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EFFECT OF TREATED *CONOCARPUS ERECTUS L.* LEAVES WITH *KLEBSIELLA PNEUMONIAE* AND *ACINETOBACTER* AS TANNIN-DEGRADING BACTERIA ON DIGESTION ACTIVITY OF RUMEN MICROORGANISMS

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Abstract: The current research focuses on the effect of *Conocarpus erectus L.* leaves (CL) processed with two tannin-degrading bacteria *Klebsiella pneumoniae* (*K. p*) and *Acinetobacter (A. b)* on digestion activity and fermentation parameters of rumen bacteria and fungi in Arabian sheep. These isolates capable of utilizing tannic acid as sole carbon and energy source. Eight species of *Klebsiella pneumoniae* (A1, A2, A3, A4, A5, A7, A8, A9) follow by an *Acinobacter* sp. were used for biological treatment of CL for ten days. Then, digestion activity and some rumen fermentation parameters of ruminal bacteria and fungi in specific culture medium (SCM) were determined in Arabian sheep. Treated CL by bacterial isolates, reduced total tannin (TT) compared with unprocessed groups ($P < 0.05$). Dry matter disappearance (DMD) was affected by biological treatment for both rumen bacteria and fungi in different incubation times, except for 3 and 6 h of incubation in SCM of mixed rumen fungi, which indicated same pattern with untreated CON group ($P > 0.05$). The NDF disappearance (NDFD) and CP disappearance (CPD) in SCM of mixed rumen bacteria and fungi were increased during all incubation times ($P < 0.05$) due to biological prosses of CL compared to CON treatment. Biologically treated CL increased gas production from the fermentable fraction (b) pattern in both SCM of mixed rumen bacteria and fungi during all incubation times ($P < 0.05$), but, the gas production rate constant (c) was increased only at SCM of mixed rumen fungi ($P < 0.05$). Overall, the data indicated that bacterial inoculation of *K. p* and *A. b.* sp. could improve digestion activity and some fermentation parameters of rumen bacteria and fungi in Arabian sheep.

Key words: Conocarpus leaves, tannin degrading bacteria, biological processing, rumen microorganisms

Introduction

The role of fodder trees and shrubs in the diet of animals as good sources of proteins is important in countries like Iran where small land holdings and large ruminant densities cause main problem of feed availability from traditional green fodder like Lucerne to feed their animals (*Mohammadabadi and Jolazadeh, 2017*). In some arid and semi-arid countries, *Conocarpus erectus L.* leaves (CL) could be suitable for ruminants as a source of green fodders which used as an energy source for host animal and microbes in the rumen (*Al-Koaik et al., 2014*). It is widely distributed on shorelines in tropical and subtropical regions of the earth, and is popularly known as button mangrove (*Nascimento et al., 2016*). Phenolic compounds especially tannins are the major secondary metabolites of this species which often considered important factors limiting their use (*Abdel-Hameed et al., 2012*). The harmful consequences of the high percentage of tannin in the diet of ruminants can reduce feed intake, have a negative effect on the activity of rumen bacteria, reduce the efficiency of digestive enzymes in the livestock and also reduce the availability of nutrients, which ultimately reduce growth and production of these animals (*Garg et al., 1992; Frutos et al., 2004; Salinger et al., 1996*) .

Using suitable and practical processing methods for improving the nutritive value of feedstuff in ruminant nutrition have an important role in reducing production costs and improving production performance of ruminant animals. Various chemical and physical methods such as sodium bicarbonate, polyethyleneglycol, soaking in water, cooking or steaming, have been reported to reduce the amount of tannin and its harmful side effects (*Frutos et al., 2004*). Recently, bioprocessing has vast applies in livestock and poultry feed, using the enzymatic capabilities of microorganisms (*Motamedi et al., 2019; Bahaeddini et al., 2016*). It is a profitable method and can break down toxic compounds to innocuous products (*Azadi et al., 2014*). Biodegradation mainly utilizes microbes such as fungi and bacteria to improve the suitability of by-products for enzymatic hydrolysis (*Lotfi and Rouzbehan, 2012; Tahmourespour et al., 2016*). Condensed tannins in *Quercus incana* oak leaves were effectively degraded by *Sporotrichum pulverulentum* as a fungi specie (*Makkar et al., 1994*). Additionally, *Curiel et al. (2009)* reported that *Lactobacillus plantarum* is a safe microorganism that capable to produce tannase to degrade and eliminate tannin compounds.

Contrary to tannin antimicrobial properties, many microbes, especially bacteria can resist and develop different mechanisms for the tannin degradation in their habitats. So, Bacteria with the ability to grow in the presence of tannins as a sole source of carbon and energy are commonly considered tannin-degrading and

degradation like resistance is not limited by species or geographical barriers (*Tahmourespour et al., 2016*). Previously, many researchers reported that *Klebsiella pneumoniae* and *Acinetobacter* sp. were able to degrade tannic acids, they had to adapt in the presence of high concentrations of tannic acid. So, these isolates have a good potential for reduction of tannins antinutritional effects in animal feeds (*Tahmourespour et al., 2016; Arunachalam et al., 2003*). Little information (particularly involving bacteria) is available about the biological treating of taniniferous plants as ruminant feed. In the one case, *Motamedi et al. (2019)* reported that nutritive value of oak leaves was increased following to biological treatment with *Klebsiella pneumoniae*. Hence, this experiment was therefore planned to assess the chemical composition, digestion activity and fermentation parameters of CL leaf species following treatment with *K. p* and *A. b* as tannase producing bacterium isolated from deer rumen on digestion activity of sheep rumen microorganisms.

Materials and Methods

Sampling and chemical composition

Conocarpus leaves were harvested and cleaned to remove any foreign substances from Khuzestan province which is located southwest of Iran. Then, leaf samples were transported to the laboratory and oven-dried to a constant weight at 65 °C. The dried samples were then ground in a hammer mill with a 1-mm screen and stored in bags for later determination of chemical composition and *in vitro* incubations. The content of dry matter after drying in the oven, organic matter, Ash and crude protein of CL were 783, 828.5, 171.5 and 107 g/kg dry matter (DM), respectively.

Ground samples were analyzed in triplicate for DM, ash, crude protein (CP), according to *AOAC (1990).* The neutral detergent fiber (NDF) contents was analyzed without sodium sulfite, expressing regardless of residual ash according to *Van Soest et al., (1991).* Total tannins (TT) were conducted in three replicates as described by *Makkar (2000).*

Bacterial strains

Eight species of *Klebsiella pneumoniae* (*K. p*) follow by an *Acinobacter* sp. (*A. b*) were used for biological treatment of *Conocarpus* leaves. These bacteria were isolated from deer rumen located at the Dez National Park of Dezful (Khuzestan Province, Iran) with tannase production ability (45 U/ml) which could grow on liquid medium containing tannic acid as the sole source of carbon and energy and was identified based on 16S rRNA sequencing analysis (*Gheibipour, 2017*). Figure 1 presents the electrophoresis result of PCR product (1500 bp) of the isolates. These isolates included *Klebsiella pneumonia* (*K. p*) A1, A2, A3, A4, A5, A7, A8, A9 and an *Acinetobacter* A6 sp. (*A. b*).

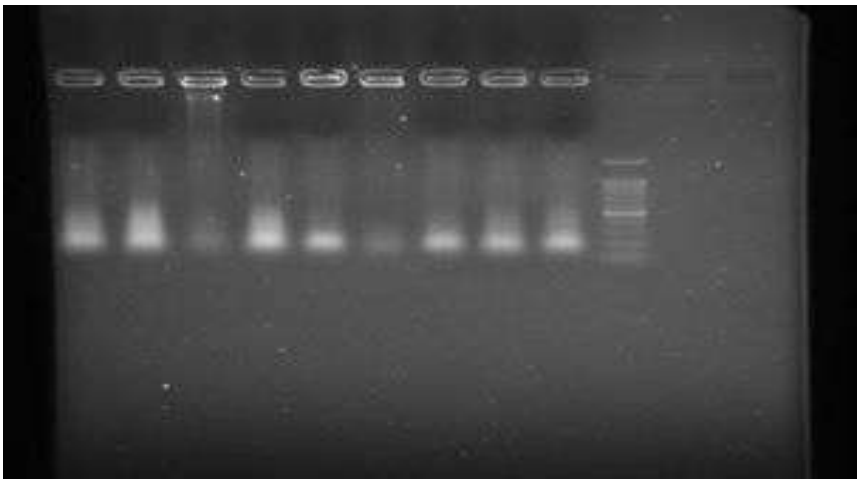

Figure 1. Amplification of 16S rRNA from i.e., A1- A9 isolates on agarose gel. M,1Kbp marker. right to left; i.e., A1- A9 (1500 bp).

Treating of leaves in liquid medium

The ground leaves were mixed with the *K. p* and *A. b*. For processing, Erlenmeyer flasks were filled with 500 ml of culture medium and 2.5% substrate (12.5 g per flask). Culture medium contained (per liter): 1.3 g $(NH_4)_4SO_4$, 1.0 g yeast extract, 0.37 g KH_2PO_4, 0.25 g $MgSO_4.7H_2O$, 0.07 g $CaCl_2.2H_2O$ and 0.02 g $FeCl_3$ (*Belur et al., 2010*). After sterilizing, corresponding flasks were inoculated with 3 ml of 24 h culture of *K. p* and *A. p* (10^7 CFU) in nutrient broth and were incubated in the shaking incubator for 10 days at 30 ^oC under aerobic conditions.

Treatments were 8 species of *Klebsiella pneumoniae* (A1, A2, A3, A4, A5, A7, A8, A9) and *Acinobacter* sp. were used for biological treatment of CL for ten days. Control was CL treated in liquid medium without bacteria. After 10 days of incubation, the contents of vials were centrifuged at 20,000×*g* for 20 min at 4 ^oC. Then were filtered and the residue collected, dried and were used for subsequent anlysis.

Rumen inoculum

Four individually housed healthy mature Arabian male sheep (body weight 50 ± 1.2 kg) were used as inoculum donor. The animals were fed alfalfa hay, wheat bran, barley grain, canola meal, mineral vitamin premix and salt twice daily, at 0900 and 1700, with free access to water. After a 14 days adaptation period to the diet, samples of ruminal fluid were collected from each sheep before morning feeding (0700) and maintained at 39 ^oC in an insulated flask during transport to the laboratory. At the laboratory, ruminal fluid was filtered through four layers of cheesecloth and maintained at 39 °C under continuous flushing with CO2.

Gas production and specific media

This experiment is part of a big study, that we performed these treatments in the other experiment by rumen fluid and in this part we specifically used rumen fungi and bacteria activity for these treatments.

Effect of treatments on digestion activity of ruminal bacteria and fungi in specific culture medium (SCM) were determined based on following methods. The bacteria SCM were performed according to *Mohammadabadi and Jolazadeh (2017).* Culture glasses containing 1 g experimental sample were autoclaved at 120 °C for 15 min. Rumen fluid centrifuged at 1000 rpm for 10 min and supernatant were added to specific culture medium of bacteria. The amounts of 36 ml of this solution as culture medium and 4 ml rumen fluid were inoculated into each culture glass. Then, the samples were cultured in incubator at 39 °C for 24, 48, and 72 h. At the end of each of the mentioned time, glasses were considered to determine the disappearance of DM, NDF and CP for each incubation time.

For fungi culture, experimental samples were cultured in specific culture of rumen fungi at 39 °C for 1, 3, and 6 days. At the end of each time, the disappearance of DM, NDF and CP was determined. Culture medium was transferred under anaerobic conditions into medium glasses and autoclaved. Rumen fluid centrifuged and supernatant were cultured in specific rumen anaerobic fungi culture.

In vitro gas production and related parameters (b and c) for two SCM were determined as described by *Blümmel et al. (1997)* in triplicate. The amount of gas produced per vial was recorded after 72 h of incubation for bacteria and 120 h (6 days) for fungi by using a digital pressure gauge (Model SDPG0015PG5, SenSym ICT, Honeywell Inc., Morris NJ) fitted with a 21 mm gauge needle. The content of each vial was centrifuged at 1500 rpm for 20 min and the residuals were collected and dried. Ruminal DMD was calculated by difference between weights of primary substrate from weight after incubation.

Statistical analysis

Data were analyzed based on a completely randomized design using to GLM option of (*SAS 2000*).

$Y_{ij} = \mu + T_i + e_{ij}$,

where Y_{ij} is the general observation, μ is the general mean, T_i is the effect of bacterial inoculation on the observed parameters, and e_{ij} is the standard error of term. Significance was declared at $P \leq 0.05$ and trends at $P < 0.10$ and $P > 0.05$ using Tukey's multiple comparison test.

Results

Treated CL with different species of *K. p* and an *A. b* sp. did not affect chemical composition, and bacterial inoculation did not change the DM, NDF or CP content of treated leaves (Table 1). However, after processing of CL by isolates, TT was decreased compared with unprocessed groups ($P < 0.05$).

Table 1. Chemical composition of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* species (g/kg DM)

Treatment	Items				
	Dry matter	CP	Ash	NDF	TT (g/100 DM)
CON	783	107	171	313	5.82[a]
K. p A8	782	105	172	314	1.67[g]
A. b A6	775	107	132	328	1.01[h]
K. p A7	759	105	142	320	2.45[f]
K. p A1	762	103	137	302	3.29[c]
K. p A4	777	104	153	320	3.62[b]
K. p A5	778	102	169	302	2.89[e]
K. p A3	763	105	163	333	2.79[e]
K. p A2	759	106	165	333	5.64[a]
K. p A9	763	107	159	343	3.11[d]
SEM	43.3	6.1	15.9	19.5	0.11
P-value	0.200	0.742	0.153	0.711	0.003

[a, d] Means with different superscripts in the same column are different (P<0.05).
CON: CL treated in liquid medium without bacteria ; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
CP, crude protein; NDF, neutral detergent fiber; TT, Total tannins.
SEM: Standard error of means

As shown in Table 2, DMD was improved by biological treatment for both rumen bacteria and fungi in SCM during different incubation times, except for 3 and 6 h after incubation in SCM of mixed rumen fungi, which all treatments indicate same pattern compared to the control diet ($P > 0.05$).

Table 2. Effect of treated *Conocarpus erectus L. leaves* with *Klebsiella pneumoniae* and *Acinetobacter* on dry matter disappearance by rumen bacteria and fungi inoculum

Incubation time	Treatment										SEM	*P*-value
	CON	*K. p* A8	*A.b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9		
Dry matter disappearance in rumen bacteria inoculum (g/kg DM)												
24 h after incubation	344^{c}	447^{bc}	359^{c}	508^{ab}	382^{c}	490^{ab}	539^{a}	523^{a}	532^{a}	488^{ab}	33.3	0.028
48 h after incubation	417^{d}	433^{d}	467^{c}	554^{ab}	486^{c}	534^{ab}	544^{ab}	569^{a}	565^{a}	543^{ab}	10.9	0.045
72 h after incubation	302^{d}	379^{c}	347^{c}	413^{b}	365^{c}	412^{b}	509^{a}	434^{b}	503^{a}	403^{b}	8.0	0.044
Dry matter disappearance in rumen fungi inoculum (g/kg DM)												
1 h after incubation	273^{e}	387^{c}	323^{d}	448^{bc}	390^{c}	406^{bc}	545^{a}	492^{ab}	534^{a}	407^{bc}	21.7	0.010
3 h after incubation	307	394	355	490	393	454	624	501	564	411	23.1	0.154
6 h after incubation	254	314	319	442	364	364	578	466	533	378	19.6	0.500

[a, f] means within a row with different superscript letters are different ($P < 0.05$).
CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
SEM: Standard error of means

Effect of biological treatment on NDFD and CPD by rumen bacteria and fungi are presented in Table 3 and 4, respectively. NDFD and CPD in SCM of mixed rumen bacteria and fungi were affected by experimental treatment during all incubation times ($P < 0.05$), and in the most case biological prosses could increase NDFD and CPD compared to CON treatment.

Table 3. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on NDF and CP disappearance by rumen bacteria inoculum (g/kg NDF and CP)

Treatment [a]	Incubation time					
	24 hours after incubation		48 hours after incubation		72 hours after incubation	
	NDF	CP	NDF	CP	NDF	CP
CON	205^{d}	199^{d}	232^{c}	206^{bc}	162^{e}	173^{bc}
K. p A8	274^{c}	282^{bc}	305^{c}	291^{bc}	253^{d}	251^{b}
*A.b*A6	244^{c}	245^{bc}	251^{c}	251^{bc}	203^{c}	194^{bc}
K. p A7	343^{b}	384^{a}	406^{b}	398^{b}	324^{c}	357^{b}
K. p A1	261^{c}	254^{bc}	268^{c}	285^{bc}	251^{d}	234^{bc}
K. p A4	298^{c}	348^{ab}	403^{b}	352^{b}	305^{c}	327^{b}
K. p A5	474^{a}	495^{a}	529^{a}	531^{a}	472.4^{a}	499^{a}
K. p A3	393^{b}	422^{a}	407^{b}	431^{a}	389^{b}	409^{a}
K. p A2	459^{a}	489^{a}	506^{a}	493^{a}	456^{a}	456^{a}
K. p A9	294^{c}	302^{bc}	402^{b}	309^{b}	271^{d}	287^{b}
SEM	26.9	29.8	24.0	34.5	22.0	45.4
P-value	0.025	0.057	0.011	0.045	0.023	0.030

[a, b] Means with different superscripts in the same column are different (P<0.05).

CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
CP, crude protein; NDF, neutral detergent fiber;
SEM: Standard error of the mean

Table 4. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on NDF and CP disappearance by rumen fungi inoculum (g/kg NDF and CP)

Treatment	Incubation time					
	1 day after incubation		3 days after incubation		6 days after incubation	
	NDF	CP	NDF	CP	NDF	CP
CON	234^{f}	184^{c}	259^{dc}	225^{c}	231^{g}	194^{c}
K. p A8	303^{e}	233^{c}	357^{c}	240^{c}	298^{e}	195^{c}
Acin A6	300^{e}	197^{c}	304^{dc}	233^{c}	264^{ef}	195^{c}
K. p A7	385^{c}	252^{bc}	407^{b}	273^{abc}	354^{c}	251^{bc}
K. p A1	301^{e}	215^{c}	328^{c}	237^{c}	276^{e}	201^{c}
K. p A4	351^{d}	251^{bc}	400^{b}	267^{abc}	341^{c}	259^{bc}
K. p A5	551^{a}	424^{a}	584^{a}	454^{a}	486^{a}	403^{a}
K. p A3	431^{b}	347^{a}	431^{b}	354^{a}	392^{b}	308^{b}
K. p A2	432^{b}	365^{a}	457^{b}	386^{a}	414^{b}	373^{a}
K. p A9	342^{d}	251^{bc}	377^{c}	263^{abc}	323^{d}	246^{bc}
SEM	15.7	47.0	23.5	34.3	13.4	24.0
P-value	0.035	0.024	0.025	0.048	0.030	0.045

[a, c] Means with different superscripts in the same column are different (P<0.05).
CON: CL treated in liquid medium without bacteria*; K. p: K. pneumonia; A. b: Acinetobacter*
SEM: Standard error of the mean

Biologically treated CL increased gas production from the fermentable fraction (b) after 24, 72 and 144 h of incubation ($P < 0.05$), but had no effect on the gas production rate constant (c) in SCM of mixed rumen bacteria (Table 5), While, these values indicate increasing pattern in both SCM of mixed rumen bacteria and fungi during all incubation times ($P < 0.05$) (Table 6).

Table 5. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on *in vitro* rumen fermentation parameters by rumen bacteria inoculum

Item	Treatment [a]											
	CON	*K. p* A8	*A. b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9	SEM	*P*-value
b, ml												
24 h after incubation	18.9^{c}	22.4^{bc}	20.5^{c}	24.8^{a}	20.2^{bc}	24.3^{a}	25.8^{a}	24.9^{a}	24.0^{a}	22.9^{bc}	1.22	0.022
48 h after incubation	19.7^{c}	20.6^{bc}	19.6^{c}	23.9^{a}	20.5^{bc}	22.9^{b}	24.4^{a}	23.8^{a}	23.7^{a}	22.2^{b}	1.90	0.040
72 h after incubation	15.2^{c}	20.6^{b}	18.2^{c}	23.1^{a}	19.9^{bc}	23.1^{a}	23.7^{a}	23.1^{a}	23.5^{a}	20.3^{b}	0.49	0.025
c, ml/h												
24 h after incubation	0.029	0.043	0.030	0.058	0.035	0.056	0.063	0.060	0.061	0.045	0.0192	0.290
48 h after incubation	0.023	0.033	0.025	0.049	0.028	0.038	0.061	0.059	0.060	0.035	0.0325	0.535
72 h after incubation	0.019	0.025	0.026	0.049	0.023	0.041	0.057	0.050	0.055	0.030	0.0255	0.140

[a] CON: CL treated in liquid medium without bacteria; *K. p: K. pneumonia*; *A. b: Acinetobacter*;
b: gas production from the fermentable fraction; c: the gas production rate constant;

[a, c] means within a row with different superscript letters are different ($P < 0.05$).
SEM: Standard error of the mean.

Table 6. Effect of treated *Conocarpus erectus L.* leaves with *Klebsiella pneumoniae* and *Acinetobacter* on *in vitro* rumen fermentation parameters by rumen fungi inoculum

Item	Treatment [a]											
	CON	*K. p* A8	*A. b* A6	*K. p* A7	*K. p* A1	*K. p* A4	*K. p* A5	*K. p* A3	*K. p* A2	*K. p* A9	SEM	*P*-value
b, ml												
1 day after incubation	9.15^{c}	16.8^{bc}	10.2^{c}	20.1^{ab}	15.2^{c}	19.8^{bc}	23.2^{a}	20.5^{ab}	22.7^{a}	18.9^{bc}	1.89	0.042
3 day after incubation	10.8^{c}	18.9^{bc}	15.6^{c}	23.1^{a}	18.9bc	20.7^{bc}	27.3^{a}	24.6^{a}	25.6^{a}	19.3^{bc}	1.15	0.035
6 day after incubation	6.31^{c}	15.5^{bc}	9.1^{bc}	18.2^{ab}	10.3^{c}	16.6^{bc}	21.0^{a}	18.5^{ab}	19.1^{a}	15.9^{bc}	1.50	0.021
c, ml/h												
1 day after incubation	0.043^{c}	0.055^{b}	0.045^{c}	0.060^{ab}	0.049^{c}	0.059^{b}	0.075^{a}	0.069^{a}	0.070^{a}	0.058^{b}	0.0169	0.025
3 day after incubation	0.045^{c}	0.059^{b}	0.048^{c}	0.065^{ab}	0.055^{c}	0.063^{ab}	0.076^{a}	0.070^{a}	0.075^{a}	0.060^{b}	0.0026	0.017
6 day after incubation	0.038^{c}	0.049^{c}	0.040^{c}	0.059^{b}	0.045^{c}	0.048^{a}	0.070^{a}	0.065^{ab}	0.069^{a}	0.050^{b}	0.0236	0.036

a CON: CL treated in liquid medium without bacteria; K. p: K. pneumonia; A. b: Acinetobacter;
b: gas production from the fermentable fraction; c: the gas production rate constant;

a, c means within a row with different superscript letters are different ($P < 0.05$).
SEM: standard error of means

Discussion

To the best of our knowledge, nutritive value of CL leaves has not been evaluated in experiments where treated by isolated bacteria. Bacterial inoculation did not change the DM, NDF or CP content of treated leaves when compared to the control (Table 1). The ineffectiveness of bacterial inoculation on the DM and NDF content of CL leaves is probably due to lack of microbial cellulase enzyme activity. Tannins form a complex with protein and lignocellulose, inhibit microbial enzyme and prevent microbial digestion, hence reducing fiber utilization by ruminants (*McSweeney et al., 2001*).

Decreasing TT content of leaves after bacterial inoculation was probably due to bacterial activity and secretion of tannase on substrates. Treatment of coffee pulp with *Bacillus* sp. improved its nutritive value by decreasing total phenol and TT content (*Ulloa Rojas et al., 2003*). Others have reported that that *Lactobacillus plantarum* were reduced phenolic compounds of pomegranate juice (*Mousavi et al., 2013*). *Rakesh et al. (2000)* reported that a 30% decrease in tannins of black

locust leaves after 30 days of incubation with tannase producer fungi caused a difference in digestibility and fermentation. Due to some differences between isolation conditions and the gastrointestinal tract of herbivores, enzymatic activity of isolates cannot indicate the true performance of microbes (*Kohl et al., 2015*). Also, the activity of tannase is induced in the presence of tannic acid that can be influenced by the season (*Dai et al., 2014*).

The observed higher DMD with biological treatment in SCM of mixed rumen bacteria and fungi, may have been due to the capacity of bacteria used to degrade anti nutritional factors present in the substrates and utilize their energy for growth and development. White-rot fungi (*Ceriporiopsis subvermispora* and *Cyathussteroreus*) as producer of tannin degrading enzymes have been reported to increase *in vitro* digestibility of *Sericea lespedeza* leaves by threefold (20–60%), but digestibility in the case of oak leaves was decreased. This could be due to different compositions of leaves, different white-rot fungi, and lower fermentation time used in studies with *S. lespedeza* leaves. An increase in fermentation time decreases cell solubility (consumed by the fungi for its growth) (*Makkar, 2003*).

Several researchers reported that *Klebsiella* strains capable of degrading phenolic compounds (*Motamedi et al., 2019; Pepi et al., 2013*). *Msimango (2018)* reported that *In vitro* dry matter degradability of *Acacia sieberiana* was affected positively by inocula from wild herbivores (giraffe, kudu, impala and consortia) in to goat microbial inoculum. *Tabacco et al. (2006)* showed that high tannin concentration in the diet caused reduction in microbial enzyme activities such as cellulase. These isolates probably enhanced pectinase and cellulase activity by changing pH and reduced toxic effects of tannins; and DM and OM degradation were improved. Researchers showed that the specific activity of tannin acyltransferase in the presence of *Selonomonas ruminantium* K2 was increased (*Babaei et al., 2015*).

Inhibitory effects and mechanisms of resistance to tannins in bacteria depend on the type and amount of tannins in the media (*Smith et al., 2005*). So, the observed maximum effects of biologically treated CL on NDFD and CPD is not unexpected, as the observed TT after processing indicate reduction pattern. In the present study, biological degradation of tannins by bacterial inoculation was led to decrease negative effect of tannin on the digestion activity of rumen microorganism which, previously reported by *Jayanegara et al. (2015)*. Tannin extracted from carob pod inhibited cellulolytic and proteolytic role of microbes in the artificial rumen technique (*Tagari et al., 1965*). Some researchers have reported reductions in NDF and CP digestibility in presence of tannin (*McAllister et al., 1994; Reed, 1995*). It is possible that improvement of the NDFD and CPD of CL leaves by bacteria processing with bacterial isolates due to the decrease of tannin content of *Conocarpus* might have increased digestibility and fermentation. Also, *Mosleh et al. (2014)* showed a reduction of the phenolic compounds by processing

of acorn with strains of *Streptococcus pneumonia* and *bovis* that increase digestibility and gas production.

Gas production from the fermentable fraction (b) were increased for both rumen bacteria and fungi in SCM for different incubation times, However, the c fractions were increased only at the SCM of fungi. These improvement in fermentation parameters of bacterial-treated leaves compared with the control may relate to better DMD, and CPD via decreasing TT in the former treatments (Table 1). The proper effect of some strains of *Klebsiella pneumoniae* on the fermentation parameters may be due to its higher enzymatic activity that can improve fermentation and digestibility. *McSweeney et al. (2001)* reported that bacterial strains of the same species can differ significantly in their tannin-degrading potential. *Elahi et al. (2012)* reported that the crude protein, fiber and phenolic compounds of the oak leaves were affected using the inoculum from two indigenous Iranian goats (i.e., Markhoz, Alam-out) which rich in tannin degrading bacteria, improve organic matter digestion and gas production. On the other hands, using the rumen fluid of Taleshi sheep fed tannin-rich diets, improved the organic matter digestion of the pistachio hulls (*Lotfi and Rouzbehan 2011*). In the similar research, *Motamedi et al. (2019)* reported that, GP and estimated parameters such as b and c were increased for *Q. infectoria* and only b, were increased for *Q. libani* following treatment with *K. pneumoniae*.

Another similar potential mechanism with NDFD and CPD values followed by breaking down the phenolic compounds of the substrates by bacterial inoculation, may occur for the gas production rate constant and thus increase nutrient digestibility. Several researchers reported that formation of tannin–macromolecule complexes which inhibit microbial enzymes and/or nutrient utilization by ruminal anaerobes (*McSweeney et al., 2001; Makkar, 2003*) could decrease cumulative gas production.

Conclusion

Biological processing of CL by *K. p* and *A. b* sp. can affect nutrients digestibility by bacteria and fungi of rumen, and processing with these bacteria decreased TT of CL. Therfore, it may be used as an alternative method for reduction tannin content of taniferus plants and prouned leaves of trees. But future studies should consider more, examining *in vitro* and *in vivo* effects of these isolates or other potent bacteria from different sources.

Uticaj listova *Conocarpus erectus* l. tretiranih sa *Klebsiella pneumoniae* i *Acinetobacter-om* kao bakterijama koje

razgrađuju tanin na digestivnu aktivnost mikroorganizama rumena

Tahereh Mohammadabadi, Alireza Jolazadeh, Zeinab Ghezi

Rezime

Trenutno istraživanje fokusira se na uticaj listova *Conocarpus erectus L*. (CL) tretiranih bakterijama koje razgrađuju tanin - *Klebsiella pneumoniae* (K. p) i *Acinetobacter* (A. b) na digestivne aktivnosti i fermentacione parametre bakterija i gljivica rumena arapskih ovaca. Ovi izolati mogu da koriste taninsku kiselinu kao jedini izvor ugljenika i energije. Osam vrsta *Klebsiella pneum*oniae (A1, A2, A3, A4, A5, A7, A8, A9) kao i *Acinobacter sp*. korišćeni su za biološki tretmane CL tokom perioda od deset dana. Zatim su u arapskim ovcama utvrđivane digestivna aktivnost, kao i neki parametri fermentacije rumena, bakterija i gljivica rumena, u specifičnom medijumu za kulturu (SCM). CL tretiran bakterijskim izolatima uticao je na smanjen ukupni tanin (TT) u poređenju sa netretiranim grupama (P <0,05). Na nestanak suve materije (DMD) uticao je biološki tretman i bakterija i gljivica rumena u različitim vremenima inkubacije, osim 3 i 6 h inkubacije u SCM mešovitih gljivica rumena, što je pokazalo isti obrazac sa neobrađenom CON grupom (P> 0,05). Nestanak NDF (NDFD) i nestanak CP (CPD) u SCM pomešanihh bakterija i gljivica rumena povećan je tokom svih vremena inkubacije (P <0,05) zbog bioloških procesa CL u poređenju sa tretmanom CON. Biološki tretirani CL povećao je proizvodnju gasa iz fermentabilne frakcije (b) obrasca u obe SCM pomešanih bakterija i gljivica rumena tokom svih inkubacionih vremena (P <0,05), ali konstantna stopa proizvodnje (c) povećana je samo kod SCM mešovitih gljivica rumen (P <0,05). Sveukupno, podaci su ukazivali da je bakterijska inokulacija *K. p* i *A. b. sp.* može poboljšati varenje i neke fermentacione parametre bakterija i gljivica rumena u arapskim ovcama.

Ključne reči: listovi konoplje, bakterije koje razgrađuju tanin, biološki tretman, mikroorganizmi rumena

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THE INFLUENCE OF BOAR BREED AND APPLIED METHOD ON THE MEAT CONTENT

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Original scientific paper

Abstract: The aim of this study was to determine the influence of the applied method of the quality carcass in 201 descendants, both sexes (n=108 male castrated animals and n=93 females), of studied boar-sires. For the determination of the meat yield (JUS1) and the share of meat (JUS 2) in carcass sides, on the basis of the performed measurements, tables for meat pigs, which are an integral part of the Rulebook on the quality of slaughtered pigs and categorization of pork meat (OG SFRY, 1985), were used. Share of meat (EC 94 and EC 06) was determined on cooled left carcass sides by method of partial dissection (according to methodology recommended by EU- Walstra and Merkus, 1996). Research was carried out on pig farm and in experimental slaughterhouse of the Institute for Animal Husbandry, Belgrade-Zemun. Housing, care and nutrition of animals were in accordance to breeding technology in investigated herd. The average share of meat determined by the application of the Rulebook (JUS2) was 43.58% with a lower absolute variation compared to the EU regulation from 1994 (EC 94; 53.56%) and from 2006 (EC 06; 56.55%). The estimated lean meat content by the application of the Rulebook (JUS 2) was by 9.98% lower compared to the EC 94 regulation and by 12.97% lower compared to EC 06. If we are talking about the selection of offspring for breeding, if we consider only the estimated leanness, we see that within the Landrace (L) breed we have high and very significant ($P<0.001$) differences between the sires for the assessed meat content according to EC 94 and EC 06. By implementing new methods of assessment of lean meat content (EC 94 and EC 06), a higher share of meat (9.98% respectively 12.97%) was determined compared to the Rulebook (1985). This research indicates the necessity of changing the current method for the establishment of meat content of pigs in the Republic of Serbia.

Key words: boar, sire, sex, season, qualification of pig carcases

Introduction

The most important factors determining the carcass quality are genetic and environmental factors. The quantitative and qualitative properties of the carcass depend on the selection methods. By cross-breeding of pigs a heterosis effect is achieved for major productive properties. Finding the best combinations of crosses is a continuous process, since the frequency of certain genes continuously changes by the selection (*Senčić et al., 2003*). It is known that certain quantitative properties of pigs are unevenly inherited, which means that the possibilities for their improvement in selection are different. The pig genotype, in addition to the nutrition, has the greatest effect on carcass quality and meatiness. The prerequisite for work on the genetic improvement of pig quality is knowledge of the variability of the production properties of the breeding animals. Regardless of the significance of the evaluation of the carcass quality and meat quality on the slaughter line, there is a problem in our country that in most slaughterhouses (except for some) no automatic or semi-automatic devices for their assessment are used. For more than 20 years the economic environment in the country has been too dynamic and insecure, which has put domestic pig breeding in difficult situations. In the process of joining the European Union, Serbia must implement the qualification of pig carcases under the (S)EUROP system. This system is obligatory for each member of EU (*Zapryanova, 2019*). In our country, the Rulebook (*OG SFRY, 1985*), according to which the total mass of muscular tissue without the belly-rib meat is determined, is still applicable. Due to the above, the content of meat determined according to the Rulebook (*OG SFRY, 1985*) is lower by 8 to 12% compared to evaluation using FOM or dissection according to the methodology recommended by the EU (*Walstra and Merkus, 1996*). Given the fact that total dissection of the carcass side is expensive and complicated, the EU has recommended a short procedure. According to this procedure, the left carcass side is cut into 12 parts, and only on 4 parts (leg, shoulder, back-loin and belly-rib part) the total dissection is performed. In the research of *Radović et al. (2009)***,** the difference in the values for share of meat established according to the Rulebook (*OG SFRY, 1985*) and dissection (according to the method recommended by the EU) was 8.83% for the genotype Large White (LW) and 10.02% for the genotype Swedish Landrace (SL).

Material and Methods

Research was carried out on pig farm and in experimental slaughterhouse of the Institute for Animal Husbandry, Belgrade-Zemun. Housing, care and nutrition of animals were in accordance to breeding technology in investigated herd. The quality of the carcass was tested in 201 descendants, both sexes (n=108 male castrated animals and n=93 females), of studied boar-sires. For the

determination of the meat yield (JUS 1) and the share of meat (JUS 2) in carcass sides, on the basis of the performed measurements, tables for meat pigs, which are an integral part of the Rulebook on the quality of slaughtered pigs and categorization of pork meat (*OG SFRY, 1985*), were used. Share of meat (EC 94 and EC 06) was determined on cooled left carcass sides by method of partial dissection (according to methodology recommended by EU-*Walstra and Merkus, 1996*). Left carcass sides were cut into 12 parts (Figure 1).

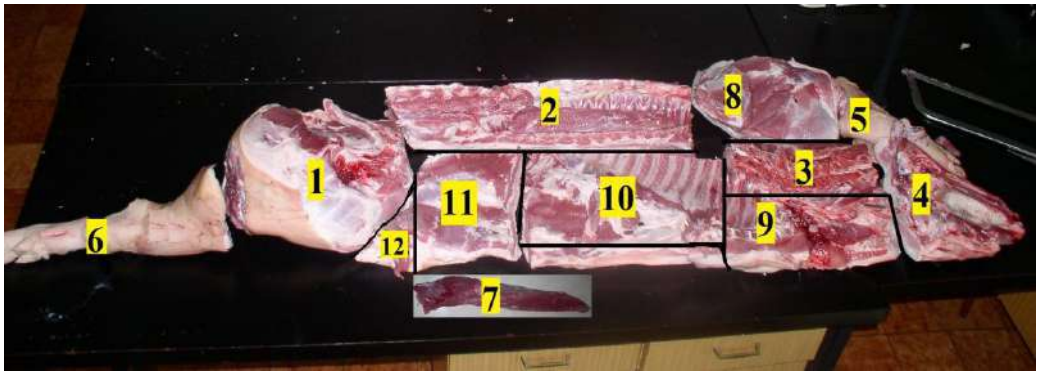

Figure 1. Scheme for cutting of carcass side into 12 parts
(Photo: Č. Radović)

Based on quantity of meat in four major parts – leg, shoulder, loin-rump and belly-rib part (skin with subcutaneous tissue, intermuscular fat and bones), which contain 75% of total musculature mass of carcass side, and mass of tender loin, percentage of meat in carcass sides was calculated using two formulas/equations:

1. Commission Regulation EC No 3127/94 (EC 94)

$$y = 1.3 \times 100 \times \frac{\text{weight of tender loin} + \text{weight of lean (fascia included) in shoulder, loin, ham and belly}}{\text{weight of tender loin} + \text{weight of dissected cuts} + \text{weight of remaining cuts}}$$

2. Commission regulation EC No 1197/06 (EC 06)

$$y = 0.89 \times 100 \times \frac{\text{weight of tender loin} + \text{weight of lean (fascia included) in shoulder, loin, ham and belly}}{\text{weight of tender loin} + \text{weight of dissected cuts}}$$

Data processing was carried out using the appropriate computer package "LSMLMW and MIXMDL, PC-2 VERSION" (*Harvey, 1990*), using the procedure of the least squares method in order to determine the significance ($P<0.05$) of the systematic effects on the growth traits, the quality of the carcass sides and meat. Properties of carcass quality were analysed by using models (1 and 2) that included the following factors: sire's race, sire, sex, offspring birth season and linear influence of warm carcass side weight.

$$Y_{ijklm} = \mu + R_i + O_{j:i} + P_k + P_{k:i} + S_l + b_3 (x_3 - \bar{x}_3) + \varepsilon_{ijklm}$$

Results and Discussion

Table 1 shows the average values for meat yield and share (JUS 1 and JUS 2) as well as the share of meat obtained using the forms of *EC No 3127/94* and *EC No 1197/06* regulations for the assessment of lean meat content. The average share of meat determined by the application of the Rulebook (JUS2) was 43.58% with a lower absolute variation compared to the EU regulation from 1994 (EC 94; 53.56%) and from 2006 (EC 06; 56.55%). The estimated lean meat content by the application of the Rulebook (JUS 2) was by 9.98% lower compared to the EC 94 regulation and by 12.97% lower compared to EC 06.

Table 1. Average values and variability of yield traits and meat share in the carcass side

Trait		$\bar{x}$ ± SD
JUS 1	Meat yield in carcass sides (*OG SFRY 1985*), kg	35.36 ± 4.33
JUS 2	Meat yield in carcass sides (*OG SFRY 1985*), %	43.58 ± 1.66
EC 94	Meat yield in carcass sides (*EC No 3127/94*), %	53.56 ± 4.48
EC 06	Meat yield in carcass sides (*EC No 1197/06*), %	56.55 ± 4.50

The effect of the sire breed, sires within breed, sex and season on the variation of meat content in carcass sides assessed under the Regulation (JUS2) and two EU Regulations (*EC 94 and EC 06*) is shown in Table 2. The general average for meat share in carcass sides determined by application the aforementioned methods was 43.62, 55.18 and 58.07%, respectively.

Quality of pig carcasses, i.e. the presence of muscle and fatty tissue, depends on the sex (*Renaudeau et al., 2005; Renaudeau and Mourot, 2007; Serrano et al., 2007; Radović et al., 2008, Carabús et al., 2017*), which was also found in our studies of fat thickness, yield and meat share. According to Wagner et al. (1999), male and female animals had almost the same share of fat tissue with 25 kg (6.92 and 7.00%), but with 100 kg it was higher in boars (16.33%) than in gilts (13.92 %). This difference between the sexes increased at a weight of 152 kg, so the share of belly fat tissue in males was 20.00 and in the gilts 16.91%. The results

of our research on the effect of sex on the quality of pig carcasses are in agreement with the results of *Latorre et al. (2003, 2004)* and *Radović et al. (2008)*. On the basis of a total dissection of four carcass parts, estimated leanness [Commission Regulation EC No 3127/94, (1994)] of genotypes 1 (SL), 6 [Px (SLxLW)], 8 [SLx (SLxLW)] and 9 [LWx (SLxLW)] in our trials was higher (+ 10.88%, + 6.66%, + 4.56% and + 5.10%) than shown by *Kosovac et al. (2009a)* for the same genotypes.

Table 2. The effect of sire breed and sires within the breed on yield traits and meat share in carcass sides (LS Mean ±S.E.)

Sources of variation		JUS 1[2)], kg	JUS 2, %	EC 94, %	EC 06, %
μ ± S.E.		35.41 ±0.20	43.62 ±0.18	55.18 ± 0.44	58.07 ± 0.45
RO[1)]	sire				
Landrace	1	35.72 ± 0.42	44.19 ± 0.39	52.82 ± 0.95	55.45 ± 0.97
	2	36.31 ± 0.45	44.87 ± 0.42	54.85 ± 1.01	57.89 ± 1.04
	3	35.52 ± 0.46	43.84 ± 0.42	50.33 ± 1.02	53.94 ± 1.05
	7	35.34 ± 0.57	43.42 ± 0.53	57.70 ± 1.28	61.21 ± 1.31
	8	34.48 ± 0.54	43.12 ± 0.50	57.93 ± 1.21	60.76 ± 1.25
	9	35.25 ± 0.58	43.85 ± 0.54	58.53 ± 1.30	62.26 ± 1.34
	15	36.14 ± 0.54	44.24 ± 0.50	53.46 ± 1.19	56.78 ± 1.23
	16	35.91 ± 0.52	44.27 ± 0.48	55.20 ± 1.15	58.72 ± 1.18
	17	36.22 ± 0.50	44.84 ± 0.46	58.16 ± 1.12	61.58 ± 1.15
	18	36.66 ± 0.48	44.33 ± 0.44	55.29 ± 1.06	58.63 ± 1.09
	Average	35.75 ± 0.24	44.10 ± 0.22	55.43 ± 0.54	58.72 ± 0.55
Large White	4	34.94 ± 0.46	43.66 ± 0.43	52.63 ± 1.03	55.53 ± 1.06
	5	32.78 ± 0.46	41.17 ± 0.43	46.14 ± 1.03	49.06 ± 1.05
	6	34.33 ± 0.46	42.68 ± 0.42	51.13 ± 1.02	53.60 ± 1.04
	Average	34.02 ± 0.29	42.50 ± 0.27	49.97 ± 0.65	52.73 ± 0.66
Pietrain	14	36.49 ± 0.33	44.85 ± 0.31	56.32 ± 0.74	60.46 ± 0.76
	19	36.38 ± 0.61	44.25 ± 0.56	64.92 ± 1.35	65.78 ± 1.39
	20	36.51 ± 0.62	43.67 ± 0.57	59.17 ± 1.38	62.06 ± 1.42
	Average	36.46 ± 0.39	44.26 ±0.36	60.14 ±0.86	62.77 ±0.89
MTP (b)		0.357***[3)]	-0.006[NS]	-0.039[NS]	-0.068*

[1)]SB-sire breed; MTP (b) -linear effect of warm carcass side weight (MTP = 81.20 kg); [2)] JUS 1-yield of meat in carcass sides, JUS 2 - share of meat in carcass sides, EC 94 - share of meat in carcass sides, EC 06 - share of meat in carcass sides [3)] ***=P<0.001; *=P<0.05; NS=P>0.05

The highest yield (JUS 1) and the share of meat in the carcass sides (JUS 2, EC 94 and EC 06) were determined in offspring of sires of Pietrain breed (Table 2). In these animals, the largest difference in the estimation of lean meat between JUS2 and EC94 was found (difference was 15.88%) and between JUS 2 and EC 06 (the difference was 18.51%). In regard to the share of meat in the carcass side using the *Rulebook* (JUS 2), a smaller difference was found between offspring of

sires of P and L breeds (0.16%), and P and LW (1.76%). The offspring originated from the sires of Pietrain breed had 60.14% of the meat in carcass side determined by the application of Regulation EC 94. The determined mean value was by 10.17 and 4.71% higher than for the offspring of the LW and L sires, respectively. By using the equation for the calculation of the lean meat content using EC 06, it was established that the offspring of the sires of Pietrain breed had a higher share of meat than the offspring of L sires (4.05%) and LW sires (10.04%).

The variation of the meat content by the application of the Rulebook (JUS 2) between the sires of L and P breeds was low (from 43.12 to 44.87% and from 43.67 to 44.85%) and was not statistically significant (Table 2). Between the descendants of three LW boars, the variation in the lean meat content was greater (from 41.17 to 43.66%) and statistically very significant ($P<0.001$; Table 4). The highest estimated lean meat content with the application of EU regulations (EC 94 and 06), within the L breed, was established for sire No. 9 (58.53% and 62.26%), while the lowest in offspring of sire No. 3 (50.33 and 53.94%). The determined difference between the offspring of these sires amounts to 8.20% and 8.32% of the muscle tissue in the carcass sides. The lowest mean values of these traits were recorded in animals originating from sire No.5 of the LW breed (46.14 and 49.06%), while the highest values were determined for the offspring of sire No.19 of Pietrain breed (64.92 and 65.78%). The difference in mean values of the meat content in the carcass sides between the offspring of these two boars-sires was 18.78% (EC 94) and 16.72% (EC 06).

Female animals had higher values (Table 3) for yield and meat share in carcass sides compared to male castrated animals. The meat content of carcass sides in female animals was estimated to be by 1.18 (JUS2), 4.22 (EC 94) and 4.62% (EC 06) higher compared to male castrated animals. The differences were statistically significant at the level of 99.9% (Table 4). When we observe the season of birth we see that the animals born in the winter period had higher average values of meat content in carcass sides determined by using the EC 94 (57.45%) and EC 06 (61.07%) Regulations compared to animals born in other seasons.

The sire breed, sires within the LW breed and the sex/gender had a very high statistically significant influence ($P<0.001$) on the yield and share, i.e. the meat content of carcass sides (Table 4). Sires within the L breed showed no influence only on the lean meat content of the carcass sides determined by the application of the *Rulebook* (JUS2), while the other properties were influenced ($P<0.01$ and $P<0.001$).

Table 3. The effect of sex/gender, sex/gender within the sire breed and the year of birth (Model 1) on the yield traits and the share of meat in carcass sides (LS Mean ±S.E.)

Sources of variation		JUS 1[2)], kg		JUS 2, %		EC 94, %		EC 06, %	
Sex	M[1)]	34.94	± 0.22	43.03	± 0.21	53.07	± 0.50	55.98	± 0.51
	F	35.89	± 0.23	44.21	± 0.22	57.29	± 0.52	60.16	± 0.54
Season	Winter	35.94	± 0.65	44.53	± 0.61	57.45	± 1.46	61.07	± 1.50
	Spring	34.80	± 0.25	43.10	± 0.24	50.63	± 0.57	53.51	± 0.58
	Summer	35.67	± 0.31	43.56	± 0.28	55.78	± 0.68	58.38	± 0.70
	Autumn	35.23	± 0.28	43.28	± 0.26	56.85	± 0.62	59.33	± 0.64

[1)]M-male castrates, F-females; [2)] JUS 1- yield of meat in carcass sides, JUS 2 – share of meat in carcass sides, EC 94 - share of meat in carcass sides, EC 06 - share of meat in carcass sides

The yield traits and the share of meat in the carcass sides, determined by the application of the *Rulebook* (JUS1 and JUS2), did not vary between the sires of Pietrain breed and the season of birth (P> 0.05). However, the estimated values for leanness obtained by applying the EC 94 and EC 06 regulations varied between the boars – sires of Pietrain breed (P<0.001). The interaction between the sex within the sire race for JUS 1 and JUS 2 was statistically significant (P <0.001), but in case of share of meat in carcass sides according to EC94 and EC06 it was not significant (P>0.05).

Table 4. Statistical significance of the influences included in the model on the tested properties

Sources of variation	JUS 1[2)]	JUS 2	EC 94	EC 06
SB	***[3)]	***	***	***
S:L	**	NS	***	***
S:LW	***	***	***	***
S:P	NS	NS	***	***
Sex	***	***	***	***
Season	NS	NS	***	***
Sex:SB	***	***	NS	NS

[1)]SB-sire breed; S:L-sires within Landrace breed; S:LW- sires within Large White breed; S:P- sires within Pietrain breed; Sex:SB- sex of offspring within sire breed; [2)] JUS 1- yield of meat in carcass sides, JUS 2- share of meat in carcass sides, EC 94 - share of meat in carcass sides, EC 06 - share of meat in carcass sides; [3)] ***=P<0.001; **=P<0.01; NS=P>0.05

By comparing the two regulations EC94 and EC06, the average estimated meat content of the tested fatteners in our trials was 53.56% and 56.55%, which means that the difference between them is 2.99% of the meat. The established difference in the average estimated meat content by applying the above regulations is lower (4.13%) than results presented by *Kosovac et al. (2009b)*. For hybrid combinations of crosses with three [(LWxL) xJ and (LxD) xJ] and four breeds

[(LWxL) x (JxP) and (LWxL) x (HxP)], *Bahelka et al. (2005)*, for both sexes (f = 61 animals and m = 62 animals), assessed the average meat content [*Commission Regulation EC No 3127/94, (1994)*] on the basis of a total dissection of four carcass parts of 55.54%, which is by 1.98% higher value compared to our result (53.56%).

Conclusions

In general, on the basis of the previously mentioned, as well as the results in their entirety, it can be concluded that it is of the utmost importance that the sires are evaluated on the slaughter line both for the production of fattening animals and for future breeding animals, since the share of heritability (h^2) for traits of carcass quality is very high. When we talk about the production of fatteners, the best results were achieved by offspring originated from the sires of Pietrain breed. If we are talking about the selection of offspring for breeding, if we consider only the estimated leanness, we see that within the L breed we have high and very significant ($P<0.001$) differences between the sires for the assessed meat content according to EC94 and EC06. By implementing new methods of assessment of lean meat content (EC 94 and EC 06), a higher share of meat (9.98% respectively 12.97%) was determined compared to the Rulebook (1985). This research indicates the necessity of changing the current method for the establishment of meat content of pigs in the Republic of Serbia.

Uticaj rase nerasta i primenjene metode na sadržaj mesa

Čedomir Radović, Marija Gogić, Dragan Radojković, Vladimir Živković, Nenad Stojiljković, Nenad Parunović, Radomir Savić

Rezime

Cilj ove studije bio je da se utvrdi uticaj primenjene metode kvaliteta trupa kod 201 potomka, oba pola (n = 108 kastriranih mukih grla i n = 93 ženskih), ispitivanih očeva nerastova. Za određivanje prinosa mesa (JUS1) i udela mesa (JUS 2) u polutkama, na osnovu izvršenih merenja, korićene su tabele za mesnate svinje, koji su sastavni deo Pravilnika o kvalitetu zaklanih svinja i kategorizaciji svinjskog mesa (SG SFRJ, 1985). Udeo mesa (EC 94 i EC 06) određen je na ohlađenim levim polutkama metodom delimične sekcije (prema metodologiji preporučenoj od strane EU - Walstra i Merkus, 1996). Istraživanje je sprovedeno na svinjarskoj farmi i u eksperimentalnoj klanici Instituta za stočarstvo Beograd-Zemun. Smeštaj,

nega i ishrana životinja bili su u skladu sa tehnologijom uzgoja u ispitivanom zapatu.
Prosečan udeo mesa utvrđen primenom Pravilnika (JUS2) iznosio je 43,58% sa nižom apsolutnom varijacijom u poređenju sa uredbom EU iz 1994. (EC 94; 53,56%) i iz 2006 (EC 06; 56,55%). Procenjeni sadržaj mesa primenom Pravilnika (JUS 2) bio je za 9,98% niži u odnosu na uredbu EC 94 i za 12,97% niži u odnosu na EC 06. Ako govorimo o izboru potomstva za uzgoj, ako uzmemo u obzir samo procenjenu mesnatost, vidimo da unutar rase L imamo visoke i veoma značajne (P <0,001) razlike između očeva za procenjeni sadržaj mesa u skladu sa EC 94 i EC 06. Implementacijom novih metoda procene sadržaja mesa (EC 94 i EC 06), utvrđen je veći udeo mesa (9,98%, odnosno 12,97%) u poređenju sa Pravilnikom (1985). Ovo istraživanje ukazuje na neophodnost promene postojeće metode utvrđivanja sadržaja mesa svinja u Republici Srbiji.

Ključne reči: nerast, otac, pol, sezona, kategorizacija svinjskih trupova

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ASSOCIATION BETWEEN AGE AT FIRST CALVING AND MILK PRODUCTION IN FIRST LACTATION ON LONGEVITY TRAITS IN HOLSTEIN COWS

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Original scientific paper

Abstract: The objective of this research was to determine the association between age at first calving (AFC) and milk production in the first lactation of longevity traits for Holstein cows. The research was conducted on 2307 cows raised on 4 farms in AP Vojvodina. The cows were culled in the period from 2017 to 2018. The cows belonged to the group of Holstein Friesian breed of cattle. Cow longevity was observed using three parameters: number of lactations (NL), lifetime milk yield (LMY) and length of productive life (LPL). The research involved the influence of the farm on which the animals realized their production, the season of first calving. A mean value obtained for the length of productive life in the analysed population was 1207 days (3.30 years on average). During this period the animals on average realized 2.45 lactations and produced on average 18798 kg milk. All studied traits showed a high level of variability. From these data, the study sought to determine the influence of factors on the traits of longevity, our research present that studied factors showed high statistical significance on these traits except in the case of the season of first calving where a statistical significance effect wasn't observed. The season of first calving did not affect the animal traits for longevity and, generally, didn't affect the calving interval. Regarding the obtained correlation data between age at first calving and length of productive life, it can be concluded that belated AFC leads to reduced reproductive performance.

Key words: Age at first calving, longevity, Holstein, milk yield in first lactation

Introduction

There are many factors affecting profitability in dairy production, one of the most important factors is longevity. Increased longevity reduces the direct costs of raising or replacement increases in total profit. Age at first calving (AFC),

calving intervals, length of each lactation and success in surviving to another lactation are effective on longevity (*Van-Raden and Klaaskate, 1993; Gröhn and Rajala-Schultz, 2000*). An important factor in the cost of raising dairy replacements is AFC in these factors. Reproductive traits, such as AFC and calving interval (CI) affect economically important traits like longevity, productive life and profitability of cows *(Do et al., 2013; Singh et al., 2017)*. Longevity is one of the most important traits in dairy cow breeding. There are strong correlations between cow reproductive traits, in this case, age at first calving have significant influence of the length of CI not only in first, but also in later lactations *(Janžekovič et al., 2009)*. Age at first calving not only has strong coherence with cow longevity but also can affect the future reproductive performance *(Nilforooshan and Edriss, 2004; Riecka and Candrák, 2011).*

In the previous studies, it was determined that the optimal first calving age for reaching maximal lifetime and daily production level and also for keeping cows in herds for as long as possible, is 24 – 27 months *(Pirlo et al., 2000; Brickell et al., 2009). Nilforooshan and Edriss (2004)* stated that the AFC of 24 months was optimal for lifetime milk production. The higher values of AFC are associated with poorer fertility in the first lactation and the shorter LPL of the cow *(Zavadilová and Štípková 2013).* The first calving between 22 and 26 months maximized the number of lactations and the number of lactation days in an animal's life *(Froidmont et al., 2013). Pirlo et al. (2000)* found that there were small differences in the longevity of cows when the AFC ranged between 671 (22.4 months) and -823 days (27.4 months).

The objective of this study was to estimate association between age at first calving and milk production in first lactation on longevity traits, using three parameters such as number of lactations (NL), lifetime milk yield (LMY) and length of productive life (LPL) for longevity in Holstein cows of the Vojvodina Province, Serbia.

Materials and Methods

A research was conducted on the animals raised on 4 farms in AP Vojvodina. Trial included 2307 cows culled in the period from 2017 to 2018. The cows belonged to the group of Holstein Friesian breed of cattle. Longevity in cows was observed using three parameters: number of lactations (NL), lifetime milk yield (LMY) and length of productive life (LPL). Length of productive life is defined as a time period starting from the date of the first calving up to the date of culling. Lifetime milk yield (LMY) was calculated using the data of individual milk recordings in different lactations.

Cows were housed in two types of housing systems (free-stall and tie-stall dairy barns) in 4 dairy farms with different sizes.

To study the effect of AFC, cows were classified in 7 groups: (group I – from 20 to 22 months of age, group II – from 22 to 24 months of age, group III – from 24 to 26 months of age, group IV – from 26 to 28 months of age, group V – from 28 to 30 months of age, group VI – from 30 to 32 months of age, group VII – from 32 to 34 months of age).

Season of calving was split into 4 seasons according to the local environmental conditions (1st: December-February; 2nd: Mart-May; 3rd: June-August and 4th: September-November). Feeding of cows included maize silage, lucerne hay and concentrate feed along with mineral supplements. The diet formulation was according to the milk production of cows.

The study included the effect of the farm on which the animal realized its production, age at first calving and the season of first calving of longevity and calving interval using of a following linear model:

$Yijkl = \mu + Fi + AFCj + Sk + SEZl + eijkl,$

Where:

Yijkl - phenotypic manifestation of a studied trait, μ - population general average,
Fi - fixed effect of i farm (i=1,2,3,4),
Sk - fixed effect of system of rearing (k=1-free stall barn, 2-tied housing system), SEZl - fixed effect of the season of first calving (l=1-4), eijkl -random error.

The average values and variability of examined traits as well as the effect of factors on these traits were studied by means of the procedures PROC UNIVARIATE and PROC GLM within SAS software package (SAS 9.3, 2012). The relationship between the examined traits was determined using the PROC CORR procedure within the same software program package.

Results and Discussion

Table 1 shows the statistical indicators of the analyzed traits. A productive life in the analysed population was 1207 days (3.30 years on average). During this period the animals realized 2.45 lactations and produced 18798 kg of milk on average. The average value for length of productive life (LPL) in research was (1207 days / 3.30 years) higher in relation to the value obtained by *Stanojević et al. (2015)* in the Serbian group of Black and White cattle improved by Holstein (3.14 years). Determined average value for length of productive life is lower in relation to the value obtained by *Jenko et al. (2015)* in the population of Slovenian Brown cattle breed (1544 days). The average number of lactations (NL) obtained from the current study (2.45) was relatively similar (2.64) with the results by *Stanojević et al. (2015)*. The number of lactations (Table 1) was lower (2.45) than the average of 2.94 noted by *Hare et al. (2006)* in the United States between 1980 and 1994.

Table 1. Phenotypic manifestation and variability of the analysed traits

Traits	n	$\bar{X}$	SD	CV (%)	min	max
LPL	2307	1207	425	35	162	2287
NL	2307	2.45	1.1	45	1	6
LMY	2307	18798	12021	64	306	79367
MY1L	2307	6427	874	13.6	4867	8379
CI	2307	438	65	14.8	347	580
AFC	2307	26.92	3.90	14.4	17.4	41.2

LPL - length of productive life (day), NL - number of lactation, LMY - lifetime milk yield (kg), MY_1L - milk yield in 1st lactation (kg), CI_1- length of calving interval among 1st and 2nd lactation (day), AFC - age at 1st calving (mo).

Obtained results for the AFC were 26.92 mo which is in accordance with those reported by *Nilforooshan and Edriss (2004)*, 26.84 months on average. In Table 1 is showed on average that cows were produced 6427 kg in the first lactation (MY1L) which is in accordance with results obtained by *Kučević et al. (2019).* Lower values in research by *Teke and Murat (2013)* for the population of Turkish Holsteins of the Mediterranean region in Turkey was established for a mean of milk yield in first lactation (5541 kg). Mean age at first calving of this population (26.92 mo) was less than the estimated mean of 29.81 mo in Turkey *(Teke and Murat, 2013).*

The research also included the analysis of the effect of farm on which the animals realized its production, the AFC, a season of 1st calving and the system of rearing on longevity. The values of F-test and their significance are shown in Table 1. The season of first calving did not affect the animal traits for longevity and, generally, didn't affect the calving interval. Similar results in their research paper published *Froidmont et al. (2012)* who established that season at first calving did not affect the animal's longevity and generally had a very little effect on the calving interval, number of lactations or average lactation length.

Table 2. Effect of fixed factors on length of productive life, number of lactations, lifetime milk yield and length of calving interval

Traits	Farm	Season of 1st calving	System of rearing	AFC
LPL	8.96^{*}	0.16^{ns}	0.77^{ns}	117.60^{**}
NL	16.46^{**}	0.01^{ns}	1.73^{ns}	92.30^{**}
LMY	5.06^{*}	0.1^{ns}	7.78^{**}	1.68^{ns}
CI	16.46^{**}	0.01^{ns}	1.73^{ns}	92.27^{**}

LPL - length of productive life, NL - number of lactation, LMY - lifetime milk yield; CI- length of calving interval between 1st and 2nd lactation; ns - no statistically significant effect, (P>0.05) * - statistically high significant effect, (0.05>P>0.01); ** - statistically very high significant effect, (P<0.01).

Age at first calving has a significant effect (P<0.01) for the length of productive life, a number of lactations but no significant effect for the lifetime milk yield (P>0.05). Age at first calving significantly determines the length of the calving interval (P<0.01). As age at first calving increases, also does length of calving interval in first and later lactations. Two types of housing systems showed no significant effect on length of productive life, a number of lactations and calving interval between 1st and 2nd lactation (P>0.05), except the lifetime milk yield (P<0.01). Results in the present study are consistent with other reports *(Stanojević et al., 2015; Kučević et al., 2019; Nilforooshan and Edriss, 2004; Teke and Murat, 2013; Janžekovič et al. 2009).*

The relationship between the examined traits such as length of productive life, number of lactations, lifetime milk yield and age of first calving of Holstein cows are given in Table 3.

Table 3. The relationship between the examined traits

Traits	AFC	MY_1L
LPL	-0.23^{**}	0.0023^{ns}
NL	-0.027^{ns}	0.0091^{ns}
LMY	-0.025^{ns}	-0.088^{ns}

ns - no statistically significant effect, (P>0.05); * - statistically high significant effect, (0.05>P>0.01); ** - statistically very high significant effect, (P<0.01)

Values of the correlation between examined traits were low and did not have statistical significance (P>0.05), except in the case of correlation between the length of productive life and age at first calving which was weak negative (-0.23) and statistically significant. This indicates that animals which were calved later had a shorter productive life. According to that, as age at first calving increased, productive life decreased. This trend shows the importance of reducing the age at first calving, which is supported by the negative phenotypic correlation between age at first calving and productive life obtained in a study by *Nilforooshan and Edriss (2004).*

In the study from *Teke and Murat (2013),* there was a slight positive correlation between the AFC and first lactation milk yield. *Moore et al. (1991)* and *Pirlo et al. (2000),* reported a positive effect of increasing age at first calving on milk yield and our results were in contrast to the results of their studies.

Bewley et al. (2001) reported a negative effect of increasing age at first calving on milk yield. They also reported that a 1-month increase in average age at first calving was associated with 102.5 kg reduction in milk yield. Also, the results obtained by *Zavadilová and Štípková (2013),* indicated that high age at first calving is related to a shortened length of productive life which confirmed our result in this case.

Conclusion

The age of first calving is one of the most important factors that have a significant impact on cow productivity in different lactations and its whole cow's life. Results from this study confirm that age at first calving can significantly affect length of productive life, number of lactations and length of calving interval. As age at first calving increases, also does calving interval in first and later lactations. Correlation between the length of productive life and age at first calving was weak negative (-0.23) and statistically significant so according to that, as age at first calving increased, productive life decreased. It can be concluded that belated AFC leads to reduced reproductive performance and it is one of the main reasons for reduced longevity.

Povezanost između uzrasta pri prvom teljenju i proizvodnje u prvoj laktaciji sa osobinama dugovečnosti kod mlečnih krava

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Rezime

Sprovedeno istraživanje imalo je za cilj da utvrdi povezanost između uzrasta pri prvom teljenju i proizvodnje mleka u prvoj laktaciji sa osobinama dugovečnosti za krave holštajn-frizijske rase. Istraživanjem je obuhvaćeno 2307 krava, sa 4 različite farme sa teritorije AP Vojvodine. Krave su izlučene u periodu od 2017 do 2018 godine. Dugovečnost krava je posmatrana kroz tri pokazatelja: broj ostvarenih laktacija (NL), životna količina proizvedenog mleka (LMY) i dužina produktivnog života (LPL). Prosečno trajanje produktivnog života za sva grla uključena u analizu iznosilo je 1207 dana (3.30 godine u proseku). Grla obuhvaćena analizom u toku svog produktivnog života su prosečno proizvela 18798 kg mleka. Broj laktacija koje je svako grlo prosečno ostvarilo u toku trajanja svog produktivnog života iznosio je 2.45, pri čemu su svi ispitani parametri pokazali visok nivo varijabilnosti. U istraživanju su ispitani fiksni uticaji farme na kojoj je grlo proizvodilo, uzrasta pri prvom teljenju, sistema proizvodnje i sezone pri prvom teljenju na osobine dugovečnosti i međutelidbeni interval, korišćenjem linearnog modela. Na osnovu rezultata dobijenih istraživanjem, fiksni faktori su pokazali visoku statističku značajnost za ispitivane osobine, osim u slučaju sezone pri prvom teljenju, gde nije ispoljena statistička značajnost za osobine obuhvaćene istraživanjem. Na osnovu rezultata, utvrđena je povezanost između uzrasta pri prvom teljenju i dužine produktivnog života, pri čemu se može zaključiti da su kraći produktivni život imale životinje koje su se kasnije telile.

Ključne reči: Uzrast pri prvom teljenju, dugovečnost, holštajn, proizvodnja mleka u prvoj laktaciji

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EFFECT OF SEX ON BIOMETRY AND MORPHOLOGICAL INDICES OF JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*)

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Original scientific paper

Abstract: The objective of this study was to determine the effect of sex on biometry and morphological indices of Japanese quails. A total of one hundred and fifteen finisher quails (115) comprising 30 males and 85 females of ten (10) weeks of age were used for this study. The quails were procured from the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria. They were managed in a deep litter system of housing from day old to finisher phase (10 weeks). The sex of the quails were identified by the production of cloacal foam following the standard protocol. Female quails recorded higher ($P<0.05$) body weight, massiveness and appears to be better for long leggedness than their male counterpart. The lower values observed for the female quails for long leggedness is an indication of blockier appearance a characteristics for meatiness. The results of the Pearson correlation for the male quails indicates that there were strong positive correlation for Body weight(BW) and Body length(BL)($r=0.465$ at $P<0.01$), BW and Breast circumference(BC), BL and BC, Foot length(FL) and Wing length(WL) ($r=0.577$, 0.429 and 0.451 at $P<0.05$). For the female quails, strong positive correlation were observed for BC and WL, FL and WL ($r=0.339$, 0.332 at $P<0.01$), BL and FL, FL and TLL ($r=0.270$, 0.263 $P<0.05$). There was also a strong negative correlation observed for Thigh circumference (TC) and FL ($r=0.406$ at $P<0.01$). The result of χ^2 for the sex was also significant ($P<0.05$). This findings would aid in the selection and breeding programme for quails' improvement.

Key words: Sex**,** traits, morphological indices, rectal temperature, quails

Introduction

Japanese quail have competitive advantages over other minor poultry species such as early sexual maturity, short generation interval, rapid growth rate, and high reproductive capacity (*Varkoohi et al., 2011)*. Also, the environmental

requirements are few and production costs are rather low. Quails plays an important role in poultry production as meat and egg production. The human tendency to consume protein coming from animals has made quail production even more important (*Balcıoğlu et al., 200*5). Factors, such as a short generation interval, suitability for genetic improvement and also due to the facts that the birds cover a small space and consume a small amount of feed have led to a frequent use of quails for breeding purposes (*Koçak and Özkan, 2000)*. The Japanese quails are sexually dimorphic birds with the female having a larger body size than males. Unlike other poultry species. Accordingly, females require more time to reach sexual maturity than males (*Reddish et al., 2003*). The differences in growth pattern between the sexes are also a well-known phenomenon (*Sezer and Tarhan, 2005*). At an early age, gender determination according to the morphological characteristics of Japanese quail is not easy. After hatching, live weight increases continuously up to puberty. The sexual maturity of Japanese quails lasts 4-6 weeks. In female birds the increase in live weight goes on until the 6^{th} week of age and they generally start laying eggs during this period (*Alkan et al., 2008b*). For this reason, the first six weeks are critical in order to determine values indicating information about certain yield traits (*Cerit and Altınel, 1998).* Although it is easier to discriminate the sex from the 6th week of age due to the larger body shape of female birds as well as through observing typical behaviour of male birds (*Brunström et al., 2009*). This is too late since quails reach a weight suitable for sales markets after 5-6 weeks. It is of advantage that there are specific differences between the sexes in quails. These differences can be determined at a rate of 99% in a day-old chicks. A remarkable protrusion or a cavity at the edge of the dorsal cloaca are characteristic features of male birds. This characteristic is typically well developed in male birds. The colouring of the chest feathers in the 2^{nd} and 3^{rd} week of age is also a reliable indicator for sex discrimination. Spotted and reddish-brown feathers start sprouting in this period. From approximately the 6^{th} week on the large body shape of female birds and distinct behaviour of male birds can be recognized. During this period the production of cloacal foam can be observed in male quails (*Tservem-Gouss and Yannakopoulos, 1986*). Therefore this study aimed at determining the effect of sex on biometry and morphological indices of Japanese quails

Material and Methods

Experimental location

This experiment was carried out at the Livestock Section of the Teaching and Research Farm of the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus. It is located at guinea savannah zone of North Central Nigeria. It lies at latitude $08^{0}35$N and longitude $08^{0}33$E.

Lafia enjoys two separate seasonal periods namely raining season (April-September) and dry season between (October-March). Annual rainfall figures range from 1100 to 2000 mm. The mean monthly temperatures in the State range between 20 and 34ºC, with the hottest months being March/April and the coolest months being December/January *(Lyam, 2000).*

Experimental birds and management

A total of one hundred and fifteen finisher quails (115) comprising 30 males and 85 female of ten (10) weeks of age were used for this study. The quails were procured from the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria. They were managed in a deep litter system of housing from day old to finisher phase (10 weeks). At the end of the 10^{th} week of the experiment data were obtained for body weight, biometric [body length (BL), breast circumference (BC), thigh circumference (TC), foot length (FL), total leg length (TLL), wing length (WL)] as well as rectal temperature respectively. Each bird was tagged with an identification number for easy data collection. The sex of the quails were identified by the production of cloacal foam as described by *Tservem-Gouss and Yannakopoulos (1986*). In male there is production of cloacal foam when pressed while non in female. The birds were fed commercial conventional feed from day old to finisher phase. Feed and fresh clean water were supplied *ad libitum.* Routine vaccination and other management practices were strictly followed. There was also a routine administration of antibiotics, vitamins and coccidiostat (Amprolium) in the drinking water.

Body weight and biometric data collection

Body weight (BW) and six primary biometric traits: body length (BL), breast circumference (BC), thigh circumference (TC), foot length (FL), total leg length (TLL) and wing length (WL) were taken on each of the quail at the end of the10^{th} week of the experiment as adopted by *Teguia et al., (2008)*. Body weight (BW):- 10-kg digital weighing balance scale was used for the individual weight measurement, Body length (BL):- Body length was taken between the tip of the Rostrum maxillare (bill) and that of the Cauda (tail, without feathers), Breast circumference (BC):- This was taken under the wings at the edge of the sternum, Thigh circumference (TC):-The circumference of the thigh was taken as the circumference of the drumstick at the coxa region, Foot length (FL):- This was taken as the distance from the shank joint to the extremity of the *Digitus pedis*, Total leg length (TLL):- This was taken as the length of the femur, shank and metatarsal, Wing length (WL):- This was taken from the shoulder joint to the extremity of the terminal phalanx, digit 111. The biometric traits measurements (cm) were taken using flexible tape rule.

Morphological indices and rectal temperature data collection

The morphological indices were determined following the procedure of *Oblakova (2007).* Massiveness (MAS):- Ratio of live body weight to body length × 100, Stockiness (STK):- Ratio of breast circumference to body length × 100, Long leggedness (LLN):- Ratio of total leg length to body length × 100, Condition index (CI):- Ratio of live body weight to wing length × 100. Rectal Temperature: Was measured using a clean clinical thermometer inserted into the vent for one minute after which the readings were taken (˚C) as described by *Yahav and McMurtry, (2001).*

Statistical analysis

The effect of sex at the end of the 10th week of the experiment were assessed using the General Linear Model (GLM) of Statistical package for social science (SPSS.22). Pearson correlation matrix was performed to explain the relationship of body weight and biometrics characteristics among individual male and among female quails. Chi-square analysis of sex using goodness of fit was also performed to show significant or non-significant relationship between the sexes. The following model was employed:

$Y_{ij} = \mu + S_i + e_{ij}$

Y_{ij} = Individual mean observation

μ = General mean

S_i = Effect of sex (male and female at the 10th week of the experiment)

e_{ij} = Error term.

Results and Discussion

Table 1. Effect of Sex on the body weight and biometric traits of quails

	Sex (n=115)		
Traits	Male (n=30)	Female (n=85)	Sig
Body weight (kg)	0.147±0.008^{b}	0.168±0.005^{a}	**
Body length (cm)	13.183±0.144^{a}	13.429±0.086^{a}	ns
Breast circumference (cm)	16.333±0.149^{a}	16.470±0.089^{a}	ns
Thigh circumference (cm)	4.233±0.095^{a}	4.149 ±0.057^{a}	ns
Foot length (cm)	3.733±0.066^{a}	3.756±0.039^{a}	ns
Total leg length (cm)	11.883±0.156^{a}	11.714±0.093^{a}	ns
Wing length (cm)	10.783±0.271^{a}	11.304±0.162^{a}	ns

^{ab} Means on the same rows bearing different superscripts are significantly different (P<0.05)

** Significant at 95%, ns- not significant.

BW=Body weight, BC= Breast circumference, TC= Thigh circumference, FL= Foot length, TLL= Total leg length, WL= Wing length

Effect of sex on the body weight and biometric traits of quail is presented in Table 1. The result indicates that only body weight recorded significant effect (P<0.05) on the quails. Female quails recorded higher (P<0.05) body weight than their male counterpart. No significant difference were recorded for the other parameters measured (body length, breast circumference, thigh circumference, foot length, total leg length and wing length).

Table 2. Effect of Sex on the morphological indices and rectal temperature of quails

	Sex (n=115)		
Traits	Male (n=30)	Female (n=85)	Sig
Massiveness (MAS)	1.113±0.063[b]	1.262±0.037[a]	**
Stockiness (STK)	125.462±16.403[a]	139.445±9.803[a]	ns
Long-leggedness (LL)	90.134±1.505[a]	86.435±0.900[b]	**
Condition index (CI)	1.369±0.112[a]	1.534 ±0.067[a]	ns
Rectal temperature (RT) (^{0}C)	40.480±0.550[a]	41.312±0.329[a]	ns

[ab] Means on the same rows bearing different superscripts are significantly different (P<0.05)
** Significant at 95%, ns- not significant.

Effect of sex on the morphological indices of quails is presented in table 2. The results were significant (P<0.05) for massiveness (MAS) and long leggedness (LL). Female quails recorded higher (P<0.05) significant value for massiveness and appears to be better for long leggedness. The lower values observed for the female quails for long leggedness is an indication of meat characteristics and this is observed by a blockier appearance. However there were no significant difference (P<0.05) recorded for stockiness (SK), condition index (CI) and rectal temperature (RT).

Correlation matrix of the body weight and biometrics characteristics among individual male and among female (hen) quails at 10th week of age is presented in table 3. The results for the male quails indicated that there were strong positive correlation for BW and BL (r=0.465 at P<0.01), BW and BC, BL and BC, FL and WL (r=0.577, 0.429 and 0.451 at P<0.05). For the female (hen), strong positive correlation were observed for BC and WL, FL and WL (r=0.339, 0.332 at P<0.01), BL and FL, FL and TLL (r=0.270, 0.263 P<0.05) whereas there was a strong negative correlation observed for TC and FL (r=0.406 at P<0.01).

Table 3. Correlation matrix of body weight and biometrics characteristics among individual male quails and among female quails at 10th week of age.

	Male						Female					
	BL	BC	TC	FL	TLL	WL	BL	BC	TC	FL	TLL	WL
BW	0.465**	0.577*	0.259	-0.043	0.340	-0.016	0.002	-0.017	0.115	-0.035	0.003	-0.127
BL		0.429*	0.060	0.051	0.214	0.213		0.171	-0.126	0.270*	0.050	0.185
BC			0.114	0.059	0.063	0.315			-0.125	0.105	0.088	0.339**
TC				-0.112	0.098	-0.294				-0.406**	0.019	-0.168
FL					0.055	0.451*					0.263*	0.332**
TLL						0.237						0.161

: P<0.01; *: P<0.05. BW=Body weight, BC= Breast circumference,** TC= Thigh circumference, FL= Foot length, TLL= Total leg length, WL= Wing length

Table 4. Test of homogeneity for the effect of sex on body weight, biometric and morphological indices of quails.

Parameters	F	df1	df2	Sig.
BW	0.602	1	112	0.440
BL	0.000	1	112	0.985
BC	4.456	1	112	0.037
TC	0.608	1	112	0.437
FL	0.683	1	112	0.410
TLL	0.074	1	112	0.786
WL	0.055	1	112	0.814
MAS	0.746	1	112	0.390
STK	1.714	1	112	0.193
LL	0.528	1	112	0.469
CI	0.122	1	112	0.728
RT	0.044	1	112	0.834

df1 and df2-degree of freedom, F- calculated value.

Results of the test of homogeneity for the effect of sex on body weight, biometric and morphological indices of quails (Table 4) indicates that all the parameters were

homogenous except for BC (sig values > 0.05 indicates not significant or homogenous and <0.05 indicates significant or heterogenous).

Table 5. Chi-Square analysis of Sex using goodness of fit

Sex	Frequency	Percentage	Mean	SD	χ^2	Df	P-Value
Male	30	26.100					
			1.739	0.441	26.304	1	0.000**
Female	85	73.900					

SD- standard deviation, df- degree of freedom, χ^2- Chi-square

There result of chi-square for the goodness of fit is presented in table 5. The result of the sex was significant (P<0.05). The χ^2 – value of 26.304 was recorded.

Discussion

The sexual differences in quails are believed to evolve under the pressure of natural and sexual selection, which implies that genes controlling sexually dimorphic characteristics differ between males and females (*Mignon-Grasteau et al., 2004*). It is important to have a wider knowledge of genetic correlations between live weight and other important traits for quail production to develop an optimal total merit index. This will also complement our understanding of the evolutionary consequences of the sexual dimorphism. The result of this study indicates that only body weight recorded significant effect (P<0.05) on the quails. Female quails recorded higher (P<0.05) body weight than their male counterpart. No significant difference were recorded for the other parameters measured (body length, breast circumference, thigh circumference, foot length, total leg length and wing length). The significant difference (P<0.05) recorded for the body weight in this study agreed with the work of *Alkan et al. (2008a)* who report that live weight and body length in Japanese quails are critical from week 2 on for discriminating the sex. However our study do not recorded significant difference (P<0.05) in the body length for the male and female quails. The result of this study on the significant difference recorded for the body weight is congruous with the report of *Faith et al. (2018)* and *Genç et al. (2009)* who observed significant effect for live weight in chickens and quails respectively. It is observed in other literatures that female quails are slow in attaining sexual maturity and however still appears to gain more body weight than the males (*Alkan et al., 2008a; Genç et al., 2009; Türkmut et al., 1999*). Female quails recorded higher (P<0.05) significant value for massiveness and appears to be better for long leggedness. The lower values

observed for the female quails for long leggedness is an indication of blockier appearance, a good characteristics for meatiness. Generally, a higher phenotypic variation of traits indicates a higher genetic variation, which guarantees a sufficient selection response. This is important because of directional selection on morphological traits and commonly occurs in natural populations (*Kingsolver et al., 2001*). The results of the correlation matrix for the male quails indicated some forms of strong positive correlation for BW with some of the biometric traits. The estimates of correlation in the present study are comparable to those reported by earlier workers for chicken (*Mancha et al., 2008*).

Conclusion

Female quails recorded higher body weight, massiveness and appears to be better for long leggedness than their male counterpart. The lower values observed for the female quails for long leggedness is an indication of blockier appearance a characteristics for meatiness. The results of the Pearson correlation for the male quails indicates that there were strong positive correlation for Body weight(BW) and Body length (BL), Body weight (BW) and Breast circumference (BC), Body length (BL) and Breast circumference (BC) as well as Foot length(FL) and Wing length (WL). For the female quails, strong positive correlation were observed for Breast circumference (BC) and Wing length (WL), Foot length (FL) and Wing length (WL), Body length (BL) and Foot length (FL) as well as Foot length (FL) and Total leg length (TLL) respectively. There was also a strong negative correlation observed for Thigh circumference (TC) and foot length (FL). Body weight and other biometric parameters could jointly be used in the assessment of quails in times of sexual differences and meat characteristics. The result of χ^2 for the sex was also significant ($P<0.05$). The results of this findings will aid in the selection and breeding for quails improvement programme.

Uticaj pola na biometriju i morfološke indekse japanskih prepelica (*Coturnix coturnix japonica*)

Faith E. Akumbugu, Musa U. Obakpa, Aya V. Ebuga, Thomas S. Esson

Rezime

Cilj ove studije bio je utvrditi uticaj pola na biometriju i morfološke indekse japanskih prepelica. Za ovu studiju korišćeno je ukupno sto petnaest prepelica (115) koje čine 30 mužjaka i 85 ženki starosti deset (10) nedelja. Prepelice su nabavljene od Nacionalnog instituta za istraživanje veterine (NVRI) Vom, država Plateau Nigeria. Držani su u sistemu duboke prostirke, od starosti jednog dana do završne faze (10 nedelja). Pol prepelica identifikovan je proizvodnjom kloakalne pene sledeći standardni protokol. Ženke prepelica zabeležile su veću ($P<0,05$) telesnu masu, veličinu i čini se da su bolje za osobinu dužina nogu od muških ptica. Niže vrednosti koje se primećuju kod prepelica ženskog pola zbog dugih nogu pokazatelj su blokerijevog izgleda što je karakteristika za mesnatost. Rezultati Pearsonove korelacije kod muških prepelica pokazuju da je postojala snažna pozitivna korelacija za telesnu masu (BW) i dužinu tela (BL) ($r = 0,465$ na $P <0,01$), BW i obim grudi (BC), BL i BC, dužina stopala (FL) i dužina krila (WL) ($r = 0,577$, $0,429$ i $0,451$ pri $P <0,05$). Za ženske prepelice primećena je snažna pozitivna korelacija za BC i WL, FL i WL ($r = 0,339$, $0,332$ na $P <0,01$), BL i FL, FL i TLL ($r = 0,270$, $0,263$ $P <0,05$). Takođe je primećena snažna negativna korelacija za obim bataka (TC) i FL ($r = 0.406$ na $P <0.01$). Rezultat χ^2 za pol takođe je bio značajan ($P <0,05$). Ovi nalazi bi pomogli u programu selekcije i uzgoja za poboljšanje prepelica.

Ključne reči: pol, osobine, morfološki indeksi, rektalna temperatura, prepelice

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THE QUALITY OF TRADITIONALLY SMOKED TENDERLOINS OBTAINED FROM MEAT OF NATIVE PIG BREEDS

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Original scientific paper

Abstract: The domestic (homeland, native) populations - breeds of pigs are grown in certain region and/or country as local, primitive breeds. In Poland following 3 pig breeds are considered as native: Pulawska, Zlotnicka White and Zlotnicka Spotted. The aim of this study was the assessment of the quality of meat and traditionally smoked tenderloins obtained from meat of native pigs breeds bred in a traditional way. Meat analysis showed that the highest fat content values were present in Zlotnicka Spotted (4.60 ± 0.33) and White (3.63 ± 0.41) while the lowest in Pulawska (2.78 ± 0.29). Smoked tenderloins shear force differed statistically. The hardness values were the highest for Zlotnicka White (65.66 ± 6.46) and the lowest for Zlotnicka Spotted (33.27 ± 4.12). Similar shear force values were recorded: the highest in Zlotnicka White (51.90 ± 7.11) and the lowest in Zlotnicka Spotted (30.43 ± 5.54). Regardless of the results obtained, meat products have good acceptance of sensory assessment test and low level of polycyclic aromatic hydrocarbons. We conclude that the meat of Pulawska, Zlotnicka White and Spotted breeds of pigs is a good quality raw material used for production of traditional and regional meat products gaining high sensory scores and good recognition among consumers. The traditionally smoked tenderloins produced from meats of above breeds, were characterized by very good quality parameters.

Key words: fatteners, native breeds, meat, tenderloins, quality

Introduction

The farm animals have accompanied humans more than 10 thousand years. Over the centuries, man has created about 8 thousand animal breeds. In the FAO database, 7,616 breeds are registered, of which over 85% are so-called local breeds *(Litwińczuk, 2017*). They have given not only food but also helped with farmer field work, were the components of agri-landscape playing the immanent part of nature, of culture and even ethnography. Nowadays, according to data obtained for 2016 in Poland, the altogether number of registered and preserved with *in situ* programs, is 83 breeds, varieties and lines, where 43 are mammals, 35 domestic birds and 5 bees lines. They are breed on 3259 farms (*Krupiński et al., 2017*).

The native populations breeds of pigs are grown in the certain region and/or country as local, primitive breeds, for example, Mangalitsa, Moravka, Resavka, Iberian, Casertana, Alentejana, Bazna, Mora Romagnola, Krskopolje, Turopolje (*Čandek-Potokar, 2019; Popova et al., 2015; Pugliese and Sirtori, 2012; Radović et al., 2017; Radović et al., 2019*). These breeds are characterized by lower fattening, slaughter and breeding yield parameters but their advantages are good quality raw meat, lower feeding demands, natural resistance to bad environmental conditions and higher resistance against illnesses and stress in comparison to regular industrial breeds (*Čandek-Potokar, 2019; Pugliese and Sirtori, 2012; Radović et al., 2019*). In Poland following 3 breeds are included in the group of native breeds: Pulawska, Zlotnicka White and Zlotnicka Spotted pig breeds (*Szyndler-Nędza et al., 2011*). The breeds are outstanding for high reproduction traits and well survival ability in difficult environmental conditions (*Litwińczuk, 2017*). Also they are known for good maternal traits (*Szyndler-Nędza et al., 2011*). The pigs of Polish homeland breeds can be used for breeding of heavy fatteners, because even after obtaining high body weight the meat shows very good quality (*Babicz et al., 2009*; *Martyniuk, 2010*; *Szyndler-Nędza, 2012*). The asset of meat of these breeds is advantageous muscle fibrils structure and the amount of intramuscular fat favourably influencing the meat marbling and sensory properties. Thanks to above the meat of these pigs breeds is used for production of regional meat products (*Hammermeister and Blicharski, 2007*; *Szyndler-Nędza et al., 2011*).

The aim of the study was the assessment of quality of meat and traditionally smoked tenderloins obtained from meat of native pigs breeds bred in a traditional way.

Material and Methods

Tenderloins

In scope of the project "The uses and the conservation of farm animal genetic resources under sustainable development" co-financed by the National

Centre for Research and Development within the framework of the strategic R&D programme "Environment, agriculture and forestry" – BIOSTRATEG, contract number: BIOSTRATEG2/297267/14/NCBR/2016 meat products of native Polish pig breeds were analysed.

The experimental material were 18 traditionally smoked tenderloins prepared using the *m. longissimus dorsi*, bought in factories processing meats of native pig breeds (Pulawska, Zlotnicka Spotted and White) – 6 items of each native pig breed. The recipes of smoked loins production were reserved by meat factories. But general technological scheme of production was stated as (according to producer's statements):

- Choice of raw material – loin
- Brining (multi-needle injection of brine)
- Massage (cylindrical shape rotating massage apparatus; 3 hours in vacuum with 6 rotations per 1hour)
- Deposition and ageing (drying of surface, colour adjustment; smoking trolley; 1 hour)
- Smoking (traditional smoking chamber; beech-alder woodchips; to obtain internal bar temp. of 72°C)
- Chilling and cooling (to product temp. below 10°C).

The obtained samples of meat were subjected to physical-chemical analyses.

Tenderloins quality

The tenderloins were minced and average samples obtained were subjected to chemical analyses.

The following items were estimated in the tenderloins samples:

- water content according to the standard (*PN-ISO 1442:2000)*,
- fat content according to the standard (*PN-ISO 1444:2000)*,
- protein content by Kjeldahl method *(PN-75/A-04018)*,
- total ash content according to the standard (*PN-ISO 936:2000)*,
- total carbohydrates content was calculated assuming that the all total solids and water stand for 100%,

- The measurements of colour of meat and tenderloins samples were obtained in CIELab system. Lightness [L*], redness [a*] and yellowness [b*] of meat were determined using a Konica Minolta CM–600d spectrophotometer,

- polycyclic aromatic hydrocarbons (PAHs) (benzo(a)pyrene and sum of benzo(a)pyrene, benzo(a)antracene, benzo(b)fluorantene and chrysene) according to the HRGC-HRMS method (CZ_SOP_D06_06_180 - except chap. 11.3.3.1 - 11.3.3.7, 11.3.3.9 l, 11.3.4 (US EPA 429, ISO 11338); PAH16: Determination of polyaromatic hydrocarbons by isotope dilution method using HRGC-HRMS),

- Texture profile analysis (TPA) of smoked loin was performed with TA-XT2 Stable Microsystem texturometer. There were obtained texture profile parameters: hardness, springiness, cohesiveness, chewiness and resilience with double pressing test of 10 samples (15 mm high and 15 mm diameter) of each meat and loin lot. The samples were squeezed up to 50% of their height. The test speed of 50mm cylindrical probe was 1,5mm with 3 s pause between squeezes. The measurement of shear force was performed with Warner-Bratzler triangle edge knife. From each lot were cut out 5 cylindrical samples (diameter 15mm, height 15mm) and the force needed for rectangular samples cutting was measured. The test blade speed was 2 mm/s.

- There was also performed consumer sensory analysis. There were assessed 9 quality characteristics with 5 point hedonic scale (PN-ISO 6658. 1998). The assessment was made by the 10-person panel consisting of employees of the Department of Animal Product Technology, University of Agriculture in Krakow. The panel had years of experience in sensory evaluation practice and were trained theoretically and practically for the methods applied (ISO 8586: 2014).

Statistical analysis

All samples were obtained at least in duplicates. All results were analysed with ANOVA and present as means with standard deviation. The calculations were performed with Statistica 6.0 *(StatSoft, 2003)*.

Results and Discussion

In Table 1 the chemical composition and colour of traditionally smoked tenderloins bought in factories processing meat of native pig breeds (Pulawska, Zlotnicka Spotted and White) is presented. The traditionally smoked tenderloin obtained from Pulawska fatteners' meat was characterised by statistically significant lower fat content in comparison to tenderloins produced from Zlotnicka White and Spotted fatteners' meat. Admittedly the high intermuscular fat content influences negatively the assessment of meat by consumers, but the fat determines the taste and texture meat traits. The level of intermuscular fat especially determines the quality of raw ripened and raw smoked hams (*Szulc and Skrzypczak, 2016*). The traditional smoked tenderloins obtained from Zlotnicka breeds were the raw ripened smoked products so their higher fat content was influencing positively their quality. *Wood et al. (1999), Daszkiewicz et al. (2005*) and *Tyra and Mitka (2015*) revealed that the optimal taste, tenderness and juiciness is observed in meat containing 2.5 – 3.0 % of fat. The contents of proteins and ash were also higher in tenderloins obtained from Zlotnicka breeds meat in comparison to Pulawska breed.

Table 1. Chemical composition and colour of traditionally smoked tenderloins obtained of Pulawska, Zlotnicka White and Zlotnicka Spotted fatteners (mean±SD)

Chemical component [%]	Breed of fatteners		
	Pulawska	Złotnicka White	Złotnicka Spotted
Water	68.84 ± 3.84	64.91 ± 2.79	63.90 ± 3.06
Protein	26.31^{a} ± 1.82	28.12^{b} ± 1.61	28.90^{b} ± 1.49
Fat	2.78^{a} ± 0.29	3.63^{ab} ± 0.41	4.60^{b} ± 0.33
Ash	1.89^{a} ± 0.08	3.13^{b} ± 0.10	2.47^{a} ± 0.08
Carbohydrates	0.18^{a} ± 0.008	0.21^{a} ± 0.01	0.13^{b} ± 0.009
Colour			
L*	56.73^{a} ± 4.71	48.01^{b} ± 3.91	50.51^{b} ± 5.12
a*	7.79 ± 1.77	8.99 ± 1.85	8.76 ± 1.73
b*	8.65^{a} ± 1.57	5.84^{b} ± 1.16	5.02^{b} ± 1.62

^{a,b} Mean values in rows marked with a different letters differ statistically significantly at $P \leq 0.05$

Janiszewski et al. (2015) assessed smoked loins and cooked hams produced using meat of Zlotnicka Spotted pigs and of crossbreds of that breed with Duroc and PLW (Polish Large White). The quality of meat smoked products obtained from assessed fattener groups did not differ significantly in regard to the physic-chemical and sensory parameters. The assessed products differed in chemical composition because the loins lowest in fat were obtained from crossbred fatteners: (Zlotnicka Spotted x Polish Large White - ZSxPLW), whereas the hams lowest in fat were obtained from purebred fatteners. *Kędzior et al. (2014)* revealed that the

traditional products have a higher nutritional value than conventional ones. They contain significantly more protein, less water and salt, and thus less total ash. Table 2 contains the content of polycyclic aromatic hydrocarbons in traditional tenderloins.

The tenderloins obtained from Pulawska pigs were characterized by higher values of parameter b*(yellow). According to *Szulc and Skrzypczak (2015)* the maet of native pig breeds is characterized by darker colour which is preferred nowadays by a part of consumers. The dark colour of meat is characteristic both for European wild boar and other European native pig breeds (*Szulc and Skrzypczak, 2015*).

Table 2. Content of polycyclic aromatic hydrocarbons in traditional tenderloins (µg/kg)

Polycyclic aromatic hydrocarbons	Traditional tenderloins of native pigs breeds:		
	Pulawska	Zlotnicka White	Zlotnicka Spotted
Naphtalene	24.0 ± 7.20	21.0 ± 6.30	51.0 ± 15.3
Acenaphtylene	35.0 ± 10.5	27.0 ± 8.10	21.0 ± 6.30
Acenaphthene	1.20 ± 0.36	3.10 ± 0.93	2.90 ± 0.87
Fluorene	11.0 ± 3.30	20.0 ± 6.00	1.30 ± 0.39
Phenanthrene	52.0 ± 15.6	60.0 ± 18.0	47.0 ± 14.1
Anthracene	7.40 ± 2.20	15.0 ± 4.50	10.0 ± 3.00
Fluoranthene	16.0 ± 4.80	13.0 ± 3.90	7.70 ± 2.31
Pyrene	13.0 ± 3.90	12.0 ± 3.60	6.90 ± 2.07
Benzo(a)anthracene	1.30 ± 0.39	<0.90	<0.87
Chrysene	1.10 ± 0.33	<0.90	<0.87
Benzo(b)fluoranthene	<0.81	<0.31	<0.43
Benzo(k)fluoranthene	<0.54	<0.31	<0.43
Benzo(a)pyrene	<0.54	<0.31	<0.65
Indeno(1,2,3-cd)pyrene	<0.75	<0.31	<0.43
Dibenzo(a,h)anthracene	<0.30	<0.21	<0.23
Benzo(g,h,i)perylene	<0.59	<0.34	<0.43

The analysed tenderloins were safe as contents of polycyclic aromatic hydrocarbons. The loins obtained of Pulawska breed meat contained larger amounts of benzo(a)antracene and chrysene in comparison to loins obtained from Zlotnicka breed pig. All tenderloins fulfilled the demands listed in EC *Commission Regulation no 835/2011 (Migdał et al., 2015)*. The results have revealed that traditionally smoked products have high quality and safety, especially as PAHs contents which could result from smoking using the traditional methods. In Table 3 the texture profile and the shear force of analysed tenderloins are presented, whereas in Table 4 the results of consumers' assessment.

Table 3. Texture profile and shear force for traditionally smoked tenderloins obtained from fatteners of Pulawska, Zlotnicka White and Spotted breeds

Breed of fatteners	Texture profile					Shear force [N]
	Hardness [N]	Springiness	Cohesiveness	Chewiness [N]	Resilience	
Pulawska	45.59[a] ±5.84	0.457 ±0.06	0.272 ±0.04	5.66[a] ±0.62	0.125 ±0.02	34.99[a] ±6.04
Zlotnicka White	65.66[b] ±6.46	0.36 ±0.04	0.339 ±0.04	8.01[b] ±0.74	0.121 ±0.02	51.90[b] ±7.11
Zlotnicka Spotted	33.27[a] ±4.12	0.438 ±0.05	0.367 ±0.05	5.346[a] ±0.60	0.125 ±0.018	30.43[a] ±5.54

[a,b] Mean values in columns marked with a different letters differ statistically significantly at $p \leq 0.05$

The tenderloins produced from Zlotnicka Spotted breed meat were characterized by better hardness and shear force parameters and in consumer assessment obtained the highest total score. *Grześkowiak et al. (2007)* when analysing the raw smoked loins obtained from Zlotncka breeds pigs meat observed higher values of shear force – for Zlotnicka White: 58.23 (N) and for Zlotnicka Spotted: 45.71 (N).

Table 4. The quality characteristics and average scores for consumer assessment of traditionally smoked tenderloin of Pulawska, Zlotnicka White and Spotted fatteners

Quality characteristics		Fattener breeds		
		Pulawska	Zlotnicka White	Zlotnicka Spotted
Appearance		4.80 ± 0.17	4.70 ± 0.15	4.80 ± 0.15
Slice cut colour		4.60 ± 0.19	4.60 ± 0.15	4.70 ± 0.12
Slice structure		4.70 ± 0.20	4.60 ± 0.15	4.80 ± 0.15
Slice cohesiveness		4.80 ± 0.12	4.75 ± 0.20	4.80 ± 0.15
Odour	Intensity	4.90 ± 0.11	4.65 ± 0.15	4.90 ± 0.12
	Desirability	4.80 ± 0.12	4.60 ± 0.15	4.80 ± 0.12
Tenderness		4.75 ± 0.14	4.50 ± 0.18	4.80 ± 0.15
Juiciness		4.70 ± 0.13	4,80 ± 0,17	4.80 ± 0.12
Saltiness		4.60 ± 0.16	4.00 ± 0,15	4.60 ± 0.15
Taste	Intensity	4.75 ± 0.17	4.60 ± 0,11	4.80 ± 0.12
	Desirability	4.80 ± 0.15	4.80 ± 0.12	4.90 ± 0.11
Total score		4.75 ± 0.11	4.64 ± 0.10	4.815 ± 0.11
		Very good quality	Very good quality	Very good quality

The consumer panel estimated higher the traditionally smoked tenderloin obtained from Zloltnicka Spotted fatteners than loins produced from Zlotnicka White and Pulawska breeds. The loins produced of Zlotnicka Spotted pigs obtained higher scores for odour/aroma, tenderness, juiciness and flavour – the characteristics directly related to the content of intermuscular fat. In the work of *Szulc et al. (2011)* slightly higher scores are obtained for raw, smoked loin produced from Zlotnicka White breed meat in comparison to Zlotnicka Spotted breed meat. To obtain the optimum sensory quality the authors list different amounts of intramuscular fat. *Łyczyński et al. (2007)* revealed as the most favourable amount of intramuscular fat the levels 2-3 %, whereas *Wood et al. (1999)* the levels from 2.0 to 3.5 %. *Olkiewicz et al. (2006)* as most favourable have chosen the level of at about 3 %. In our experiment, the loins obtained from Zlotnicka Spotted breed meat contained 4.60 % of intermuscular fat, the loins obtained from Zlotnicka White contained 3.63 % and the loins produced of Pulawska breed pigs contained 2.78 %, respectively, on average. *Florowski et al. (2006)* described that the fatteners' breed is the factor which significantly influences and differs many of meat quality characteristics. Among all pig breeds kept in Poland, the advantageous characteristics are observed with Duroc breed, the native breeds, here also good scores for Pulawska breed, whereas lower scores are granted to Pietrain breed meat. Paralely with growth of fatness of fatteners the losses during preservation and preparation for consumption of meat are also growing (*Trombetta et al., 1997)*. The intermuscular fat content above 2.5 % can influence the lower score of meat granted by consumers because of meat marbling (*Czarniecka-Skubina et al., 2007*). But on the other side, the higher intermuscular fat content of the traditionally smoked tenderloins produced using such meat have better scores for aroma, tenderness, juiciness and flavour/taste.

Above described characteristics are observed in meat of all estimated, native pig breeds - Pulawska, Zlotnicka White and Zlotnicka Spotted. The meat of these fatteners is widely used for production of traditional products high in demand by consumers, so they have to be bred and preserved. The practical protection rules of each native breed depend directly from each breed market position i.e. the quality of raw slaughter material, the traditional and regional products possible to obtain and of the promo-marketing activities. For above reason there is lead a campain "Wieprzowina regionalna – doceń smak tradycji" (regional pork meat-appreciate the taste of tradition).

The fatteners of native breeds, despite of lower content of meat in the carcass, are characterized by a high meat quality with the proper meat myofibrils structure, good marbling and very good sensory characteristics. These assets of native breed meats cause that they are the basic raw material for regional meat products (*Hammermeister and Blicharski, 2007*; *Szyndler – Nędza et al., 2011*). The meat of Zlotnicka Spotted breed is especially suited for production of long ripened meat products (*Szyndler-Nędza et al., 2012; Szulc et al., 2012*).

According to *Kušec et al. (2015)* protection of small local breeds from extinction through direct payments from governments may help, but it is not sustainable, so efforts should be made sustainable by marketing.

Conclusions

The meat of Pulawska, Zlotnicka White and Spotted breeds of pigs is a good quality raw material which is used for production of traditional and regional meat products gaining high sensory scores and good recognition among consumers. The meat of Zlotnicka Spotted breed deserves the special attention because of advantages intramuscular fat content, lower thermal losses and smaller shear force.

The pigs of Pulawska, Zlotnicka White and Spotted belong to native preserved breeds. Even though these breeds are characterized by lower parameters of fattening and slaughter yields, their asset is a good quality raw material used for traditional and regional meat products gaining the recognition from consumers. The traditionally smoked tenderloins produced using the meat of above mentioned breeds, are characterized by very good quality parameters. The analysed smoked loins were safe due to the low content of polycyclic aromatic hydrocarbons. The consumer panel assessed as the best the tenderloin produced from Zlotnicka Spotted pig meat. The high scores granted by consumers to tenderloins obtained from native pig breeds hold the good promise for their future. Promotion of traditional products obtained from above assessed breeds will favour the development of their breeding and stocks rising.

Kvalitet tradicionalno dimljene pečenice dobijenih od mesa autohtonih rasa svinja

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Rezime

Domaća (nativna) populacija - rase svinja su rase nastale u određenom regionu i/ ili zemlji kao lokalne, primitivne rase. U Poljskoj to su 3 rase svinja: pulavska, zlotnička bela i zlotnička šarena svinja. Cilj rada bio je procena kvaliteta mesa i tradicionalno dimljenih pečenica dobijenih od mesa autohtonih rasa svinja koje se uzgajaju na tradicionalan način. Analiza mesa pokazala je da je najveći udeo masti u zlotničkoj šarenoj svinji (4,60 ± 0,33) i beloj (3,63 ± 0,41), dok je najmanji u

pulavskoj (2,78 ± 0,29). Vrednosti dobijene za silu presecanja dimljene pečenice se statistički razlikuju. Vrednosti tvrdoće bile su najviše kod zlotničke bele (65,66 ± 6,46), dok su najniže bile kod zlotničke šarene (33,27 ± 4,12). Slične vrednosti sile presecanja utvrđene su kod zlotničke bele (51,90 ± 7,11), odnosno kod zlotničke šarene rase (30,43 ± 5,54). Bez obzira na dobijene rezultate, mesni proizvodi su dobili dobre ocene na testu senzorne procene i nizak nivo policikličkih aromatskih ugljovodonika. Zaključujemo da je meso pulavske, zlotničke bele i šarene rase svinja kvalitetna sirovina koje se može koristiti za proizvodnju tradicionalnih i regionalnih mesnih proizvoda čime se postižu visoki senzorni rezultati i dobro prepoznaju kod potrošača. Tradicionalno dimljene pečenice proizvedene od mesa navedenih rasa odlikuju su vrlo kvalitetnim parametrima.

Ključne reči: tovljenici, autohtone rase, meso, žitarice, kvalitet

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PHYSICOCHEMICAL CHARACTERISTICS OF ACACIA AND MEADOW HONEY FROM DIFFERENT REGIONS OF THE REPUBLIC OF SRPSKA/BOSNIA AND HERZEGOVINA WITH AN EMPHASIS ON THE ENVIRONMENT OF BEEKEEPING ZONES

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Original scientific paper

Abstract: Honey is a thick, sweet, syrupy substance, the product of the honey-bee, *Apis mellifera L.*, obtained from the collected fruit juices and other, processed in the stomach of bees and is a pure product with no additives of any other substance. The paper present physical-chemical analysis for the following parameters, performed on 20 honey samples: sugar content, sucrose content, moisture content, free acidity, electrical conductivity, mineral content, the content of HMF and content of matter insoluble in water. As important indicators of the environment, this paper emphasises the analyses on the presents of antibiotic residues in two types of honey. The importance of establishing these indicators is to protect nature, which is the basis of agricultural development in the Republic of Srpska. The work suggests that the described bee region presents qualitative honey produced by using the natural resources of a designated area as an economic sector for the survival and development of those parts of the Republic of Srpska.

Keywords:physicochemical characteristics, honey, antibiotic, environment

Introduction

Honey as a primary product of the honey-bee, is a natural product, variable not only in colour, taste and smell but also in its chemical composition and depends on the ecological environment and the vegetation of the honey plants (*Bogdanov, 2009*). Considering the number of possible sources of honey grazing, it is understandable that there is no completely identical honey from different localities (*Ivanovic et al., 2015; Matović et al., 2018*). While this is an under-clarified issue from the market demand perspective, at the same time, this diversity makes it possible to choose the type of honey with the best features. Scientifically, this

variability can be the result of various factors, but in most cases, it is related to the botanical origin of honey and agroecological conditions (*Kaškonienė and Venskutonis, 2010*).

In the European Union, the results of the analysis of honey on the content of heavy metals and pesticide residues serve as an indicator of the degree of environmental contamination, that is, the state of product quality of the area (*Al-Waili et al., 2012*).

From a chemical point of view, honey is a highly concentrated solution of a complex sugar mixture (*Codex Alimentarius, 2001*). Its composition depends largely on the plant species from which the nectar or honeydew was collected, as well as the environmental and climatic conditions. In addition to sugar, honey has a wide range of less present compounds, many of which including polyphenols, have anti-oxidant effects. Honey is considered a potential complete food, in terms of dietary standards, as a natural product, rich in simple and highly assimilable sugars, enzymes, amino and organic acids, vitamins, volatile oils, minerals, carotenoids, phenolic acids and flavonoids (*Belitz et al., 2009; Milojković Opsenica et al., 2015*).

The Codex Alimentarius, the EU Legislation and the National Standards of the States prescribe the authenticity of honey. Authenticity in terms of production and authenticity in terms of geographical origin and botanical originality are two major aspects of the general authenticity of honey (*Lazarević, 2016*).

The presence of established honey types will be an indicator of the variety and originality of honey, which will result in the separation of monofloral from polyfloral honey, as well as suppression of counterfeiting. The assumption is that this established network of the most important grazing areas of the Republic of Srpska lays the foundation for the determination of ecologically clean zones in our area. This paper points to the possibilities of economic and agricultural justification in the production of ecologically quality honey in 4 different regions. The research should point to the environment in which we are dealing with agriculture, ie the possibilities of sustainability of our products, through quality, in the world market.

Materials and Methods

Samples were collected during 2011, at the end of honey collection season. An investigation of samples was done at the Institute for public health of the Republic of Srpska (RS), with a place in Banja Luka and the Institute for hygiene and technology of meat in Serbia, with a place in Belgrade. A total of 20 samples of different species of honey (acacia and meadow honey) was taken from 4 different parts of RS. From Hercegovina region were collected 5 samples of meadow honey, the same was for Romanija region, for both, Doboj and Krajina were taken 5 samples of acacia honey. For a determinate of botanical region of honeys, we used their declarations, sensory characteristic and geographical origins.

Samples of honey were kept in their original packages at 20-21°C in the dark before laboratory analysis. A honey investigation was done using the International Honey Commission (2009), recommended standard methods of official analyses methods from the Association of Official Analytical Chemists (*AOAC, 1990*). Chemical analyses measured the following perimeters: total inverted sugars, reducing sugars, sucrose, water, acidity, electrical conductivity, mineral materials, hydroxymethylfurfural (HMF) and water-insoluble content.

The content of sugars was measured by aperture Meopta (Meopta, Germany), testing was applied with refractometric detection (HPLC-RI) and automatic compensation of temperature between 10-30 °C (*Rybak-Chmielewska, 2007*), concentration of sugar is presented in the form of percentage (%).

Water content was estimated from the refractive index measured with refractometric detection (HPLC-RI) by aperture Meopta (Meopta, Germany), testing was applied and automatic compensation of temperature on 20°C, after waiting for 6 min for equilibrium and results were converted in the form of a percentage (%). Form determination of free acidity was used via the titrimetric methodology with 0.1 of sodium hydroxide. The solution was titrated with 0.05 NaOH and parameters are presented in mEq/kg. Electrical conductivity (EC) measured at 20°C in a 20% of honey solution in water with a KIT consort conductometer (Wissenschaftlich-Technische Werkstätten, Germany) and expressed as mS/cm. Mineral materials and water-insoluble content were measured by gravimetric method determinated by Labmaster-a apparatus (Novasina**,** Switzerland) on 25 °C, results are expressed in precent (%).

The HMF (hydroxymethylfurfural) content was done on a HPLC apparatus/method (Hewlett Packard, USA). A detailed procedure of HMF determination described by *Zappala et al. (2005)*, parameters are expressed in mg/kg, according to the International Honey Commission *(2009).*

Both laboratories of Institutes have implemented accreditation by BAS-EN-ISO/IEC 17025-2006; LI-40-01, for good quality system results of physical – chemical analyses. Results were compared with Codex Alimentarius, BiH regulations (*Codex Alimentarius 2001, Rulebook BiH, 2009, 2011*) and European Council Directive 2001/1100/EC (2002).

For determination of antibiotic residues microbiological method "Modified method 4 plates" was used (Heitzman, 1994). Determination was done with a test MUVA PROFICIENCY TESTING apertures (Kempten, Germany). The main principle of the test is on detection of microorganism growth inhibition during the presence of antibiotics, test consist a layer of inoculated nutrient agar (*Apić et al., 2015*). In BiH, the use of antibiotic to prevent bacterial diseases is prohibited and accordingly no MRL (maximum residue limits) for antibiotic in honey, in this paper we use relevant provisions for of certain ordinances and prescribed methods for quality control (*Rulebook, BiH 2009).*

Statistical data processing

Data were analysed by one-way analysis of variance (effect of experimental group) using GLM procedures in the statistical software SPSS Statistics (version 17.0). Data are presented as LS means with respective standard errors. In case that *F*-test was significant (P<0.05) differences between samples were evaluated using the Tukey test.

Results and Discussion

Physicochemical characteristics of acacia and meadow honey

Results of physicochemical characteristics of two types of honey were presented in table 1 and 2.

Table 1. Results of physico-chemical parameters of Acacia honey from Krajina and Doboj regions of RS (LS means ± SE).

Type of honey	**Acacia**	**Acacia**	***p* – Value**
Region	**Krajina**	**Doboj**	
Number of honeys	**5**	**5**	
Total inverted sugars (%)	76.25 ± 1.32	77.77 ± 1.14	0.42
Reducing sugars (%)	73.10 ± 1.26	73.55 ± 0.29	0.74
Sucrose content (%)	2.97 ± 0.29	4.05 ± 1.15	0.40
Water (%)	16.50 ± 0.58	16.62 ± 0.50	0.87
Free acidity (milieqv kis/g)	11.70 ± 1.51	9.62 ± 0.62	0.25
Electrical Conductivity (mS/cm)	0.28 ± 0.04	0.23 ± 0.01	0.28
Mineral materials (%)	0.04 ± 0.00	0.04 ± 0.01	1.00
HMF (mg)	5.67 ± 1.09	7.65 ± 2.80	0.53
Water-insoluble content (%)	0.01 ± 0.00	0.01 ± 0.00	0.35

Carbohydrates are the main constituent of honey with content of 73-83%, so honey can be defined chemically as a saturated solution of sugar. It makes up more than 95% of the dry matter in honey, fructose and glucose monosaccharides about 85-95% of the total sugar content (*Lazarevic, 2016*). Statistically differences between the regions of acacia honey, for the content of total inverted sugars were not observed (Table 1). Environmental factors probably had no influence on a variation of redacted sugars in honey. Also, water and humidity as well as wind can affect the honey-bee and the physical and chemical properties of honey (*Semkiw et al., 2008*). Other authors report similar results (*Marghitas et al. 2009; Ciric et al., 2018; Đogo Mračević et al. 2019*). Furthermore, higher content of the invert is present in the Herzegovina region (Table 2), because the external environmental

factors (humidity, sunlight) provided good bee pasture. The weather conditions were ideal, the flowering period of the plants is much longer than in the Romani region. Also, the type of honey plants affects the sugar content of honey, in Herzegovina, honey plants with a higher sugar content (sage, chestnut) are more prevalent. Similar results were reported by *El Sohaimy at. al.*, (2015), where is explanted that content of sugar is indicator of ability for honey crystallisation. Parameters are in accordance with international standards established by the *Codex Alimentarius Commission (2001)* (with a level of not less than 60g/100g of reducing sugars in honey).

Table 2. Results of physico-chemical parameters of meadow honey from the Hercegovina and Romanija region of RS (LS means ± SE).

Type of honey	**Meadow honey**	**Meadow honey**	
Region	**Hercegovina**	**Romanija**	***p* - Value**
Number of honey	**5**	**5**	
Total inverted sugars (%)	78.50 ± 0.94^{a}	70.76 ± 2.49^{b}	0.02
Reduced sugars (%)	75.12 ± 1.41^{a}	68.40 ± 2.58^{b}	0.05
Sucrose content (%)	3.25 ± 0.70	2.30 ± 0.34	0.26
Water (%)	16.54 ± 0.80	17.34 ± 0.40	0.40
Free acidity (milieqv kis/g)	23.76 ± 1.95	28.76 ± 1.98	0.11
Electrical Conductivity (mS/cm)	0.62 ± 0.04^{a}	0.77 ± 0.04^{b}	0.05
Mineral materials (%)	0.22 ± 0.07	0.22 ± 0.01	0.94
HMF (mg)	7.40 ± 1.49	7.40 ± 4.54	1.00
Water-insoluble content (%)	0.02 ± 0.01	0.02 ± 0.01	1.00

Notes: [a,b]Statistically significant differences between the groups are denoted by different superscript letters.

The allowed water content in honey, according to Codex Alimentarius and BiH regulations (*Codex Alimentarius 2001, Rulebook, 2011*) should not be more than 20%.

The water content of honey determines its stability and quality (*Abdulkhaliq and Swaileh, 2017*). The most suitable would be to store honey containing less than 18% water without a risk of fermentation, although the ordinance provides for up to 20% (*Bilić, 2013*). Water content is the most important parameter of honey quality, as it plays a key role in preserving stability and preventing microbial processes that result in fermentation and the spoilage of honey (*Lazarevic et al., 2013*).

The water content of acacia honey of the Herzegovina region is 16.54 % and in the region of Romania 17.34, 16,50 % for the meadow honey in Krajina and 16.25 %

in the Doboj region. Results are in accordance with another published research (*Mirjanić and Mladenović, 2012; El Sohaimy et al., 2015; Vranić et al., 2017; Ciric et al., 2018*).

The water content affects the significant physical properties of honey, such as crystallisation and viscosity (*Bilić, 2013*). The higher content of water in honey, follows a fermentation process caused by the osmophilic yeast (*Saccharomyces spp*.), the fermentation process leads to changes in the chemical composition and organoleptic properties of honey (because fermentation of glucose and fructose produces ethanol and carbon dioxide) which creates a foam on the surface of the product (*Isengard and Daniela 2003; Gallina et al., 2010*).

Very important factors for the classification of honey analyses according to their geographical origin are water content, electrical conductivity and free acidity (*Ciric et al. 2018*).

Differences between 4 regions of two types of honey were not observed for free acidity. Results are according to the Codex Alimentarius and BiH regulations (not more than 50 meq/kg). While electrical conductivity was only statistically different between the Hercegovina and Romanija regions for meadow honey (p - 0,050). Some authors state that EC is in relationship with measurements of ash and acid (El Sohaimy et al. 2015). In our research free acids were higher in meadow honey compared to acacia honey, which agrees with other published papers (*Cirić et al. 2018, Vranić et al., 2017*). The hydroxymethylfurfural parameter in honey represents damage caused by heating, it is usually absent in fresh and untreated honey (*Matović et al., 2018*). Our results agree with maximum limited concentration of 40 mg/kg, recommended by the European Union (*EU Directive 110/2001*). The HMF between two groups of meadow honey was 7.40 mg/kg, between the acacia range from 54.67 to 7.65 mg/kg. Results indicated that honey from different regions was of a good quality; results are not in agreement with *the Ciric et al* (2018) research. Similar results were obtained for acacia honey, published by *Vranić et al., (2017).* Minerals are present in honey in very small quantities, mostly potassium. In addition to potassium, honey contains other chemical elements such as sodium, calcium, magnesium, iron, copper, manganese, aluminum, molybdenum, cobalt, zinc, chlorine, phosphorus, sulfur, etc (*Bilić, 2013; Czipa et al., 2019*). Average values do not differ statistically significantly between measured groups. The same results were obtained for acacia and linden honey in a research in Serbia (*Vranić et al., 2017, Matović et al., 2018*). A research published by *Grujić and Komić* (2012) states that with a different content of minerals materials can prove the botanical origin of different species of honey.

The maximum permissible reference value for the content of substances insoluble in water for meadow and acacia honey is 0.1 g /100g, the values obtained in all four beekeeping regions vary between 0.010 g/100g and 0.028 g100/g, which is in accordance with the criteria for physico-chemical parameters of honey established by regulations (*Official Gazette BiH, 2009, 2011*). Results are in accordance with

results published for research in the Balkan region (*Vranić et al., 2017, Ciric et al., 2018. Matović et al., 2018*).

Table 3. shows the results of the presence of antibiotics in honey, it demonstrates that antibiotic residues were not found in all 20 samples honey. The results are in accordance with SFRJ regulation for the pesticides residues, metals and metalloids, chemotherapeutics. anabolic and other substances that may be present in foods (*Rulebook SFRJ, 2002*).

Table 3. Results of laboratory test of honey samples for the presence of residues of antibiotic

Antibiotics residues	**Meadow honey**	**Acacia honey**	**Prescribed value**
Units	mg/kg		
Number of honeys	10	10	
Amoxycillin	not determined	not determined	not allowed
Ampicillin	not determined	not determined	not allowed
Benzil penicilin G	not determined	not determined	not allowed
Chlortetracycline	not determined	not determined	not allowed
Cloxacillin	not determined	not determined	not allowed
Dicloxacillin	not determined	not determined	not allowed
Dihydrostreptomycin	not determined	not determined	not allowed
Doxicycline	not determined	not determined	not allowed
Erythromycin	not determined	not determined	not allowed
Gentamycin	not determined	not determined	not allowed
Kanamycin	not determined	not determined	not allowed
Lincomycin	not determined	not determined	not allowed
Neomycin	not determined	not determined	not allowed
Oxacillin	not determined	not determined	not allowed
Oxytetracycline	not determined	not determined	not allowed
Spectinomycin	not determined	not determined	not allowed
Tetracycline	not determined	not determined	not allowed
Tylosin	not determined	not determined	not allowed

In the honey and its product, antibiotics are often used for veterinary setting, such as streptomycin, sulfonamide, and chloramphenicol (*Al-Waili et al., 2012*). Maximum residue limits were not allowed for honey and its products by regulation of European Union (*EC Directive 1990, 2009 and 2010*). High levels of antibiotics in honey exported from India to the EU and the US have been reported by agricultural food producers to the Development Agency from 2005 onwards (*Solomon et al., 2006)*. In 2006, about 14% of samples were contaminated with

tetracyclines and between 2007-2008 about 28% of the samples were contaminated with the same antibiotic. In the period between 2009-2010, 462 honey samples were analysed, 29.2% of the samples had more than the prescribed limit for the presence of antibiotics. In 2000-2001, streptomycin was detected in 4/248 samples, tetracycline in 2/72 samples, and sulfonamides in 1/72 samples (*Solomon et al., 2006, Forsgren, 2010*). Our presented results are not in accordance with published results by authors *Ortelli et al. (2004), Saridaki-Papakonstadinou et al., (2006) and Johnson et al., (2010),* where a large number of studies cited in the last 20 years have identified antibiotics in wax, pollen, bees and honey. With these presented results, we can confirm ecological clean beekeeping zones in the RS.

Conclusion

An examination of the physico-chemical parameters has determined a fulfilment of requirements regarding the satisfaction of the quality of monofloral and polyfloral honey of the Republic of Srpska in accordance with domestic and European Regulations. Counterfeits in the work have not been proven. It is obtained that mountain honey in comprising with honey from lowland plains, was of a higher quality. The microbiological safety of the tested honey species (presence of antibiotics) in the Republika Srpska region was determined.

The results of the analyses presented in this paper, confirm that two types of honey from different parts of the Republika Srpska, due to the diversity of honey and medicinal plants, climatic conditions and that type of production represents a great potential for the investigated areas and deserves a great commitment from competent institutions.

Fizičko-hemijske karakteristike bagremovog i livadskog meda iz različitih područja Republike Srpske / Bosne i Hercegovine s naglaskom na okruženje pčelarskih zona

Diana Bilić-Šobot

Rezime

Med je gusta, slatka, sirupasta supstanca, proizvod medonosne pčele, *Apis mellifera L.*, dobijen od prikupljenih voćnih sokova i drugog, prerađenog u stomaku pčela i čisti je proizvod bez dodataka bilo koje druge supstance. Fizičko-hemijska analiza za sledeće parametre izvedena je na 20 uzoraka meda: sadržaj

šećera, saharoze, sadržaj vlage, slobodna kiselina, električna provodljivost, sadržaj minerala, sadržaj HMF i materija nerastvorljivih u vodi. Kao važan indikator životne sredine, u ovom radu se ističu analize zastupljenosti ostataka antibiotika u dve vrste meda. Važnost uspostavljanja ovih pokazatelja je zaštita prirode, što je osnova razvoja poljoprivrede u Republici Srpskoj. Rad sugeriše da opisani pčelinji kraj predstavlja kvalitativni med proizveden korišćenjem prirodnih resursa određenog područja, kao ekonomskog sektora za opstanak i razvoj tih delova Republike Srpske.

Ključne reči: fizičko-hemijske karakteristike, med, antibiotici, životna sredina

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NATURAL TOXIGENIC FUNGAL AND MYCOTOXIN OCCURRENCE IN MAIZE HYBRIDS

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Abstract: The objective of the present study was to investigate the susceptibility of maize hybrids to the natural occurrence of toxigenic fungal species, in particular toxigenic *Aspergillus* and *Fusarium* species, and mycotoxins (aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins B_1, B_2 and B_3 (FBs)). Grain samples of six commercial maize hybrids (MAS 34.B – FAO 300, MAS 40.F, MAS 48.L, KWS Konfites and ZP 427 – FAO 400, and MAS 56.A – FAO 500) were collected at harvest in 2018. A total of seven fungal genera, *Acremonium, Alternaria, Epicoccum, Fusarium, Nigrospora, Penicillium* and *Rhizopus*, were identified of which only species from the genus *Fusarium* were present on maize grains of all hybrids tested. The incidence of *Fusarium* spp. was higher in the hybrids MAS 48.L (54.6%), and MAS 56.A (53.3%), compared to MAS 40.F (37.3%), KWS Konfites (28%), MAS 34.B (22.6%) and ZP 427 (12%) hybrids. Among the identified *Fusarium* species (*F. graminearum*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides*), *F. proliferatum* was present in all hybrids, ranging from 9.3% (ZP 427) to 30.7% (MAS 48.L), whereas *F. subglutinans* was present in two hybrids, MAS 40.F (16%) and MAS 56.A (9.3%). The incidence of *F. graminearum* ranged from 0% (KWS Konfites) to 9.3% (MAS 34.B), while the incidence of *F. verticillioides* ranged from 0% (MAS 34.B and ZP 427) to 21.3% (MAS 48.L). In the samples, *Aspergillus* species were not identified. The effect of maize hybrids was significant on the level of mycotoxins. MAS 34.B hybrid had a statistically significantly higher levels of AFB_1 and DON than other hybrids. The FBs level was the highest in the hybrid MAS 34.B (1202 μg kg^{-1}) and the lowest in the hybrid KWS Konfites (88.33 μg kg^{-1}). However, the FBs level did not differ between hybrids MAS 34.B, MAS 40.F, and MAS 56.L, MAS 40.F, MAS 48.L, and MAS 56.A, and KWS Konfites and ZP 427. In all hybrids, AFB_1, DON, and FBs levels were below the maximum permissible levels stipulated by the legislation of the European Union and the Republic of Serbia in unprocessed maize.

The agro-ecological conditions in 2018 favored the development of *Fusarium* species on maize grains of the hybrids tested, especially fumonisin producing species.

Key words: toxigenic fungal species, mycotoxins, maize hybrids

Introduction

Maize is the main cereal crop in Serbia, grown on about one million hectares (*Statistical Yearbook of the Republic of Serbia, 2019*). It is used for human and animal nutrition and industrial processing. However, under stress abiotic and biotic factors, the maize grains can be infected by mycotoxigenic fungi. The most common mycotoxigenic fungi isolated from harvested and stored maize grains belong species from the genera *Fusarium*, *Aspergillus* and *Penicillium* (*Krnjaja et al., 2015*). These fungal species produce secondary metabolites (mycotoxins) which cause adverse effects on animal and human health, and economic losses. In particular, species from genera *Aspergillus* and *Fusarium*, causers of ear rot, cause serious risk of mycotoxin accumulation in maize (*Masiello et al., 2019*). Recently, aflatoxins, which are secondary metabolites produced by *Aspergillus flavus* Link and *A. parasiticus* Speare have been isolated in high levels on the maize grains in Serbia (*Kos et al., 2018; Obradović et al., 2018*). Also, mycotoxins such as type B trichothecenes (deoxynivalenol) and zearalenone produced primarily of *Fusarium graminearum* Schwabe and fumonisins produced mainly of *F. verticillioides* (Sacc.) Nirenberg and *F. proliferatum* (Matsush.) Nirenberg have been detected on maize grains in Serbia (*Jajić et al., 2008; Krnjaja et al., 2015; Obradović et al., 2018; Jakšić et al., 2019*).

Farm animals are sensitive to higher mycotoxin levels. Among the four main aflatoxins (B_1, B_2, G_1, and G_2), aflatoxin B_1 (AFB_1) is the most toxic and causes liver damage in animals. The toxic effects of deoxynivalenol (DON) on pigs are feed rejection, vomiting, reproductive, and neurological disorders *(Biagi, 2009; Reddy et al., 2017)*. Leukoencephalomalacia of horses and porcine pulmonary edema of pigs are diseases caused by fumonisins B_1, B_2 and B_3 (FBs) *(Dohnal et al., 2010; Leggieri et al., 2015)*.

Maize grains can be contaminated with toxigenic fungi and their mycotoxins before and after harvesting. High temperatures and levels of humidity from silking to maturity stages are favourable conditions for fungal ear colonization by *Fusarium* species and synthesize of *Fusarium* mycotoxins (*Logrieco et al., 2002*), while drier growing seasons suitable for the growth of *Aspergillus* spp. and aflatoxin accumulation in maize crops (*Giorni et al., 2019*). The occurrence of mycotoxins in maize grains is also dependent on hybrid susceptibility (*Blandino et al., 2017*).

The control strategy for the prevention of fungal and mycotoxin contamination of maize grains includes pre- and post-harvest measures. The most important preventive measures in the pre-harvest time are good agricultural crop practices and the utilization of tolerant maize hybrids (*Blandino and Reyneri, 2008*). The drying grains to below 15% moisture content, insect control, the application of detoxification methods are the most common post-harvest measures in storages (*Di Gregorio et al., 2014; Kumar and Kalita, 2017*).

Since the selection and sowing of the tolerant and less susceptibility maize hybrids as one of the success measures in reduce fungal contaminants, the research purpose was to determine the natural occurrence of toxigenic fungal species, in particular, toxigenic *Aspergillus*, and *Fusarium* species, and mycotoxins, AFB_1, DON, and FBs, in five foreign and one domestic maize hybrids in agro-ecological climate conditions in Serbia.

Materials and Methods

Fungal and mycotoxin contamination of maize grains was evaluated in six commercial hybrids. Four French hybrids (MAS 34.B - FAO 300, MAS 40.F and MAS 48.L - FAO 400, and MAS 56.A - FAO 500), one German hybrid (KWS Konfites - FAO 400) and one Serbian hybrid (ZP 427 - FAO 400) were investigated.

Hybrids were grown in 2018 in the experimental field of the Institute for Animal Husbandry, Belgrade-Zemun. The sowing and harvesting date of the hybrids was consistent with the FAO maturity groups. The plot size was 440 m x 50.4 m, sub-plot was 440 m x 8.4 m. Each maize hybrid sown in 12 rows, with a 0.7 m inter-row spacing. Crop densities were in accordance with manufacturers recommendations.

Maize grain samples were collected at harvest time. The sub-plot divided into three parts. A total of 30 ears were randomly taken per each hybrid (sub-plot), 10 ears from each part, then put in the paper bags and transferred to the laboratory. The maize grains of 10 ears (sub-sample) manually removed. A total of 18 maize grain sub-samples, each sub-sample approximately of 500 g weight, were kept at 4°C until analyses.

The moisture content of maize sub-samples was determined using OHAUS MB35 (USA) moisture analyser. In mycological analyses, maize grains were disinfected in 1% NaOCl (sodium hypochlorite) for a few minutes, rinsed in distilled water, and dried on the filter paper. Per each hybrid, 300 grains were plated on potato dextrose salt agar, 100 grains per sub-sample, 5 grains per plate (*Krnjaja et al., 2019*). After14 days of keeping plates on the room temperature, fungal species were identified using fungal keys of Leslie and *Summerell (2006)*

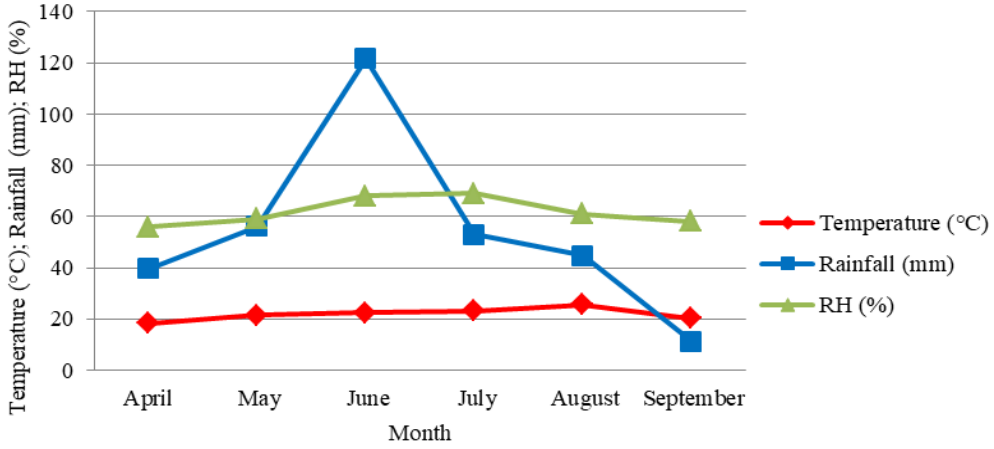
and *Watanabe (2002)*. The incidence of fungal species on maize grains was calculated according to *Lević et al. (2012)*.

Before the mycotoxicological analysis, ground maize sub-samples dried during 72 h at 60°C. Enzyme-Linked Immunosorbent Assay (ELISA) for determining AFB_1, DON, and FBs levels was applied according to the manufacturer's instructions Celer Tecna® ELISA kits in three repetitions. The limit of detection for AFB_1. DON and FBs were 1 μg kg^{-1}, 40 μg kg^{-1} and 750 μg kg^{-1}, respectively.

Effect of different maize hybrids on the mycotoxin levels was determined by one-way analysis of variance (One-Way ANOVA) using SPSS software (IBM, Statistic 20). Comparing means values with significant differences at $P \leq 0.05$ was done using the Tukey's test. The Pearson correlation coefficient was used in correlation analyses.

Results and Discussion

In a Belgrade area, according to the meteorological data of the Republic Hydro Meteorological Services of Serbia, in 2018, the mean monthly temperatures (> 20°C), total monthly rainfall (> 40 mm) and mean monthly relative humidity (RH) (> 60%) at the flowering stage (July) and milk stage (August) were suitable for fungal maize colonization (Graphic 1).



Graphic 1. Mean monthly temperatures, total monthly rainfall and mean monthly relative humidity (RH) in Belgrade area from April to September in 2018

The average moisture content of maize grain sub-samples was the highest in the hybrids MAS 34.B and MAS 56.A (16.4%), followed by hybrids MAS 48.L

(14.8%), ZP 427 (14.3%), KWS Konfites (13.9%) and MAS 40.F (12.5%) (data not presented).

In the mycological analysis, the seven fungal genera were identified: *Acremonium*, *Alternaria*, *Epicoccum*, *Fusarium*, *Nigrospora*, *Penicillium* and *Rhizopus* (Table 1.) The genus *Fusarium* was present on grains of all maize hybrids. The high incidence of *Fusarium* spp., on the maize grains, was found in the hybrids MAS 48.L (54.6%) and MAS 56.A (53.3%), followed by hybrids MAS 40.F (37.3%), KWS Konfites (28%), MAS 34.B (22.6 %) and ZP 427 (12%). *Aspergillus* species were not identified in maize grain samples. Similar, *Tančić Živanov et al. (2019)* have identified 16 fungal genera, with the *Fusarium* species as the dominant pathogens in two Serbian commercial dent maize hybrids from FAO 600 maturity group, while *Aspergillus* and *Penicillium* species were established in low incidence. Also, in Italy, *Covarelli et al. (2011)* have found that the genus *Fusarium* was the most frequent on maize grains, followed by *Aspergillus* and *Penicillium* genera. Contrary, in Tunisia, the fungal genus *Aspergillus* was the predominant genus in the harvest maize grain samples in 2011 (*Jedidi et al., 2018*).

Table 1. The incidence (%) of fungal genera in maize hybrids tested in 2018

	Fungal genus						
Maize hybrid	*Acremonium*	*Alternaria*	*Epicoccum*	*Fusarium*	*Nigrospora*	*Penicillium*	*Rhizopus*
MAS 34.B	0	0	13.3	22.6	38.7	0	18.7
MAS 40.F	30.7	4	0	37.3	16	2.7	0
MAS 48.L	0	10.7	0	54.6	1.3	8	2.7
KWS Konfites	18.7	4	0	28	29.3	0	0
ZP 427	1.3	6.7	0	12	13.3	10.7	0
MAS 56.A	0	0	0	53.3	2.7	0	4

Among isolated *Fusarium* species, *F. proliferatum* was identified in all hybrids in range from 9.3% (ZP 427) to 30.7% (MAS 48.L) (Table 2). *F. subglutinans* was identified in MAS 40.F (16%) and MAS 56.A (9.3%) hybrids. The incidence of *F. graminearum* was ranged from 1.3% (MAS 48.L) to 9.3% (MAS 34.B), but not isolated in the KWS Konfites hybrid. The incidence of *F. verticillioides* was ranged from 1.3 (MAS 40.F) to 21.3% (MAS 48.L), but not isolated in the hybrids MAS 34.B and ZP 427. Sterile mycelia was isolated only in two hybrids MAS 34.B (6.7%) and KWS Konfites (2.7%), and from 0% (MAS 34.B) to 56% (ZP 427) grains were without mycelia (data not presented).

The incidence of *Fusarium* species on the maize grains was similar to the reported data of *De Curtis et al. (2011)* and *Covarelli et al. (2011)*. Analyzing the susceptibility of three maize hybrids to *Fusarium* and FBs contamination, in Southern Italy, *De Curtis et al. (2011)* have isolated *F. proliferatum* (up to 81.5%) and *F. verticillioides* (up to 26.5%) as the predominant fungal species in both

investigated years (2005-2006). Then, *Covarelli et al. (2011)* were obtained that the *F. verticillioides* was the most predominant species isolated from the maize grains in 2006 (40.2%) and 2007 (65.2%) in central Italy, followed by *F. proliferatum* (up to 7.8%), *F. subglutinans* (up to 5.7%), while *F. graminearum* (2.6%), *F. culmorum* (0.9%), and *F. sporotrichioides* (0.9%) were isolated only in 2006. A recent study of *Tančić Živanov et al. (2019)* demonstrated that 11 *Fusarium* species were isolated from the grains of two commercial maize hybrids grown in Serbia, of which *F. verticilioides*, *F. graminearum*, and *F. proliferatum* were predominant.

Table 2. The incidence (%) of *Fusarium* species in maize hybrids tested in 2018

Maize hybrid	*Fusarium* spp.			
	F. graminearum	*F. proliferatum*	*F. subglutinans*	*F. verticillioides*
MAS 34.B	9.3	13.3	0	0
MAS 40.F	6.7	13.3	16	1.3
MAS 48.L	1.3	30.7	0	21.3
KWS Konfites	0	14.7	0	13.3
ZP 427	2.7	9.3	0	0
MAS 56.A	6.7	18.7	9.3	18.7

Levels of mycotoxins were significantly affected by hybrids (Table 3). The levels of AFB_1 and DON were significantly higher in the hybrid MAS 34.B compared to other hybrids. AFB_1 and DON levels found were below the detection limits in the hybrids KWS Konfites, ZP 427, and MAS 56.A, and KWS Konfites and MAS 56.A, respectively. There were no significant differences between hybrids MAS 34.B, MAS 40.F, and MAS 56.A, MAS 40.F, MAS 48.L, and MAS 56.A, and KWS Konfites, and ZP 427 for FBs levels. The highest FBs level was found in the hybrid MAS 34.B (1202 μg kg^{-1}), while the lowest FBs level was in the hybrid KWS Konfites (88.33 μg kg^{-1}).

Mycotoxin analyses showed that in the hybrids tested, the mean levels of AFB_1, DON and FBs did not exceed maximum limits of 5, 1750, and 4000 μg kg^{-1}, respectively, prescribed by European Commission (*EC, 2007; 2010*) and Serbian Regulation (*Službeni Glasnik RS, 2014*) for unprocessed maize.

Similar to our results, *Blandino et al. (2017)* have also established that FBs and DON levels in maize grains influenced by the type of the hybrids from FAO 500 and 600 maturity groups and environmental conditions. In addition, *Van Rensburg et al. (2016)* concluded that seven South African maize genotypes were differed in susceptibility to natural fungal and FBs contamination. *Leggieri et al. (2015)* found that there were no significant differences in the aflatoxins level between maize hybrids from different FAO maturity groups in two-year trials (2009-2011). Also, in Serbia, in 2013, *Krnjaja et al. (2016)* have demonstrated that there were no significant effects of hybrids and the interaction effect of hybrids and

location on the level of AFB_1 in the maize hybrids from FAO 300, 400, 500, and 600 maturity groups.

Table 3. The effect of maize hybrids on the level of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and fumonisins (FBs)

Factor	AFB_1 (μg kg^{-1})	DON (μg kg^{-1})	FBs (μg kg^{-1})
MAS 34.B	1.52^{a}	64.67^{a}	1202.00^{a}
MAS 40.F	1.02^{b}	46.78^{b}	1181.89^{ab}
MAS 48.L	1.14^{b}	42.56^{b}	1088.78^{b}
KWS Konfites	< 1	< 40	88.33^{c}
ZP 427	< 1	40.89^{b}	115.33^{c}
MAS 56.A	< 1	< 40	1093.56^{ab}
F test	**	**	**
Mean	1.11	45.82	794.98

Means followed by the same letter within a column are not significantly different according to Tukey's multiple comparison test ($P \leq 0.05$); ** - significant at the 0.01 level of probability, * - significant at the 0.05 level of probability, ns – not statistically significant

Using the Pearson correlation analyses, a statistically significant positive correlation ($P<0.05$) was established among the incidence of *F. verticillioides* and *F. proliferatum* ($r = 0.56$) (data not presented). No significant positive correlations were established among the incidence of *F. graminearum* and *F. subglutinans* ($r = 0.15$), the incidence of *F. verticilioides* and *F. proliferatum* and the FBs level ($r = 0.22$ and $r = 0.08$, respectively), and the incidence of *F. graminearum* and the DON level ($r = 0.40$). The incidence of *F. verticillioides* and *F. proliferatum* was in no significant positive correlations with the moisture grain content ($r = 0.22$ and $r = 0.24$, respectively). The coefficients of correlation indicate that FBs and DON levels were influenced by the incidence of their *Fusarium* producers. In the studies of *Balconi et al. (2014)*, it was also confirmed that the FBs level was depended on the incidence of *F. verticillioides*.

Conclusion

In this study, fungal species of the genus *Fusarium*, as economically important toxigenic species, were isolated from the grains of all maize hybrids, while *Aspergillus* species were not detected in any samples. Climatic factors in the growing season in 2018 were very favourable for the development of *Fusarium* species. Among *Fusarium* species, fumonisin producing species were dominant. The levels of mycotoxins, AFB_1, DON, and FBs, were influenced by hybrids.

These results confirmed that in addition to climatic factors (temperature, rainfall, and RH), the susceptibility of hybrids was also one of the important risks for the appearance of toxigenic fungi and their mycotoxins. Therefore, investigation of susceptibility of hybrids should also be the focus of further studies with an aim for advancing integrated pest management control in the maize production.

Prirodna pojava toksigenih gljiva i mikotoksina u hibridima kukuruza

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Rezime

Cilj rada je bio da se ispita osetljivost različitih hibrida kukuruza na prirodnu pojavu toksigenih vrsta gljiva, posebno toksigenih *Aspergillus* i *Fusarium* vrsta, i mikotoksina (aflatoksina B_1 (AFB_1), deoksinivalenola (DON) i ukupnih fumonizina B_1, B_2 i B_3 (FBs)). Uzorci zrna šest komercijalnih hibrida kukuruza (MAS 34.B – FAO 300, MAS 40.F, MAS 48.L, KWS Konfites i ZP 427 – FAO 400 i i MAS 56.A – FAO 500) sakupljeni su u vreme berbe 2018. godine.

Analizom mikobiota identifikovane su vrste iz sedam rodova, *Acremonium*, *Alternaria*, *Epicoccum*, *Fusarium*, *Nigrospora*, *Penicillium* i *Rhizopus*, od kojih su samo vrste iz roda *Fusarium* bile prisutne na zrnu kukuruza svih ispitivanih hibrida. Veća učestalost *Fusarium* spp. na zrnu kukuruza bila je kod hibrida MAS 48.L (54,6%) i MAS 56.A (53,3%) u poređenju sa hibridima MAS 40.F (37,3%), KWS Konfites (28%), MAS 34.B (22,6%) i ZP 427 (12%).

Među identifikovanim *Fusarium* vrstama (*F. graminearum*, *F. proliferatum*, *F. subglutinans* i *F. verticillioides*), vrsta *F. proliferatum* bila je prisutna kod svih ispitivanih hibrida u rangu od 9,3% (ZP 427) do 30,7% (MAS 48.L), dok je vrsta *F. subglutinans* bila prisutna kod dva hibrida MAS 40.F (16%) i MAS 56.A (9,3%). Učestalost *F. graminearum* je bila od 0% (KWS Konfites) do 9,3% (MAS 34.B), dok je učestalost *F. verticillioides* bila od 0% (MAS 34.B i ZP427) do 21,3% (MAS 48.L). U uzorcima zrna, *Aspergillus* vrste nisu bile identifikovane.

Hibridi kukuruza statistički su značajno uticali na sadržaj ispitivanih mikotoksina. Hibrid MAS 34.B imao je statistički značajno viši sadržaj AFB_1 i DON u odnosu na druge hibride. Sadržaj FBs bio je najviši kod hibrida MAS 34.B (1202 μg kg^{-1}), a najmanji kod hibrida KWS Konfites (88,33 μg kg^{-1}). Međutim, sadržaj FBs nije se razlikovao između hibrida MAS 34.B, MAS 40.F i MAS 56.A, MAS 40.F, MAS

48.L i MAS 56.A i KWS Konfites i ZP 427. Kod svih ispitivanih hibrida, sadržaji AFB_1, DON i FBs bili su ispod maksimalno dozvoljenih količina propisanih zakonskom regulativom Evropske Unije i Republike Srbije u neprerađenom kukuruzu.
Agroekološki uslovi u 2018. godini pogodovali su razvoju *Fusarium* vrsta na zrnu kukuruza ispitivanih hibrida, i to posebno fumonizin producenata.

Ključne reči: toksigene vrste gljiva, mikotoksini, hibridi kukuruza

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EFFECT OF VARIETY AND SEED RATE ON HYDROPONIC MAIZE FODDER BIOMASS YIELD, CHEMICAL COMPOSITION, AND WATER USE EFFICIENCY

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Abstract: Maize varieties BH540, BH660, BH661, and MVFG (unknown variety as local check) were evaluated at low (5.6 kg m^{-2}), medium (7.6 kg m^{-2}), and high (9.6 kg m^{-2}) seed rate for hydroponic fodder productivity. A 3 × 4m wide and 3 m height low-cost plastic house made of translucent plastic and a plastic trays made by bisecting a 25 liter capacity plastic oil container into two equal parts were used for growing the hydroponic fodder. The bottoms of the trays were drilled to open holes to drain excess water during irrigation and placed on shelves.The BH661 exhibited significantly ($p<0.01$) higher dry fodder yield (6.63 kg) per square meter and per kg seed than the other varieties. Among the seed rates, the high seed rate has a higher ($P< 0.01$) Dry Mater (DM) fodder yield, but the medium and low seed rates had greater DM fodder conversion efficiency and lower cost per kg DM fodder production. Water use efficiency was lower for BH540 (64 kg DM fodder per cubic meter water) as compared to the other varieties that had similar values (90 to 95kg DM fodder per cubic meter water). Medium and high seed rates exhibited similar water use efficiency, and it is higher than the low seed rate. Therefore, the use of BH661 variety at medium seeding rate is recommended for maize hydroponic fodder production.

Key words: maize, sprout, variety, hydroponic

Introduction

According to the Central Statistical Agency of Ethiopia *(CSA, 2018),* improved forage covers only 0.32% of the total feed resources under the smallholder production system. Conventional forage production practices in Ethiopia and elsewhere have been constrained by many factors. Long dry period,

unavailability of water for irrigation and competition for land with food crop production are some of the challenges that make the use of improved forage crops still at its low level *(Yayneshet, 2010; Naik et al., 2013)*. As a result, hydroponic fodder production technology has been advocated as a solution in order to overcome the challenges faced by conventional green fodder production and for climate change adaptation *(Muthuramalingam et al., 2015; Saidi and Omer, 2015)*.

However, *Sneath and McIntosh (2003)* and *Dung et al. (2005)* argued that profitable use of sprouting grain as a feed source for commercial cattle production to appear unlikely due to a reduction in dry matter (DM) weight and increase in cost as a result of sprouting. Nevertheless, some other authors noted that it is the ultimate animal performance relative to the alternative costs that determine the profitability and usefulness of hydroponic fodder *(Muela et al., 2005)*. Although such dialogue exists among authors, hydroponic fodder production technology was introduced in some parts of Ethiopia without any preliminary study.

The survey conducted in northwestern part of Ethiopia after four years of its introduction showed that production was based on low-cost hydroponic unit with barley seed and nutrient solution *(Getachew et al., 2018)*. The average cost of a kg of barley seed and a liter of nutrient solution was 0.37 and 3.72USD, respectively. Even though about 75.7% of the materials needed for hydroponic fodder production are available in the area at an affordable price, almost all respondents have the feeling to quit producing hydroponic fodder production because of the high cost of barley seed indicating that searching for an alternative low-cost cereal seed is required.

The cost of maize (*Zea maize*) is much less than barley in Northwestern part of Ethiopia. Maize has also been used by Indian farmers for hydroponic fodder production *(Naik et al., 2015)*. Studies *(Naik et al.,2015, Weldegerima et al., 2015)* showed that about 6 kg of fresh fodder per kg seed with crude protein (CP) content of 13.57 and water efficiency of 1.7 to 4, in as fresh basis, or 0.22 to 0.60 DM fodder per liter water was produced from a kg of maize grain in India. Similarly, barley produced 4.1 to 6.55kg fresh fodder per kg seed with CP content of 9.16 to 13.2% *(Emam, 2016)* and water efficiency of 1.55, in as fresh basis, or 0.11 kg barely fodder per kg water *(AI-Karaki, and AI-Hashimi, 2012)*.

However, the variety of maize available and the environment (especially humidity) differs between countries requiring the evaluation of hydroponic fodder production from different varieties under the prevailing natural environment. Therefore, the present study is conducted to evaluate the potential of locally available maize varieties for hydroponic fodder production at different seed rates on biomass yield, chemical composition, water use efficiency, and cost of production.

Materials and Methods

Experimental Site. The experiment was conducted at the University of Gondar, Atse-Tewodros campus located at 12°36' N latitude, 37°28' Longitude *(Worldatlas, 2016)* and at an altitude of 2133 meter above sea level. The average annual rainfall of the area is 1772 mm and average annual minimum and maximum temperatures are12.3ºC and 26.4ºC, respectively*(NMA, 2013)*.

Hydroponic System and Fodder Production. Hydroponic fodder was produced in 3 × 4m low-cost plastic house (greenhouse) made of translucent plastic. Plastic trays with 46 cm length, 23cm width, and 8cm depth were made by bisecting a 25L capacity plastic oil container into two equal parts. The bottoms of the trays were drilled to open holes at thirteen points to drain excess water from irrigation. The trays were placed on shelves made of eucalyptus tree timber.

Three widely cultivated varieties of maize (BH660, BH661 and BH540) were used. An unknown, possibly mixed variety of maize available in the local market was used as a check. After cleaning the foreign materials the seed of each variety were weighed as per the treatment plan, i.e., low seed rate (5.6 kgm^{-2}), medium (7.6 kgm^{-2}), and high (9.6 kgm^{-2}). Seeds were sterilized by soaking in a 1% sodium hypochlorite solution (household bleach) for one hour separately. Planting trays and other equipments were also cleaned and disinfected with similar solution of 1% sodium hypochlorite. Then the seeds were washed and soaked in tap water (1.5 mlg^{-1} sample) for about 12 hours. We did not find literature on seed to water ratio for soaking. From our preliminary trial conducted, we found that about 1.2 ml water per gram of seed is required to fully submerge the seed in water during soaking. Since maize absorbs 30% of its weight water before germination *(Shaban, 2013)*, we soaked 1 gram of seed with 1.5 ml water. After washing, the seeds were placed in a cotton cloth bag; water was sprinkled over it and kept for about 24 hours to initiate the emergence of radicles. Then the seeds were sown in a plastic tray as mentioned earlier and tap water was sprinkled over the seedlings four times a day. The seedlings were grown for seven days and harvested. This study was conducted in a 4 × 3 factorial experiment in a Complete Randomized Block Design (CRBD) with three replications, considering the position of a tray on a shelf as a block.

Fodder Biomass and Chemical analysis. In the morning of the eight day of growing fodder biomass yield was measured by difference (the weight of the tray with the fodder – the tray weight). The fodder cake were dismantled and mixed to take a representative sample. The feed conversion efficiency was measured by dividing the weight of hydroponic fodder produced by weigh of seed used. Representative fresh samples were weighed and air-dried in a well-ventilated room spreaded on plastic sheet. After air drying, each replicated sample were weighed, packed in a labeled polyethylene bags, dried in the oven at 60 ^{O}C for 72

hours, milled in a Wiley mill at 1 mm sieve size. The samples were then chemically analyzed.Dry matter, nitrogen (N), and ash were determined using the standard procedures of *AOAC (2005)*. Crude protein (CP) was calculated as N x 6.25. Organic matter was calculated by subtracting ash from 100%. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined following the standard procedures of *Van Soest and Robertson (1985)*. Calcium, phosphorus, potassium, and magnesium were determined by Atomic Absorption Spectrophotometer following Perkin-Elmer AAS 2380procedure *(Perkin-Elmer, 1996)*.Sulfur was determined using Turbidimetric method after digestion with HNO_3-$HCLO_4$ *(Tabatabai and Bremner, 1970)*.

Water use efficiency measurement. Throughout the experimental period, the total water added to and drained out of the trays was recorded every day to compute the total water use and water use efficiency. The total water used by plants (liters/tray) was computed as Total water use = Total added water in irrigation − Total drained water out of the trays *(FAO, 1982; AI-Karaki and AI-Hashimi, 2012)*. Water use efficiency (WUE), kgm^{-3} was computed by the following equation:

$$\text{WUE} = \frac{\text{Total green fodder produced (kg/tray)}}{\text{Total water used (liter/tray)}}$$

Production cost of maize hydroponic fodder. The costs considered for hydroponic fodder production were seed, water, chemical, and material (i.e. depreciation of materials for hydroponic fodder unit and other materials like perforating needle, Jeri can, plastic tray and saw blade). The cost for seed, water and chemical was taken from the information collected from the respondents during the survey conducted by *Getachew et al. (2018)*. The depreciation of materials was estimated based on the material cost and their life span. The total yearly depreciation was divided to the number of days in a year (365) and the result multiplied by 8 to find depreciation per production cycle. Labor was not included in cost analysis since smallholder dairy farms practiced as a part-time activity with family labor. The total cost was tested for sources of costs, cost per varieties, and seed rates.

Statistical Analysis. The data were analyzed using SAS (2009) and when the existence of difference between treatment means was declared, Tukey's multiple range test was employed to detect differences between treatments. The model used for data analysis was $Yijk=\mu+Bi+Vj+Sk+(VjSk)+\varepsilon ijkl$; where:Yijk = an observation in block i, variety j and seed rate k; μ = the overall mean; Bi=Block effect; Vj= the effect of Variety j; Sk= the fixed effect of seed rate k; (VjSk) = Interaction effect of variety and seed rate and εijkl = random error.

Results

Fodder biomass yield. Maize fresh hydroponic fodder biomass yield varied significantly ($p<0.01$) among varieties and seed rates (Table 1). The variety BH661 has the highest and that of the check (MVFG) has the lowest fodder yield ($p<0.05$). The fresh and dry fodder yield increased with increasing seed rate. Contrary, fodder conversion efficiency decreased ($p < 0.01$) with increasing seed rate. Interaction between variety and seed rate was not evident for all parameters. Fresh biomass yield showed a variation of 3.85 (BH660) to 5.03 (BH540) and 4.40 (medium seed rate) to 4.47 (high seed rate).The variations on DM basis were 0.61 (BH540) to 0.88 (BH661) and 0.68 (high seed rate) to 0.74 (medium seed rate).

Table1. Biomass of maize hydroponic fodder yield as affected by variety and seed rate

Parameter	Fodder yield (kgm^{-2})		FCE (Fodder weight/seed weight)	
	Fresh	DM	Fresh	DM
Variety				
BH540	38.03^{a}	4.58^{c}	5.03^{a}	0.61^{c}
BH660	29.29^{b}	5.59^{b}	3.85^{b}	0.74^{b}
BH661	37.78^{a}	6.63^{a}	4.95^{a}	0.88^{a}
MVFG	29.93^{b}	4.82^{c}	3.92^{ab}	0.64^{c}
SEM	1.23	0.23	0.15	0.03
Seed rate (kgm^{-2})				
5.6 (Low)	24.92^{c}	4.11^{c}	4.45	0.74^{a}
7.6 (Medium)	33.41^{b}	5.60^{b}	4.40	0.74^{a}
9.6 (High)	42.94^{a}	6.50^{a}	4.47	0.68^{b}
SEM	1.23	0.23	0.15	0.03
P-value				
Variety	***	***	***	***
Seed rate	**	***	ns	**
Variety× seed rate	ns	ns	ns	ns

$^{a-c}$Means with different superscripts within column and under variety or seed rate differ significantly; **P<0.01; ***P<0.001; ns = non significant; FCE=Fodder conversion efficiency; DM= Dry matter; SEM=Standard error of mean.

Chemical composition. Chemical composition of the hydroponic fodder was unaffected by the interaction of variety and seed rates ($p>0.05$; Table 2). Among varieties, BH540 has the lowest (12.14%) and BH660 has the highest (19.23%) DM content.The high seed rate has lower DM content than the other seed rates that had similar DM content. The maize varieties differ ($p< 0.01$) in their CP content and ranked BH661>BH540>BH660>MVFG. The low seed rate has significantly higher CP content than medium and high seed rates. The NDF content differs only between BH540 and MVFG. There was no significant difference

among seed rates in NDF content. The ADF content of the varieties ranked BH540>BH661>BH660 ($p<0.05$); while the value for MVFG was similar with BH540 and BH661 ($p>0.05$). The medium seed rate has significantly higher ADF content than low seed rate, while the high seed rate has similar value with other seed rates.

The ADL content did not differ among varieties ($p>0.05$), while for seed rate ADL content was in the order of medium>low>high ($p<0.05$). Varieties BH660 (4.25%) and BH661 (4.23%) had significantly higher EE content compared to BH540 (4.08%) and MVFG (4.02%). The medium seed rate has lower EE content than low seed rate but was similar with the EE content of the high seed rate. The P and K content of the varieties were similar, while the contents of Ca differed among varieties. The low and high seed rates had significantly higher mineral contents than the medium seed rate.

Table 2. Chemical composition of maize hydroponic fodder

Parameter	DM	Ash	CP	NDF	ADF	ADL	EE	Ca	P	K
Variety	(%)	---------- (as%DM) ----------						------- (gkg^{-1}DM) -------		
BH540	12.14^{d}	2.60	10.37^{b}	36.28^{a}	12.17^{a}	1.50	4.08b	1.81^{a}	4.79	5.00
BH660	19.23^{a}	2.54	9.90^{c}	32.67^{ab}	6.89^{c}	1.65	4.25a	1.75^{ab}	4.73	4.94
BH661	17.72^{b}	2.43	10.93^{a}	35.41^{ab}	9.27^{b}	1.44	4.23a	1.64^{b}	4.62	4.83
MVFG	16.23^{c}	2.45	8.01^{d}	31.65^{b}	10.96^{ab}	1.45	4.02b	1.66^{ab}	4.64	4.63
SEM	0.26	0.10	0.20	1.75	0.85	0.23	0.03	0.08	0.08	0.57
Seed rate$^{\Theta}$										
5.6 (Low)	16.77^{a}	2.70^{a}	10.13^{a}	32.82	8.79^{b}	1.5^{b}	4.18^{a}	2.35^{a}	5.33^{a}	5.37^{a}
7.6 (Medium)	16.95^{a}	2.07^{b}	9.49^{b}	35.32	10.97^{a}	2.18^{a}	4.11^{b}	0.42^{b}	3.40^{b}	3.61^{b}
9.6 (High)	15.27^{b}	2.74^{a}	9.78^{b}	33.87	9.71^{ab}	0.77^{c}	4.14^{ab}	2.39^{a}	5.37^{a}	5.58^{a}
SEM	0.26	0.10	0.20	1.75	0.85	0.23	0.03	0.08	0.08	0.57
P value										
Variety	***	ns	***	***	***	ns	***	**	ns	ns
Seed rate	***	***	**	ns	**	***	**	***	***	***
Var. × SR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

$^{a-d}$Means with different superscripts within column and under variety or seed rate differ significantly;**P<0.01; ***P<0.001; ns = non significant; CP= Crude Protein; NDF= Neutral Detergent Fiber; ADF= Acid detergent Fiber; ADL= Acid Detergent Lignin; EE= Ether extract; Ca =Calcium; P= Phosphorus; K= Potassium; SEM=Standard error of mean; Θ = Seed rate is $kgm^{-2.}$

Water use efficiency. The water consumption of maize varieties hydroponic fodder ranged from 1.83 (MVFG) to 2.07 (BH660) liter per kg fresh fodder produced or 10.62 (BH661) to 15.71(BH540) liter per kg DM fodder (Table 3).

Table 3.Water use efficiency of maize hydroponic fodder as affected by variety and seed rate

Parameter	Total water use			Water use efficiency	
	Liters/tray	Liters/kg fresh fodder	Liters/kg DM fodder	Fresh fodder (kgm^{-3})	DM fodder (kgm^{-3})
Variety					
BH540	7.82[a]	1.91[b]	15.71[a]	531.41[ab]	64.22[b]
BH660	6.47[b]	2.07[a]	10.74[b]	491.70[b]	94.19[a]
BH661	7.60[a]	1.88[b]	10.62[b]	539.91[a]	95.23[a]
MVFG	5.78[b]	1.83[b]	11.22[b]	561.34[a]	90.66[a]
SEM	0.20	0.07	0.53	19.64	3.36
Seed rate (kg/m2)					
5.6 (Low)	6.07[c]	2.23[a]	13.61[a]	450.34[c]	75.02[b]
7.6 (Medium)	6.92[b]	1.88[b]	11.46[b]	533.74[b]	90.39[a]
9.6 (High)	7.77[a]	1.65[c]	11.15[b]	609.20[a]	92.81[a]
SEM	0.20	0.07	0.53	19.64	3.36
P- value					
Variety	***	**	***	**	***
Seed rate	***	**	***	***	***
Variety X seed rate	ns	ns	ns	ns	ns

[a-c]Means with different superscripts within column and under variety or seed rate differ significantly; **P<0.01; ***P<0.001; ns = non significant

The water use efficiency of maize varieties hydroponic fodder ranged from 491.70 (BH660) to 561.34 (MVFG) kg fresh fodder per m^3 or 64.22 (BH540) to 95.23 (BH661) kg DM fodder per m^3 water (Table 3).Water use efficiency of DM fodder was lower in the variety BH540 (64.22 kg m^{-3}) compared to the other varieties that had similar values. The medium and high seed rates had higher ($p< 0.05$) water use efficiencyof DM fodder than low seed rate.

Production cost of maize hydroponic fodder. The total cost of hydroponic fodder production was 34.34 USD per 100kg DM without labor, and labor cost being 0.83 USD per 100 kg DM (Table 4). Seed cost accounted for the highest share of the cost of hydroponic fodder production. Materials depreciation, water, and chemical were the second, third, and fourth source of cost for hydroponic forage production, respectively.

Table 4. Relative cost of maize hydroponic fodder production (per 100kg DM)

Source of cost	Without labor		With labor	
	Cost (USD)	%	Cost (USD)	%
Seed	31.56	91.90	31.56	89.74
Water	0.28	0.82	0.28	0.80
Chemical	0.12	0.35	0.12	0.34
Material	2.38	6.93	2.38	6.77
Labor	-	-	0.83	2.36

When labor is considered, labor cost took third place following seed and material depreciation costs.The less expensive variety for production of hydroponic forage was BH661 and the more expensive variety was MVFG (Table 5). Among seed rates, the medium seed rate has lower ($p<0.05$) cost of production per kg DM fodder when compared with the high seed rate.

Table 5. Maize hydroponic fodder cost of production (WoL) as affected by variety and seed rate

Parameter	Cost per 100 kg fresh fodder (USD)	Cost per 100 kg DM fodder yield (USD)	HpF to Grain Cost ratio
Variety			
BH540	4.55^{c}	37.66^{b}	2.09^{b}
BH660	5.96^{b}	31.06^{c}	1.73^{c}
BH661	4.62^{c}	26.11^{d}	1.46^{d}
MVFG	6.90^{a}	42.54^{a}	2.37^{a}
SEM	0.22	1.47	0.08
Seed rate (kg/m^2)			
5.6 (Low)	5.69	34.34^{ab}	1.91^{a}
7.6 (Medium)	5.46	32.79^{b}	1.83^{b}
9.6 (High)	5.38	35.89^{a}	2.00^{a}
SEM	0.22	1.47	0.08
P-value			
Variety	***	***	***
Seed rate	ns	**	***
Variety x Seed rate	ns	ns	ns

$^{a-c}$Means with different superscripts within column and under variety or seed rate differ significantly; **P<0.01; ***P<0.001; ns = non significant

Changing maize grain to hydroponic fodder increased the cost of feed by 1.46 (BH661) to 2.37 (MVFG) per kg DM for variety and by 1.83 to 2.00 for seed rates. Using variety BH540, MVFG and high seed rate for hydroponic fodder production increased cost by more than double the cost of feed per kg DM. Variety BH661 increased the cost of feed by only 31.51%.

Discussion

The fresh biomass yield productivity (3.85 to 5.03 fold) per initial seed used observed in the present experiment was comparable to that reported by *Naik and Singh (2013)* and *Jemimah et al. (2018)* who obtained 5 to 6 kg and 4.6 kg of hydroponic maize fodder per kg seed used, respectively. *Al-Ajmi et al. (2009)* and *Lamnganbi and Surve (2017)* also reported 2.76 and 5.7 kg green fresh fodder yield per kg of barley seed, which is almost similar with the result obtained in the present work. However, it was less than the value (6 to 10 kg of fodder per kg of maize seed) reported by *Sneath and McIntosh (2003)*. The variation in fodder yield

among studies could be attributed to the differences in the varieties of maize used or differences in the extents to which the environmental factor such as humidity and temperature might have been fully controlled since they had used commercial fodder units. The decrease in a DM recovery (fodder conversion efficiency) when seed rate increased from 7.6 kg m^{-2} to 9.6 kg m^{-2} agreed with the report of *Naik et al. (2017).* These authors, also reported that the yield per kg decreases with increase in seed rate.

Considering the total net productive area of the shade (3 × 4m area with three floor shelves that accommodate 126 trays of 0.11m^2) and 8 days cycle of hydroponic fodder production at a productivity potential of 29.29 to 38.03kg m^{-2}, a total of 1.14 to 1.48 tons of fresh hydroponic fodder can be harvested from the present hydroponic system in nine dry months of the area. Under conventional farming, the average fresh forage biomass produced from maize was reported to be 28.43 and 30.67 tons ha^{-1} at planting space of 75 and 35.5 cm, respectively *(Dicu et al., 2016)*, which is equivalent to 2.24 to 3.07 kg m^{-2} of land area. With three cycles a year production, only about 6.84 to 9.21 kg fresh fodder can be produced per m^2 of land under conventional farming indicating high efficiency of hydroponic fodder production in terms of land utilization. Based on the observed productivity in the present experiment, an area of 4 to 6m^2 land is sufficient to produce 10 kg fresh fodder required for a cow per day indicating even such a small size hydroponic fodder units is enough for saving expense on material depreciation and opportunity cost of the space. The area requirement can be reduced if production per unit area is more maximized. In this regard, *Kamanga (2016)* reported that one square meter space was enough to produce fodder for two cows per day.

The DM percentage of hydroponic fodder was comparable with the values of 18.48 %, 16.53 %, and 17.21 to 23.25 % DM content of maize hydroponic fodder reported by *Gebremedhin (2015)*, *Dadhich (2016)* and *Jemimah et al. (2018)*, respectively. Varieties differ in DM content, and the reason for variation among varieties in DM percentage of hydroponic fodder may be due to the difference in growth rate which is also related to the rate of conversion of starch stored in the seed into a simple sugar, which produces energy and gives off carbon dioxide and water *(Bakshi et al., 2017).*

The CP of hydroponic fodder in this study was comparable with the value (8.72 to 17.55) reported by *Jemimah et al. (2018)* but lower than the 13.3 % reported by *Naik et al. (2014)* and 14.56 % reported by *Gebremedhin (2015)*. The variation may be due to the differences in variety of maize used for hydroponic fodder production. The reduction in dry biomass yield due to changing the grain to hydroponic fodder was also reported by *Sneath and McIntosh (2003)*, *Dung et al. (2010)* and *Putnam et al. (2013)* for different crops. The loss in weight may be due to leaching of soluble carbohydrates and respiration. The conversion of starch stored in the seed by seed soaking activated enzymes in endosperm to a simple sugar produces energy and gives off carbon dioxide and water. This process leads

to loss of DM with a shift from starch in the seed to fiber and pectin in the roots and green shoots *(Bakshi et al., 2017).*

The amount of water required (1.65-2 liter if water is recycled and 2-3.3 liter if water is not recycled) to grow one kg of hydroponic maize fodder reported by *Naik et al. (2013)* agreed with the net water consumption of maize hydroponic fodder in the present experiment (1.52-2.29 l kg^{-1}fresh fodder).Our finding also agreed with the value of 1.5-2 liter of water required per kg fodder reported by *Al-Karaki (2010).* Under conventional farming 73, 85 and 160 liters of water is required to produce one kg green fodder of barley, alfalfa, and Rhodes, respectively *(Bakshi et al., 2017). Naik et al.(2015)* and *Naik and Singh (2013)* noted that 90% and 90-98%, respectively of the hydroponic fodder production cost is associated with cost of seed, which agreed with the finding of the present study. The cost of pasture grass hay per kg in Ethiopia from 2010 to 2014 was between 0.70 to 4.44 Birr *(Mesfin et al., 2014; Adugna et al., 2014)*; equivalent to 0.02 to 0.14 USD at current exchange rate of 0.031 USD per Birr. Pasture grass hay contains on the average 6.54 % CP (SSAFeed, 2019) and from 6.95 to 9.83 MJ ME per kg DM *(Fekede et al., 2014).* Hydroponic maize fodder contains 13.23 to 13.31 MJ ME per kg DM *(Getachew et al., 2019)*. This indicates, in terms of CP cost hydroponic fodder is better due to the cost per kg CP as compared to pasture grass hay. A kg CP in hay costs 0.34 to 2.09 USD whereas in hydroponic fodder it is 0.02 to 0.05 USD per kg CP. In terms of energy pasture grass hay costs 0.001 to 0.02 USD per MJ ME as compared to 0.01 to 0.03 US dollars MJ^{-1}ME for hydroponic fodder.

Conclusion

Among the varieties tested BH661 is better for hydroponic fodder production due to higher DM fodder yield with relatively low cost of production. The 9.6 kg seed rate m^{-2} produced higher DM hydroponic fodder m^{-2} and has low hydroponic fodder to grain cost ratio. Nevertheless, its cost per kg DM was high. In comparison to this, seed rate of 7.6 kg m^{-2} is high in its fodder conversion efficiency. Changing maize grain to hydroponic fodder reduced the DM weight of the initial grain and increased the cost of feed per kg DM. This means that hydroponic fodder production *per se* has no yield advantage. However, quality advantage together with its effect on the profitability of livestock production and the need for green fodder under the scenarios of climate change need to be considered to use hydroponic fodder production.

Uticaj sorte i setvene stope na prinos biomase krme hidroponskog kukuruza, hemijski sastav i efikasnost upotrebe vode

Getachew Assefa, Mengistu Urge, Getachew Animut, Getnet Assefa

Rezime

U ovom istraživanju, ispitan je uticaj sorte kukuruza BH540, BH660, BH661 i MVFG (nepoznata sorta kao lokalna provera) u uslovima niske (5,6 kg m^{-2}), srednje (7,6kg $m^{-}2$) i visoke (9,6kg $m^{-}2$) količine semena po jedinici površine/setvenoj stopi, na produktivnost hidroponskog krmiva. Za uzgoj hidroponskog krmiva korišćena je jeftina plastična kućišta dimenzija 3×4 i visine 3 m, izrađena od prozirne plastike i plastičnih ležišta napravljenih odvajanjem posude od plastike zapremine 25 litara u dva jednaka dela. Dna ležišta su izbušena da bi se izlivala suvišne voda tokom navodnjavanja i postavile na police. BH661 je pokazao značajno ($p < 0,01$) veći prinos suve krme (6,63 kg) po kvadratnom metru i po kg semena od ostalih sorti. Od svih korišćenih setvenih stopa, visoka stopa je imala veći ($P<0,01$) prinos suve materije (DM), ali srednje i niske stope imale su veću efikasnost konverzije DM krme i nižu cenu po kilogramu proizvodnje DM krme. Učinkovitost upotrebe vode bila je manja za BH540 (64 kg krme po kubnom metru vode) u poređenju s ostalim sortama koje su imale slične vrednosti (90 do 95 kilograma krmiva po kubnom metru). Srednja i visoka količina semena pokazala je sličnu efikasnost upotrebe vode, i viša je od niske setvene stope. Zbog toga se za proizvodnju hidroponske krme za kukuruz preporučuje upotreba sorte BH661 u srednjoj setvenoj stopi.

Ključne reči: kukuruz, izdanci, sorta, hidroponik

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ASSESSMENT OF YIELD, QUALITY AND NITROGEN INDEX OF *AGROSTIETUM CAPILLARIS* GRASSLAND AS AFFECTED BY FERTILIZATIONS

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Abstract: Managing N, P and K inputs in semi-natural meadow production systems is important for achieving maximum yields in livestock farming. The objective of the present study was to estimate the effect of different NPK levels ($N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ kg ha^{-1} yr^{-1}) on the yield, quality and nitrogen nutrition index (NNI) in a grassland community of *Agrostietum capillaris* (semi-natural meadow) in western Serbia. The study was conducted during the seasons of 2005-2008. The values of the investigated parameters, except for the unit N uptake, were the highest in 2004/2005 due to favorable climate conditions. The levels of nitrogen significantly increased all of the studied parameters compared to the control treatment, except for unit N uptake. Mineral fertilizers at $N_{200}P_{150}K_{150}$ provided the highest green forage yield (25.12 t ha^{-1}), dry matter yield (8.12 t ha^{-1}), crude protein yield (876.3 kg ha^{-1}), nitrogen uptake (140.2 kg ha^{-1}) and nitrogen nutrition index (70.2%), and the lowest unit N uptake (0.0022 kg N kg DMY^{-1}). The use of mineral fertilizers increased green forage yield, dry matter yield and crude protein yield, increasing fertilizer from lowest to highest rate increased fresh and dry matter yield, as well as protein yield. Based on the results of the study, monitoring of nutrition indices would be necessary in order to increase productivity and economic benefits.

Key words: *Agrostietum capillaris*, fertilization, nitrogen indices, quality, yield

Introduction

The impacts of climate and biodiversity on biomass are well known, but the relative significance of these two factors with regard to management and utilization has not been studied extensively *(Bernhardt-Römermann et al., 2011)*. In order to recommend suitable agrotechnical measures that will maximize biomass yields, the individual effects of climate drivers and the functional diversity of the flora on the biomass after different treatments of grasslands needs to be examined.

There are abandoned grasslands in many mountain villages in Serbia, due to population migration and consequent reduction in livestock headcounts. Anthropogenic and zoogenic activities (such as irrigation, fertilization, grass cutting, and grazing) in the abandoned grasslands have ceased, such that apart from the dominant plants there are numerous weed and ruderal species. In addition, the dry mass yield of used meadows and pastures is much lower than the production potential of the grassland *(Simić et al., 2015)*. A large number of factors affect the uptake of nutrients by plants, such as the total nutrient concentration, soil pH, organic substances, redox potential, plant species, stage of growth, weather conditions, and interaction of different chemical elements *(Halvin et al., 2005)*. The interpretation of the content of plant macronutrients and, based on them, the quality of grassland for animal feed is a highly complex issue due to varying botanical compositions, nutrient concentration changes during growth, and interactions among the elements *(Liebisch et al., 2013)*. These authors have investigated the use of P nutrient indices (PNI) for grass fractions and proposed the following ranges for grassland fertilization: N:P 5.5–9.0 and K:P 6.0–10.5. They also concluded that the differences in biomass between the management approaches were much more pronounced than between the years of use. The application of nitrogen fertilizers to grasslands in Turkey had a negative impact on the proportion of protein in animal feed because it reduces the share of legumes from 47% on untreated plots to 5% on those treated with high doses of nitrogen *(Aydin and Uzun, 2005)*. According to them, adding P can compensate for the negative impact of nitrogen. Economically optimal levels were achieved with the highest rates of P and K (52 kg P ha^{-1} + 180 kg N ha^{-1}), which resulted in 4810 kg ha^{-1} of dry forage mass, with a raw protein concentration of 124 g kg^{-1} and a 12% share of legumes. However, smaller amounts of nutrients should be applied to grasslands to conserve biodiversity *(Isselstein et al. 2005)*. Fertilization can cause major changes in the vegetation cover and lead to a rapid decrease in biodiversity *(Păcurar et al. 2014)*. Generally speaking, fertilization of grasslands with nitrogen is on the decline in Europe, due to restrictive EU policies (EU Directive 91/676/EEC), as is grazing in dairy farming for various reasons, but cut grass is becoming increasingly important. There are different opinions about critical values and economically viable NPK ratios for grassland fertilization, depending on the

type of grassland, the approach to maintenance and use, and local weather conditions *(Whitehead, 2000)*.

The goal of the present research was to assess the effect of added nutrients on the plant biomass of a permanent grassland of *Agrostietum capillaris*, which was managed to arrive at recommended critical values of N, P and K. The paper aims to highlight the significance of mineral fertilizers in terms of the productivity, quality, and optimal use of typical permanent grassland.

Materials and Methods

Experiment details and treatments

The study was carried out during a period of four years (2005-2008), in type of semi-natural meadow dominated by *Agrostis capillaris* in western Serbia, near the City Valjevo (44°10'40.1" N and 19°49'38.5" E, at 750 m altitude). The following species were represented on the meadow: *Holcus lanatus, Cynosurus echinatus, Trisetum flavescens, Arrhenatherum elatius* and *Anthoxanthum odoratum*. The experiment was a set up by randomized block design, with four replications. In 2004, 24 plots of 3x4 m each were arranged in a multifactorial design, combining five levels of N (0, 50, 100, 150 and 200 kg ha^{-1} $year^{-1}$), four levels of P (0, 50, 100, 150 kg ha^{-1} $year^{-1}$) and four levels of K (0, 50, 100, 150 kg ha^{-1} $year^{-1}$) in following ratios ie. the six fertilization treatments ($N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ kg ha^{-1} yr^{-1}) per year. The fertilizer NPK 15:15:15 was applied in autumn, and additional amount of KAN (27%) in spring after the snow melt (early April), as a spring N application (50 kg N) in treatments $N_{100}P_{50}K_{50}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$.

At the beginning of the fourth year, after regular grass cutting and fertilizer application, the botanical composition was determined and compared with a control variant. The identified plant species were classified by their quality and forage importance into three categories: grasses, legumes and forbs (useless or conditionally useful plant species from other plant families) *(Tomić et al., 2011)*.

Soil properties and climate conditions

The soil was analyzed before the experiment was set up. The *Agrostietum capillaris* grasslands in the experiment is formed on shallow to medium-deep soils, skeletal to varying degrees, with a high organic content (5.4%) and an acidity of 4.9 in KCl. The soil features medium potassium concentrations (18.4 mg $100g^{-1}$) and very low phosphorus concentrations (2.8 mg $100g^{-1}$).

A modified climate diagram *(Walter and Lieth, 1967)* shows that the season of 2004/2005 (total seasonal rainfall 895.0 mm) was not a period of drought (Figure 1). Contrary, in 2005/2006 (total seasonal rainfall 833.4 mm) there were

droughts in July and September, in 2006/2007 (total seasonal rainfall 706.6 mm) in April and July, and in 2008 (total seasonal rainfall 799.3 mm) in August.

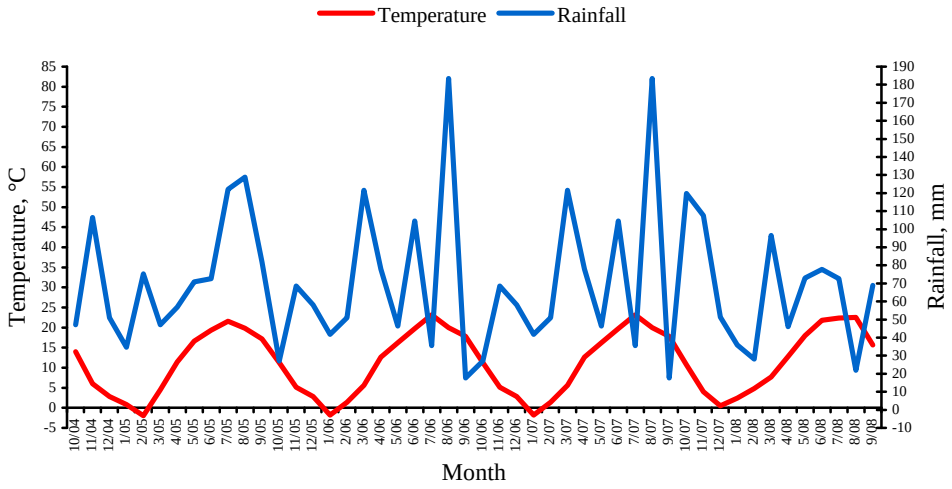

Figure 1. Modified climate diagram of the study site

Data collection

The plots were harvested during flowering of the dominant meadow plants. The green forage yield (GFY) was weighed immediately after harvest. During the study period only one harvest was in July, because of no summer regrowth, caused by a period without rainfall. Samples of 1 kg were collected from all the plots to determine dry matter yields (DMY). DMY was the difference in mass before and after oven drying to constant mass at 60°C. The botanical composition was determined in fresh biomass samples, collected from 1 m^2 per experimental plot. Nitrogen content was determined according to Kjeldahl *(AOAC, 1990)*. Crude protein (CP) was calculated as N × 6.25 and crude protein yield (CPY) as CP × DMY. The nitrogen uptake (NU) is the nitrogen absorbed by a plant and was calculated as a percentage of the amount of N accumulated in the plant (except roots) × DMY (kg ha^{-1}) / 100 *(Kabir et al., 2012)*. The unit nitrogen uptake (UNU) was calculated as the amount of N accumulated in the plant (except roots) / DMY *(Hermanson et al., 2000)*. The nitrogen nutrition index (NNI) was determined using the formula: $NNI = N / 4.8 \times (DMY)^{-0.32}$ *(Lemaire and Meynard, 1997)*. A value of NNI from 80 to 100% indicated normal nitrogen levels, NNI < 80% indicated nitrogen insufficiency and NNI > 100% indicated excessive nitrogen.

Statistical analysis

The data were analyzed using ANOVA with Statistica 10 (version 10). The significant differences among the treatments were estimated by Duncan's Multiple Range Test at $P \leq 0.05$. The significances levels were set at $P \leq 0.05$ and $P \leq 0.01$. Pearson's correlation coefficients were used to determine direct correlations between the studied parameters.

Results

Seasonal effect on the studied parameters

The seasons had a very significant effect on GFY, DMY, NC, NU, UNU and NNI ($P<0.05$), and a highly significant effect on CPY ($P<0.01$) (Table 1). GFY (20.23 t ha^{-1}), NC (18.78 g kg^{-1} DM), NU (113.57 kg ha^{-1}) and NNI (68.62 %) were the highest in the season of 2004/2005. DMY was significantly lower in 2006/2007 (4.43 t ha^{-1}) than in 2004/2005 (5.88 t ha^{-1}), 2005/2006 (5.72 t ha^{-1}) and 2007/2008 (5.70 t ha^{-1}). UNU was the highest in 2006/2007 (0.0043 kg N kg DMY^{-1}). Generally, the rainfall distribution was unfavorable in 2006/2007 because in April high temperatures and a small amount of rainfall shortened the regrowth stages of grass.

Table 1. Season and NPK fertilization rate effects on meadow productivity

Factor		GFY*	DMY	NC	CPY	NU	UNU	NNI
Season (A)	2004/2005	20.23^{a}	5.88^{a}	18.78^{a}	709.8^{a}	113.57^{a}	0.0037^{b}	68.62^{a}
	2005/2006	16.85^{b}	5.72^{a}	14.64^{c}	540.5^{a}	86.48^{b}	0.0030^{c}	52.90^{b}
	2006/2007	12.04^{c}	4.43^{b}	14.84^{b}	415.6^{b}	66.49^{c}	0.0043^{a}	48.87^{c}
	2007/2008	15.93^{b}	5.70^{a}	12.83^{d}	460.7^{b}	73.72^{c}	0.0029^{c}	45.74^{d}
NPK fertilizer rate, kg ha^{-1} (B)	$N_0P_0K_0$	5.15^{e}	1.96^{e}	14.22^{d}	175.5^{e}	28.08^{e}	0.0075^{a}	36.69^{f}
	$N_{50}P_{50}K_{50}$	12.67^{d}	4.43^{d}	13.68^{f}	382.4^{d}	61.18^{d}	0.0032^{b}	45.77^{e}
	$N_{100}P_{50}K_{50}$	16.95^{c}	5.75^{c}	13.99^{e}	497.6^{c}	79.62^{c}	0.0025^{c}	50.68^{d}
	$N_{100}P_{100}K_{100}$	17.57^{c}	5.75^{c}	14.95^{c}	532.4^{c}	85.19^{c}	0.0027^{c}	54.24^{c}
	$N_{150}P_{100}K_{100}$	20.50^{b}	6.58^{b}	17.50^{a}	725.7^{b}	116.11^{b}	0.0027^{c}	66.59^{b}
	$N_{200}P_{150}K_{150}$	24.73^{a}	8.12^{a}	17.29^{b}	876.3^{a}	140.20^{a}	0.0022^{d}	70.22^{a}
F test	A	**	**	**	*	**	**	**
	B	**	**	**	**	**	**	**
	A × B	ns	ns	**	**	*	**	**
M		16.60	5.43	15.27	531.65	85.06	0.0035	54.03

*GFY – green forage yield (t ha^{-1}), DMY – dry matter yield (t ha^{-1}), NC – nitrogen content (g kg^{-1} DM), CPY – crude protein yield (kg ha^{-1}), NU – nitrogen uptake (kg ha^{-1}), UNU – unit nitrogen uptake (kg N kg DMY^{-1}) and NNI – nitrogen nutrition index (%); Means followed by the same letter in a column are not significantly different according to Duncan's Multiple Range Test at the 5% level ($P<0.05$); NS = not significant P value 0.05; * = P value < 0.05; ** = P value <0.01.

Effect of NPK fertilization rate on the studied parameters

The NPK fertilizers had a highly significant effect on all the investigated parameters. Compared to the other treatments, $N_{200}P_{150}K_{150}$ (with nitrogen content 17.29 g kg^{-1} DM) resulted in significantly higher values of GFY (24.73 t ha^{-1}),

DMY (8.12 t ha^{-1}), CPY (876.3 kg ha^{-1}), NU (140.20 kg ha^{-1}) and NNI (70.22 %), and a significantly lower UNU (0.0022 kg N kg DMY^{-1}). NC was significantly higher in the $N_{150}P_{100}K_{100}$ treatment (17.50 g kg^{-1} DM), compared to the other treatments. Treatments $N_{100}P_{50}K_{50}$ and $N_{100}P_{100}K_{100}$ did not differ with regard to GFY, DMY, CPY, NU and UNU.

The interaction of the season and the nitrogen fertilization level had a highly significant effect on NC, CPY, NU, UNU and NNI (data are not shown). The highest nitrogen content (22.79 g kg^{-1} DM), crude protein yield (1172.8 kg ha^{-1}), nitrogen uptake (187.65 kg ha^{-1}) and nitrogen nutrition index (93.10%) were recorded with $N_{200}P_{150}K_{150}$ in 2004/2005. The control treatment had the highest UNU in 2006/2007.

Botanical composition

The control treatment, which was not cut or fertilized, exhibited the largest biodiversity, including 45 different species of which 73% were forbs, 18% grasses 9% legumes (Table 2). Cutting reduced biodiversity (29 species), but the proportions of the grasses, legumes and forbs did not really change (76% forbs, 17% grasses and 7% legumes). Biodiversity decreased with increasing fertilizer rates. The proportion of useful grass species increased, and those of the legumes and forbs decreased.

Table 2. Selected botanical composition parameters of the studied treatments

Treatment	Proportion (%)			Number of species				Dominant species
	G	L	F	G	L	F	Total	
Uncut meadow $N_0P_0K_0$	18	9	73	8	4	33	45	**Ac, Hl, Ce, Tf, Ae, Ao, Fr, Sm, Tc, Lc*
$N_0P_0K_0$	17	7	76	5	2	22	29	*Ac, Fr, Ae, Hl, Pl, Vc, Vo*
$N_{50}P_{50}K_{50}$	35	5	60	7	1	12	20	*Ae, Ac, Hl, Tf, Fr, Ce, Ca*
$N_{100}P_{50}K_{50}$	35	6	59	6	1	10	17	*Ae, Ac, Hl, Tf, Fr, Fp, Pl*
$N_{100}P_{100}K_{100}$	26	16	58	5	3	11	19	*Ac, Hl, Ae, Tf, Fr, Ea, Vc, Sm*
$N_{150}P_{100}K_{100}$	35	6	59	6	1	10	17	*Ae, Ac, Hl, Tf, Fr, Pl, Sg, Dg*
$N_{200}P_{150}K_{150}$	86	14	0	6	1	0	7	*Ac, Hl, Ae, Tf, Fr*

**Ac-Agrostis capillaris, Ao - Anthoxanthum odoratum, Ae - Arrhenatherum elatius, Ca - Convolvulus arvensis, Ce - Cynosurus echinatus, Dg - Dactylis glomerata, Ea - Erigeron annuus, Fp - Festuca pratensis, Fr - Festuca rubra, Hc - Holcus lanatus, Lc - Lotus corniculatus, Pl - Plantago lanceolata, Sm - Sanguisorba minor, Sg - Stellaria graminea, Tc - Trifolium campestre, Tf - Trisetum flavescens, Vo - Veronica officinalis, Vc - Vicia cracca, G- grasses; L – Legumes; F – Forbs*

Correlations among the studied parameters

The results in Table 3 show that CPY exhibited a very strong positive correlation with NU, NNI, GFY and DMY, and GFY with DMY and NU and NU with NNI and DMY, as did NC with NNI, CPY and NU and NNI with GFY and DMY. There was a weak positive correlation between GFY and NC, and between DMY and NC. UNU strongly and negatively correlated with GFY and DMY, and

had a moderate negative correlation with CPY, NU and NNI, and a very weak to negligible negative correlation with NC. Correlation coefficients showed that many of the parameters were inter-related.

Table 3. Pearson correlation coefficients (r) of green forage yield (GFY), dry matter yield (DMY), nitrogen content (NC), crude protein yield (CPY), nitrogen uptake (NU), unit nitrogen uptake (UNU) and nitrogen nutrition index (NNI) in different seasons and at different NPK fertilization rates.

	GFY	DMY	NC	CPY	NU	UNU
DMY	0.96**					
NC	0.54**	0.34**				
CPY	0.96**	0.90**	0.71**			
NU	0.96**	0.90**	0.71**	1.00**		
UNU	-0.75**	-0.83**	-0.03^{ns}	-0.62**	-0.62**	
NNI	0.86**	0.73**	0.89**	0.95**	0.95**	-0.46**

*GFY – green forage yield, DMY – dry matter yield, NC – nitrogen content, CPY – crude protein yield, NU – nitrogen uptake, UNU – unit nitrogen uptake and NNI – nitrogen nutrition index; Means followed by the same letter in a column are not significantly different according to Duncan's Multiple Range Test at the 5% level ($P<0.05$); ns – not significant; ** – significant at $P<0.01$

Discussion

Seasonal effect

Among the seasons, significant differences were recorded in all the studied parameters. GFY, NC, NU and NNI were significantly higher under the favorable environmental conditions in 2004/2005. GFY was significantly higher in 2004/2005, whereas in 2005/2006 and 2007/2008 there was no difference. Contrarily, DMY did not differ in 2004/2005, 2005/2006, and 2007/2008.

In spring, during early growth, plants of the semi-natural meadow type *Agrostietum capillaris* are in a vegetative stage, with emerging leaves. Also, roots and shoots begin to develop. The formation of new leaves and stems depends on the extent of tillering, caused by climate conditions. The *Agrostis* tiller density peaked in late summer and varied in spring due to tiller birth rate variations (*Bullock, 1994)*. Year-to-year weather variation affected the dynamics.

Favorable weather conditions in the second half of November and in April can improve tillering rates of grass, whereas high temperatures and little rainfall lower tillering rates. Generally, tillers formed in autumn and spring are important for winter and summer survival, respectively. Also, in Serbia's climate, meadow species begin to grow intensively in April. Drought stress significantly reduces productive tillering and total biomass due to a decrease in the rates of photosynthesis and dry matter accumulation (*Tomaškin, 2013)*. Unfavorable climate conditions can shorten this stage and the growing season of plants, inducing jointing (internode elongation) and rapid aging (drying of leaves). In

general, meadow species reduce the formation of new leaves because of stopped regrowth. As a result, the drought stress in April 2007 led to poor regeneration of grasses and legumes, which was attributable to lower GFY (12.04 t ha^{-1}) and DMY (4.43 t ha^{-1}). By contrast, in the other seasons there was drought stress in July, when the meadow was cut. Favorable spring and summer weather conditions improved grassland productivity, especially in 2004/2005. A timely first cut favored regrowth, but summer heat and drought stress in July had an adverse effect on tillering. Basically, the drought stress in July had a negative impact on the regrowth of grass and there was no second cut. As a result, grassland productivity was poor.

NC, CPY, NU, UNU and NNI depended on climate factors during the season. The highest values of NC, CPY, NU and NNI were recorded in 2004/2005, the season with the highest GFY and DMY. The highest values of UNU were recorded in 2006/2007. The NNI index ranged from 45.74 % in 2007/2008 to 68.62 % in 2004/2005 and the nitrogen nutritional status was lower than normal, ranging from 80 to 100 %.

In general, favorable climate condition in the vegetative state of plants, especially the large amount of rainfall in the growing season of 2005, had a positive effect on nutrient uptake and therefore resulted in the highest productivity of green forage, dry matter and CPY. In 2006/2007, dry weather conditions caused a reduction in the nutritional value of forage because of slower plant growth and a larger percentage of synthetized fibers.

Effect of NPK fertilization rate

In western Serbia, and across Serbia in general, fertilizers are not applied on meadows in spring. However, our results show that fertilization has benefits as it boosts GFY and DMY, and can thus maximize yield per unit area. It is very important for the development of livestock farming in this region. GFY and DMY significantly increased with increasing NPK levels. The increase in GFY ranged from 12.67 t ha^{-1} (246.0 %) in the $N_{50}P_{50}K_{50}$ treatment to 24.73 t ha^{-1} (480.2 %) in the $N_{200}P_{150}K_{150}$ treatment, compared to the control treatment (5.32 t ha^{-1}). DMY increased from 2.47 t ha^{-1} (223.5%) in the $N_{50}P_{50}K_{50}$ treatment to 6.16 t ha^{-1} (414.7%) in the $N_{200}P_{150}K_{150}$ treatment, relative to the control treatment (1.96 t ha^{-1}). GFY and DMY of treatments $N_{100}P_{50}K_{50}$ and $N_{100}P_{100}K_{100}$ did not differ significantly. Accordingly, GFY and DMY depended on N because the same amount of nitrogen (100 kg ha^{-1}) and different amounts of P (50 and 100 kg ha^{-1}) and K (50 and 100 kg ha^{-1}) resulted in similar yields of green mass and dry matter. Further increases of nitrogen, from 100 to 150 kg ha^{-1}, and from 150 to 200 kg ha^{-1}, increased GFY and DMY. This confirmed that nitrogen application had the greatest effect on GFY and DMY. Therefore, high-yielding meadows need large amounts of N for regrowth and proper development of plants. Nitrogen is the crucial nutrient that limits meadow yield. However, in Serbia meadows are generally

degraded, with low yields, poor quality and little or no fertilizers used. The application of 1kg of NPK fertilizer increased hay yield by 16.2 kg in the case of natural grassland, type *Agrostietum capillaris (Vučković et al., 2010)*. Also, the application of NPK fertilizer significantly increased GFY and DMY of an *Agrostietum capillaris* meadow.

Nitrogen levels significantly increased CPY. The highest CPY (876.3 kg ha^{-1}) was recorded in the $N_{200}P_{150}K_{150}$ treatment, with the highest DMY, and the lowest in the $N_0P_0K_0$ treatment (175.5 kg ha^{-1}), with the lowest DMY. The proportion of forage crude protein in ewe, lamb and cow nutrition should be 9.4%, 12.8% and 11%, respectively *(National Research Council, 1996)*. In our research, the CP content resulting from $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ treatments met protein requirements for livestock. Many researches have shown that NPK fertilization of natural *Agrostietum capillaris* grasslands increased the ratio of high-quality plants and hence the level of protein production *(Đurić et al., 2007; Tomić et al., 2009; Radić et al., 2014, Radić et al., 2017).* It should be emphasized that nitrogen application had a positive effect on forage production and CPY, even under drought condition in 2007. Accordingly, nitrogen fertilizer input is a limiting factor for the production of high quality forage. The availability of N limited forage production of rangeland and pastures more than rainfall in southeastern Alberta, in arid regions *(Blonski et al, 2004).*

NU and NNI significantly increased, while UNU significantly decreased with increasing nitrogen rates. The minimum values of NU and NNI and the maximum value of UNU were recorded in the $N_0P_0K_0$ treatment. There was a wide variation in NU and UNU due to different fertilizer treatments. The average NU and UNU of all the fertilization rates were 85.06 kg ha^{-1} and 0.0035 kg N kg DMY^{-1}, respectively. DMY of the control treatment was low compared to the other treatments, so NU was minimal. By contrast, in the $N_{200}P_{150}K_{150}$ treatment DMY was higher, as was NU. Lower values of NNI, less than 80%, indicated that the nutritional status of the plants was too low. Also, the nitrogen rates were low and needed to be increased. Similarly, in the Romanian mountain grasslands of *Festuca rubra* L. and *Agrostis capillaris* L. increased NNI with increasing N fertilization, and that low values of NNI indicated nutrient deficiencies in the soil *(Samuil et al., 2018).*

Botanical composition

In the untreated and uncut meadow there were 45 different plant species, of which 73% of the total count were forbs. The dominant species was *Agrostis capillaris*, followed by *Holcus lanatus, Cynosurus echinatus, Trisetum flavescens, Arrhenatherum elatius* and *Anthoxanthum odoratum.* The proportions of grasses and legumes were relatively small: 18% and (9%), respectively. Cutting decreased biodiversity (29 species), but the proportions of the grasses (17%), legumes (7%) and forbs (76%) remained virtually the same. Fertilizer treatments increased the

number of grasses but decreased the number of legume and forb species. The proportion of the tall grass *Arrhenatherum elatius* and other useful species increased, while the overall biodiversity decreased to less than 20 species. At the maximum nutrient rate, the grass species became absolutely dominant, with only one legume and no significant representatives of forbs. This is attributable to the fact that nitrogen favors faster and ampler growth of grasses than of the other identified species. In general, legumes are a weak competitor of grasses *Andreata-Koren et al., 2009)*. Furthermore, an increase in the amount of nitrogen from 40 to 120 kg ha^{-1} increased the proportion of grasses and significantly reduced those of the legumes and other plant species in a natural meadow of the *Agrostietum capillaris* type *(Stevović et al., 2011)*. Moreover, the fertilization of natural grasslands with nitrogen increased the share of C3 plants, decreased biodiversity and reduced the proportions of C4 plants and legumes *(Gough et al., 2000; Stevens et al., 2004)*. It is believed that this is a consequence of more competition for light *(Xia and Wan, 2008)*.

Conclusions

Managing NPK fertilization of semi-natural *Agrostietum capillaris* meadows is important for achieving yields of satisfactory quality for nutrition of small ruminants in the mountainous regions of Serbia. The *Agrostietum capillaris* association had the highest GFY, DMY, NC, CPY, NU and NNI in the season of 2004/2005, when the rainfall distribution was the most favorable, and had a positive effect on grass tillering and accelerated plant growth. As the nitrogen level increased, GFY, DMY, CPY, NU and NNI tended to increase as well. By contract, UNU exhibited a downward trend. However, the value of NNI indicated that the nutritional status of the soil was too low. Consequently, the nitrogen rate of 200 kg ha^{-1} was low and needed to be increased

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Prinos, kvalitet i indeks ishrane azotom livade tipa *Agrostietum capillaris* pod uticajem đubrenja

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Rezime

Unošenje N, P i K inputa u poluprirodnim livadama je važno za postizanje maksimalnih prinosa na stočarskim farmama. Cilj ove studije bio je procena uticaja različitih nivoa N, P i K ($N_0P_0K_0$, $N_{50}P_{50}K_{50}$, $N_{100}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{100}K_{100}$ and $N_{200}P_{150}K_{150}$ kg ha^{-1} yr^{-1}) na prinos, kvalitet i indeks ishrane azotom (NNI) u livadskoj zajednici *Agrostietum capillaris* (poluprirodna livada) u zapadnoj Srbiji. Studija je sprovedena tokom perioda 2005-2008. Vrednosti ispitivanih parametara, izuzev usvajanja azota po jedinici mase, bile su najviše u 2004/2005 zbog povoljnih klimatskih uslova. Nivoi azota su značajno povećali sve ispitivane parametre u poređenju sa kontrolnim tretmanom, osim usvajanja azota po jedinici mase. Đubrenje sa $N_{200}P_{150}K_{150}$ obezbedilo je najveći prinos zelene krme (25.12 t ha^{-1}), prinos suve materije (8.12 t ha^{-1}), prinos sirovih proteina (876.3 kg ha^{-1}), usvajanje azota (140,2 kg ha^{-1}) i indeks ishrane azotom(70,2%), kao i najmanji (0,0022 kg N kg PSM^{-1}). Upotreba mineralnih đubriva povećala je prinos zelene krme, prinos suve materije i prinos sirovih proteina, povećavanjem đubriva od najmanje do najveće količine je povećalo prinos sveže i suve mase, kao i prinos proteina. Na osnovu rezultata studije, praćenje indeksa ishrane azotom bi bilo neophodna mera u cilju porasta produktivnosti i ekonomske efikasnosti.

Ključne reči: *Agrostietum capillaris*, đubrenje, azotni indikatori, kvalitet, prinos

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LIVESTOCK PROTECTIVE FENCING (LPF) TO PROTECT DAIRY CATTLE AGAINST VECTORS IN SERBIA - PROJECT PROCESSES AND METHODOLOGY

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Short communication

Abstract: Lumpy skin disease (LSD) is among a number of vector-borne diseases (VBDs) threatening the Balkans and therefore, preventing feeding of insects on cattle would reduce the spread of VBDs. In order to test the efficiency of Livestock Protective Fences (LPF) in the protection of dairy cattle from insect bites, a case-control study was conducted, in the districts of Nišava, Pirot and Pčinja, Southern Serbia. It consisted in comparing the number of biting flies collected within time, between 10 farms protected with LPF and 10 non protected ones. The insects were collected using two types of traps; the monoconical Vavoua trap set outside in between forested areas or rivers and the actual farm, and the BG-sentinel trap baited with CO_2, placed in proximity of the cattle but outside the stable. Vectors were collected every 15 days for 48 hours from May to October 2018 and kept in vials containing 70% of ethanol. Catches per trap were separately stored and for each trap, insects were classified according to species and sex and then counted. Data on milk parameters were analyzed separately, on data collected within protected farms, before and after the LPF deployment, and on data without protection at all. It was not possible to detect a direct impact of LPF on vector densities but the number of bacteria colonies (CFU) values were reduced. Some corrections/adaption in the methodology used may lead to better impact.

Key words: Serbia, lumpy skin disease, vectors borne diseases, Livestock Protective Fence, milk quality

Introduction

Lumpy skin disease (LSD) is among a number of vector-borne diseases (VBDs) threatening the Balkans. This notifiable disease has dramatic effects on rural livelihoods and the effect at national level is also devastating due to strict trade restrictions (*Casal et al., 2018; Molla et al., 2017*). The situation worsens with the arrival of summer's higher temperatures, favouring insect multiplication and, hence, disease dissemination. Therefore, preventing feeding of insects on livestock would also reduce the spread of VBDs (*Bauer et al., 2006; Bauer et al., 2011*). Unfortunately, vector control strategies used so far are either costly, time consuming or environmentally unacceptable. The large-scale and indiscriminate use of insecticides, mostly pyrethroids, constitutes the mainstay of vector control efforts. Pyrethroids are all upsetting the function of the sodium channels. Resistance against one pyethroid will lead to resistance against the whole class. This has led to widespread pyrethroid insecticides resistance in target vectors insects like *Aedes sp., Culex sp*. or *Culicoides sp.* (*Laveissiere and Grebaut,1990; Caputo,2018; Pichler et al., 2018; Bengoa et al., 2017*) Stable flies (*Stomoxys* spp.) have found to be less or no longer susceptible, (*Reissert-Oppermann et al., 2019*). However, there is a highly effective technology, the use of insecticide-incorporated knitted textile screens (known as livestock protective fences – LPF), which has been very successfully used in sub-Saharan African countries for the control of several VBDs (*Heilmann et al., 2017; Maia et al., 2012; 2010; Bauer et al., 2011*). The fences are deployed in the vicinity of resting places near to cattle or alongside milking parlors, preventing insects from alighting on the animals.

The technology, never used in the Balkans, could be very useful in preventing VBDs. Another externality is stress reduction associated with insect bites of dairy cattle, an improvement of animal welfare and an increase in milk production (*Maia et al., 2010*). Moreover, significant reductions in mastitis cases have been recorded in the past, as insect vectors commonly transmit bacteria from animal to animal.

The current study aims to scientifically prove the efficiency of LPFs in the protection of dairy cattle from insect bites, thus reducing the transmission of VBD such as LSD, and reducing the stress of the animals due to insect bites.

If proven successful, the pilot study could be expanded within Serbia and to other affected and at-risk countries in the region (*Allepuz et al., 2018*).

Materials and Methods

Study location

Twenty farms were selected for a case-control study over one vector season, from mid-May to October 2018. Half of the farms were protected by LPF (case group), while the other half was considered as a control group, enabling to assess the trends of insect numbers and species. Half (50%) of the selected farms was located in the Nišava District with almost 100% of the case farms, and in Pirot and Pčinja districts, where control farms were mainly located (Figure 1). All farms were dairy production ones and were mostly maintained on a zero-grazing scheme, with cattle number not exceeding 40 in total.

The farms were characterized through a questionnaire covering the production system, dung management, insect control treatments, etc.

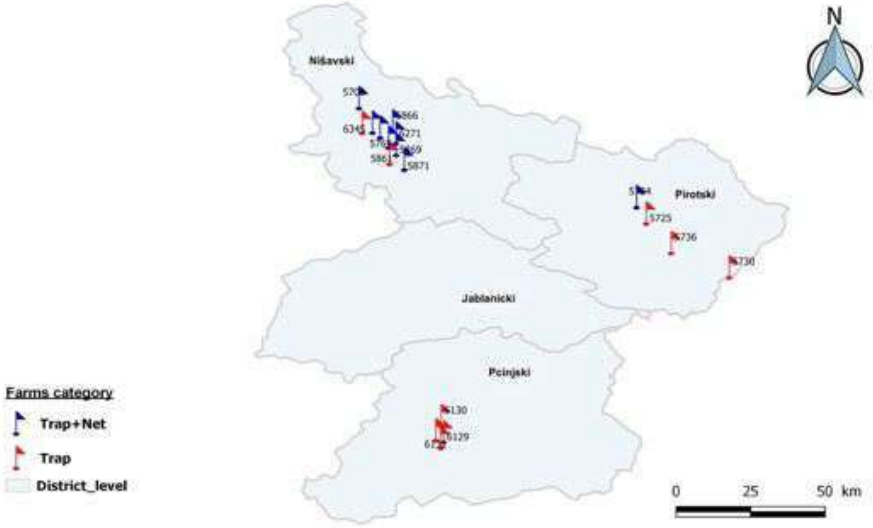

Figure 1. Location of the farms selected for the monitoring of the impact of LPF on the densities of LSD vectors

T0 data collection and monitoring of the LPF impact on vector densities

Once a farm was selected, and before the deployment of the LPF, two types of traps were used to estimate the presence of target insect species: Monoconical Vavoua (*Laveissière et al., 1990*) (Figure 2) and BG-sentinel trap (Figure 3). The Monoconical traps were set outside in between forested areas or

rivers and the actual farm, while the BG-sentinel trap, baited with CO_2 (to enhance their efficacy) were placed in proximity of the cattle, but outside the stable, connected to a power source and attached to a CO_2 bottle. All trap locations were geo-referenced and visibly labelled. Throughout the intervention the positions of the traps were not supposed to be modified unless in a situation of emergency (change of pen location, bushfire, threat of theft). Continuous trapping would also allow assessing the LPF impact before and during intervention.

Figure 2. Monoconical Vavoua trap

Figure 3. BG-Sentinel trap

Likewise, data on milk parameters, notably, the CFU (colony-forming units; i.e. the number of viable bacterial/fungal cells/ml) and the number of somatic cells were collected from the dairy association, for comparison. These data related to milk production should allow to assess the effects of LPF on animal welfare and common dairy associated diseases such as mastitis.

Impregnation of LPF

Lambda-Cyhalothrin in a suspension concentrate (S.C.) in a concentration of 0.6% supplied from Changzhou Biochemical Co. Ltd, China, in 10 litre canisters was used for the impregnation. For each impregnation, the netting material (100 m *0.8m; 1-2 mm diameter holes) was soaked in 2.7 litres of solution of active ingredient and water, to thoroughly moisten the fabric with as little as possible relic

solution remaining. After impregnation, the netting was first dried and then attached to horizontal pieces of wood to facilitate deployment (Figure 4).

In case farms, LPF were deployed covering all windows and openings in the stable, except for those needed for the operation of the farm, e.g. to get manure/feed in/out of the stable, and doors for animals and personnel to enter/exit/move around (Figure 5). In addition, LPF are also deployed around dung pits or ponds when not covered.

Figure 4. Netting preparation prior to deployment

Figure 5. Impregnated netting deployed around a farm to prevent insects from neighbouring forest

Vector collection and identification

Vectors were collected every 15 days for 48 hours from May to October 2018 by the farmers and then sent to the Scientific Veterinary Institute in Belgrade in vials containing 70% of ethanol. Any collected insect was duly labeled and identified based on morphological characteristics, using keys given (*Wall and Shearer, 1997; Cedric, 2005; Capinera 2008*). Catches per trap were separately stored and, for each trap, insects were divided/classified according to species and sex and then counted.

Data analysis

An ANOVA was first applied to the data, and for each treatment, (control or case), the period (month) was used to explain the variation of insect numbers random effects. Data were then submitted to a multiple comparison of means (Tukey contrast) using statistical package software available online (R Development Core Team, Vienna, Austria). Because (case and control) are very

different in terms of geographical distribution, and so cannot be compared by pairs, this comparison was done within each type of treatment (case and control). The insects were first pooled by genus (Mosquitoes, Stomoxys, Tabanids, etc.) before being submitted to analysis.

For the data on milk parameters, analysis was made separately, on data collected within protected farms, before and after the LPF deployment, and on data without protection. As for cell numbers, CFU data were analyzed with a negative binomial generalized linear mixed model. The treatment was used as explanatory variable, and the farm ID and months were considered as random effects

Results

Insects collected

A total of 3,007 insects were collected during the 5-month monitoring period (Table 1), with up to 46% of *Musca spp* and 20% of *Ceratopogonidae*. Mosquitoes constitute up to 12% of the total insects caught, with a predominance of the genus *Culex* (54%)

Table 1. Total number of vectors collected during the 5 months monitoring

	Anopheles	*Culex*	*Aedes*	*Phlebo - tominae*	*Cerato- pogonidae*	*Tabani dae*	*Stomo- xyinae*	*Musca spp*	*Fan- niidae*
Mono	13	20	0	0	190	49	8	483	90
Mono net	30	11	0	0	157	33	7	376	80
BG	28	85	17	9	125	16	77	250	74
BG net	54	82	27	35	131	29	59	263	99
Total	**125**	**198**	**44**	**44**	**603**	**127**	**151**	**1372**	**343**

Aedes and *Phlebotominae* were only by BG traps (inside and closer to cattle) and none by the monoconical traps deployed outside and far from the cattle. Likewise, BG traps caught up 4-8 folds more *Culex* than monoconical traps.

Impact of LPF on vector densities

As indicated previously, this has been assessed in comparing the evolution of the densities within each type of treatment. Regardless of the natural variations, vector populations are expected to be decreasing within the time in farms where LPF was deployed. Numbers of different species of mosquitoes were pooled for this purpose.

For each of the species caught by both type of traps, no significant difference was observed between the two periods (before and after LPF deployment) in terms of insects' number.

Impact of LPF on milk quality parameters

In a first step, comparison was made within the 10 case farms before and after, showing a significant decrease of the number of CFU (a mean number of 319 to 122); p=0.028, figure 5.

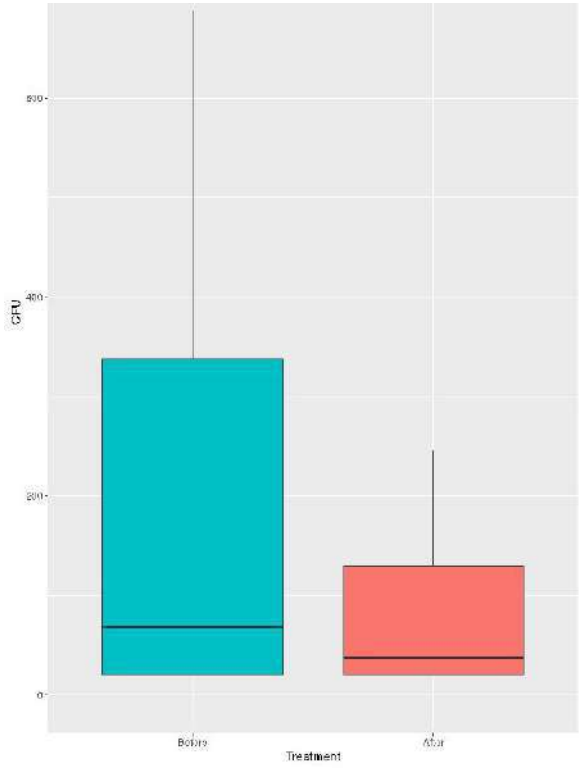

Figure 5. Mean number of CFU in protected farms, before and after the LPD deployment

For the somatic cells, however, the decrease is not significant; 241 *vs.* 200, p=0.519 (figure 6).

In a second step, the comparison was made within the 10 control farms, no significant evolution was observed for both CFU (275 *vs.* 181, p=0.795) and cells (253 *vs.* 296, p=0.442).

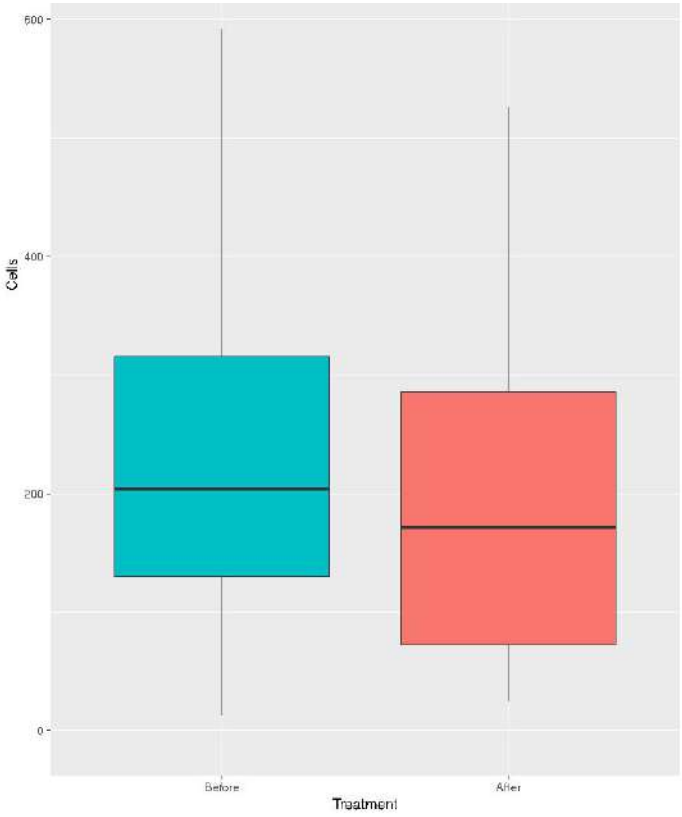

Figure 6. Mean number of cells in protected farms, before and after the LPD deployment

Discussion

Target insect densities were recorded and compared on 20 farms in southern Serbia. Ten farms served as controls and 10 farms benefited from a protection with insecticide-impregnated netting material that had been strategically deployed, closing windows and entrances. This approach was aiming at the control of putative vectors for LSD and other VBDs. Vector control may serve as a prevention measure against VBDs.

Insect densities were monitored by using a total of forty traps – i.e. two trap types per farm. Mono-conical ("Vavoua") traps were deployed outside the farm buildings but still within the premises of the respective farm. Mono-conical farms are particularly suited for catching horse flies and muscid flies – including stomoxyine biting flies, commonly known as stable flies.

BG sentinel traps are known for their efficacy of catching mosquito populations. Also, they have been used on a large scale for assessing other

hematophagous vectors such as *ceratopogonidae* (biting midges) and *phlebotominae* (sandflies). BG traps were deployed in proximity of the cattle inside the farms. All traps were controlled at fortnightly intervals and all catches were transferred to Belgrade (Scientific Veterinary Institute) for identification and counting.

A cotton wool netting material was impregnated with lambda-cyhalothrin, a pyrethroid formulation. Previous work using this approach had shown great potential of controlling target insect populations in various African countries (*Bauer et al., 2011*) and in Germany. The ready-to-use fence was then attached to a wooden frame, reducing the space for entering target insect species. The trial lasted for five months during 2018.

As in previous studies, no impact on vector densities was found. Usually, it would take at least two years for any detectable reduction of target vector species (*Maia et al., 2010*). However, when controlling tsetse flies in a forest area of South-Eastern Ghana significant differences between protected and unprotected pens were detected (*Bauer et al., 2011*).

The year 2018 was particular in much of Europe with temperatures reaching or exceeding 40°C and a long, dry season. The climate may have had a negative impact on the survival rate of many insects. All the same, the initial figures of insect catches should have been high. However, at no time the numbers of collected insects corresponded to what has been recorded elsewhere. Indeed, during the Bluetongue outbreak in Germany, more than 1,000 Culicoides/trap/night were caught. It is puzzling and not well understood from our point of view why the total insect numbers were so low. For instance, it would be beyond any expectation from previous studies if a total of 603 biting midges were caught during five months with 40 traps. It is also puzzling to observe that the catches of midges with monoconical traps exceeded those ones recorded with BG sentinel traps. At the same time the catches of stable flies with mono-conical traps were widely below current expectations and cannot be explained at our level. It was also noted with surprise and cannot be explained why BG sentinel traps caught the vast majority of stable flies.

As already stated, any detectable impact on target vector populations would appear premature. However, it would be important and it is recommended to monitor and evaluate the efficacy of the netting material: immediately after impregnation and then at regular intervals up to the termination of this study.

During a study on dairy farms in Kenya, significant reductions of mastitis cases in milking cows due to their protection with LPF were shown. Similar assessment should be performed during an eventual follow-up study.

In view of the particular animal husbandry management system – all cattle were mostly kept inside the pens on a zero-grazing scheme, the putative vector populations can be narrowed down to stable flies, mosquito species, biting midges and sandflies. Arguably, the role of house flies should be considered as irrelevant

in the transmission of LSD. Should there be a follow-up study, it might be worthwhile to record (with cameras) and compare defensive cattle movements on protected and control farms. Benefits would be more convincing if it were shown that the cattle well-being is greatly enhanced by a partial protection of their pens. Horse flies are exophile and exophagic. Their preference of feeding on horses and cattle while on pasture has been frequently observed. In reaction to this, it is observed for instance, that horses were trying to protect themselves by retreating under an open but roofed hut, whenever peak densities of horse flies were occurring. Stable flies, on the other hand readily feed on their hosts inside as well as outside the pens. Camera recordings of defensive movements would therefore go a long way in producing the required evidence for the benefits of protection.

Conclusion

The objective of this study was to explore the possibility of using LPF to protect cattle, in order to minimize risks of contracting VBD, notably LSD, and, also, to improve their wellbeing and milk quality. Although it was not possible to capture the direct impact of LPF on vector densities, the CFU values have improved. Some corrections/adaption in the methodology used may lead to better impact.

Zaštitna mreža za stoku (lpf) u zaštiti mlečnih krava od vektora prenosioca bolesti – projekat i metodologija

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Rezime

Bolest kvrgave kože (LSD) spada među brojne bolesti koje se prenose vektorskim putem (VBD) koji prete Balkanu, pa bi sprečavanje hranjenja insekata stokom smanjilo širenje VBD-a. Da bi se testirala efikasnost stočnih zaštitnih mreža (LPF) u zaštiti mlečnih goveda od uboda insekata, sprovedena je studija njene efikasnosti. Ogledi su rađeni u Nišavskom, Pirotskom i Pčinjskom okrugu (južna Srbija). Sastojao se u poređenju broja hematofagih insekata prikupljenih u šestomesečnom periodu , na 10 farmi zaštićenih LPF i 10 nezaštićenih. Insekti su sakupljeni koristeći dve vrste zamki; monokonična zamka Vavoua koja je postavljena napolju između šumovitih područja ili reka i stvarne farme, i BG-sentinel zamka sa CO_2,

smeštenih u blizini stoke, ali izvan štale. Vektori su prikupljeni svakih 15 dana tokom 48 sati od maja do oktobra 2018. godine i držali su se u bočicama sa 70% etanolom. Ulovi po zamci odvojeno su smešteni i za svaku zamku su insekti klasifikovani prema vrsti i polu, a zatim su brojani. Podaci o parametrima mleka analizirani su odvojeno. Podacima na zaštićenim farmama prikupljani su pre i posle primene LPF i sa farmi gde nije postavljena zaštita. Nije bilo moguće detektovati direktan uticaj LPF na gustinu vektora, ali je broj kolonija bakterija (CFU) u mleku smanjen. Neke korekcije / prilagođavanja u korišćenoj metodologiji mogu dovesti do boljeg uticaja primene LPF-a.

Ključne reči: Srbija, bolest kvrgave kože (LSD), vektorski prenosive bolesti, zaštitna mreža za stoku

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Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[1]

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

Example 2

EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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