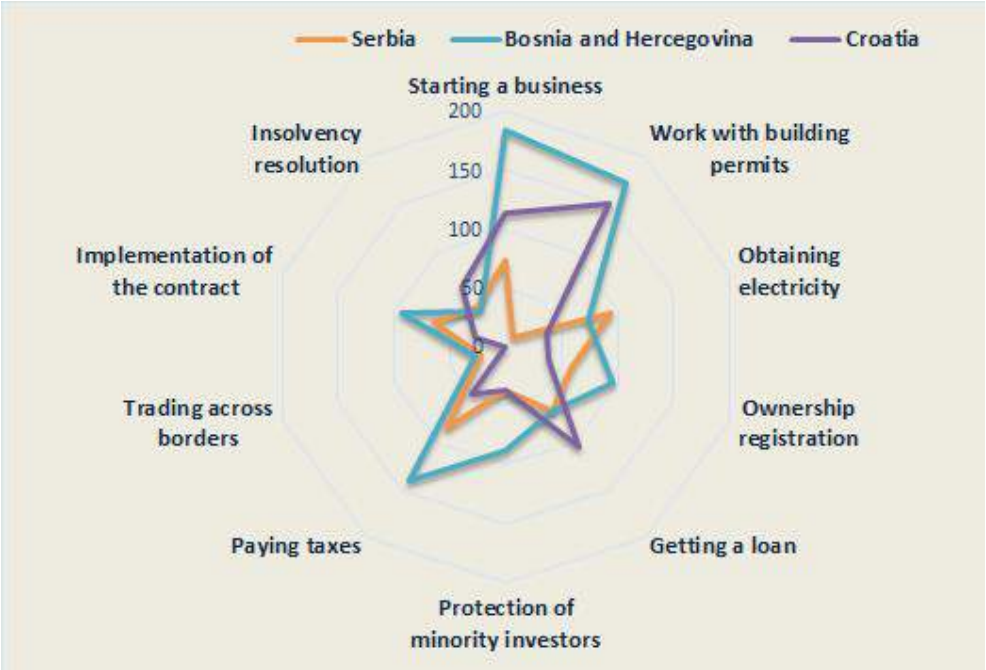

Fig. 1. Indicators of the ease of starting a business in the analyzed countries

In order to get the overall picture and determine the interdependence of the analyzed indicators and the implementation of the investment policy in the direction of attracting investments in agricultural production, below in Table 2, based on the data of statistical reports (BHAS, 2022; DZS, 2022; RZS, 2022), the accumulation of investments in the year 2020 will be presented and compared with the year 2019.

Tab. 2. The volume of investments in agricultural production in 2020 and 2019

State	The volume of investments in agricultural production		The index of change
	2020 (Eur)	2019 (Eur)	
Serbia	204,351,872	202,996,527	1.007
Croatia	187,450,133	187,018,548	1.002
Bosnia and Hercegovina	134,996,901	134,516,573	1.004

The data from the above table 2 show that all three countries achieved an increase in investments in agricultural production, that is, that the index of change ranges from 1.002 in the case of Bosnia and Herzegovina to 1.007 in the case of Serbia.
Although there are positive results in the volume of investments, it is necessary to carry out reforms in those segments that make it difficult to start and develop economic activities.

CONCLUSIONS

The results of the conducted research showed that the concept of decentralization is well designed, considering that each local authority can create the conditions for operating economic policy based on its natural resources and local capacities. In today's era of globalization, for investors, insight into the indicators that affect the complexity of business is very important because they can easily move their business from one area to another. Accordingly, to the value of the indicators, all three analysed states were better positioned in the year 2020 compared to 2019. The reforms that were carried out, which relate to obtaining building permits, protecting minority investors, paying taxes, and improving infrastructure, were evaluated positively, which resulted in an improvement in the ranking list. The implemented reforms had positive implications on the index of changes in the volume of investments in agricultural production. The general conclusion can be drawn that the decentralization process enabled local self-governments to create a positive investment environment that contributes to overall economic development.

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DECENTRALIZACIJA VLASTI, MOGUĆNOSTI I PREPREKE ZA PRIVLAČENJE ULAGANJA U POLJOPRIVREDNU PROIZVODNJU NA PRIMJERU SRBIJE, BOSNE I HERCEGOVINE I HRVATSKE

Rezime

Cilj ovog istraživanja bio je, na temelju stvarnih nadležnosti jedinica lokalne samouprave, dodijeljenih nakon decentralizacije središnje države, analizirati moguće pretpostavke koje utječu na privlačenje investicija u poljoprivrednu proizvodnju te ukazati na trenutne slabosti pojedinih segmenata ekonomske politike.
U radu se analitičkom metodom analiziraju pojedini pokazatelji koji, prema Doing business listi, olakšavaju pokretanje poslovanja. Istraživanje je provedeno za Srbiju, Bosnu i Hercegovinu te Hrvatsku za razdoblje 2019. i 2020. godine. Nadalje, fokus istraživanja je bio na onim pokazateljima koji su odgovornost lokalne politike, a koji mogu doprinijeti boljoj konkurentskoj poziciji na tržištu.
Opći zaključak iz iznesenih podataka je da prostora za reforme ima te da se na svim razinama vlasti, a posebno na lokalnoj, moraju poduzeti dodatne mjere za poboljšanje i olakšavanje uvjeta poslovanja. Analizirani pokazatelji vrlo su značajni jer pokazuju spremnost lokalnih samouprava da se prilagode tržišnim trendovima. Nadalje, lokalni planovi, studije i strategije moraju omogućiti postupke koji bi prioritetno davali razvoj i unapređenje poljoprivredne proizvodnje.

Ključne riječi: *decentralizacija, prostorni plan, lokalna politka, investiciona politika, investicije*

IMPACT OF THE ECONOMIC CRISIS ON THE APPLE PRODUCTION PRICE IN THE REPUBLIC OF NORTH MACEDONIA*

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Original scientific papers

Summary

Apple production has the greatest significance among all types of fruit crops in Macedonia, sharing 56% of the total fruit production. Due to the economic crisis caused by Covid-19 and the war in Ukraine, the prices of agricultural inputs have increased greatly. To that end, the main goal of this paper is to evaluate the impact of the global economic crisis on the apple production price. The production costs calculation is based on calculation of variable and fixed costs of apple production for 2020 and 2021 as reference years and 2022 as the year when the production price change is considered. Data for the normative calculation and production price for 2020 and 2021 are obtained based on the expert knowledge and confirmed with the semi-structured interview with 3 farmers and 2 apple producing companies. Based on this normative calculation and market prices for inputs in 2020, 2021 and 2022 collected from the 10 input suppliers, AMIS and SSO, the apple production price is calculated. The results show that the economic crisis has a large negative economic impact on apple production, as the estimated full production price of apples in 2022 ha increased by 37% compared with that of 2020 & 2021.

Keywords: *impact assessment, socio-economic crisis, apple producers, production price, production costs.*

INTRODUCTION

The economic consequences of the COVID-19 pandemic, the military conflicts in Ukraine and severity of climate change are exposing global food supply chains to high levels of fragility. Russia's military aggression against Ukraine has raised a widespread international concern of a global food crisis similar, or worse, to the one the world faced in 2007-2008. These two countries are key agricultural players which, combined, export nearly 12% of the food calories traded globally, and are major providers of basic agro-commodities, including wheat, maize and sunflower oil (Capril & Pichon, 2022). Russia is also the world's largest exporter of fertilisers. Russia's war against Ukraine has

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demonstrated that the EU's and many other countries depend on Russian coal, oil and gas. Since the invasion started, energy prices have risen dramatically, forcing consumers to pay more for petrol, heating and electricity. This resulted in huge disruptions in the supply of agri-food products, which have relatively begun to recover from the stresses and disruptions of the Covid-19 virus pandemic and limited supplies after 2020. This caused volatility and increased commodity prices, and consequently, an increase of food prices to levels not seen since the 2007-2008 crisis (WB, 2022). The FAO Food Price Index, tracking monthly changes in international commodity prices, indicates an increasingly difficult situation, it averaged 140.7 points in February 2022, its highest point ever (FAO, 2022). In the EU, food prices have increased 5.6% compared to February 2021. Concerning fertiliser, prices were already on the rise before the war, reaching levels unseen since the global financial crisis, mostly due to higher gas prices. The FAO forecasts that the global reference price of fertiliser would undergo an additional 13 per-cent increase in 2022/23, relative to its already elevated baseline level, in response to the more expensive production inputs implied by the higher crude oil price, but also by higher crop prices. This increase would influence production costs for the 2022/23 growing seasons (Capril & Pichon, 2022).

Countries that have weak institutions and/or societies with ingrained inequalities, or high dependency on imports of inputs and food, already face grievances that can be intensified by price and availability of necessities, like food. The socio-economic impact of the disruption of supply chains is particularly related to food security, development of agriculture and the well-being of rural households.

Since apple production in Republic of North Macedonia is the main fruit crop in terms of exports value, production and land areas, and has a large socioeconomic impact in the regions where it is grown, it is of particular importance to determine the impact on the price of apple production in conditions of disrupted value chains.

The areas under fruit plantations in the country in 2020, take about 3.3% of the total arable area or 17.095 ha, sharing 56% of the total fruit production. Apple plantations in 2020 amounted to 5,900 ha, taking up 34.5% of the total area of orchards (17,095 ha). Compared to the previous year, 2019, there was an increase in the total areas under fruit plantations by 311 ha. The most important region for apple production is the lake region (Prespansko-Ohridski), where about 75% of the total amount of apples is produced. The production of apples in 2020 was about 105.940 tons, which is an increase of about 19.3% compared to the year before. The increase in production is mainly the result of increased production per tree (on average 3 kg additional yield compared with 2019). About 25% of the total production of apples is sold on the domestic market, while the remaining 75% are processed or exported. The value of exported apples in 2020 was around 7.7 million EUR and is significantly higher compared with the exported value of 5.4 million EUR in 2019.

The orchards production is occupation of around 35,549 rural households, mostly apple growers, out of which only 82 are business entities.

MATERIALS AND METHODS

The production costs calculation is based on calculation of variable and fixed costs of apple production for 2020 and 2021 as reference years and 2022 as the year when the production price change is considered. Data for the normative calculation and production price for 2020 and 2021 are obtained based on the expert knowledge and confirmed with the semi-structured interview with 3 farmers and 2 apple producing companies. Based on this normative calculation and market prices for inputs in 2020, 2021 and 2022 collected from the 10 input suppliers, Agriculture Market Information System (AMIS) of the Ministry of Agriculture, Forestry and Water Economy (MAFWE) and the State Statistical Office (SSO), the apple production price is calculated.

As an economic indicator, cost of production (CoP) is the average cost of production for producing one unit of apple (kg product). At the same time CoP is presenting the minimum selling price of 1 kg apple (break-even price) as a break-even point in order to manage production without losses covering all costs of production. The apple CoP are calculated based on standard (normative) production on 1 hectare apple plantation and methodology used in different relevant literature (for example, see Ciaian *et al*., 2013; Kay *et al*., 2014; FAO, 2016), also customarily applied in the local context (Milanov and Martinovska, 2002; Martinovska *et al.*, 2009).

$$CoP = TC / Y$$

CoP - Cost of production (in EUR/kg)[2]
TC - Total cost (in EUR)
Y - Yield (produced apple in kg)

The CoP of apple production are calculated based on the total cost of production, calculated as used quantities for inputs and other resources for apple production, formed upon pre-defined normative costs of apple production, multiplied by their real market values in 2020/2021 and 2022.[3] In addition, the fixed costs (depreciation) of apple plantation are included in the total costs.

Apple yields normatively are fixed on average yearly expected yields of 30,000 kg apples per hectare.

The total costs represent the sum of variable and fixed costs of apple production.

$$TC = VC + FC$$

VC - Variable cost (in EUR)
FC - Fixed cost (in EUR)

[2] All values are presented in EUR. The average exchange rate for 2020/2021 is calculated as average yearly exchange rates in 2020 and 2021 (61.651 MKD/EUR) and average daily exchange rates in 2022 from 1 January to 15 June 2022 (61.677 MKD/EUR).

[3] The difference in market prices in 2020 and 2021 is minor. Modest increase in the value of the fertilizers can be noticed, which does not have huge impact on costs of production and unit price.

The variable yearly costs are the sum of the direct costs used for materials (fertilizer, pesticides) and resources consumed, irrigation and machinery costs for soil cultivation and additionally the labour used for apple production.
The value of fixed costs of apple production is calculated based on the costs of depreciation of the assets for apple production. The depreciation and value of fixed costs are calculated based on the value of apple plant establishment (cost of investment).

$D = VA \times DR$

D – Annual depreciation (in EUR)
VA – Value of the asset (Investment in apple plantation establishment in EUR)
DR – Depreciation rate (in %, DR = 1 ÷ Years of assets utilization)

The value of machinery, equipment and other assets used for apple producing are calculated as services provided and included in the variable cost.
According the methodology for investment in perennial crops, the value of apple plantation establishment as an investment asset is calculated as the sum of costs for plantation establishment, including the costs for regular yearly maintenance/production (in case of apples - 4 years), reduced by the gain income in this period. The period of investment and yearly maintenance costs are calculated until the yearly maintenance costs are higher than the yearly gained income form the archived yields.

RESULTS AND DISCUSSION

The comparative analyses of the production costs provide solid ground to describe the economic impact on apple production. In regards to the investment costs between the reference years 2020 and 2021 with 2022, presented in the Tables from 1 to 4, show that the investment costs for establishing an apple orchard have increased greatly.

Table 1. Investment costs in the first year of establishment of 1 ha apple plantation, average values for the reference years 2020/2021 and 2022 (in EUR).

Costs	Unit		2020/2021		2022		Change (%)
		Quantity	Unit price	Total	Unit price	Total	
Planting material	seedlings	1,000	1.95	1,946	2.11	2,108	8
Deep ploughing (80-100 cm)	number	1	567.71	568	616.11	616	9
Soil cultivation before planting (25-30 cm)	number	1	51.91	52	72.96	73	41
Levelling planting site	number	1	51.91	52	72.96	73	41
Marking of planting distance	days	2	24.33	49	32.43	65	33
Opening holes	ha	1	60.83	61	81.07	81	33
Planting	days	2	24.33	49	32.43	65	33
Cultivation	number	4	32.44	130	56.75	227	75
Mulching	number	4	32.44	130	56.75	227	75
Fertilizer application	number	2	32.44	65	32.43	65	0
Plant protection	number	10	32.44	324	40.53	405	25
Manipulations during the year	number	3	32.44	97	40.53	122	25

Pruning after planting	days	1	24.33	24	32.43	.32	33
Three row cultivation	days	1	24.33	24	32.43	32	33
Irrigation	days	1	24.33	24	32.43	32	33
Pruning	days	1	24.33	24	32.43	32	33
Total costs 1 year				**3,620**		**4,256**	**18**

Table 2. Investment costs in the second year for 1 ha apple plantation establishment 2020/2021 and 2022 (in EUR)

Costs 2 year	**Unit**	**Quantity**	**2020/2021**		**2022**		**Change (%)**
			Unit price	**Total**	**Unit price**	**Total**	
Fertilization	total	1	567.71	568	891.74	892	57
Mulching	number	4	56.77	227	72.96	292	29
Plant protection	number	15	89.21	1,338	100.52	1,508	13
Herbicides	number	2	81.10	162	154.03	308	90
Irrigation	ha	5	24.33	122	32.43	162	33
Pruning	days	2	24.33	49	32.43	65	33
Harvest	days	6	24.33	146	32.43	195	33
Transport and manipulation	kg	5,000	0.02	81	0.03	162	100
Mechanization operators	days	20	56.77	1,135	72.96	1,459	29
Other	total	1	97.32	97	97.28	97	0
Total costs 2 year				**3,925**		**5,140**	**31**

Table 3. Investment costs in the third year for 1 ha apple plantation establishment 2020/2021 and 2022 (in EUR)

Costs 3 year	**Unit**	**Quantity**	**2020/2021**		**2022**		**Change (%)**
			Unit price	**Total**	**Unit price**	**Total**	
Fertilization	total	1	567.71	568	891.74	892	57
Mulching	number	4	56.77	227	72.96	292	29
Plant protection	number	15	89.21	1,338	100.52	1,508	13
Herbicides	number	2	81.10	162	154.03	308	90
Irrigation	ha	5	24.33	122	32.43	162	33
Pruning	days	4	24.33	97	32.43	130	33
Harvest	days	10	24.33	243	32.43	324	33
Transport and manipulation	kg	8,000	0.02	130	0.03	259	100
Mechanization operators	days	20	56.77	1,135	72.96	1,459	29
Other	total	1	97.32	97	97.28	97	0
Total costs 3 year				**4,120**		**5,432**	**32**

Table 4. Investment costs in the fourth year for 1 ha apple plantation establishment 2020/2021 and 2022 (in EUR)

Costs 4 year	**Unit**	**Quantity**	**2020/2021**		**2022**		**Change (%)**
			Unit price	**Total**	**Unit price**	**Total**	
Fertilization	Total	1	567.71	568	891.74	892	57
Mulching	number	4	56.77	227	72.96	292	29
Plant protection	number	15	89.21	1,338	100.52	1,508	13
Herbicides	number	2	81.10	162	154.03	308	90
Irrigation	Ha	5	24.33	122	32.43	162	33
Pruning	Days	4	24.33	97	32.43	130	33

Harvest	Days	19	24.33	462	32.43	616	33
Transport and manipulation	Kg	15,000	0.02	243	0.03	486	100
Mechanization operators	Days	20	56.77	1,135	72.96	1,459	29
Other	Total	1	97.32	97	97.28	97	0
Total costs 4 year				**4,452**		**5,950**	**34**

The total costs for establishing an apple plantation have increased for 18% in the first year (Table 1), 31% in the second (Table 2), 32% in the third (Table 3) and 34% in the fourth year of investment (Table 4). The main increase in investment costs can be seen in the increase in the cost of transportation and manipulation of apple with 100% and use of herbicides 90%. Other investment costs increased on average around 33%.
The value of 1ha apple plantation establishment in 2022 has increased by 42% compared with the 2020/2021 period. The total value of apple plantation establishment as the asset and value as basis for calculation of depreciation in 2022 is 15,691 EUR/ha, compared with 11,022 EUR/ha in 2020/2021 (Table 5).

Table 5. Value of 1 ha apple plantation establishment for the reference years 2020/2021 and 2022 (in EUR)

Costs 1 year	**2020/2021 l**	**2022**	**Change (%)**
Total costs 1 year	3,620	4,256	18
Total costs 2 year	3,925	5,140	31
Total costs 3 year	4,120	5,432	32
Total costs 4 year	4,452	5,950	34
Total costs for apple plantation establishment (∑ investment costs 1, 2, 3 and 4 year) in EUR	**16,117**	**20,778**	**29**
Total income (∑ income 2, 3 and 4 year) in EUR	**5,096**	**5,094**	**0**
Total value of apple plantation as asset value for depreciation (Total costs apple plantation establishment – Total income)	**11,022**	**15,691**	**42**

The increase in apple plantation establishment means additional 311 EUR fixed costs as an annual depreciation of the apple plantation or rise of annual depreciation cost from 735 EUR in 2022/2021 up to 1,046 EUR in 2022.
The average annual costs - the annual variable costs of apple production have increased for 37% from 5,182 EUR in 2020/2021 up to 7,085 EUR in 2022 or apple producers this year have to pay an additional 1,903 EUR per hectare (Table 6).

Table 6. Average annual cost of apple production for the reference years 2020/2021 and 2022 (in EUR)

Cost of Production	**Unit**	**Quantity**	**Unit price 2020/2021**	**Total 2020/2021**	**Unit price 2022**	**Total 2022**	**Change (%)**
Fertilization	total	1	568	568	892	892	57
Mulching	number	4	57	227	73	292	29
Plant protection	number	15	89	1,338	101	1,508	13
Herbicides	number	2	81	162	154	308	90
Irrigation	ha	5	24	122	32	162	33

Pruning	days	5	24	122	32	162	33
Harvest	days	38	24	925	32	1,232	33
Transport and manipulation	kg	30,000	0.02	487	0.03	973	100
Mechanization operators	days	20	57	1,135	73	1,459	29
Other	total	1	97	97	97	97	0
Total variable costs				**5,182**		**7,085**	**37**
Yearly depreciation (fixed costs)				**735**		**1,046**	**42**
Total costs in normal year				**5,917**		**8,131**	**37**

The increase of variable costs is primarily due to the increase in fuel and labour costs, which result in an increase of transport and manipulation (100%) and impact the increase of mechanisation processes. In addition, the increase of protection materials, especially herbicides (90%), fertilization (57%) and other materials and operations (in average for 33%), contribute to increased variable costs of apple production (Table 6).
These increases of apple production costs, resulted in an increase of apple production price calculated on the basis of variable costs from 0.17 EUR/kg in 2020/2021 to 0.24 EUR/kg in 2022 or additional 0.07 EUR per kilogram apples (Table 7).
The increase of total costs of apple production (increased by 37% in 2022 with increase from 5,917 EUR/ha in 2020/2021 up to 8,131 EUR/ha in 2022 or additional 2,214 EUR per hectare) results in increases of the full apple production price (CoP at total costs) calculated based on total costs from 0.20 EUR/kg in 2020/2021 to 0.27 EUR/kg in 2022 or additional 0.07 EUR per kilogram apples and 37% increase (Table 7).

Table 7. Cost of apple production 2020/2021 and 2022 (in EUR)

Cost of Production normal year		**Quantity**	**Unit price**	**Total**	**Unit price**	**Total**	**Change (%)**
Yields apple	kg	30,000					
CoP (at variable costs)	**EUR/kg**			**0.17**		**0.24**	**37**
CoP (at fixed costs)	**EUR/kg**			**0.02**		**0.03**	**42**
CoP (at total costs)	**EUR/kg**			**0.20**		**0.27**	**37**

CONCLUSION

The combined impact of the Covid-19 pandemic and the war in Ukraine has created a number of disruptions to the country's apple production, markets and supply chains that have been ongoing for the past several years. The main impact refers to the price of apple production, due to an increase in investments and input costs, mainly due to increase of transportation and manipulation costs, herbicides by 90% and fertilizers by 57%. The estimated production costs of apples show that the negative impact of the economic crisis results in an increase in the total cost of apple production by 37% i.e. apple producers have to invest 0.07 EUR for production of 1kg apples more in 2022 compared to 2020/2021.

Under the influence of the crises, the actual wholesale prices of apple in Macedonia have risen from 0.38 EUR/kg in 2020/2021 up to 0.49 EUR/kg in 2022 or additional 0.11 EUR/kg.

Even though the wholesale prices increase is higher than the increase of production cost prices, this situation suggests that we should rethink and adopt more sustainable management and production practises on agricultural farms in relation to the new conditions. The Government and apple producers should introduce more efficient and effective measures and production systems for reducing production costs and increasing the efficiency of production, such as implementing new and modern technology, innovation and digitalization and strengthening of cooperative work and cooperatives.

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UTICAJ EKONOMSKE KRIZE NA PROIZVODNU CIJENU JABUKE U REPUBLICI SJEVERNOJ MAKEDONIJI

Rezime

Proizvodnja jabuka ima najveći značaj među svim vrstama voćarskih kultura u Makedoniji, sa 56% ukupne proizvodnje voća. Zbog ekonomske krize izazvane Covid-19 i rata u Ukrajini, cijene poljoprivrednih inputa su značajno porasle. U tom cilju, osnovni cilj ovog rada je da se procijeni uticaj svjetske ekonomske krize na proizvodnu

cijenu jabuke. Obračun troškova proizvodnje zasniva se na kalkulaciji varijabilnih i fiksnih troškova proizvodnje jabuke za 2020. i 2021. kao referentne godine i 2022. kao godinu kada se uzima u obzir promjena proizvodne cijene. Podaci za normativni obračun i proizvodnu cijenu za 2020 i 2021 godinu su dobijeni na osnovu ekspertsku procenu, potvrđeni putem polu-strukturiranim intervjuom sa 3 poljoprivrednika i 2 kompanije za proizvodnju jabuka. Na osnovu ovog normativnog obračuna i tržišnih cijena inputa u 2020, 2021 i 2022 godini prikupljenih od 10 dobavljača inputa, AMIS i SSO, izračunava se proizvodna cijena jabuke. Rezultati pokazuju da ekonomska kriza ima veliki negativan ekonomski uticaj na proizvodnju jabuka, jer je procijenjena puna proizvodna cijena jabuka u 2022. godini porasla za 37% u odnosu na onu iz 2020. i 2021. godine.

Ključne riječi: *procjena uticaja, socio-ekonomska kriza, proizvođači jabuka, proizvodna cijena, troškovi proizvodnje.*

ANTIOKSIDATIVNA AKTIVNOST KOMBUCHA FERMENTIRANOG MLIJEČNOG NAPITKA

Tijana Brčina[1], Milica Vilušić[1], Melisa Zahirović[1], Dijana Miličević[1]

Originalan naučni rad - *Original scientific papers*

Rezime

Kombucha se odlikuje bogatim kemijskim sadržajem i zdravim svojstvima. Sadrži organske kiseline, minerale i vitamine koji potiču uglavnom iz čaja, aminokiseline i biološki aktivna jedinjenja - posebno polifenole, koji utječu na antioksidativnu aktivnost.
Cilj rada je bio ispitivanje sadržaja polifenola i antioksidativne aktivnosti proizvedenih fermentiranih mliječnih napitaka upotrebom čajne gljive Kombucha kao netradicionalne starter kulture i inokuluma, koji su dobiveni nacjepljivanjem gljive na crnom čaju i mlijeku, u ovisnosti o korištenoj starter kulturi i zaslađivaču.
Kao izvor ugljika korištena su dva zaslađivača, saharoza i med. Proizveden je i uzorak sa liofiziranom kulturom Lyofast Y 452 E. Eksperiment je pokazao da se čajna gljiva može uspješno koristiti za proizvodnju fermentiranih mliječnih napitaka i da se dobro razvija i na mlijeku. Sadržaj ukupnih fenola se kretao u intervalu od 24,48 do 75,08 mg GAK/L. Antioksidativni kapacitet kombucha fermentiranog mliječnog napitka je određen sa $ABTS^+$ metodom i DPPH. Rezultati antioksidativnog kapaciteta određenog $ABTS^+$ metodom kretali su se u intervalu od 16,29 do 104,58 $IC_{50,}$ a DPPH metodom od 110,22 do 251,56 IC_{50}, i dobar su pokazatelj biološke vrijednosti napitka.

Ključne riječi: *fermentirani mliječni napitak, čajna gljiva Kombucha, fenoli, antioksidativni kapacitet.*

UVOD

Kombucha se u literaturi često naziva čajnom gljivom (Mo i sur., 2008), ali je zapravo simbiotska asocijacija bakterija octene kiseline (BOK) rodova *Acetobacter* i *Gluconobacter,* bakterija mliječne kiseline rodova *Lactobacillus* i autohtonih vrsta kvasaca rodova *Saccharomyces, Torulaspora, Zygosaccharomyces, Saccharomycodes, Candida, Kloeckera/Hanseniaspora, Pichia, Brettanomyces/Dekkera* (Sievers i sur., 1995; Yamada i sur., 1997; Chen i Liu, 2000; Wu i sur., 2004; Dutta i Gachhui, 2006; Trovatti i sur., 2011; Zhang i sur., 2011; El-Salam, 2012; Marsh i sur., 2014; De Filippis i sur., 2018). Kao podloga za fermentaciju čajne gljive najčešće se koriste crni ili zeleni čaj, koji se zasladi sa saharozom (5-10% (w/v)), ohladi i inokulira sa inokulumom iz prethodne serije (10-20% u odnosu na ukupan

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volumen). Fermentacija se odvija na sobnoj temperaturi (20°C) na tamnom mjestu (Teoh i sur., 2004; Malbaša i sur., 2011; Jayabalan i sur., 2014). Osim čaja, i mlijeko može poslužiti kao supstrat za fermentaciju (Lončar i sur., 2001; Milanović i sur., 2002; Duraković i sur., 2008; Malbaša i sur., 2008; Malbaša i sur., 2009a,b). Fermentirani mliječni proizvodi obuhvataju veliku grupu proizvoda koji se međusobno razlikuju prema vrsti, tipu fermentacije, sadržaju mliječne masti, konzistenciji, vrsti dodataka i dr. (Carić, 1997; Tamime i Robinson, 2004). Svaka vrsta fermentiranog mlijeka uključuje specifične mikroorganizme, optimalne uvjete rasta starter kulture (mezofilne i termofilne), kao i određenu vrstu i kvalitetu mlijeka od kojeg su dobijeni (Surono i Hosono, 2011). Upotreba kombuche kao nekonvencionalne starter kulture u tehnologiji fermentiranih mliječnih proizvoda je ispitivana sa aspekta utjecaja vrste čaja, temperature fermentacije, koncentracije i vrste inokuluma, utjecaja sadržaja mliječne masti i dr. (Kanurić i sur., 2011; Vitas i sur., 2013; Villarreal-Soto i sur., 2018; Vitas i sur., 2021; Iličić i sur., 2021; Kruck i sur., 2021). Fermentacija mlijeka kombuchom traje znatno duže nego pri proizvodnji klasičnog jogurta kao posljedica specifičnog mikrobiološkog sastava inokuluma i složenog sastava mlijeka (Milanović i sur., 2002; Malbaša i sur., 2009a).

Veliki broj studija su potvrdile da ishrana bogata antioksidansima doprinosi poboljšanju zaštite ljudskog organizma od prekomjerne proizvodnje i negativnog djelovanja slobodnih radikala (Ozyurt, 2020), te na taj način smanjuje rizik od mnogih bolesti, uključujući određene vrste raka, kardiovaskularne poremećaje i dr. (Chandan i Shah, 2006; Jayabalan i sur., 2008; Jiménez i sur., 2008; Cossu i sur., 2009; Jayabalan i sur., 2014; Najgebauer-Lejko i Sady, 2015). Primijećeno je da kombucha čaj ima veću antioksidativnu aktivnost nego nefermentirani čaj. Razlog tome je proizvodnja niskomolekularnih spojeva i strukturnih modifikacija polifenola iz čaja enzimima koje proizvode bakterije i kvasci tijekom fermentacije (Bhattacharya i sur., 2011). Rezultati istraživanja pokazuju da uvjeti prerade imaju značajno veći utjecaj na vrijednosti antioksidativnog djelovanja na DPPH i hidroksil radikale kao i na vitamin C u krajnjim proizvodima, zbog dobre metaboličke aktivnosti mikroorganizama u napitku (Malbaša i sur., 2009b).

Cilj rada je bio ispitivanje sadržaja polifenola i antioksidativne aktivnosti proizvedenih fermentiranih mliječnih napitaka upotrebom čajne gljive Kombucha kao netradicionalne starter kulture i inokuluma, koji su dobiveni nacjepljivanjem gljive na crnom čaju i mlijeku, u ovisnosti o korištenoj starter kulturi i zaslađivaču.

MATERIJALI I METODE

Za proces fermentacije je korištena kultura kombuche koja je uzgajana na crnom čaju. Čaj je pripremljen tako što je 8 g čaja dodato u 2 L vode temperature 95°C. Nakon 5 minuta, čaj je filtriran, ohlađen na sobnu temperaturu i podijeljen u 2 jednaka dijela. U jedan dio je dodato 70 g/L šećera (saharoze), a u drugi dio 61,25 g/L livadskog meda, te dobro izmiješani. U oba dijela je dodato po 10 vol % startera (fermentiranog čaja) i

gljiva (tablica 1). Posude za fermentaciju su pokrivene tankom pamučnom tkaninom. Fermentacija je provedena na tamnom mjestu (7 dana/25°C) do formiranja novog biofilma kulture kombuche koji se kasnije odvojio. Nakon formiranja dovoljnog broja kombucha gljiva pristupilo se procesu fermentacije mlijeka.

Tablica 1. Uzorci fermentiranog mliječnog napitka
Table 1. Sample of fermented milk drink

Oznake uzoraka	Udio inokuluma netradicionalnog startera (%)	Starter kultura	Zaslađivač	Udio kombucha fermentiranog mliječnog napitka kao inokuluma (%)	
				kb1	kb2
kb1	10	-	saharoza	-	-
kb2	10	-	med	-	-
kb3	-	-	-	10	-
kb4	-	-	-	-	10
kb	-	Lyofast Y 452	-	-	-

Za proizvodnju fermentiranog mliječnog napitka korišteno je UHT mlijeko sa 2,8% m.m. proizvođača "Meggle" koje je nacijepljeno sa 10% inokuluma netradicionalne starter kulture u odnosu na ukupnu količinu mlijeka (kb1 i kb2) i nacijepljenog mlijeka (kb3 i kb4) sa 10% kombucha napitka.
Fermentirani mliječni napitak kb proizveden je pomoću liofizirane (FD-DVS, Frozen dried Direct Vat Set) kulture (proizvođača Sacco Clerici, Italija) Lyofast Y 452 E (u sastavu: *Lactobacillus delbrueckii* ssp. *bulgaricus* i *Streptococcus thermophilus*), uz poštivanje svih parametara proizvođača kulture.
Fermentacija uzoraka nacijepljenog mlijeka je provedena, uz ponavljanje tri puta, u vodenoj kupelji na 34ºC, uz praćenje pH vrijednosti do 4,6. Potom su uzorci hlađeni u ledenoj vodi, pakirani u sterilnu staklenu ambalažu i skladišteni na temperaturi +4ºC.

Određivanje ukupnih fenola

U postupku određivanja ukupnih fenola korištena je Folin-Ciocalteu-ova modifikovana metoda (Ozyurt, 2020).
U staklenu epruvetu otpipetira se redom 2,5 mL F.C. reagensa (10x razrijeđen destiliranom vodom), 2 mL 7,5%-tnog natrijeva karbonata i 0,5 mL uzorka fermentiranog mliječnog napitka (10x razrijeđen otapalom). Uzorci se promiješaju, zagriju u vodenoj kupelji 15 minuta pri temperaturi 45°C, te mjeri apsorbancija pri valnoj duljini 765 nm.

Na isti način se pripremi i slijepa proba, ali se umjesto uzorka fermentiranog mliječnog napitka uzima otapalo za ekstrakciju.
Iz izmjerenih vrijednosti apsorbancije nacrta se baždarni pravac pomoću programa Microsoft Excel pri čemu su na apscisi nanesu koncentracije galne kiseline (mg/L), a na ordinati izmjerene vrijednosti apsorbancije pri 765 nm.

Određivanje antioksidativnog kapaciteta

Za određivanje antioksidativnih svojstava fermentiranih napitaka, korištene su dvije različite metode: DPPH (DPPH: Sigma-Aldrich. Inc. ST. Louise) i ABTS (ABTS: 2,2'-azinobis(3-etilbenztiazolin-sulfonska kiselina, Sigma-Aldrich CH-9471 Buchs, Germany)) metode kao sptektrofotometrijske metode.

Antoksidativna aktivnost sa DPPH metodom
Antioksidativna aktivnost (DPPH) je određena prema modifikovanoj metodi (Živković i sur. (2009)). Kod DPPH testa, uklanjanje DPPH radikala je praćeno smanjenjem apsorbancije pri 515 nm, do koje dolazi zbog smanjenja količine antioksidansa ili reakcije sa radikalima (Brand-Williams i sur., 1995).
Postupak: otpipetira se 0,2 mL uzorka, 2 mL metanola i 1 mL otopine DPPH, dobro promiješa i reakcijska smjesa se ostavi mirovati 15 minuta. Nakon toga mjeri se apsorbancija pri 517 nm. Za slijepu probu umjesto uzorka korišten je metanol. Antioksidativna aktivnost je izračunata iz kalibracijske krive.

Antoksidativna aktivnost sa ABTS metodom
ABTS metodom prati se raspadanje radikala $ABTS^{\cdot+}$ koji nastaje oksidacijom 2,2`-azinobis (3-etilbenzotiazilin-6-sulfonat) djelovanjem fenolnih tvari. U odsustvu fenolnih tvari, $ABTS^{\cdot+}$ je relativno stabilan, ali brzo reaguje u prisustvu.
Koristi se za određivanje sposobnosti flavonoida i fenola da uklanjaju slobodne radikale. Osjetljivija je od DPPH metode. Primjenjuje se kod različitih pH u organskim i vodenim otopinama.
ABTS reagens se priprema od dva rastvora:

- 7 mM 2,2´-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt
- 2,4 mM kalijpersulfat

Oba rastvora se prave sa destilovanom vodom.
Radni rastvor se priprema miješanjem jednakih zapremina ova dva rastvora. Nakon što se rastvori pomiješaju ostavi se na tamnom mjestu 16 sati. Dobiveni radni rastvor je tamnozelene boje .
Zbog intenziteta boje potrebno ga je razblažiti tako da njegova absorbanca mjerena na 734 nm bude oko 0,7 do 0,9.
Postupak: pomiješa se 5 ml uzorka i 5 ml metanola i centrifugira se. Za analizu se koristi neonatant, prave se različite koncentracije. Volumen uzorka je od 0,1 ml do 0,5 ml a dopunjava se sa metanolom do 3 ml. Na svaki od ovih pripremljenih rastvora doda se

0,1 ml ABTS reagensa (kontrolnog), dobro se promućka i ostavi na tamnom mjestu 6 minuta. Nakon toga se očitava absorbanca na talasnoj dužini 734 nm.

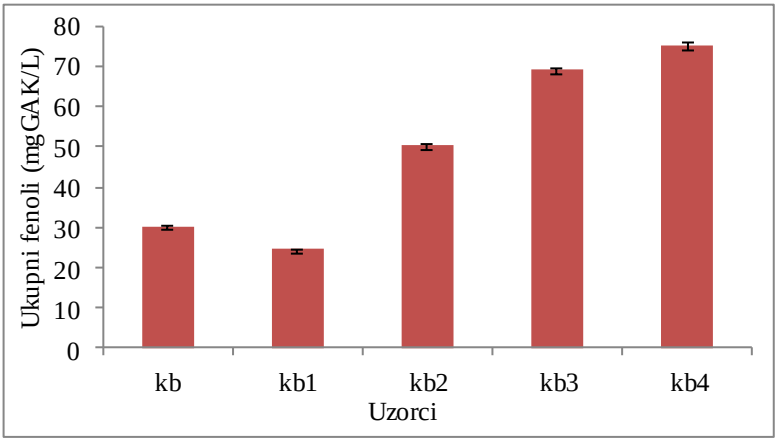
Statistička analiza

Korelacija i analiza varijance su urađene upotrebom SPSS softvera (verzija 22). Svi eksperimentalni rezultati su izraženi kao srednje vrijednosti s odgovarajućim standardnim devijacijama. Dunnett test je korišten za procjenu statističke značajnosti između grupa ($p<0,05$).

REZULTATI I DISKUSIJA

Rezultati sadržaja ukupnih fenola u uzorcima fermentiranog mliječnog napitka prikazani su na dijagramu 1. Na dijagramima 2 i 3 su prikazani rezultati antioksidativne aktivnosti izražene kao aktivnost na $ABTS^{\cdot+}$ radikale i antioksidativne aktivnosti izražene kao sposobnost hvatanja DPPH slobodnih radikala uzoraka fermentiranih kombucha mliječnih napitaka. Analiza varijance je pokazala da postoji statistički značajna razlika između grupa ($p<0,05$) za ukupne fenole i antioksidativni kapacitet (DPPH i ABTS metoda).

Polifenoli su jedna od najbrojnijih i najprisutnijih spojeva biljnih metabolita u ljudskoj ishrani. Zadnjih godina interes za njima je znatno porastao zahvaljujući njihovoj antioksidativnoj aktivnosti, odnosno sposobnosti neutraliziranja (zasićenja) slobodnih radikala (Bravo, 1998). Polifenoli pokazuju i značajnu zaštitu od oksidativnog stresa in vitro (Weichselbaum i Buttriss, 2010).



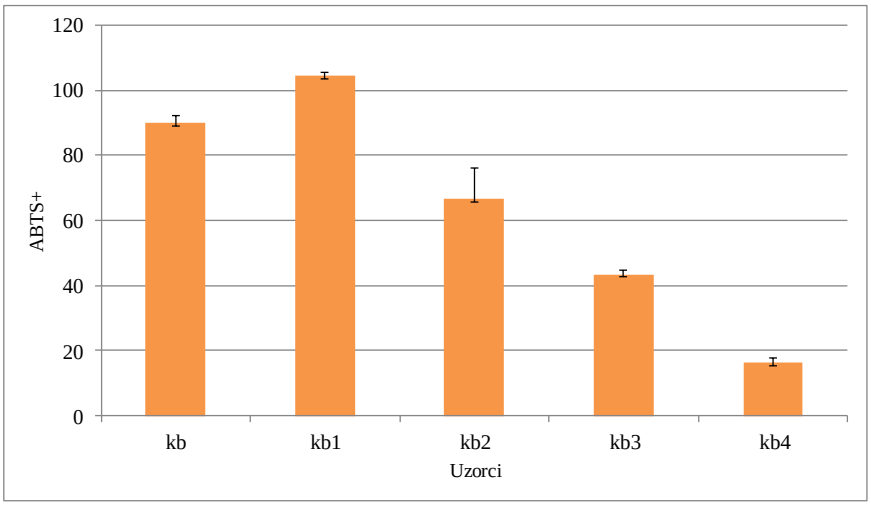
Dijagram 1. Sadržaj fenola u uzorcima fermentiranih kombucha mliječnih napitaka
Diagram 1. Phenol content in samples of fermented kombucha milk drinks

Dunnett-ov test je pokazao da postoji statistički značajna razlika u sadržaju fenola između i anktioksidativne aktivnosti (ABTS i DPPH metoda) kontrolnog uzorka Kb i uzoraka kod kojih se korsitila kombucha kao netradicionlani starter (Kb1, Kb2, Kb3 i Kb4).

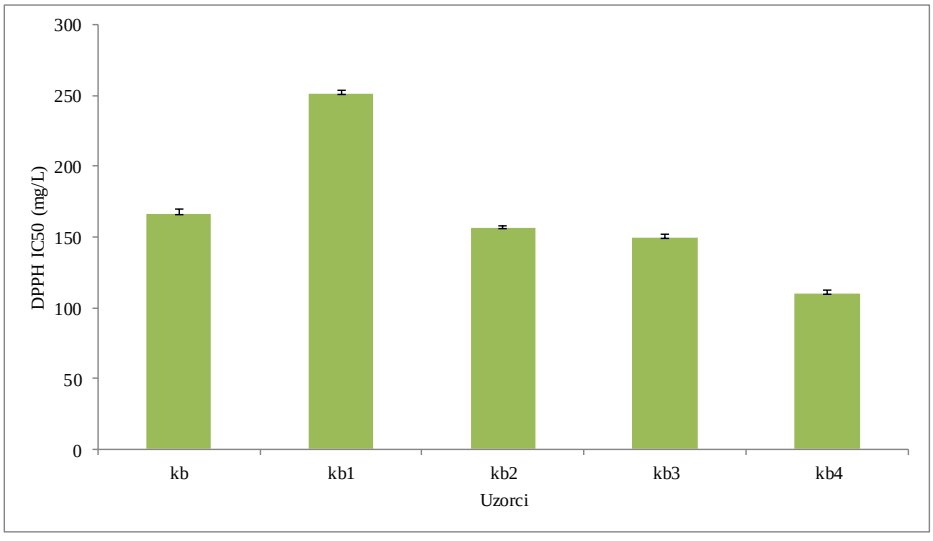
Kod uzorka Kb2 je korišten med kao zaslađivač, od kojeg i potječe veći sadržaj fenola sa utvrđenom koncentracijom od 50,21 mg/L. Med posjeduje snažno antioksidativno djelovanje, jer je bogat izvor fitokemikalija poput flavonoida i fenola. Antioksidativni kapacitet je mjera kojom se pokazuje sposobnost reduciranja i zaustavljanja štetnih oksidativnih reakcija, kako u hrani, tako i u organizmu. Za antioksidativna svojstva meda su uglavnom odgovorni fenolni spojevi (Al-Mamary i sur, 2002; Kesić i sur., 2009). Antioksidativne tvari meda vežu metalne ione u komplekse i time sprječavaju katalizu reakcija prilikom kojih nastaju slobodni radikali. Veliki dio protuupalnog djelovanja meda je sadržan u antioksidativnom djelovanju, ali i kad ne djeluje direktno na upalu, antioksidativni sastojci meda pronalaze postojeće slobodne radikale u tijelu, hvataju ih i time smanjuju količinu štetnog učinka (Manyi-Loh i sur.,2011). Antioksidativna aktivnost, odnosno sposobnost hvatanja jedinica slobodnih radikala vodenih ekstrakata fermentiranih mliječnih napitaka, ispitana je na $ABTS^{+}$ i DPPH radikale. Obje analize ukazuju na mogućnost supstrata da donira elektrone ili H atome u reakcijama slobodnih radikala. Sposobnost kombucha čajnog napitka kao hvatača različitih slobodnih (hidroksi, superoksid anion, itd.) radikala pripisuje se polifenolnim spojevima, vitaminu C i vitaminima grupe B prisutnim u inokulumu (Jayabalan i sur., 2008).

Razlika dobivena u rezultatima antioksidativne aktivnosti leži u činjenici da su primijenjene različite metode kod kojih se za određivanje antoksidativne aktivnosti koriste različiti slobodni radikali te dolazi do drugačijih reakcija između slobodnih radikala i antioksidanasa u uzorcima, što ovisi o strukturi fenolnih tvari (Singleton i Rossi, 1965). Rezultati istraživanja pokazuju da uzorci koji su imali manji sadržaj polifenola u datim uzorcima fermentiranog mliječnog napitka imali su više vrijednosti antioksidativne aktivnosti mjerene ABTS i DPPH metodom.

Rezultati dobiveni DPPH metodom izraženi su kao IC_{50} (mg/L), tj. kao koncentracija potrebna za 50%-tno smanjenje početne vrijednosti DPPH. To znači da je antioksidativni kapacitet veći što je manja vrijednost IC_{50} analiziranog uzorka. Čajna gljiva kombucha povećava sposobnost fermentiranog mliječnog napitka da stabilizira DPPH radikale tijekom skladištenja najvećim dijelom zahvaljujući sopstvenom antioksidativnom kapacitetu (Jayabalan i sur., 2008; Vitas i sur., 2013).



Dijagram 2. Antioksidativni kapacitet uzoraka fermentiranih kombucha mliječnih napitaka mjeren po $ABTS^+$
Diagram 2. Antioxidant activity expressed as ability to capture $ABTS^+$



Dijagram 3. Antioksidativni kapacitet uzoraka fermentiranih kombucha mliječnih napitaka mjeren po DPPH
Diagram 3. Antioxidant activity expressed as ability to capture DPPH free radicals

Najveću vrijednost antioksidativne aktivnosti nakon proizvodnje pokazali su uzorci fermentiranog kombucha mliječnog napitka sa saharozom kao zaslađivačem (Kb1), a potom slijedi uzorak Kb sa starter kulturom Lyofast Y 452E.

Dobijeni rezultati ukazuju na veću aktivnost na DPPH nego na ABTS slobodne radikale i to kod svih uzoraka.

Općenito, dobijeni rezultati antioksidativne aktivnosti fermentiranih mliječnih napitaka mjerene ABTS+ i DPPH metodom ukazuju na značajan utjecaj korištenog zaslađivača u procesu fermentacije mliječnog napitka. Antioksidativni kapacitet je veći što je manja vrijednost IC_{50} analiziranog uzorka i veći sadržaj ukupnih fenola. Uzorak koji je imao najmanju sposobnost gašenja slobodnih radikala, a najveći antioksidativni potencijal je Kb4 koji je ujedno i imao najveći sadržaj ukupih fenola. Analizirajući Pearsonov koeficijent korelacije uočena je veoma visoka negativna korelacija između sadržaja ukupnih fenola i antioksidativnog kapaciteta određen $ABTS^+$ metodom ($r=-0,981$; $p<0,01$) i visoka negativna koreacija sa antioksidativnim kapacitetom određen DPPH metodom ($r=-0,834$; $p<0,05$).

ZAKLJUČAK

Na osnovu dobijenih rezultata, može se zaključiti da su uzorci fermentiranog kombucha napitka imali visok sadržaj ukupnih fenola, osobito uzorci inokulirani sa 10% inokuluma koji je dobiven fermentacijom mlijeka sa kombucha gljivom. Uzorci fermentiranog mliječnog napitka zaslađeni sa medom imali su statistički značajno najveći sadržaj fenola i antioskidativni kapacitet u odnosu na ostale uzorke. Kombinacija meda i kombucha gljive su povećali antioksidativno djelovanje fermentiranih mliječnih napitaka. Uzorak sa najvećim sadržajem fenola Kb4 je ujedno imao i najveći antiokidativni kapacitet bez obzira na korištenu metodu. Statistička obrada podataka je pokazala da postoji statistički signifikantna razlika između uzoraka i u sadržaju ukupnih fenola i antioksidativnog kapaciteta, u ovisnosti o upotrebljenom inokulumu i zaslađivaču.

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ANTIOXIDANT ACTIVITY OF KOMBUCHA FERMENTED MILK DRINK

Summary

The study aimed to produce a fermented milk beverage using Kombucha tea fungus as a non-traditional starter culture and inoculum, which were obtained by inoculating the fungus on black tea and milk. Two sweeteners, sucrose, and honey, were used as the carbon source. A control sample with lyophilized culture Lyofast Y 452 E was also produced. The study included testing the content of total phenols and antioxidant capacity depending on the starter culture and sweetener used. The experiment showed that tea fungus can be successfully used for the production of fermented milk beverages. The content of total phenols ranged from 24.48 to 75.08 mg GAK / L. The antioxidant capacity of kombucha fermented milk beverage was determined with ABTS + method and DPPH. The results of antioxidant capacity determined by the ABTS + method ranged from 16.29 to 104.58 IC50, and the DPPH method from 110.22 to 251.56 IC50, and are a good indicator of the biological value of the beverage.

Key words*: fermented milk drink, tea mushroom Kombucha, phenols, antioxidant capacity.*

INULIN AS A FAT-REDUCTION INGREDIENT IN CHICKEN FRANKFURTERS WITH A REDUCED LEVEL OF SODIUM - TECHNOLOGICAL PROPERTIES AND CONSUMER ACCEPTANCE*

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Original scientific paper

Summary

Cooked sausages (frankfurters) are one of the most popular meat products in Serbia and the world. However, high contents of saturated fatty acids and sodium, along with the lack of complex carbohydrates, such as dietary fibers, contribute to the unfavorable health perception of these products. In today's market, a wide variety of additives and substitutes are being used as a replacement for animal fat and sodium in meat products that have little to no impact on processing loss and/or the sensory quality of the final product. Research in this field usually goes toward partial or complete fat replacement with different hydrocolloids or vegetable oils, while KCl is generally recommended for partial replacement of NaCl. However, there is limited data regarding frankfurters that have reduced contents of fats and sodium, along with reduced caloric value with additional prebiotics function. The results of this paper suggest that half of the pork backfat and a third of the NaCl could be replaced in chicken frankfurters with inulin gel and potassium salt to improve their nutritive characteristics and to obtain a product that would have characteristics of functional food. The experimental formulation (INK^+) analysis showed characteristical traits for this group of meat products in terms of technological quality and chemical composition and received high sensory evaluation scores for taste, odor, texture, and juiciness. The improved chicken frankfurter group has also shown exceptional nutritional value, considering that it contains a significantly reduced fat content (the main contributor to the caloric value of the final product) and prebiotic function (originating from inulin).

Key words: *inulin, sodium, fat substitution, chicken frankfurters*

INTRODUCTION

In recent years, the meat industry, as well as other branches of the food industry, following consumer demands and modern scientific knowledge, has begun to produce products with functional food properties. Functional food refers to foods that, in addition to essential nutritional components (proteins, fats, carbohydrates, vitamins, and

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minerals), also contain ingredients that positively affect human health. These ingredients include probiotics, prebiotics, antioxidants, omega-3 fatty acids, dietary fiber, additional micronutrients, and vitamins (Jiménez-Colmenero *et al.*, 2001; Arihara, 2006). Also, functional foods are considered foods in which ingredients considered harmful and traditionally found in those products have been reduced/replaced. The most common ingredients in meat products considered harmful to health and whose excessive intake is linked to various diseases are saturated fat, cholesterol, and sodium from table salt (Horita *et al.*, 2014).

Cooked sausages are one of the most popular meat products (Lu *et al.*, 2021). In Serbia, they are classified and marketed as hot dogs, frankfurters or boloney sausage (Official Gazette of RS, 50/2019). Frankfurters usually contain 20 to 30% of added pork backfat as a typical representative of this product category. This high content of animal fat affects the high caloric value and, as a rule, the high level of saturated fatty acids and cholesterol. Consequently, this affects the negative health perception of these products on the market (Kang *et al.*, 2016).

However, since fat plays a vital role in the technological process of production and obtaining the characteristic sensory properties of this group of sausages, such as texture, taste, and juiciness, its reduction would cause lower quality of the finished product (Colmenero, 2000; Choi *et al.*, 2009; Wu *et al.*, 2009). Complex carbohydrates, such as dietary fiber, are known to have many health effects (Kaur and Sharma, 2019), and their incorporation into products with high energy potential, such as meat products, significantly improves the nutritional characteristics of the finished product. In addition to having positive health effects, dietary fibers also serve numerous technological functions in meat products, such as binding and retaining water, emulsifying fats, improving the texture of finished products, etc. Present research follows the direction of using different vegetable fibers as ingredients used in the production of frankfurters. The fibers form a "gel-like" network when hydrated with water. This functionality shows promising results, as they contribute to water retention and the characteristic texture of sausages (Lundberg *et al.*, 2014). Several studies have been carried out on the approach of improving the quality of cooked sausages by using vegetable fibers as additives in production, most often to reduce the fat content (Cofrades *et al.*, 2000; Cengiz and Gokoglu, 2005; Ayo *et al.*, 2007; Kim *et al.*, 2015). Research has established that inulin has good potential as a substitute for fat tissue since it forms stable gels with water and has a neutral smell and taste. At the same time, inulin fibers have prebiotic properties, so with the addition of inulin suspension, the sausage becomes enriched with prebiotics.

In addition to the high-fat content, hot dogs on the Serbian market usually contain a relatively high sodium chloride content, between 1.28 and 2.03% (Vranić *et al.*, 2009), as the primary source of sodium in meat products. In addition to the sensory and antimicrobial function, sodium chloride in meat products has many technological effects. One of the most critical effects is on the solubilization of functional proteins of myofibrils, which results in an increase in hydration and the capacity of binding and retaining water of activated proteins and, consequently, in improving the product's

texture (Ruusunen and Puolanne, 2005). Also, since adding NaCl enhances the taste, its reduction would negatively impact the final product's sensory acceptability (Stamenić *et al.*, 2021). However, as excessive sodium intake is one of the leading causes of hypertension and cardiovascular diseases (WHO, 2007), its reduction in meat products would positively affect human health. Recent studies have reported that meat products are accountable for approximately 20-30% of daily sodium intake, justifying the meat industry's attempt to reduce sodium chloride as the primary source of sodium (Horita *et al.*, 2014). There are various strategies to reduce NaCl content in meat products, one of the most common ways is replacement with KCl, which is widely used due to similar ionic strength and chemical properties with NaCl (Geleijnse *et al.*, 2003). However, substitution with a KCl concentration higher than 30–40% (depending on the formulation) results in an unpleasant bitter and metallic taste in the final product. Partial replacement of NaCl with $CaCl_2$ may be a healthier alternative, as it may provide additional calcium in the diet (Cáceres *et al.*, 2006). However, many studies have shown that divalent salts such as $CaCl_2$ can reduce the functionality of meat proteins, leading to unstable emulsions (Piggot *et al.*, 2000; Ma *et al.*, 2013).

In order to be able to use the health claim "reduced sodium content" for a product, it is necessary that, compared to the traditionally sold product, the sodium content is reduced by at least 25% (Regulation EC No 1924/2006). In this regard, the World Health Organization recommends an increased intake of potassium in order to prevent cardiovascular diseases. For a product to be able to use the nutritional statement "good source of potassium", that product must contain at least 30% of the recommended daily amount, which is 2000 mg (Official Gazette of RS, 19/2017).

Based on everything mentioned above, it can be concluded that reducing the fat and sodium content without lowering the finished product quality is currently a big challenge for the meat industry. Fat and NaCl improve cooked sausage's sensory and technological properties, and their reduction is not easily achievable. The problem that this paper tackle is the production of cooked sausages of satisfactory sensory quality, with the characteristics of a multifunctional product: reduced content of saturated fat, caloric value, and sodium, with increased content of potassium and with the addition of prebiotic fibers, which have been found to have positive effects on human health.

MATERIAL AND METHODS

Preparation of chicken frankfurters

The frankfurters were manufactured in the meat processing plant of the Institute of Animal Husbandry (Belgrade, Serbia) in adherence to industrial processing protocol. Fresh chicken breast meat (Piljan komerc, Belgrade, Serbia) and pork backfat (Institute for animal husbandry, Belgrade, Serbia) were used as raw materials in production. Two different batches of frankfurters were made, each of 5 kg. Batch 1 was used as a control (CF) with a traditional formula (percentages in formulation add up to 100%): chicken breast (53%), pork backfat (22%), 22.3% water (ice form), 1.5% sodium chloride

(commercially bought combined 99,5% NaCl + 0,5% $NaNO_2$), 0,5% polyphosphates ("Tari K2"), 0.2% soy isolate ("Supro 548"), 0.48% commercially bought spices and 0.02% vitamin C. The second batch (InK^+) was formulated by replacing 50% pork backfat using inulin gel and replacing a third of sodium chloride with potassium chloride. Inulin suspension (inulin gel) was prepared by mixing inulin (Cosucra, Belgium) and distilled water in a 1:2 ratio in the blender (CombiMax 600, Braun, Germany) and placed at 4°C until added to the meat batter. The experimental group (InK^+) was prepared as follows: chicken breast (53%), 22.3% water (ice form), pork backfat (11%), inulin gel suspension (11%), 1.0% sodium chloride (99,5% NaCl + 0,5% $NaNO_2$), 0.5% potassium chloride, 0,5% polyphosphates ("Tari K2"), 0.2% soy isolate ("Supro 548"), 0.48% commercially bought spices and 0.02% vitamin C. Inulin content in the InK+ group was calculated at 3.67% (to 100% of formulation).

Both batches were prepared on the same day, identically: chilled chicken breast meat and pork backfat were grounded at 8 mm diameter in a meat grinder (Laska W 130-H, Austria) and then mixed with water (ice) and previously prepared inulin suspension (InK^+ group), salt and condiments in cutter (Seydelmann K60, Germany) until the smooth batter was obtained. Meat batter was then stuffed into 22 mm diameter collagen casings and manually linked (to approximately 80 g) and placed on the sticks, which were then hung in the chamber for smoking/cooking and underwent the following regime: 10 min drying at 50°C, 30 min smoking at 60°C and heating at 85°C until the temperature in the central part of the product has reached 72°C
After heat treatment, all frankfurters were showered with cold water and stored in a cooling chamber at 4°C. After 24 hours of storage, frankfurters were sampled from each group for analysis. The proximate chemical analysis and technological quality were determined on ten samples of frankfurters from each group. Sensory analysis was performed on eight frankfurters from each group. The rest of the frankfurters were vacuum-packed in plastic bags and stored at 4°C for 21 days.

Proximate composition and technological properties of frankfurters

The mass of the frankfurters was determined prior to and after heat treatment and after the 21. days of storage in a cooling chamber at 4°C (± 0.001 g) to calculate the process and purge loss (expressed in %). The stability of the emulsion was determined by the method described by Bolger *et al.* (2018). After 2 days of storage in the refrigerator, plastic cuvettes containing approximately 25 ml of the meat batter were heated in a water bath at 98º C for 45 min. Then they were cooled in ice water for 10 min to room temperature, opened, and turned into pre-measured glaas containers for 1 h to drain the liquid (fat and water) released during the heat treatment (HT). The separated liquid, expressed as a percentage of the mass of the filling before HT, represents the total loss of HT. The cooking loss was determined from the difference in the weight before and after cooking in distilled water (at 80°C for 10 min) and expressed as a percentage of the weight of the sample before cooking.

Prior to chemical analyses, the casings were removed from all samples, and the frankfurters were homogenized in a blender (CombiMax 600, Braun, Germany). The proximate chemical composition was determined as follows: water content by drying the samples to a constant mass at 102 ± 2°C (SRPS ISO 1442, 1998); protein content by the Kjeldahl method (SRPS ISO 937, 1992) on the Kjeltec system 1026 apparatus (Foss Tecator, Denmark); fat content, by the Soxhlet method with petroleum ether as a solvent (SRPS ISO 1444, 1998) on a Soxtherm Multistat (Gerhardt, Germany); ash content, by mineralization of samples at 550 ± 25°C (SRPS ISO 936:1999); the portion of carbohydrates was calculated from the difference to 100%. The content of sodium and potassium was determined by atomic spectrophotometry (PinAAcle 500, Perkin Elmer) after microwave digestion, according to the AOAC method (Rachida *et al.*, 2010).

The pH value was measured with a pH-meter Hanna, HI 83141 (Hanna Instruments, USA), with a penetration electrode previously calibrated using standard buffer solutions (SRPS ISO 2917:2004).

The caloric value was calculated based on the proximate chemical composition of frankfurters, where the amount of protein, carbohydrates, and fat has a caloric value of 4 kcal, 4 kcal, and 9 kcal per gram of product, respectively (Garcia-Santos *et al.*, 2019).

Sensory analysis was performed on samples stored for seven days in vacuum bags at 4°C by a panel of 12 assessors. Prior to sensory evaluation, samples were prepared by heating in water at 80°C/10 min. After heat treatment, the samples were identically presented to the evaluators: cut into pieces on white marked plastic plates. For each evaluated parameter, a quantitative-descriptive scale of 5 points was used (from 1 - extremely unacceptable to 5 - extremely acceptable). The following parameters were assessed: taste, smell, texture, and juiciness.

Statistical analysis

The obtained data were processed by analysis of variance in the one-way ANOVA program SPSS Statistics 22, and all results are displayed as the mean value ± standard deviation. The statistical significance of the difference between mean values was determined by a t-test.

RESULTS AND DISCUSSION

Technological quality of frankfurters

The ability to bind and retain water is an important factor in determining the technological quality of frankfurters (Lu *et al.*, 2021). In this experiment, the InK+ group of frankfurters had a significantly higher mass loss after heat treatment (process loss) and after cooking of the finished product. This was probably the result of a higher amount of water in the meat batter due to the replacement of fatty tissue with an aqueous

suspension of inulin (table 2). On the other hand, no change in purge loss (after the 21st day of vacuum storage at 4°C) was found between the tested groups. Also, no significant difference was found in the final pH value of the product. The commercial group of frankfurters had better emulsion stability (expressed as % liquid released) than the InK+ group. Similar results were obtained by Horita *et al.* (2014), as they determined that the partial replacement of NaCl with KCl led to an increase of released fluid (water and fat) and a decrease in the emulsion stability of frankfurter sausages. Despite slightly increased water loss during production and storage, it can be concluded from the obtained data that frankfurters with partially replaced fat (with inulin suspension) reduced-sodium content had satisfactory technological characteristics, very similar to the commercial group of products.

Table 2. Technological characteristics of chicken frankfurters

Parameter	CF n=10	InK^+ n=10	Statistical Significance
Process loss (%)	7,08 ± 0,67	7,45 ± 0,54	*
Purge loss (%)	1,59 ± 0,63	1,46 ± 0,50	ns
pH	6,34 ± 0,05	6,31 ± 0,05	ns
Cooking loss (%)	4,17 ± 0,77	5,20 ± 0,63	*
Emulsion stability (%)	6,71 ± 0,72	9,26 ± 0,86	*

ns–not significant; * significant at the level of $p<0.05$

Chemical composition and caloric value of frankfurters

The proximate chemical composition, the content of sodium, potassium, and the nutritional quality of the frankfurters are shown in Table 3. As expected, the frankfurters in which a part of the pork backfat was replaced by an inulin suspension (InK+ group) had a significantly higher water content and a significantly lower content of extracted fat (by about 7%). As a result, the caloric value of InK+ frankfurters is reduced considerably compared to the commercial group - by about 22%. The protein and mineral matter (ash) content was similar between the groups.

Similar results were achieved by Šojić *et al.* (2011). Their research states that replacing fat tissue with 5% inulin affected a significantly higher water content and a significantly lower fat content. In contrast, the protein content was not statistically significantly different compared to the control group. Also, they state that the content of carbohydrates was statistically significantly higher in sausages with 5% added inulin and that the caloric value was reduced by 30% compared to the control group. There were no significant differences in moisture, protein, and ash content when adding inulin in amounts of 2.5, 5.0, and 7.5% in powder or gel form in the research of Selgas *et al.* (2005). On the other hand, this replacement contributed to a decrease in fat content and caloric value. Similar results were obtained by Mendoza *et al.* (2001) in fermented sausages. The results of the research by Vasilev *et al.* (2011) showed that cooked sausages with inulin suspension contained 2.6 to 3.7% less fat than conventional ones,

corresponding to the amount of fat replaced by the inulin suspension. The same authors report that cooked sausages with inulin suspension contained about 3% more moisture than traditional sausages.

According to the results obtained by Alaei *et al.* (2018), decreasing the level of fat with inulin (the treatment of 25%, 50%, 75 %, and 100% substitution of inulin) increased the content of moisture, protein, and ash, salt, carbohydrates, and lowered the content of fat. However, the research conducted by Huang *et al.* (2011) confirmed that an increase in the level of inulin (from 3.5% to 7%) significantly reduced the fat content in the sausage. In contrast, the moisture content in the sausage samples was reduced, which conflicted with our research results. This difference probably results from the different formulations of the sausages, as well as the type of inulin. Menegas *et al.* (2013) also confirmed that inulin supplementation reduced fat content, which is in line with the results of our study.

Replacing 1/3 sodium salt with potassium salt in the InK+ group resulted in a significant decrease in total sodium level (by about 28%) and an increase in potassium level in the product (nearly 225%). Additionally, the InK+ group of frankfurters was enriched with inulin (about 3.67 g in 100 g of product), which has been shown to have many positive health effects (Yousefi *et al.*, 2018).

Based on the data obtained, the InK+ group of frankfurters can be declared as a product with "reduced sodium content" and "good source of potassium" with reduced caloric value and enriched with prebiotics (Regulation EC No 1924/2006; Official Gazette of RS, 19/2017). The InK^+ frankfurter formulation also complies with the FDA-approved health claim: “Foods that are a good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke” (FDA, 2016). The notion states that foods qualifying for the proposed claim must contain 10% or more of the Daily Value for potassium to be considered a "good source of potassium" and be "low in sodium." Therefore, the new frankfurter must contain at least 350 mg of potassium per reference portion customarily consumed, which is a request that InK^+ frankfurter meets (884,4 mg/ 100 g).

For a product to be able to use the nutritional statement "good source of potassium", that product must contain at least 30% of the recommended daily amount, which is 2000 mg (Official Gazette of RS, 19/2017).

Table 3. Chemical composition and caloric value of chicken frankfurters

Parameter	CF n=10	InK^+ n=10	Statistical Significance	Difference (%)[3]
Water (%)	58,86 ± 1,03	63,50 ± 0,85	*	7,88
Fat (%)	20,11 ± 0,74	12,63 ± 0,88	*	-37,20
Protein (%)	17,05 ± 1,94	17,73 ± 1,62	ns	3,99
Carbohydrates (%)	0,97 ± 0,21	3,36 ± 0,39	ns	246,39
Ash (%)	2,52 ± 0,11	2,46 ± 0,14	ns	-2,38
Na (mg/100g)	774,7 ± 14,09	553,1 ± 8,06	*	-28,60

K (mg/100g)	271,7 ± 54,8	884,4 ± 37,2	*	225,51
Caloric value (Kcal/100g)[1]	252,51 ± 12,46	197,23 ± 15,11	*	-21,89

[1] Caloric value – fat (9 Kcal), protein (4 Kcal), carbohydrates (4 Kcal);
[2] ns – not significant; * significant at the level of $p < 0.05$;
[3] Difference (%) = (InK^+ – KF) / KF · 100

Sensory evaluation of frankfurters

Sensory evaluations of taste, smell, texture, and juiciness are shown in Table 4. The 5-point system was used in the evaluation. The taste of commercial chicken frankfurters was rated as highly acceptable (>4.5), with a significantly higher rating than the InK+ group. This difference is probably due to the reduced fat and sodium salt content, which have been proven to correlate positively with the finished product's taste. On the other hand, the InK+ group of frankfurters' texture was rated higher than the commercial group. Juiciness is positively correlated with the product's water content (Aaslyng *et al.*, 2003), which is probably the reason for the higher-rated texture of the InK+ group of frankfurter sausages.

Table 4. Sensory quality of chicken frankfurters

Sensory properties	CF n=8	InK^+ n=8	Statistical Significance
Taste	4,57 ± 0,68	4,09 ± 0,51	*
Smell	4,00 ± 0,64	4,11 ± 0,77	ns
Texture	3,52 ± 0,50	4,23 ± 0,53	*
Juiciness	3,86 ± 0,79	4,03 ± 0,61	ns

[1] 1 – extremely unacceptable to 5 – extremely acceptable;
[2] ns – not significant; * significant at the level of $p < 0.05$;

Research data on sensory evaluation are inconclusive. Some of the literature data reveal that the texture of sausages with the addition of inulin was sensorial assessed as optimal and that the addition of inulin suspension can be done without adversely affecting the product's sensory properties (Šojić *et al.*, 2011; Nitsch, 2006; Zamora *et al.*, 2015). In contrast, Makala (2003) results on the sensory properties of functional cooked sausages containing inulin have lower general acceptability scores than control samples and that products with higher inulin content had lower general desirability. Further, obtained results on taste disability evaluation agree with Makala (2003) research.

CONCLUSION

The results of this work suggest that half of pork backfat and a third of NaCl in frankfurters made from chicken meat could be replaced by a suspension of inulin and potassium salt to improve their nutritional characteristics and obtain a product with functional food characteristics. The analysis of INK^+ frankfurters showed that they have characteristic features for this group of products in terms of technological quality and chemical composition, as well as high sensory scores for taste, smell, texture, and juiciness. This product also has an exceptional nutritional value, considering that it contains significantly reduced fat content, as the primary source of calories, with the addition of prebiotic fibers (inulin). Additionally, these sausages can be declared as a product "reduced in sodium" and a "good source of potassium."

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INULIN KAO ZAMENA ZA MAST U PILEĆIM VIRŠLAMA SA SMANJENIM SADRŽAJEM NATRIJUMA – TEHNOLOŠKE KARAKTERISTIKE I PRIHVATLJIVOST POTROŠAČA

Rezime

Fino usitnjene barene kobasice jedan su od najpopularnijih proizvoda od mesa, kako u Srbiji tako i u svetu. Međutim, visok sadržaj zasićenih masti i natrijuma, uz nedostatak složenih ugljenih hidrata, poput biljnih vlakana, doprinosi negativnoj zdravstvenoj percepciji ovih proizvoda. Danas na tržištu postoje različiti aditivi i dodaci koji se koriste kao zamena životinjskih masti i natrijuma u proizvodima od mesa, a koji nemaju veliki uticaj na gubitak mase tokom proizvodnje i/ili na promene senzornog kvaliteta finalnog proizvoda. Većina istraživanja u ovoj oblasti ide u pravcu delimične ili potpune zamene masti različitim hidrokoloidima ili biljnim uljima, dok se za delimičnu zamenu NaCl uglavnom preporučuje KCl. Međutim, postoji malo proizvoda na tržištu, iz grupe fino usitnjenih barenih kobasica, kod kojih je zamenjena mast i natrijum, a koji imaju i smanjenu kalorijsku vrednost uz dodatne prebiotike.

Rezultati ovog rada sugerišu da bi se 1/2 svinjskog masnog tkiva i 1/3 NaCl u viršlama proizvedenih od pilećeg mesa, mogla zameniti dodatkom suspenzije inulina i kalijumove soli, kako bi se poboljšale njihove nutritivne karakteristike i dobio proizvod sa karakteristikama funkcionalne hrane. Analizom INK+ viršli utvrđeno je imaju

karakteristične osobine za ovu grupu proizvoda, u pogledu tehnološkog kvaliteta i hemijskog sastava, a takođe i visoke senzorne ocene za ukus, miris, teksturu i sočnost. Ovaj proizvod takođe ima i posebnu nutritivnu vrednost, s obzirom da sadrži značajno smanjen sadržaj masti, kao glavnog izvora kalorija, uz dodatak prebiotskih vlakana (inulina).

Ključne reči: *inulin, natrijum, zamena masti, pileće viršle*

TESTING OF DEVIATIONS OF PATE FILLING SYSTEM USING PARETO METHOD*

Almir Toroman[1], Senita Isaković[2], Enver Karahmet[1]

Original scientific paper

Summary

The main goal of this research study was to find the causes of the deviation system for filling chicken pate in samples of different weights, with 27 g and 45 g. By applying the recognized methods of determining the level of process control for the calculation of the potential index (Cp) and the capacity index (Cpk) of the process, it was necessary to determine whether the filling process is under control.
The research was performed in the plant for the production of chicken pate, and the samples needed for the research were collected on the technological line for the production of chicken pate, at the process steps for filling and closing. Samples were taken from the filling line periodically at intervals of 30 minutes to 60 minutes. For each group, 500 measurements were performed over a period of several days depending on the production plan for a given group of samples.
The results of the research show that the examined filling processes in both groups meet the requirements on filling quantities prescribed by the Internal Regulations, as well as the EU Directive on prepacked products (76/211 / EEC, ANEX 1) and (75/106 / EEC). Calculations of process ability indicators indicated that according to the values of Cp and Cpk index (precision and adjustment), the process of filling 27 g of chicken pate belongs to the group of precise and adjusted process, while the process of filling 45 g of chicken pate belongs to the group of precise but unadjusted processes. CpU and CpL centering indicators indicate the non-centering of both processes, i.e. the measured values in both, group I and group II go towards the upper limit of the specifications. The results showed in the first process there is no defect at all, so there are no measured values outside the specifications, while in the second process the percentage of defects is 31.4%. Determining the causes of deviation using the Pareto method indicated the fact that overload dosing, lack of weight in the dosing can and damage to the packaging cause the largest number of defects (89.5%).

Key words**:** *filling system, chicken pate, Pareto method, potential index, capacity index*

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INTRODUCTION

The technological production process of chicken pate is a complex process consisting of a series of operations, the last step is filling or dosing the pate into packaging. Although there is an opinion, especially among manufacturers, that this step in the production of pate is less risky in terms of impact on the quality of the finished product than, for example, the preparation of raw materials or making the mass and here mistakes are possible (Burr, 1990). In the process step of filling, special attention should be paid to the compliance of the net quantity of the product with the nominal quantities, i.e. compliance with the requirements according to legal regulations (Rampersad, 2000; Novak and Vukasović, 2016).

The prepared pate is packed, hermetically sealed and heat-treated in the shortest possible time. This ensures sterility and durability, and the quality and microbiological correctness of the product. Much attention is paid to the packaging, which must be safe and preserve the quality and durability of the product. Dosage (weight control) as well as the other parameters in the filling process (speed, number of filling per hour, pressure, temperature, etc.) are automatically adjusted on the filler depending on the type of product (Rampersad, 2000). After the packaging is filled, the aluminum lid is automatically placed on the can, and under pressure and high temperature, the polyethylene mass of the lid and the can melt so that they stick together and create an impermeable layer, and it is hermetically sealed. The correctness of the closure (tightness) as well as the net weight of the product is checked every 30 minutes (Novak and Vukasović, 2016).

Control is not limited to the control of raw materials, semi-finished products and the finished product. It refers to the entire production process, so that the risk of food malfunction and other food quality indicators is reduced to a minimum (Harry and Schroeder, 2000). Production process control can be divided into two basic systems:

1. Off-line control system in which the control is performed outside the production line. Measurement samples can be taken at any time, and the results of the analyzes do not follow the course of the production process, but these results are obtained even after the completion of the production process.
2. The online control system provides current quality indicators. Critical factors are measured continuously during the process at given time intervals. Information is obtained during the process (Harry and Schroeder, 2000).

The ability of the process that creates quality is most often examined (measured, controlled), analyzed and evaluated using capability indicators such as: process potential or precision index Cp (process dissipation measure) and ability index - accuracy Cpk (process adjustment measure). The detection system detects non-compliant products after production, i.e. in the "after production" phase. The system was applied to control input components and control the finished product in order to discard or modify non-compliant products (Harry and Schroeder, 2000). Within the prevention system, the focus of quality management is shifting from the "after production" phase to the "on line" phase, ie to the production process itself. This

approach includes planning, control and quality assurance, as well as improving the quality of individual processes. Process capability is an industrial term that refers to the relationship between the "tolerance" of a product specification according to the bias and variation (standard deviation) of the process. The high "capability" process is more likely to produce products within the permitted specification, and conversely, the low "capability" process has a greater chance of obtaining defective products. The "ability" of the process is most often expressed through Cpk, which is calculated as Cpk = specification tolerance (deviation) / 3 Sd (Ignjatović, 2004).
The assessment of the capabilities (possibilities) of the process is the assessment of the scattering and tuning of the process in the state of statistical control. When the data follow a normal distribution, the ability is defined by the term "scattering" of the process and measured by the natural tolerance $Tp = 6 \cdot \sigma$ where the scattering contains 99.73% of the matched products. Ability analysis is performed to answer the questions: "Should the process be improved?" And "If so, by how much?" The analysis of process capabilities aims to show which processes are not under normal conditions, where variations occur, and how much the obtained values deviate from the required quality measures (Karahmet *et al*., 2021; Lazić, 2011).
The basic role of the Pareto method is to enable management to distinguish the essential from the irrelevant for the improvement of quality or the overall economy (Chase, 1993). Using this method, management obtains information on the relative contribution of each factor to the overall consequence (Perović and Krivokapić, 2007).

MATERIAL AND METHODS

The material for this study consisted of two groups of chicken pate samples: Group I - chicken pate filled in aluminum cans of 27.0 g, Group II - chicken pate filled in aluminum cans of 45.0 g. Samples were taken from the production line at the process step for filling and closing for a period of 10 days, or 100 samples per day. A total of 1,000 samples were collected, or 500 per each group.
The collected samples were weighed on an analytical balance, after which the weight of the empty packaging (dose + lid) was taken from the weighed mass in order to obtain the mass of the product. Weighing 50 samples of empty packaging, the mean value was obtained, which is:
- Group I - for packaging of 27 g - 2.1112 g and
- Group II - for packaging of 45 g - 2.5535 g.

The standard deviation in group I was 0.0200, and in group II 0.0145, which shows that the weight of the packaging is not standard, but also that the variations in weight are negligibly small. For group I, variations in the weight of packaging were from 2.0982-2.1130 g, while for group II they were from 2.5286-2.5760 g.
The obtained results were entered and processed in Excel. For statistical processing of data obtained by measurements, the software package (software) Excel SPC Software - QI Macros was used, which was used to calculate the potential index (Cp) and capability index (Cpk) of the process and other indicators of process capability (CpU,

CpL, Cpm, Pp, PPK, etc.). All measurements are presented using histograms also with the QI Macros software package.

RESULTS AND DISCUSSION

Determining the causes of deviations in the system of filling and closing the packaging and testing the ability of this phase of filling the pate is of great importance from the manufacturer's point of view to reduce waste, i.e. costs and improve the process by removing the causes of deviations. If the process is not controlled, adjusted and centered, there may be deviations in the weight of the finished products, which leads to unnecessary costs, deviations from legal regulations.

Group I - chicken pate filled into aluminum cans of 27 g.

The first group of samples was chicken pate weighing 27 g packed in aluminum packaging.

500 samples were taken from the line by random sampling and for each of the samples was calculated:

• net weight in g.,
• deviations from the nominal quantity in g.,
• deviations from the nominal quantity in percent.

After that, statistical data processing was performed, which is shown in Table 1. From the data presented in the table, it can be seen that for 500 tested samples of pate, the net weight varied from 27.02 to 28.00 g. The mean net weight of the pate was 27.49 g, which is relatively close to the nominal amount of filling (27.00 g). The allowed deviation from the nominal weight according to the Quality Specification of 27 g chicken pate is -0.5 g and +1.0 g. Therefore, the allowed variation of the weight of the pate of 27 g is in the range of 26.5-28.0 g. The mean deviation from the nominal filling quantity was only 0.49 g or 1.82%. These results indicate that the obtained values of mass, ie the mean value of the deviation in grams and the mean value of the deviation in percent meet the prescribed criteria.

Table 1. Results of statistical processing for I and II group of samples

Indicators	**I group – 27 g**	**II group – 45 g**
Number of samples	500	500
The total sum of the masses in g	13.746,73	22.693,31
Mean mass in g	27,49	45,39
Minimum value of mass in g	27,02	44,88
Maximum value of mass in g	28,00	46,00
Standard deviation	0,19	0,2016
Nominal quantity in g	27,00	45,00

Permitted deviation in g	from 26,5-28,0 g	from 44,1 g to 45,5 g
Minimum value of deviation from nominal quantity in g	0,02	0,12
Maximum value of deviation from nominal quantity in g	1,00	1,00
Mean value of deviation from nominal quantity in g	0,49	0,39
Minimum value of deviation from nominal quantity in%	0,07	0,27
Maximum deviation value from nominal quantity in%	3,70	2,22
Mean deviation from nominal quantity in%	1,81	0,87

The obtained results show that the process, in addition to meeting the above regulations, has a low standard deviation (0.19), ie that the range between the minimum (27.02 g) and maximum (28.00 g) of the measured mass is small, it was 0,98 g. Such results indicate great precision and tuning of the controlled process. Calculations of the process potential index (Cp), process capability index (Cpk), and other process capability indicators are presented graphically using histograms (Figure 1).

Based on the data shown in the histogram, it can be seen that the scattering of the process ($\pm 3\sigma$) is within the limits of specifications (lower tolerance limits / LSL - 26.50 and upper tolerance limits / USL - 28.00), which is the first sign that the process is capable. Based on the values of process capability indicators in a longer time interval, process potential index (precision) Cp = 2.52> 1.33 and capability index (accuracy, setting) Cpk = 1.70> 1.33, which have relatively high values, it can be concluded that the process of filling pate weighing 27 g belongs to the group of precise and accurate (adjusted) processes.

The ability ratio Cr = 0.40 confirms that the process is capable (for a capable process the Cr index should be less than 1).

The values of CpL = 3.34 and CpU = 1.70 are not equal, which indicates the non-centering of the process, which can be seen from the presented histogram. Namely, the values tend towards the upper limit of tolerance (USL). With ideal centering of the process, the values of CpL and CpU should be equal.

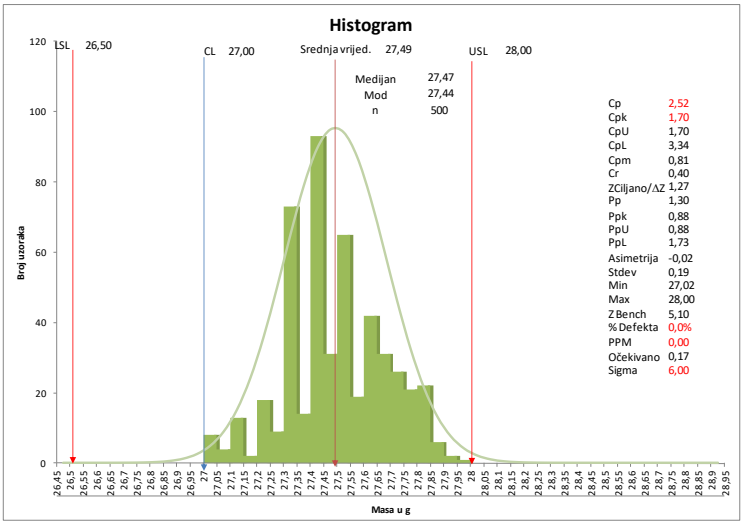

Figure 1. Histogram for group I samples - 27 g pate

Preliminary assessment of process capability is conducted at the beginning of the process or after a relatively short process monitoring time. The capable process should have Pp and Ppk values ≥ 1.67. Since the obtained indicators (Pp = 1.30 and Ppk = 0.88) are less than 1.67, there is a danger that an anomaly will occur in a large number of samples, which will bring the process to the limit of ability.

The indicator of process capability in a short time interval is Z targeted / ΔZ. It shows how far the mean deviates from the target value, and it is recommended that it be less than 0.5. Z targeted / ΔZ for the pate filling process weighing 27 g was 1.27.

The obtained percentage of defect is 0.0%, which shows that all measured values of net weight of finished products are within the limits of the specification (LSL and USL). This indicator is also in line with the PPM (number of errors per million possibilities) and sigma level. Calculations of the process capability indicated that the process works at the 6-sigma level, which is confirmed by the obtained results, primarily PPM and% of defects, as well as the values of Cp, Cpk and others. It can be concluded that the obtained results of process capability indicators are extremely good and rank the examined process at the highest level of capability.

Group II - chicken pate filled in aluminum cans of 45 g

The second group of samples consists of chicken pate with a net weight of 45 g, filled in aluminum cans. For the research, 500 samples were taken at random from the production line and for each of the samples the net weight in g, deviations from the nominal quantity in g., and deviations from the nominal quantity in percent were calculated.

From the data given in the table 1 it can be seen that for 500 tested samples of pate of the second group the net weight varied from 44.88 to 46.00 g. The mean value of the net weight of the pate was 45.39 g., which is relatively close to the nominal amount of

filling (45.00 g.). The permissible deviation from the nominal weight according to the Quality Specification of 45 g of chicken pate is -0.9 g. and +0.5 g. Therefore, the allowed variation of the weight of the pate of 45 g. is in the range of 44.1 to 45.5 g. The mean value of deviation from the nominal amount of filling was only 0.39 g. or expressed as a percentage of 0.87. These results indicate that the obtained values of mass, i.e. the mean value of the total deviation meet the criteria.
The obtained results show that the process, in addition to meeting the above regulations, has a low standard deviation (0,2016), i.e. that the range between the minimum (44.88 g.), and maximum (46.00 g.) of the measured mass is small and amounts to 1,12 g. These results indicate, as in the first group of samples, the high precision and adjustment of the tested process.

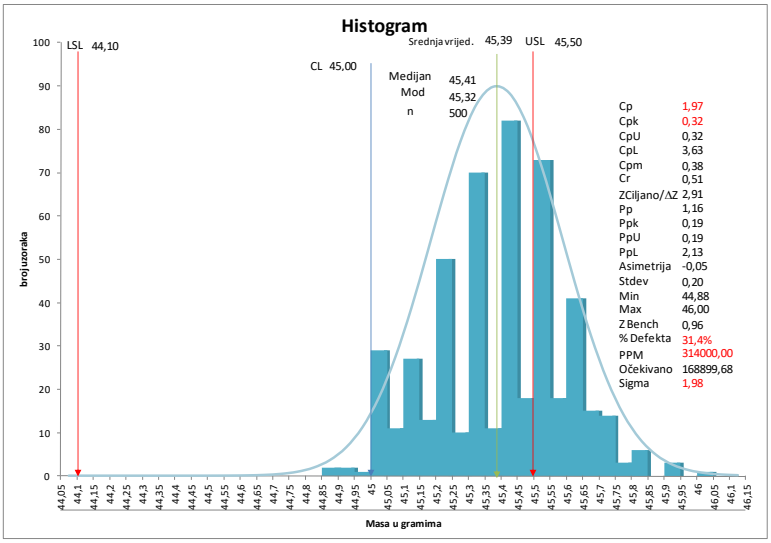

Figure 2. Histogram for group II samples - 45 g pate

Based on the data shown in the histogram, it can be concluded that the scattering of the process (± 3σ) is within the limits of specifications (lower tolerance limits / LSL - 44.10, and upper tolerance limits / USL - 45.50), which is the first sign that the process is capable. Based on the values of process capability indicators in a longer time interval, process potential index (precision) Cp = 1.97> 1.33, and capability index (accuracy, setting) Cpk = 0.32> 1.33, which have relatively high values, it can be concluded that the process of filling pate weighing 45 g. belongs to the group of precise but not accurate (adjusted) processes.
The ability ratio Cr = 0.51 confirms that the process is capable (for a capable process the Cr index should be less than 1).
The values of CpL = 3.63 and CpU = 0.32 are not equal, which indicates a small lack of centering of the process, which can be seen from the presented histogram. Namely, the values tend towards the upper limit of tolerance (USL). With ideal centering of the process, the values of CpL and CpU should be equal.

Process performance indicators Pp and Ppk ≥ 1.67 indicate the ability of the process to meet consumer needs. Preliminary assessment of process capability is conducted at the beginning of the process or after a relatively short process monitoring time. The obtained values (Pp = 1.16 and Ppk = 0.19) are lower than 1.67 which means that the process is shifted towards the ability limit.
The process ability indicator in the short time interval Z targeted / ΔZ (recommended to be less than 0.5), for the process of filling pate weighing 45 g. was 2.91.
The obtained percentage of defects is 31.4%, which shows that all measured values of net weight of finished products are within the limits of the specification (LSL and USL). This indicator is in line with both the PPM (number of errors per million possibilities) and the sigma level. Calculations of the process capability indicated that the process works at the 1.98 sigma level, which is confirmed by the obtained results, primarily PPM, and % of defects and the values of Cp, Cpk and others. It can be concluded that the obtained results of process capability indicators are extremely good, and rank the examined process at the highest level of capability.
The results of calculations for both groups of samples show high values of indicators process capability, especially Cp and Cpk (indices of precision and setting), which examined the processes classified as extremely precise and set processes. This can be explained by a small deviation of the net weight of pate samples (mean deviation for group I samples - 0.49 g. or 1.83%, and 0.39 g. or 0.86% for group II) in relation to the legally prescribed tolerances of the nominal quantity. That is why the tested processes have high values of process capability indicators, no defects, PPM is zero, and work at the 6-sigma level.

Determining the causes of deviations in the charging system of the examined groups of samples. The causes of deviations were determined during the sampling period, when problems that occurred during production at the process step for filling and closing were monitored and recorded. Table two lists the possible causes of deviations in the process step for filling and closing, on which the net weight of the finished products depends, i.e. the level of accuracy of the filling system.
The causes of deviations were determined during the sampling period, when problems that occurred during production at the process step for filling and closing were monitored and recorded. Table two lists the possible causes of deviations in the process step for filling and closing, on which the net weight of the finished products and the level of accuracy of the filling system depend.

Table 2. Causes of deviations in filling systems

Type of cause	**Corrective measures**
Dosing overload, due to high filling and closing speed	Correction of machine parameters - loading speed.
Lack of mass in the dosing bowl	Expert inspection of the machine and probe for repair and training for continuous operation. If necessary, replace the faulty probe in the filler hopper.
Damage to the can or lid	A corrective measure may be a visual inspection of the cans and lids before placing them on the filling line.
Closing dose at closing	Mass filling pressure correction. If necessary, temper the filling mass.
Heater temperature during gluing	Heater temperature control when gluing the cover. Visual inspection of welds.
Calibration	Regular calibration
Human error	Strengthen supervision and training of employees

In samples of group I, results showed no defects (values outside the specification limits). The standard deviation between the measured values is only 0.19, which is exceptional for the examined process. However, there is a very pronounced lack of centering of the process, i.e. the tendency of the measured values towards the upper limit of the specifications (this is indicated by CpU and CpL).
Overloading due to the high rate of filling and closing in the tested process can cause problems that do not manifest themselves by creating defects that affect the setting and centering of the process that are slightly worse than other indicators of process capability.
In samples of group II results showed that there is a deviation, and the resulting defect is 31.4% (values outside the specifications).
In group II of samples, the main cause of deviations is overload dosing (high filling speed), which is explained by the large amount of daily production (over 60 thousand pieces), and the need for faster filling. The following figure graphically presents the listed causes of deviations and ranks them according to significance (areas A, B and C) based on the Pareto principle.

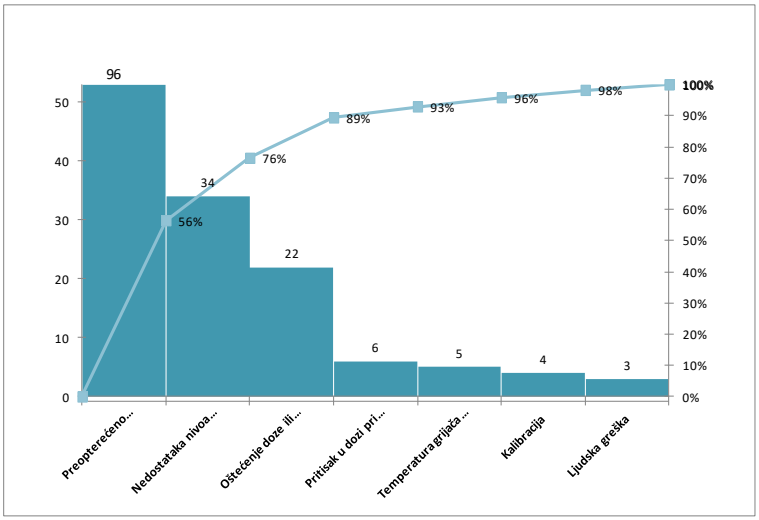

Figure 3. Parreto for second group of samples

Determining the causes of deviations in these two groups of samples (chicken pate filled in packages of 27 and 45 g), showed that in both groups there is a small deviation between the measured values (net weight of finished products). This indicates that the process is very precise and moderately adjusted, especially for the group of samples of 45 grams, which is indicated by the calculations of the process performance indicators. Within the first group of samples (27 grams) the process has no defects at all while in the second group (45 grams) there is a significant number of errors that can be reduced by interpreting Pareto diagrams by eliminating the cause of errors in area A in this process. The reason for the appearance of errors is the high specifications in relation to the variations in the charging process. However, the process capability indicators very much point to the fact that the process can be centered and undoubtedly one of the main reasons for the high process capability is the filling machine and the setting to shut down automatically when a defect occurs.

DISCUSSION

The results of the measurement of the net weight of the finished products show that the processes of filling chicken pates of 27 g., and 45 g. meet the Internal Regulations in Company ltd. (specification of quality chicken pate 27 g., and 45 g., no. 7040 and 7041). Namely, in both groups of samples the measurements showed that the mean values of deviations from the nominal mass in grams (0.49 in group I and 0.39 in group II), and the mean values of deviations from the nominal mass in percent (1.83% in group I) and 0.86% for group II) meet the deviations prescribed by the above internal regulations, according to which deviations in grams and percentages are allowed for net quantities of 27 g., and 45 g. as follows:

- for packages of 27 g.: -0.5 and +1.0 g., or 1.85% for negative and 3.70% for positive deviation;
- for packages of 45 g.: -0.9 and +0.5 g., or 2% for negative and 1.11% for positive deviation.

The results of the calculation of the standard deviation show that the value in both groups is low (0.19 in group I and 0.20 in group II), which indicates small ranges between the measured values. Such data indicate the high precision of the examined process. Oslić [11] states that sigma (in statistics, standard deviation) is a measure of process dissipation or a measure of quality. Therefore, the obtained sigma values initiate a small scattering of the examined processes.

According to the results for the potential index (precision) Cp, and the ability index (setting) Cpk, obtained in this paper, the examined processes are classified into the following groups:

- the process of filling pate in aluminum cans of 27 g. belongs to the group of precise and adjusted processes. The Cp value is 2.52 and the Cpk value is 1.70.
- The process of filling pate in aluminum cans of 45 g. belongs to the group of precise but not adjusted processes. The values of the Cp and Cpk indices are 1.97 and 0.32, respectively.

As can be seen from the obtained results, both groups have acceptable values of the Cp index, so in both groups there is a small scatter between the measured values, ie the processes are precise. Regarding the adjustment index (Cpk) in group II there is a much higher unadjustment of the process, which is indicated by the value of Cpk (0.32).

By comparing the values of the CpU and CpL indices, the position of the process can be determined (Runje, 2003).

• identical amounts indicate complete centering of the process (the value of the index Cpk is equal to the value of the index Cp),
• an amount less than 1 indicates the occurrence of a mismatch and
• The process has moved towards the specification limit of the smaller index amount.

According to calculations performed on the basis of the Pareto method, the three main causes of deviations in the tested processes are overload dosing due to high filling and closing speed, lack of mass levels in the dosing vessel and damage to the dose or lid, which cause as much as 89% of problems in the 45 g pate filling process. Kilibarda (2008) points out that there are three areas according to the degree of significance of the given causes for the problem analyzed by the Pareto diagram. The stated causes that belong to area A represent the area of the largest increase in the observed quantities (Donevski, 2011). This is actually an area with a group of causes that most influence the occurrence of the analyzed problem and it is a group of causes that, according to the theory of the Pareto method, should be addressed as a priority.

Overloaded dosing (high filling speed) was singled out as the main cause of deviations, which is explained by the large amount of daily production (over 60 thousand pieces), and the need for faster filling. It is estimated that removing 20% of the causes can solve 80% of the problems (Burr, 1990). Therefore, special attention should be paid by the management and those responsible for quality to solving or eliminating the stated

causes of deviations, which would eliminate 89% of the problems, with the aim of improving the examined processes.
Determining the causes of deviations showed that the same causes occur in both processes. However, the second process is the one in which the percentage of defects is 31.4% and in which these causes occur in a much higher intensity compared to the first process. The reason for this is primarily a slightly higher tolerance of tolerances when filling pate of 27 g, where a positive deviation is allowed (since deviations occur in both processes in the positive direction) 3.70% in contrast to the process of filling pies of 45 g, a positive deviation of 1.11% is allowed.
According to the European Community directive in which a deviation of (negative) 9% is allowed, the examined processes work at six sigma level and other indicators of process capability have extremely high values, which means that processes according to this standard are extremely adjusted, precise and under control.

CONCLUSIONS

During this research work, it was confirmed that the tested filling processes in both groups meet the requirements for nominal filling quantities prescribed by the Internal Regulations, as well as by the EU Directive on prepackaged products (76/211/EEC, APPENDIX 1) and (75). /106 / EEC). The checked weights of the samples showed that the packaging is not of standard weight, and that the weight of the packaging is not constant, there are deviations, but they are still minimal and insignificant. Values of process ability Cp and Cpk index (accuracy and adjustment) the process of filling 27 g. of chicken pate belongs to the group of precise and adjusted processes, while the process of filling 45 g. of chicken pate belongs to the group of precise but unadjusted processes. Centering indicators CpU and CpL indicate non-centering of both processes, that is, the measured values in both group I and group II tend to the upper limit of the specification. In this case, consumers receive slightly more product than declared, but do not exceed the upper limit of the specification. The research determined the causes of deviations which are insignificant in the filling of pate of 27 g., while in the filling of 45 g., they occur much more intensively and cause 31.4% of defects. Determining the causes of deviations using the Pareto method indicated the fact that three groups of causes (dosing overload, lack of weight in the dosing container and packaging damage) cause the largest number of defects (89.5%). Analysis of determining the causes of deviations in the filling system of the tested samples indicates the need to solve the problem in the 45 g. pate filling system by eliminating primarily the causes in area A, which are three groups of causes on the Pareto diagram, which would eliminate 89.5% of failures. For this purpose, it is necessary to give recommendations for the improvement of the tested processes, first of all their placement, centering, and in general to bring the systems under a higher level of control. In this way, a balance between the processes would be achieved and the causes of deviations in the examined processes would be eliminated or reduced to an acceptable level.

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TESTIRANJE ODSTUPANJA SISTEMA PUNJENJA PAŠTETE PARETO METODOM

Osnovni cilj ovog istraživanja bio je pronaći uzroke odstupanja sistema punjenja pileće paštete u uzorcima različite težine, od 27 g i 45 g. Primjenom priznatih metoda određivanja nivoa kontrole procesa za proračun indeksa potencijala (Cp) i indeksa kapaciteta (Cpk) procesa bilo je potrebno utvrditi da li je proces punjenja pod kontrolom.
Istraživanje je obavljeno u pogonu za proizvodnju pilećih pašteta, a uzorci potrebni za istraživanje prikupljeni su na tehnološkoj liniji za proizvodnju pašteta u procesnim fazama punjenja i zatvaranja. Uzorci su uzimani sa linije za punjenje periodično u intervalima od 30 minuta do 60 minuta. Za svaku grupu izvršeno je 500 mjerenja u periodu od nekoliko dana u zavisnosti od plana proizvodnje za datu grupu uzoraka.
Rezultati istraživanja pokazuju da ispitivani procesi punjenja u obje grupe ispunjavaju zahtjeve o količinama punjenja propisane Internim pravilnikom, kao i Direktivom EU o prethodno upakovanim proizvodima (76/211/EEC, PRILOG 1) i (75/106). / EEZ). Proračuni pokazatelja procesne sposobnosti pokazali su da prema vrijednostima Cp i Cpk indeksa (preciznost i podešavanje) proces punjenja 27 g pileće paštete spada u grupu preciznih i prilagođenih procesa, dok proces punjenja 45 g pileće paštete spada u grupu preciznih i prilagođenih procesa. spada u grupu preciznih, ali neprilagođenih procesa. CpU i CpL indikatori centriranja ukazuju na necentriranje oba procesa, tj. izmjerene vrijednosti u grupi I i grupi II idu prema gornjoj granici specifikacije. Rezultati su pokazali da u prvom procesu nema nikakvog defekta, tako da nema izmjerenih vrijednosti van specifikacija, dok je u drugom procesu postotak grešaka 31,4%. Utvrđivanje uzroka odstupanja Pareto metodom ukazalo je na činjenicu da preopterećenje doziranja, nedostatak težine u doziranju i oštećenje ambalaže uzrokuju najveći broj nedostataka (89,5%).

Ključne riječi: *sistem punjenja, pileća pašteta, Pareto metoda, index potenciala, index kapaciteta*

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