UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

Original scientific paper

Communication

VOL 34, 1
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2018

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2018

Biotechnology in Animal Husbandry 34 (1), p 1-126, 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

Address of the Editor's office
Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164;
e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

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According to CEON bibliometrical analysis citation in SCI index 212, in ISI 9, impact factor (2 and 5) of journal in 2012: 0,667 and 0,467, - M51 category

Annual subscription: for individuals -500 RSD, for organizations 1200 RSD, -foreign subscriptions 20 EUR. Bank account Institut za stočarstvo, Beograd-Zemun 105-1073-11 Aik banka Niš Filijala Beograd.

Journal is published in four issues annually, circulation 100 copies.

The publication of this journal is sponsored by the Ministry of Education and Science of the Republic of Serbia.
Printed: "Mladost birošped", Novi Beograd, St. Bulevar AVNOJ-a 12, tel. 381 11 2601-506

Biotechnology in Animal Husbandry 34 (1), p 1-19 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 631.563
https://doi.org/10.2298/BAH1801001G

MODIFICATION OF THE PROPORTION OF EXTRACTABLE AND BOUND CONDENSED TANNINS IN BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS*) AND SAINFOIN (*ONOBRYCHIS VIICIFOLIA*) DURING WILTING, ENSILING AND PELLETING PROCESSES

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Original scientific paper

Abstract: Condensed tannins (CT) in legume forages vary not only in concentration and structure, but also in the portion of soluble and protein- and fibre-bound fractions. This study aimed to assess the changes in the total CT level as well as relative abundance of the three CT fractions from fresh to wilted, ensiled or pelleted legumes like in birds foot trefoil (two cultivars) and in sainfoin (one cultivar). Each legume underwent three consecutive harvests, of which the first two were wilted. Additionally, wilted legumes were either ensiled (first harvest) or transformed into dehydrated pellets (second harvest). For each harvest, total CT and the percentage of soluble, protein- and fibre-bound CT differed ($P < 0.01$) among plants. The total CT content was similar after wilting but was lower ($P < 0.05$) after ensiling. After wilting, ensiling and pelleting the portion of soluble CT was lower in favour of protein-bound CT portion. However, time of harvest affected ($P < 0.05$) total CT and the percentage of soluble and protein-bound CT. Thus, measuring the bound-fraction should not be ignored in the determination of CT content since this fraction, together with the soluble fraction, might protect protein from ruminal degradation.

Keywords: condensed tannins, soluble fraction, bound fraction, wilting, ensiling, pelleting

Introduction

The use of legume forages in livestock farming decreased in Europe over the last two decades principally because of the low price of soyabean meal and the increasing use of corn silage (*Doyle and Topp 2004; Peyraud et al. 2009*).

However, in the last few years there is increasing interest for temperate legumes such as birds foot trefoil (*Lotus corniculatus*) and sainfoin (*Onobrychis viciifolia*). Apart from their crude protein content, the content on bioactive secondary metabolites like condensed tannins (CT) attracts great interest. Condensed tannins have been shown to improve health, production efficiency and product quality in ruminants. For instance, tanniferous legumes reduce bloat and parasitic burden and modify protein utilization through a reduction in N excreted in urine and milk thereby reducing the metabolic load (*Barry and Mcnabb 1999; Patra and Saxena 2011; Grosse Brinkhaus et al. 2016a*). Moreover, feeding CT can modify the quality of ruminant products by increasing n-3 polyunsaturated fatty acid levels and by reducing pastoral off-flavour (*Schreurs et al. 2007; Girard et al. 2015, 2016*).

Condensed tannins are a vast family of polymers composed of flavan-3-ol monomers present in different concentrations in plants. Within the same plant, CT are not equally distributed, leaves and flowers are richer in CT than stems (*Lees et al. 1993; Häring et al. 2007*). In addition to the concentration, the bioactive properties of CT play an important role (*Mueller-Harvey 2006; Frazier et al. 2010*). Bioactivityis mainly driven by the chemical structure of CT, including the mean degree of polymerization (mDP), chemical conformation (*cis*:*trans* ratio) and the ratio of procyanidin (PC) to prodelphidin (PD) monomers. Moreover, CT in the plant can be present in a soluble or insoluble form, the latter being principally bound to proteins or dietary fibres. Up to now, methods to quantify CT mainly focused on analysing the content and chemical structure of the soluble CT. Only few studies concentrated on the properties of the bound fraction of CT in relation to animal nutrition. For instance Kariuki and Norton (2008) showed that the portion bound to proteins is of interest in animal nutrition because protein can dissociate from CT and be available for digestion and absorption in the small intestine of ruminants. Furthermore, since in many livestock production systems forages are fed not only fresh but also after being conserved for months, the additional steps of the conservation process, such as drying or ensiling, may affect the content and composition of the CT and ultimately influence their bioactive properties (*Scharenberg et al. 2007a; Theodoridou et al. 2010*).Other ways of conservation like pelleting would offer a good compromise to include CT into the rations, to avoid feed wastage and to facilitate transport and storage (*Terrill et al. 2007*). However, the high temperature of this process used during pelleting might modify the bioactive properties of CT.

The present study was performed with CT-rich legumes from two different species known to differ in their CT content and in their chemical structure: two birds foot trefoil cultivars (birds foot trefoil Polom and birds foot trefoil Bull) and one sainfoin cultivar (sainfoin Perly).The study wanted to tackel the following objectives: firstly, monitor changes in the CT content from the fresh state through the wilting and ensiling or pelleting processes with special emphasis on the soluble,

protein- and fibre-bound CT fractions; secondly, assess whether the variation of the CT content and of the ratio of the three fractions with the conservation mode was similar in all three legumes; thirdly, compare these effects between harvests.

Material and methods

Forage legumes and harvest

The experiment was carried out at Agroscope Institute for Livestock Sciences, Posieux, Switzerland (latitude:46°46' N, longitude: 07°06' E; altitude: 650 m). Before sowing, fields were ploughed with a rotary harrow. Three CT-containing legumes were sown in March 2012 in fields of 7300 square meters each. The sainfoin (*Onobrychis viciifolia*, Perly cultivar; OvP) was provided by Delley Semences et Plantes (Delley semences et plantes SA, Delley, Switzerland) and the two birds foot trefoil cultivars (*Lotus corniculatus*, Bull and Polom; LcB and LcP) were provided by Cotswold Seeds (Cotswold Seeds Ltd, Gloucestershire, United Kingdom). The sowing density was 1.6 kg per are for the OvP and 300 g per are for the LcP and LcB. No mineral fertilizer was applied. To obtain three independent replicates (batches) per legume, fields were subdivided in three plots and sampling between two plots was performed at a distance of approximately 150 m (*Grosse Brinkhaus et al. 2016b*).

Legumes were harvested for the first time in July 2012 at early flowering stage for LcP and LcBand full flowering stage for OvP. The legumes were cut a second and third time after 50 days of regrowth each, in August and October 2012, respectively. In August, the bird's foot trefoil cultivars and the OvP were at full flowering and at the end of the flowering stage, respectively, whereas at the third cut all plants were at a vegetative stage. The poor yield of the third harvest hindered any further conservation trails.

Immediately after cutting, at 13:00 h, fresh samples of each legume were randomly collected in the three aforementioned plots (3 batches per field). The rest of the harvest was wilted for 24h. One day after cutting, three wilted samples were collected in the vicinity of each fresh sampling location. A fraction of each fresh and wilted sample was separated for the determination of dry matter (DM) content and the rest was stored at -20°C for further laboratory analysis.

Regarding the weather conditions during the whole experiment, from sowing to harvesting (Figure 1), rainfall was higher in June (127mm) and in September (142 mm) but with 25 mm markedly lower in March. The greatest average temperature was recorded in August with 18.8°C with temperatures ranging from 11° to 24.3°C.

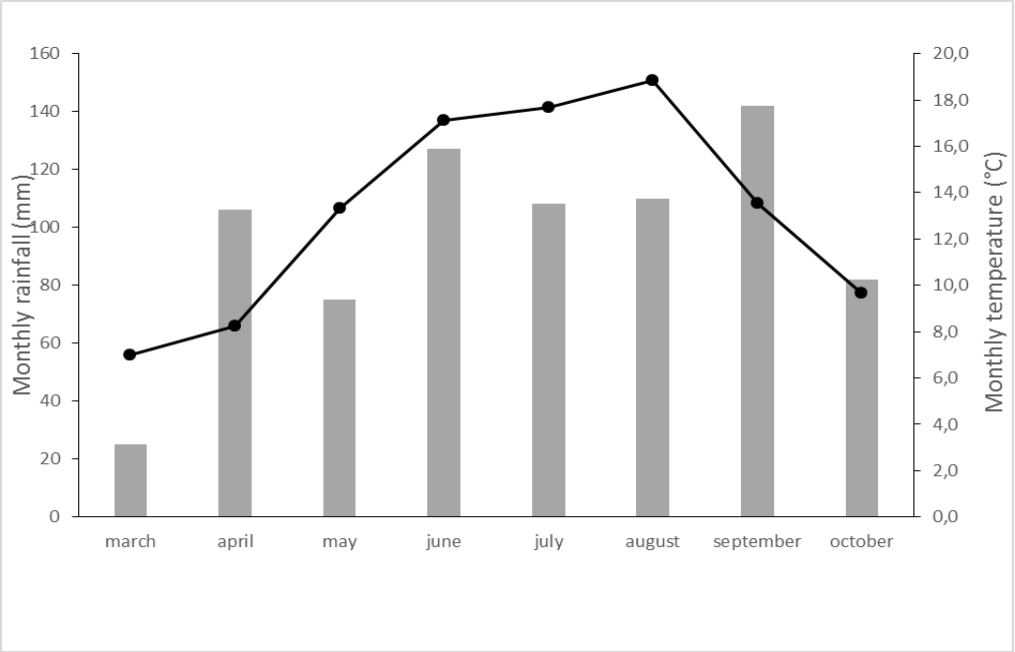

Figure 1. Monthly average temperatures (joined line) and monthly rainfall (bars) during the experimental period

Ensiling and pelleting procedures

After wilting of the first harvest, the legumes were chopped (1-2 cm) with a chaff cutter (Mex GT, Poettinger, Grieskirchen, Austria) and ensiled without additives in 1.5 L-silos. For each legume, three silos per batch were prepared. The different silos were kept at 20°C for 86 days. Afterwards, the silos were opened and pooled per batch. One subsample was used to estimate the silage DM content and a second subsample was stored at -20°C for later analysis.

The wilted forages of the second harvest were transported to a forage drying company (Trocknungsgenossenschaft des Sensebezirks, Tafers, Switzerland) for the production of dehydrated pellets. Briefly, wilted forages from the three batches of each legume were mixed together and chopped (5 to 8 cm; Neumann Würzer, Kissleg, Germany) before being dried in a rotating barrel (type 5.0, Kunz, Langnau, Switzerland). The drying process, which lasted 4 min, was a succession of heating and cooling phases repeated three times. The temperatures in the heating and cooling phases were approximately 700 and 82°C, respectively. Dried samples were finely ground (c610, Kunz, Langnau, Switzerland) and then extruded as pellets (8mm matrix, Kahl, Reinbeck, Germany).

Nutrient analysis of the samples

The DM concentration of all the samples was determined by drying at 105°C for 3 h (after a previous 24 h drying at 60°C as sample conservation means). To access chemical composition, fresh, wilted and ensiled samples were lyophilized (Christ Delta 1–24 LSC, Osterode, Germany) and ground to pass a 1-mm sieve (Brabender mill, Brabender, Duisburg, Germany). The pellets were ground to pass a 1-mm sieve. The DM content of all lyophilized and pelleted samples was quantified thermo-gravimetrically by heating at 105°C for 3 h (LECO TGA 601; Mönchengladbach, Germany). In order to determine the organic matter content, total ash content was determined by dry-ashing the samples at 550°C for 4 h. The N concentration was quantified according to the Dumas method [Association of Official Analytical Chemists (AOAC), 2000] and crude protein content was calculated (N × 6.25). The neutral (NDF) and acid detergent fibre (ADF) were analysed following standard protocols (AOAC, 1995) using an ANKOM 200⁄220 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA) where NDF was assayed with heat-stable amylase and sodium sulphite. Both NDF and ADF were expressed without residual ash after incineration at 500°C for 1 h. The nutrient composition of the fresh material used in this experiment is reported in the Table 1.

Determination of soluble, protein-bound and fibre-bound CT

The CT content was determined using an HCl-Butanol method based on the one previously described by *Terrill et al. (1992).* Thus, three consecutive fractions were prepared in duplicate to access soluble, protein-bound and fibre-bound CT. Briefly, soluble CT were extracted by mixing 500±1 mg of lyophilized plant material with a 20 ml acetone:water solution (70:30, v:v) containing ascorbic acid (1 gl^{-1}) and 10 ml of diethyl-ether. This mixture was then centrifuged at 25’000 g at 5°C for 15 minutes. The upper (organic) layer was then discarded and the acetone:water layer collected. The extraction and centrifugation steps were repeated once with the solid residue. Following the second centrifugation, the solid residue containing the insoluble part of CT was kept. After combining the two acetone:water fractions containing the soluble portion of the CT, acetone was removed in a rotary evaporator (Büchi Rotavapor R-205, Büchi Labortechnik AG, Flawil, Switzerland). The aqueous acetone-free fraction was added with ultrapure water type I (milli-Q) to a total volume of100 ml in a volumetric flask. The kept solid residue was mixed with 15 ml of sodium dodecylsulfate (SDS) and 2-mercapto-ethanol solution (10 respectively50 gin 1 l of water), heated for 45 min at 95°C and cooled in an ice-bath for 10 min before being centrifuged at 25’000 g for 15 min at 5°C. The aforementioned steps for the solid residue were repeated once and after each centrifugation, supernatants were collected into 50 ml volumetric flasks and filled up with the SDS:2-mercapto-ethanol solution to a total of 50 ml.

This solution contained the protein-bound CT. The remaining solid residue contained the fibre-bound CT.

The subsequent colorimetric determination was performed individually on each of the three fractions using HCl (37%):butanol (5:95; v:v) solution. Six ml of HCl:butanol (5:95; v:v) solution was added to 1 ml of extract containing soluble or protein-bound CT whereas the solid residue containing the fibre-bound CT was mixed with30 ml HCl:butanol (5:95; v:v) and 3 ml of the SDS:2-mercapto-ethanol solution. Subsequently, all samples were boiled under reflux for 1h. The reflux was carefully set so as to avoid any losses. Similarly, a blank for each fraction was prepared with water:butanol (5:95; v:v) instead of the HCl:butanol solution. After colour development, all samples were immediately cooled in an ice-bath. Then, for samples containing the fibre-bound CT, A centrifugation at 15'000 g for 15 min at 5°C was performed in order to remove the solid pellet. Finally, all samples were filtered through hydrophilic filter. Absorbance at a wavelength of 550 nm was readily measured against a blank HCl:butanol (5:95; v:v) solution using a UV/VIS Spectrometer (PerkinElmer instruments, Lambda 40).

Purification of CT for the calibrations curves

Since each legume has its characteristic CT profile (e.g. different polymer size, chemical composition and conformation, soluble/non-soluble fractions, etc.) a specific calibration curve was prepared for each legume and solvent (water or SDS:2-mercapto-ethanol solutions). Purified CT material from each legume was prepared as following: A sample (50 g) from each lyophilized fresh-legume was stirred for 40 min in a solution of acetone:water (70:30, v:v) containing ascorbic acid (1 g l^{-1}) and then vacuum filtrated. The filtrate was washed three times with 250 ml dichloromethane in a separating funnel in order to remove lipids and pigments. The aqueous phase was then evaporated and lyophilized. This lyophilized sample was dissolved in methanol:water (50:50; v:v),run through a Sephadex column (Sephadex LH-20,25-100 µm, Fluka n°84952, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and eluted with acetone:water (70:30; v:v). The presence of CT in the different elution fractions was detected by the vanillin/HCl test (100 g l^{-1} of vanillin in 37% HCl). Finally, the eluted fractions positive to the vanillin/HCl test were combined, concentrated in a rotary evaporator and finally freeze-dried. Calibration standards of different concentrations were prepared with each purified CT material, both in water and in SDS:2-mercapto-ethanol (10:50 g l^{-1}).A total of six calibration curves were prepared, two per legume (one in water for the soluble fractions and one in SDS:2-mercapto-ethanolfor the insoluble fractions).

Statistical analysis

Except for fresh samples, data on total, soluble, protein- and fibre –bound CT levels were analysed for each harvest time separately using the procedure

MIXED of SAS (version 9.2). With the data of the first and second harvests, the plant (OvP, LcP, LCB), the forage form (fresh, wilted, silage and fresh, wilted, dehydrated pellet, respectively) and the plant × forage form interaction were used as fixed effects. As in the first harvest the batches were ensiled separately, the three batches were used as random effect in the statistical model.

Data on total, soluble, protein- and fibre–bound CT levels determined in the fresh samples of OvP, LcP and LCB were compared between the three harvests. For the mixed model the plant, the harvest time and the plant × harvest time interaction were used as fixed effects and the three batches as random effect. Least squares means were compared using the PDIFF option with the Tukey adjustment statement. All statistical tests were considered significant at $P < 0.05$.

Table 1. Nutrient composition of the fresh material at different harvest times

Plant	Harvest	Forage form	Items*				
			DM	OM	CP	NDF	ADF
Lotus corniculatus Polom	1	fresh	160.13	901.93	195.42	411.27	321.78
	2	fresh	179.60	903.32	199.16	383.73	364.81
	3	fresh	146.07	904.19	252.19	245.17	233.14
Lotus corniculatus Bull	1	fresh	153.00	902.37	194.63	429.52	336.04
	2	fresh	149.83	903.45	224.09	355.03	341.67
	3	fresh	156.43	903.58	255.04	265.77	235.65
Onobrychis viciifolia Perly	1	fresh	169.37	916.29	132.34	395.90	388.51
	2	fresh	219.43	916.75	147.98	340.98	328.20
	3	fresh	178.30	922.17	227.98	238.46	248.52

*expressed in g kg^{-1}: DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Results

Changes in the total CT content and the relative portion of total, soluble, protein- and fibre-bound CT in the fresh, wilted and pelleted legumes at the first harvest

The CT content and the percentage of soluble and insoluble CT from the first harvest are presented in Table 2. Regardless of forage form, total CT content was on average five times greater ($P < 0.05$) in the OvP compared with the LcP and LcB. With respect to the different fractions, relative differences ($P < 0.01$) between plants in the percentage of the 3 fractions can be observed. The average relative content of the soluble and protein–bound fractions in fresh, wilted and ensiled LcP was similar counting for 45 and 43% of the CT respectively, whereas relative content of the fibre bound fraction was12% lower ($P < 0.05$). By contrast, in LcB the average relative content of the soluble CT fraction was with 70% the most

abundant fraction, whereas the relative content of the protein- and fibre-bound fractions was 18 and 12% lower ($P < 0.05$), respectively. In fresh, wilted and ensiled OvP, almost two- and one-third of the CT were present in the soluble and protein-bound fractions, respectively, whereas the level of fibre-bound CT was only 9%. Regardless of the forage form, when comparing between legumes the average percentage of soluble CT was the greatest ($P < 0.05$) in LcB, followed by OvP and the lowest in LcP. Contrarily, percentage of protein-bound CT was lower ($P < 0.05$) in LcB, followed OvP and LcP. The percentage of fibre-bound CT was greater ($P < 0.05$) in the 2 birds' foot trefoil cultivars than in OvP.

With respect to the conservation mode, the average CT content of the 3 legumes decreased by 27% between fresh and silage samples. However, during the ensiling process, the relative abundance of soluble CT progressively declined ($P< 0.05$) reaching a difference of 19% between fresh and ensiled samples. Concomitantly, the protein-bound CT fraction increased ($P < 0.05$) by 17% from fresh to silage samples. The percentage of the fibre-bound CT fraction increased ($P < 0.05$) from fresh to the wilted samples but afterwards in the silage levels were comparable to the fresh samples.

Table 2. Changes in the total condensed tannins (CT) content and the relative portion of soluble (S-CT), protein- (P-CT) and fibre-bound CT (F-CT) in fresh, wilted and ensiled *Lotus corniculatus Polom*, *Lotus corniculatus Bull*and *Onobrychis viciifolia Perly*at the first harvest[1]

	Total CT (g kg^{-1} DM)	Percentage of		
		S-CT	P-CT	F-CT
Lotus corniculatus Polom				
fresh	23.1c	51de	38ab	11abc
wilted	24.4c	41f	43ab	16a
silage	23.1c	44ef	46ab	10bc
Lotus corniculatus Bull				
fresh	34.3c	75ab	13d	12ab
wilted	33.2c	68b	17d	15ab
silage	34.8c	66bc	23cd	11abc
Onobrychis viciifolia Perly				
fresh	174.1a	79a	15d	6c
wilted	155.5a	56cd	33bc	11abc
silage	111.6b	38f	51a	11abc
SEM[2]	8.62	2.0	2.5	1.2
P-values				
plant	<0.001	<0.001	<0.001	0.002
forage form	0.027	<0.001	<0.001	<0.001
plant × forage form	0.011	<0.001	<0.001	0.033

[1]Within a column, means not sharing lowercased letters differ significantly at the $P < 0.05$ level.

[2]SEM = standard error of plant × forage form mean

A plant × forage form interaction ($P < 0.05$) existed for total CT content as well as for the percentage of soluble, protein-bound and fibre-bound CT. Regardless of the forage form, total CT content did not change in the 2 birds' foot cultivars. By contrast, total CT content was lower in the OvP silage compared with the fresh and wilted OvP (interaction plant × forage form; $P < 0.05$). The percentage of soluble CT decreased and the percentage of protein-bound CT increased in the OvP, whereas in fresh, wilted and silage samples of the 2 birds foot cultivars the contents did not change (plant × forage form; $P < 0.05$).

Changes in the total CT content and the relative portion of total, soluble, protein- and fibre-bound CT in the fresh, wilted and pelleted legumes at the second harvest

Similar to the first harvest, in the second harvest average total CT content of OvP was greater ($P < 0.05$) compared with the 2 birds foot trefoil cultivars (Table 3). Regardless of the forage form, the percentage of soluble CT decreased ($P < 0.05$) from OvP to LcB to LcP. The percentage of protein-bound CT was greater ($P < 0.05$) in LcP compared with LcB and OvP while the percentage of fibre-bound CT was greater ($P < 0.05$) for the 2 birds' foot trefoil compared with the OvP.

Table 3. Changes in the total condensed tannins (CT) content and the relative portion of soluble (S-CT), protein- (P-CT) and fibre-bound CT (F-CT) in fresh, wilted and pelleted *Lotus corniculatus Polom, Lotus corniculatus Bull* and *Onobrychis viciifolia Perly* at the second harvest[a]

	Total CT (g kg^{-1} DM)	Percentage of		
		S-CT	P-CT	F-CT
Lotus corniculatus Polom				
fresh	31.5	59d	30c	11
wilted	27.1	49e	39b	12
pellets	24.8	34f	50a	16
Lotus corniculatus Bull				
fresh	39.3	73b	17e	10
wilted	41.0	70bc	20de	10
pellets	36.4	65cd	22de	13
Onobrychis viciifolia Perly				
fresh	207.0	79a	15e	6
wilted	166.3	69bc	22d	9
pellets	140.4	69bc	21de	10
SEM[b]	16.30	1.7	1.8	1.6
P-values				
plant	<0.001	<0.001	<0.001	0.002
forage form	0.069	<0.001	<0.001	0.007
plant × forage form	0.104	<0.001	0.001	0.677

[a]Within a column, means not sharing lowercased letters differ significantly at the $P < 0.05$ level.
[b]SEM = standard error of plant × forage form mean

Regardless of the legumes, dehydrated pellets had a greater ($P < 0.05$) percentage of fibre-bound CT than the fresh and wilted samples. The average percentage of soluble CT was 20% lower ($P < 0.05$) and that of the protein-bound CT portion 50% greater ($P < 0.05$) in dehydrated pellets compared to the fresh samples. However, a plant × mode of conservation interaction existed ($P < 0.001$) for the percentage of both soluble and protein-bound CT (Figure 3). This interaction was mainly caused by the steady decrease in the portion of the soluble CT fraction and an increase in the protein-bound fraction from fresh to wilted and pelleted LcP samples. Only minimal changes in the relative portions of the 3 fractions occurred in the LcB and OvP.

Changes in the total CT content and the relative portion of total, soluble, protein- and fibre-bound CT in the fresh legumes depending on the time of harvest

In all three cuts, the total CT content and the relative portions of soluble, protein- and fibre-bound CT fractions in fresh samples differed ($P < 0.001$) among legumes (Table 4). Also when the third harvest was included, total CT content of the OvP was still 5 to 6 times greater ($P < 0.05$) compared with the 2 birds foot trefoil cultivars. Both, OvP and LcB had a greater ($P < 0.05$) relative portion of soluble and a lower ($P < 0.05$) portion of protein-bound CT than the LcP. The portion of fibre-bound CT was greater for the 2 birds' foot trefoil cultivars compared with the OvP.

Table 4. Changes from different harvest times in the total condensed tannins (CT)content and in the relative portion of soluble (S-CT), protein- (P-CT) and fibre-bound CT (F-CT) in fresh Lotus corniculatus Polom, Lotus corniculatus Bull and Onobrychis viciifolia Perly(OvP)[a]

		Total CT	Percentage of		
Plant	harvest	(g kg^{-1} DM)	S-CT	P-CT	F-CT
Lotus corniculatus Polom	1	23.1	51bc	38	11ab
	2	31.5	59b	30	11ab
	3	19.4	47c	41	12a
Lotus corniculatus Bull	1	34.3	75a	13	12a
	2	39.3	73a	17	10abc
	3	37.4	75a	17	8bcd
Onobrychis viciifolia Perly	1	174.1	79a	15	6d
	2	207.0	79a	15	6d
	3	164.5	75a	18	7cd
SEM[b]		8.33	2.0	2.3	0.8
P-values					
plant		<0.001	<0.001	<0.001	<0.001
harvest		0.031	0.020	0.031	0.220
plant × harvest		0.194	0.030	0.086?	0.003

[a]Within a column, means not sharing lowercased letters differ significantly at the $P < 0.05$ level.
[b]SEM = standard error of plant × harvest mean

The total CT content and on average the percentage of soluble CT were greater ($P < 0.05$) in the second than the third harvest with intermediate values for the first harvest. However, the differences in the portion of soluble CT was mainly observed in the LcP, but not in LcB and OvP (plant × harvest interaction: $P < 0.05$). The percentage of protein-bound CT was on average lower ($P < 0.05$) in the second compared with the third harvest with intermediate values for the first harvest. Except for LcB, where the portion of fibre-bound CT was greater in the first compared to the third harvest, the changes in the relative portion of the fibre-bound fraction in LcP and OvP were minimal (plant × harvest interaction: $P < 0.003$)

Discussion

Effect of the plant species and plant cultivar on CT content the percentage of the CT fractions

The total CT content differed primarily between plant species and only numerically between the two birds' foot cultivars (LcB >LcP). By contrast, some authors demonstrated that the CT content of birds foot trefoil as well as sainfoin cultivars differ (*Acuña et al. 2008; Azuhnwi et al. 2011*). However, the current observations are in line with results of *Scharenberg et al. (2007a)* who reported greater CT content in sainfoin than in birds' foot trefoil. The CT content of the LcP and LcB are similar to previously reported values of other cultivars using the same method *(Terrill et al. 1992; Scharenberg et al. 2007a*). The CT contents determined in the OvP were on average two times greater compared to earlier experiments from our group in which the total CT content of sainfoin accessions (cultivars such as Visnovskyor Perly, ecotypes or landraces) ranged from 50 to 100 g kg^{-1} DM (*Scharenberget al. 2007a 2007b; Azuhnwi et al. 2011*). However, others reported CT contents of 120 g kg^{-1} DM in the variety Nova of sainfoin (Li et al. 2014). Besides the cultivars, the CT content is depending on many agronomic and environmental factors such as the photoperiod, the temperature and the type of soil (*Theodoridou et al. 2011a*). The unexpected great concentrations for the OvP compared to previous studies could also result from the calibration standard used to quantify the CT content. In the present study, a standard of each plant and each cultivar was purified from the fresh material from the first harvest. Thus, 3 calibration standards were used, whereas in the study of *Azuhnwi et al. (2011)* only one calibration standard from sainfoin was used for all the cultivars and accessions. By using qualitative rather than quantitative analytical methods, such as thiolysis *(Gea et al. 2011),* it revealed the heterogenic chemical structure of CT. For instance, it has been shown that sainfoin contains more PD than PC while birds' foot trefoil contains more PC than PD (*Foo et al. 1996; Gea et al. 2011).* Similarly, the mDP can differ according to the plant. For instance, the mDP in

sainfoin is usually higher than the one in birds foot trefoil with reported mDP values up to 70 and 20 for sainfoin and birds foot trefoil respectively (*Meagher et al. 2004; Gea et al.2011*).An additional indication of the complexity of the CT is the findings that the percentage of the soluble, protein- and fibre-bound CT fraction differs between plants and the cultivars. In the OvP and LcB the soluble CT fraction represented >58% of total CT whereas this portion was only up to 48% in the LcP.

Effect of the harvest on CT content and the percentage of the CT fractions

The content of extractable CT increases only numerically in the present study between the first and the second harvest. The review of *Wang et al. (2015)* reported that this content increases usually after regrowth. The reason for this increase is not fully elucidated. Various possible explanations have been proposed. Firstly, the increase in CT content could be linked to higher and drier temperature and a longer photoperiod in the second compared to the first growth period which occurs usually in early summer *(Lascano et al. 2001; Wang et al 2008; Theodoridou et al. 2011a; Li et al. 2014).* Secondly, the increase in the plant biomass and the concomitant increase in the portion of leaves after regrowth (*Häring et al., 2007*). Finally, producing more CT could also be a defence response against herbivores and plant pathogens to dissuade them from eating the plant.

An interesting finding of the present study was the fact that harvest time point affects qualitatively the CT content of fresh legumes, the main differences being observed between the second and the third harvest. Previous studies already reported qualitative change of CT like PC:PD and *cis*:*trans* ratio between two successive harvests. *Azuhnwi et al. (2013)* found a general tendency to lower PC portion and *cis* configuration between the primary growth and regrowth. Ultimately, changes in the polymer composition can modify the properties of CT to interact with proteins (*Sarni-Manchado et al. 1999; Frazier et al. 2010*).

However, as reported by *Theodoridou et al. (2011b),* the differences observed might not solely be the result of time of harvest but probably more due to differences in the phenological stage and thus plant maturity between the harvests. In the current experiment, the third harvest was carried out at the vegetative, thus less mature stage of the three plants, whereas the first and second harvest were performed at an intermediate and very advanced stage of maturity, respectively. Thus, if the maturity of the plant is considered independently of the time of harvest, the CT content is progressively increasing from the least to the more advanced stage of maturity, especially for the LcB and the OvP. This is in line with results obtained by *Theodoridou et al. (2011a)* and could be explained by the fact that at a more mature stage, legumes are developping the flowers which are rich in CT. In addition, it seems that with advanced maturity, the portion of protein-bound CT is decreasing in favor of an increase in soluble CT, particularly for the LcP. In contradiction to *Wang et al. (2015), Theodoridou et al. (2011b)* observed a

decrease in the mDP from the first (end of flowering) to the second vegetation cycle (start of flowering) indicating that with increasing plant maturity CT polymers become shorter. Shorter polymers have a reduced affinity to bind protein as fewer numbers of active sites on the CT molecule are available (*de Freitas and Mateus 2002*). Moreover, *Theodoridou et al. (2011a)* already showed that nitrogen concentration is decreasing with plant maturity because nitrogen is mainly in the leaves (Borreani et al. 2003) and the leaf-to-whole-plant ratio is decreasing with plant maturity. Consequently, a reduced affinity to bind protein associated to a decrease in nitrogen content in the plant withadvanced maturity could explain why the protein-bound CT are decreasing in the present study.

Effect of wilting, ensiling and pelleting on CT content and the percentage of the CT fractions

Forage conservation methods not only alter the nutrient composition (*Wyss, 2013*) but also the CT content of legumes (*Lorenz et al. 2010*). Although wilting had no clear effect on the total CT content, the level was lower after ensiling and tended to be even lower after pelleting. The reason for the decrease in the CT content from fresh to ensiled or pelleted legumes might be due to oxidative processes caused by fermentation during ensiling and by high processing temperature during the pelleting. The HCl butanol method used in this study did not allow to determine the extent of oxidized CT. However, from fresh to silage and pellets a 36 and 33% decrease in the total CT content in OvP but not LcB or LcB were observed in the present study. One possible reason for this finding could be related to the nature of the CT, such as a greater PD content in OvP compared to birds' foot trefoil which could be oxidized more easily than PC (*Foo and Porter 1980*).

The current results are in line with other studies who found that compared to fresh forage, hay of *Sericea lespedeza* or sainfoin contained less extractable CT (*Terrill et al. 1990; Aufrère et al. 2008*). Nevertheless, the new approach here compared to previous studies was to monitor the changes of each CT fraction during the conservation of the forage independently of the total CT content. The insoluble portion of CT is interesting from an animal nutrition perspective as it has been shown that protein-bound CT can dissociate in the small intestine of ruminants and makes protein available for digestion (*Kariuki and Norton, 2008*). In addition, the study of *Grosse Brinkhaus et al. (2016b)* showed that an OvP silage, with 60% of protein-bound CT, supplied more duodenally utilisable crude protein relative to total crude protein than other legumes. The increase in protein- and fibre-bound CT fractions confirms results obtained on sainfoin by *Scharenberget al. (2007b)* who compared hay and silage and by *Terrill et al. (2007)* who showed that pellets of *Sericea lespedeza* contained mainly protein-bound CT. In the present study, the pelleting process has the same effect as hay making or ensiling. During the whole ensiling and pelleting process, the portion of soluble CT continuously

decreases from fresh to wilted and to silage or pellets. This decrease is accompanied by a concomitant increase in the protein-bound CT fraction and regarding pelleting process, an increase in the fibre-bound fraction. *Minnee et al. (2002)* hypothesized that during conservation, the plant cells are damaged allowing the release of previously sequestered soluble CT from the vacuole into the cytosol and form complexes with proteins and fibres. This course of possible events would be in line with the present findings.

Conclusion

The present study demonstrated that the plant species and the different modes of conservation can affect quantitatively as well as qualitatively total CT content as well as the relative portion of the three CT fractions of forages. Total CT content can be characterized in terms of chemical structure with the development of methods such as in situ thiolysis and the soluble part of the CT can be easily extracted with acetone and water and characterized as well by thiolysis or LC-MS/MS. For animal nutritionists, the interest in the soluble part of the CT comes from their ability to affect ruminal fermentation by preventing protein degradation via complex building and thus protecting both dietary and endogenous proteins and/or indirectly by affecting microbial activity. However, the present results showed that the insoluble portion of CT is with over 50% of the total CT the main fraction in silage and dehydrated pellets. In the case of conserved forages, it would be interesting to get a better understanding of the relevance of the bound CT fractions in ruminant nutrition. Hence, the question arises whether these plant protein which have been protected from ruminal degradation because they were bound to CT can dissociate from CT in the small intestine and be available for absorption. Thus, in subsequent studies a better characterization of the chemical properties of these bound portions needs to be envisaged.

Modifikacija proporcije ekstrabilnih i vezanih kondenzovanih tanina u žutom zvezdanu (*Lotus corniculatus*) i esperzeti (*Onobrichis viicifolia*) tokom procesa sušenja, siliranja i peletiranja

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Rezime

Kondenzovani tanini (CT) u leguminozama se razlikuju ne samo u koncentraciji i strukturi, već i u delu rastvorljivih frakcija koje su vezane za proteine i vlakna. Ova studija je imala za cilj da proceni promene u ukupnom nivou CT, kao i relativno obilje tri CT frakcije od svežih do osušenih, siliranih ili peletiranih mahunarki kao što su žuti zvezdan (dve sorte) i esperzeta (jedna sorta). Svaka vrsta je imala tri uzastopne žetvama, od kojih su prve dve sušene. Pored toga, osušene mahunarke su ili silirane (prva žetva) ili transformisane u dehidrirane pelete (druga žetva). Za svaku žetvu, ukupni CT i procenat rastvorljivog CT-a vezanog za protein odnosno vlakna, razlikovali su se ($P < 0,01$) među biljkama. Ukupan CT sadržaj je bio sličan nakon sušenja, ali je bio manji ($P<0,05$) nakon siliranja. Posle sušenja, siliranja i peletiranja, deo rastvorljivog CT-a bio je niži u korist CT-a vezanog za protein. Međutim, vreme žetve je uticalo ($P <0,05$) na ukupni CT i procenat rastvorljivog i CT vezanog za protein. Prema tome, merenje vezane frakcije ne treba zanemariti u određivanju sadržaja CT-a, jer ova frakcija, zajedno sa rastvorljivom frakcijom, može zaštititi protein od degradacije u rumenu.

Ključne reči: kondenzovani tanini, rastvorljiva frakcija, vezana frakcija, sušenje, siliranje, peletiranje

Acknowledgments

This study was funded by the EU Marie Curie Initial Training Network (‘LegumePlus’; PITN-GA-2011-289377). The authors gratefully acknowledge and thank the skilled technical assistance for the experimental fieldand the analytic departments of Agroscope in Posieux.

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Received 12 February 2018; accepted for publication 25 March 2018

Biotechnology in Animal Husbandry 34 (1), p 21-31 , 2018
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.2.03
https://doi.org/10.2298/BAH1801021S

YIELD AND NUTRITIONAL VALUE OF PERMANENT GRASSLAND FORAGE UNDER SIMULATED ROTATIONAL GRAZING

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Original scientific paper

Abstract: A cutting experiment was conducted to test the changes in botanical composition, yield and nutritional value of forage, obtained in conditions of simulated rotational spring grazing on permanent grassland. The experiment was carried out on permanent pasture in vicinity of Šabac, Serbia in 2015 included three cuttings as a simulated rotational spring grazing. The highest share of grasses was noted in the first cut and decreased in the second and third cut, with increased forbs participation, and relatively constant percentage of legumes. The highest dry matter (DM) yield was obtained for the first harvest, followed by the second cut, and the lowest forage production was determined for the third harvest, with only 11.04 and 17.42% of the first and second cut yield. There were not found the significant differences between cuts for herbage DM and crude protein content. Markedly lower value for non-protein N concentration ($p<0.05$) was determined in the third cut. The fiber content increased during the grazing season ($p<0.05$), with the highest value determined in the herbage obtained in the second cut. The highest energy values ($p<0.05$) had the herbage produced in the first cut (DM basis), wherein the lowest values were found in the forage from the second harvest. These results indicate that especially herbage yield of analyzed grassland as chemical composition and nutrition value are highly variable during the growing season. The accurately defined optimal period for using is necessary to provide the high-quality forage for grazing animals.

Key words: forage production, pasture quality, nutrition, ruminants, pigs

Introduction

Grassland vegetation of Serbia occupying about 1.5 million ha or 27% of the total agricultural area of the country as the most represented type of the agroecosystem *(Simić et al., 2015)*. Permanent grasslands, serve both for production and environmental purposes, as the most represented type of the

agroecosystem, and they are the main source of forage on farms with animals which are raised on pasture *(Adamović et al., 2005)*. Besides the importance of grazing in rations for ruminants – cattle, sheep and goat, in organic pig production the requirement is that the animals be allowed access to pasture. In general the permanent grasslands in Serbia are situated on soils with low natural fertility, are of low productivity and have sub-optimal botanical composition *(Simić et al., 2015)*. Perennial legumes as clovers and medics play a special role as a pasture components in the Serbia through their production, quality and ability to fix atmospheric nitrogen. However, acidic soils of low fertility on Serbian hilly-mountainous grasslands cause lack of legumes, their low share and the longevity, as well as limitations in successful forage production (*Simić and Vučković, 2014*). Forage quality encompasses many factors, including content of crude protein (CP) and nonprotein N, fiber (neutral detergent fiber-NDF and acid detergent fiber-ADF), available energy concentration, as important indicators of nutritive value for grazing forages. The DM content of pasture has a significant effect on forage intake of grazing animals *(Stojanović et al., 2016)*. Temperature and rainfall are climatic factors that can affect forage quality. Seasonal variation in environment alters forage quality, even when forages are harvested at similar maturity stages *(Buxton and Casler, 1993)*. The depressed digestibility associated with elevated temperatures is usually attributed to higher NDF concentrations, whereby the NDF of forages grown under higher temperatures is usually less digestible than that of forages grown under lower temperatures because of increased lignification *(Buxton and Fales, 1994)*. During spring growth, the effect of increasing temperatures interact with advancing maturity to cause a more-rapid decline in forage quality with time than occurs during summer growth *(Van Soest, 1994)*. According to *Mandaluniz et al. (2015)* the CP content was decreased, and NDF and ADF content increased for grazing herbage mass during the spring grazing period (April - June), with 20-25 days of resting period. *Wilkins et al. (2000)* found that the seasonal variation in protein concentration of perennial ryegrass herbage was much larger than the differences among cultivars.

To meet the nutritional requirements of grazing animals, throughout the plant growing season, beside the yield, the determination of energy and nutrients content in obtained forage is necessary. For this reason, we conducted a cutting experiment to test the changes in yield and nutritional value of herbage obtained in conditions of simulated rotational spring grazing on permanent grassland. The objective of this study was also to investigate and launch sustainable pasture exploitation in Serbia.

Material and methods

The trial was conducted on pasture in 2015 included three cuttings as a simulated rotational grazing. The field experiment was established in Western

Serbia, 11 km southern of Šabac (44°40′ N, 19°39′ E) on poor quality soils. Experimental plots were 5 × 2 m in 5 replications, designed by RCB method. Pasture was situated on poor quality soils and has been exploited permanently for dairy cattle grazing. Harvesting dates were 1 May, 24 May and 19 June. The plots were harvested at 9:00-9:30 a.m. with a rotary cutter to a residual stubble height of 7 cm. The herbage was weighed after cutting, sub-samples were taken for chemical and botanical analyses.

Identified plant species were classified by their quality into three categories: quality grasses, quality legumes and forbs (harmful, useless or conditionally useful plant species from other plant families), *(Tomić et al., 2005)*, and the percentage of these yield-contributing species per cut were noted (botanical composition by covering).

Table 1. Chemical properties of soil

Depth	pH		OM, %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g	Total C, %	Total N, %
	$CaCl_2$	H_2O					
0-20 cm	5.07	5.73	4.31	1.98	11.51	1.37	0.16

Meteorological data for this site were collected from the Sremska Mitrovica Weather Stations, respectively, located near the experimental sites.

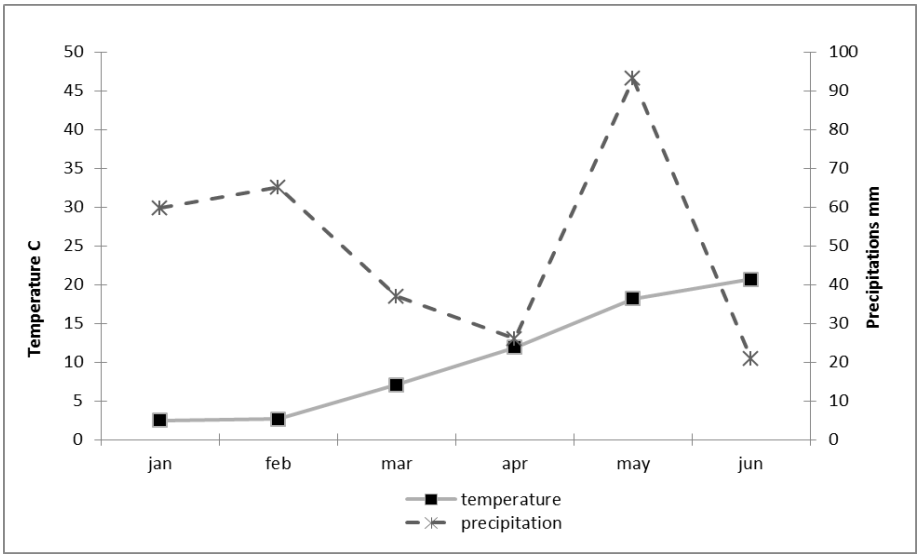

Figure 1. Average monthly temperature and monthly precipitation sum, during spring grazing season

Samples of harvested material for chemical analysis were placed in plastic bags, stored in a cooled portable refrigerator, and transported to the laboratory for processing. Chemical analysis of herbage samples was done in the Laboratory for the animal nutrition at the Faculty of Agriculture - Belgrade. All samples were dried before chemical analyzes. Parameters of proximate analysis, NDF, ADF and ADL (acid detergent lignin) were determined according to the procedure of *AOAC (2002)*. The protein fractions (true protein and NPN) were determined using standardizations of *Licitra et al. (1996)*. The energy values of forage DM for ruminants and pigs were estimated according to the *NRC (2001)*, *INRA (2004)*, *Noblet and Perez (1993)* and *NRC (1998)*:

NE_L (MJ/kg DM) = 0.703 × ME – 0.795 *(NRC, 2001)*
NEm-rum. (MJ/kg DM) = ME × (0.287 × q + 0.554) *(INRA, 2004)*
NEf-rum. (MJ/kg DM) = ME × (0.78 × q + 0.006) *(INRA, 2004)*
NEl-rum. (MJ/kg DM) = ME × ((0.60 + 0.24 × (q – 0.57)) *(INRA, 2004)*
MEswine (kcal/kg DM) = DE - 0.68 × CP, g *(Noblet and Perez, 1993)*
NEswine (kcal/kg DM) = 328 + (0.599 × ME) - (15 × Ash, %) - (30 × ADF, %) *(NRC, 1998)*

NE_L – Net energy lactation for dairy cows; NEm-rum., NEf-rum., NEl-rum. - Net energy maintenance, fattening and lactation for ruminants, respectively; MEswine – Metabolizable energy for swine; NEswine – Net energy for swine.

An ANOVA-procedure using the STATISTICA v.6 *(StatSoft, 2003)* was conducted to assess the effects of different harvests on yield, composition and nutritional value of herbage from permanent grassland. Differences among treatment means were tested for significance using LSD test. Statistical significance was determined at $p<0.05$.

Results and discussion

Soil on the experimental field had limited productive abilities, and frequent lack of precipitation during period May-June influenced grazing capacity of pasture. The total precipitation was 302.0 mm in the first part of vegetation season (Fig. 1). The average monthly temperatures registered in May and June also negatively influenced grazing capacity of pasture. Soil from experimental field had a low P content and moderately acidic pH (Table 1). *Aćić et al. (2013)* reported that the most influencing factors determining development of permanent grassland used as a pasture are the quantity of nutrients in the soil and the habitat moisture.

Three cuts were reached in the part of vegetation season, before summer drought period. The obtained results indicate that yield of forage DM depends on the cutting period (Table 2). In the first cut, the highest DM yield was obtained, followed by the second cut with markedly lower yield (36.62%), and the lowest

forage production was determined for the third harvest, only 11.04 and 17.42% of the first and second cut yield.

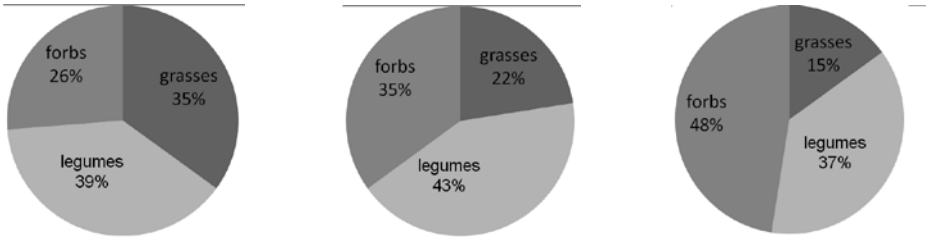

Figure 2. Botanical composition of pasture by covering

The highest share of grasses was noted in the first cut, mainly *Poa pratensis*, *Dactylis glomerata* and *Lolium multifloum*, and decreased in the second and third cut. However, a notable contribution of forbs to the canopy formation was seen (*Ranunculus sp.*, *Taraxacum officinale*, *Stellaria media*), and increased by harvests. The percentage of legume species was relatively constant for different cuts during analyzed period and main species were: *Trifolium repens*, *T. campestre T. pratense*, and *Vicia sp*. High numbers of forbs suggest high biodiversity of species, but also underline poor quality and low production for livestock farming. In the second and third cut, a combination of weather conditions had the influence on the botanical composition. According to *Simić et al. (2015)* on areas that are not permanently managed, forbs (i.e. weeds in forage production) make over half of the plant production. Due to poor fertility of the soil and relatively severe climate, pastures are often overgrown with plant species of low nutritional value. In south Eastern Europe, the growth of legumes is seriously limited by the ability of each species to grow during usually cold winters *(Simić and Vučković, 2014)*. There, the distribution of legumes on Serbian natural grasslands ranges from 6.73% to 34.12%, depending on plant nutrition *(Đurić et al., 2007)*.

This research showed significant effect of different cuts during spring growing season on a forage quality, with determined marked changes as the grazing season progresses. Results for chemical composition of herbage for three harvests from the 1 May to 19 June are presented in table 2.

Table 2. Chemical composition of permanent grassland forage from consecutive spring harvests

Cuts	DM yield kg/ha	DM, %	CP, %DM	NPN, %CP	NDF, %DM	ADF, %DM	Lign., %DM	NFC, %DM	EE, %DM	Ash, %DM
1.	770 [a]	26.28	15.30	19.55 [a]	39.09 [a]	25.54 [a]	3.19 [a]	36.15 [a]	3.39 [a]	8.87
SEM	86	1.53	0.60	0.84	1.38	0.84	0.25	0.79	0.16	0.19
2.	488 [b]	28.46	13.72	19.69 [a]	48.61 [b]	31.22 [b]	5.20 [b]	27.12 [b]	3.86 [ab]	10.00
SEM	58	1.04	0.83	1.10	1.95	0.79	0.34	1.25	0.13	0.58
3.	85 [c]	28.79	14.55	15.79 [b]	43.62 [a]	28.61 [c]	4.06 [a]	31.79 [c]	4.21 [b]	8.95
SEM	12	1.31	0.69	1.11	1.03	0.68	0.36	0.83	0.19	0.23

[1]SEM: Standard Error of the Mean. [a, b, c] Means in the same column with different superscripts differ ($p<0.05$); DM-dry matter; CP-crude protein; NPN-nonprotein nitrogen; NDF-neutral detergent fiber; ADF-acid detergent fiber; Lign.-lignin; NFC-nonfiber carbohydrate; EE-ether extract;

There was found the slightly increase in forage DM content while the CP values were lower, during the analyzed spring grazing period, where in these differences were not significant. Markedly lower value for NPN concentration ($p<0.05$) was determined in forage from the third cut. There was found the increase of fiber content (NDF and ADF) for the different cuts, during the season, with the highest values for herbage obtained in the second cut ($p<0.05$) where the lowest concentration of nonfiber carbohydrate - NFC ($p<0.05$) was determined, too. Observed results are in accordance with the research of *Rayburn (1991)* where it was found the reduction of forage quality of intensive rotationally grazed pastures (mixed mostly grass and mixed mostly legume) with increased NDF content in DM, while the NSC (nonstructural carbohydrate) and CP concentration was decreased, as the grazing season progresses (May - August interval). When temperatures rise above the optimum range for plant growth, the nutritive value of forage declines *(Reid, 1988)*. According to *Elgersma and Søegaard (2016)* for grass-legume swards, NDF and ash contents were lower in the first harvest in May, than during June − September, whereas, contents of water-soluble carbohydrates and crude fat, as *in vitro* OM digestibility were highest in the first harvest in May. Determined increase in fiber content with the advancement of vegetation, which also reported by *Elgersma and Søegaard (2016)*, is a result of lower temperatures during May and vegetative, leafy plant materials as opposed to generative, stemmy plant materials during summer. Obtained results which indicated the markedly lowest value for NPN concentration in forage from the third cut (second regrowth) are likely correlated with the lower protein solubility at greater NDF and ADF content in forage DM *(Rayburn, 1991). Wilson and Brigstocke (1981*) reports that the proportion of non-protein nitrogen in typical pastures may be 18% CP, what is in agreement with the obtained results.

The net energy concentration in herbage from the three consecutive harvests during the spring growing season are shown in table 3.

Table 3. Net energy content of permanent grassland forage, for three harvests during the May – June interval

Cuts	NE_L, MJ/kg DM	NEm-rum., MJ/kg DM	NEf-rum., MJ/kg DM	NEl-rum., MJ/kg DM	MEswine, MJ/kg DM	NEswine, MJ/kg DM
1.	6.17 [a]	8.26 [a]	5.52 [a]	6.91 [a]	9.51 [a]	3.31 [a]
SEM[1]	0.10	0.18	0.18	0.15	0.22	0.24
2.	5.36 [b]	7.19 [b]	4.41 [b]	6.01 [b]	7.64 [b]	1.40 [b]
SEM	0.04	0.07	0.09	0.06	0.11	0.13
3.	5.94 [a]	7.80 [c]	4.99 [c]	6.52 [c]	8.89 [c]	2.55 [c]
SEM	0.09	0.11	0.12	0.09	0.13	0.15

[1]SEM: Standard Error of the Mean. [a, b, c] Means in the same column with different superscripts differ ($p<0.05$); NE_L – Net energy lactation for dairy cows *(NRC, 2001)*; NEm-rum., NEf-rum., NEl-rum. - Net energy maintenance, fattening and lactation for ruminants, respectively *(INRA, 2004)*; MEswine – Metabolizable energy for swine *(Noblet and Perez, 1993)*; NEswine – Net energy for swine *(NRC, 1998)*.

Determined the greatest values ($p<0.05$) for net energy contents of the herbage produced in the first cut (DM basis) could be expected, considering the highest NFC and CP content, with the lowest NDF and ADF concentration *(Stojanović et al., 2002)*. The most prominent decrease of herbage energy content was determined for the second cut. With exception of the concentration of net energy lactation for dairy cows *(NRC, 2001)*, forage produced in the third cut had significantly lower energy values compared to the first cut. According to *Van Vuuren and Van Den Pol-Van Dasselaar (2006)* the energy value of grass pasture is highest in April, but remains rather stable throughout the year, also the highest crude protein concentrations were found in spring and autumn, wherein the sugar concentration decreases throughout the season, too. In this research, in particular, the significantly lower content ($p<0.05$) of NE for pigs in forage that was obtained from second harvest, only 42.30 and 54.90% of NE content in first cut forage, certainly could be attributed to the markedly higher fiber (NDF and especially ADF) content *(Noblet and Le Goff, 2001)*. Our findings which indicate on reduction of nutritional value of pasture during the spring grazing season are in accordance with study of *Vestergaard et al. (1995)* where dried grass meal derived from the first three cuts from the ryegrass - red clover pasture, showed reduced sugar content, increased content of dietary fiber and decreased energy value.

Conclusion

The significant reduction of forage DM yield was determined for analyzed three cuts during the growing season before summer drought period. Considering the botanical composition of pasture, it was determined the reduction of grass species portion for different cuts as the grazing season progresses, with increased percentage of forbs, while the participation of legume species was relatively

constant. In general, it can be concluded that different harvests during spring growing season, have a significant effect on nutritional value of forage from analyzed permanent grassland. With approximate DM content of herbage from different cuts, the greatest values for CP, NFC and content of net energy for ruminants, as metabolic and net energy for swine, was found in first cut forage, and these parameters have decreased as the grazing season progresses (first and second regrowth), while the concentration of NDF, ADF and ether extract have increased. These results indicate that accurately defined optimal period for using of permanent pastures, as also required regrowth interval throughout the season, are necessary to provide the high-quality forage for pasture raised animals.

Prinos i hranljiva vrednost zelene mase sa permanentnog travnjaka u uslovima simulacije prolećne ispaše

Bojan Stojanović, Aleksandar Simić, Goran Grubić, Aleksa Božičković, Ivan Krga

Rezime

Istraživanje u kome je košenjem simulirana pregonska ispaša, sprovedeno je u cilju utvrđivanja razlika u botaničkom sastavu, prinosu i hranljivoj vrednosti dobijene zelene mase sa permanentnog pašnjaka, tokom prolećne sezone. Eksperiment je izveden tokom 2015. godine na prirodnom pašnjaku u okolini Šapca, Srbija i uključivao je tri otkosa kao simulaciju pregonske prolećne ispaše. Najveći udeo trava je zabeležen u prvom otkosu, dok je zastupljenost trava u drugom i trećem otkosu bila smanjena, uz istovremeno povećanje učešća zeljanica, i relativno ujednačen udeo leguminoza po ciklusima iskorišćavanja. Najveći prinos suve materije (SM) je utvrđen u prvom ciklusu iskorišćavanja, zatim u drugom, dok je najmanja produkcija zelene mase izmerena u trećem otkosu, samo 11,04 i 17,42% prinosa u prvom odnosno drugom otkosu. Nisu nađene značajne razlike između ciklusa iskorišćavanja u pogledu sadržaja SM i sirovih proteina. Značajno manje učešće neproteinskog N ($p<0,05$) je utvrđeno u biljnoj masi dobijenoj iz trećeg otkosa. Determinisano je povećanje sadržaja vlakana ($p<0,05$) u SM biljne mase, tokom pašne sezone, pri čemu su najveće vrednosti utvrđene u drugom otkosu. Najveću energetsku vrednost ($p<0,05$) imala je zelena krma iz prvog otkosa (u SM), pri čemu je najniži sadržaj iskoristive energije utvrđen u zelenoj masi dobijenoj iz drugog ciklusa iskorišćavanja. Rezultati do kojih se došlo, ukazuju na naročito izraženo variranje prinosa, kao i hemijskog sastava i hranljive vrednosti zelene mase sa permanentnog pašnjaka, tokom prolećne sezone porasta vegetacije. U skladu sa tim, u cilju dobijanja kvalitetne paše za ishranu životinja, neophodno je

precizno definisanje optimalnog perioda za iskorišćavanje pašnjaka tokom sezone ispaše.

Ključne reči: proizvodnja zelene krme, kvalitet paše, ishrana, preživari, svinje

Acknowledgment

This study is realized by financial support of Ministry of Education, Science and Technological Development, Republic of Serbia, through the Technological Development Project TR-31086.

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Received 13 December 2017; accepted for publication 20 March 2018

Biotechnology in Animal Husbandry 34 (1), p 33-40 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 575.113:637.5'64
https://doi.org/10.2298/BAH1801033P

LIPE GENE POLYMORPHISM c.442 G>A INFLUENCE ON CARCASS TRAITS IN PIGS

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Original scientific paper

Abstract: Hormone sensitive lipase is one of three enzymes involved in lipolysis process and encoded by LIPE gene. In this study we investigated LIPE gene polymorphism c.442 G>A influence on carcass traits in hybrid pigs. Genomic DNA extracted using Chelex resin, genotypes determined using RFLP-PCR. Allele A observed with frequency 0,738, allele G – 0,262. The most common genotype was AA, genotype GG was observed with lower frequency, genotype AG was rarest. While evaluating population heterozygosity, it was noticed that observed heterozygosity was only 0,075, while expected heterozygosity was 0,387. In observed pig population allele A is associated with better animal muscularity, allele G – with greater fat content.

Keywords: LIPE gene, SNP, polymorphism, c.442 G>A, carcass traits

Introduction

According to the United Nations Food and Agriculture Organization pork is the most consumed meat *per capita* in the world. Therefore, it is very important to know about the quality of consumed meat, which can be determined by animal's age, breed, genetic or environmental factors. It is known that large intakes of saturated fatty acids can cause heart and coronary diseases, type 2 diabetes or cancer (*Schwab et al. 2014*), hence consumers are choosing meat by its juiciness, tenderness, texture and properties when cooking.

Ongoing pig selection in the world is based on lowering fat content in the meat and increasing lean carcass yield (*Zimmermann et al. 2004; Tyra et al. 2011*). To maintain valuable meat traits in all generations pig selection should be based on genetic, not phenotypical properties. Accordingly, a lot of research work was done to evaluate genes candidates and their influence on carcass traits. Selected genes candidates are carefully evaluated in various animal populations, accurate enzyme

function is determined, polymorphism influence on phenotypic traits is evaluated. Selected genes are called markers; they are included in quantitative trait locus (QTL) maps.

LIPE gene encodes hormone-sensitive lipase (HSL), this enzyme plays a key role in fatty acid metabolism, so called lipolysis *(Harbitz et al. 1999; Holm et al. 2003; Chahinian et al. 2005; Thiriet et al. 2013*). HSL along with adipose triglyceride lipase and monoacyglycerol lipase synergistically affects fats in adipocyte lipid droplet and breaks down triacylglycerol to non-esterified fatty acids and glycerol. HSL is activated by glucagon or glucagon-alike enzymes (*Lampidonis et al. 2011; Siu et al. 2013*), thus lipolysis is activated when there is a lack of energy. Intronic site human LIPE gene polymorphism causes altered lipolysis in adipocytes, obesity and type 2 diabetes (*Dahlman et al. 2007*). Zidi with a team found out that LIPE genotype can determine goat milk yield and its components, as dairy animals receive most of their energy from breaking down accumulated fats (*Zidi et al., 2010*).

After *in situ* hybridization pig LIPE gene was assigned to chromosome 6 (6q12), alongside with glucose phosphate isomerase (GPI) and calcium ion channel (CIC) genes (*Chowdhary et al. 1995*). LIPE gene structure is very conservative compared to human, mouse or rat: splicing sites are fully conservative, compatible exon and intron sites are highly conservative (*Harbitz et al. 1999; Kaminski et al 2008*). There are only few pig LIPE gene polymorphisms found: polymorphic *Alu1* sequence, c.3436G<T and c.442 G>A. This research goal was to evaluate LIPE gene c.442G>A polymorphism on carcass traits in pigs in studied pig population.

Material and methods

Genetic material for study was collected at the Lithuanian Pig Breeding station. We used 40 hybrid pigs: Yorkshire x Landrace hybrids (N=16), Yorkshire x Landrace x Landrace hybrids (N=24) and Large White x Landrace x Landrace hybrids (N=10). Research was performed at the Institute of Biology Systems and Genetics in Lithuanian University of Health Sciences. Genetic material was extracted from hair follicles using Chelex resin. For one sample we used 6-10 bristle follicles, placed them in centrifuge tubes and mixed with 200 µl Chelex resin, 7.5 µl DTT and 10.7 µl proteinase K. Tubes vortexed for 30s., centrifuged at 13500 RPM for 10s and placed in thermostat for 45min at 56^{o}C (*Miceikiene et al. 2002*). Then PCR is performed, for one reaction used 15µl of mastermix (2.95µl high quality deionized water, 3µl buffer solution without $MgCl_2$, 2µl $MgCl_2$, 2.5µl dNTP, 2µl forward primer (LIPE P1 5´-CGCACRATGACACAGTCGCTGGT-3´),

2µl reverse primer (LIPE P2 5´-CAGGCAGCGRCCRTAGAAGCA -3´) (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 0.25µl BSA, 0.3µl Taq polymerase). PCR had 30 cycles. 498bp product was generated.

For restriction fragment length polymorphism reaction, we used 10µl PCR product and 10µl mastermix (7.5µl high quality deionized water, 2µl FastDigest Green buffer solution, 0.5µl *Hinf1* restriction enzyme (Thermo Scientific FastDigest *HinfI*) (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). Tubes with the mix were shortly vortexed and centrifuged. RFLP reaction was performed in thermocycler for 5min at 37^{o}C degrees. After reaction four fragments were obtained: 308bp and 190 bp for allele A; 67bp, 190bp and 241bp for allele G.

Polymorphism influence was evaluated on carcass traits: hot carcass weight and yield; carcass without head weight and yield; age at 100kg; daily gain; 1kg gain feed intake; half carcass length; half bacon length; loin area; weight of ham; fat thickness at 6-7th rib, at 10th rib, behind last rib, at last waist vertebra; "Piglog" data: fat thickness at point Fat_1 and point Fat_2, muscle thickness, muscularity. Before slaughtering pig's muscularity and fat thickness was evaluated using ultrasound device "Piglog 105". Data, related to carcass traits was obtained from National Pig Breeding Station.

Statistical data analysis was performed using *Excel* and *IBM SPSS Statistics for Windows* software. Evaluation of allele and genotype distribution, expected and observed heterozygosity and polymorphism influence on traits mentioned above was completed.

Results

After statistical data analysis and observed polymorphism evaluation all possible allele combinations were found. Most common genotype AA, observed with frequency 0.700 in 32 animals out of 50. AA genotype most commonly found in Yorkshire x Landrace x Landrace hybrids with frequency 0.714, most rarely genotype AA observed in Large White x Landrace x Landrace hybrids with frequency 0.667. Genotype GG was observed in lower frequency (0.225): Yorkshire x Landrace hybrids carried genotype GG with 0.308 frequency, Yorkshire x Landrace x Landrace with 0.238 frequency. In Large White x Landrace x Landrace population genotype GG was not detected. Rarest observed genotype was AG, frequency 0.075. Heterozygous genotype was observed in Large White x Landrace x Landrace population with frequency 0.333, Yorkshire x Landrace x Landrace – 0.048, no heterozygotes were found in Yorkshire x Landrace population.

Allele distribution in studied population is uneven: allele A was observed almost 3 times more (0.738) than allele G (0.262) (Table 1).

Table 1. Genotype and allele distribution

Genotype	n	Frequency	Allele	Frequency
AA	32	0.700	A	0.738
AG	6	0.075	G	0.262
GG	12	0.225		
	50	1		1

When evaluating population heterozygosity, it was determined that observed heterozygosity was significantly lower than expected, which shows that genetic diversity in studied population is decreased, results statistically significant. (Table 2)

Table 2. Pig population heterozygosity

Expected heterozygosity	0.387
Observed heterozygosity	0.075
X2	26.00
P value	0.0000003

Table 3 shows statistically significant results related to LIPE gene polymorphism influence on carcass traits in pigs.

Table 3. Carcass traits of pigs

Trait	Lion area cm^2	Weight of ham, kg	Overall muscularity, %	Fat thickness at 6-7^{th} rib, mm	Fat thickness at 10^{th} rib, mm	Fat thickness behind last rib, mm	Fat thickness at last waist vertebra, mm	Fat thickness at Fat_1, mm	Fat thickness at Fat_2, mm
AA	45.4±1.04^a	12.4±0.12^a	59.5±0.25^a	14.9±0.93^a	13.2±0.85^a	13.9±0.69^a	10.3±0.50^a	12.4±0.31^a	11.2±0.27^a
AG	40.3±1.14	11.9±0.10	57.0±0.94^b	24.4±5.40^b	19.1±2.57^b	20.7±3.49^b	16.4±2.43^b	14.3±1.20^b	14.0±1.00^b
GG	36.6±1.79^b	11.6±0.30^b	56.1±0.90 ^{c}	18.8±1.76	16.4±1.30	15.0±1.48^a	13.2±1.60^b	15.1±0.82	13.4±1.04^b
P	**0.0001**	**0.014**	**0.0001**	**0.008**	**0.033**	**0.026**	**0.005**	**0.002**	**0.004**

a, b, c - different letters in the column marked averages differ with each other significantly at P <0.05.

The highest muscularity was observed in pigs with genotype AA: loin area (45.4cm2±1.04) bigger than genotype GG (36.6cm2±1.79); weight of ham (12.4kg±0.12) greater than genotype GG (11.6kg±0.30); largest overall muscularity (59.5%±0.25) compared with AG (57.0%±0.94) or GG (56.1%±0.90) genotypes. Greatest fat mass is usually determined by genotype AG. Fat thickness at 6-7^{th} rib (24.4mm±5.40) greater than genotype AA (14.9mm±0.93) at 10^{th} rib (19.1mm±2.57) greater than genotype AA (13.2mm±0.85; greatest behind last rib (20.7mm±3.49) compared with AA (13.9mm±0.69) and GG (15.0mm±1.48) genotypes; at last waist vertebra greatest fat thickness was influenced by genotype

AG (16.4mm±2.43). as well as genotype GG (13.2mm±1.60). genotype AA influenced lower fat thickness at this point (10.3mm±0.50). Greater fat thickness at point Fat_1 (Piglog data) was determined by genotype AG (14.3mm±1.20). lower – genotype AA (12.4mm±0.31. However, at point Fat_2 greater fat thickness was influenced by genotype AG (14.0mm±1.00) as well as genotype GG (13.4mm±1.04), compared with genotype AA (11.2mm±0.27).

Discussion

LIPE gene encodes hormone sensitive lipase, which is one of the most important enzymes involved in accumulated fats breakdown and energy mobilization (*Ding et al., 2000*). Modifications in gene sequence can lead to altered HSL (hormone-sensitive lipase) function, which can induce incomplete lipolysis and fat accumulation in body. Pig LIPE gene polymorphism c.442 G>A was identified by American scientist Andrew Knoll and his team. In the first exon *missens* mutation occurs, when in gene sequence guanine is substituted by adenine and in enzyme sequence isoleucine is substituted to valine. After inheritance analysis both alleles (A, G) were observed in Meishan (pig breed from China known for abundance of fat) pigs, in Pietrain pigs (lean and muscular breed) G allele was fixated, Landrace (high produce of meat, low intramuscular fat content) and Large White (higher content of muscle fiber, less meat marbling) breeds were monomorphic to allele G, however Duroc breed (high content of intramuscular fat) was polymorphic (*Knoll et al., 1998*).

Scientist Lei and his colleagues conducted study data is slightly similar with our obtained data , though their investigated pig population consisted of the Great White and Meishan crossbreds. The largest back-fat thickness and intramuscular fat content determined the AG genotype, and total muscularity of animal was similar in both AA (58.4%) and GG genotypes (58.8%) (*Lei et al,. 2005*).

Wang and other scientists were investigated two local Chinese pig breeds (Nuogu bei Luobo) and Large White and Landrace crossbreeds.This scientist maintained that the animals which have AA genotype their meat properties were significantly superior nor of GG genotype animals. The results showed that the A allele was associated with the largest muscle thin and the least fat thickness of back, compared with the G allele (*Wang et al., 2012*).

When evaluating our observed population, both alleles and all genotypes were found, hence uneven allele distribution was observed. Allele A was observed almost 3 times more than allele G. Similar tendency can be observed in genotype distribution: genotype AA was most common – 32 animals out of 50, genotype GG was less common – 12 animals out of 50, homozygous genotype AG was rarest – only 6 animals out of 50. Statistical analysis was performed to evaluate genotype influence on carcass traits in pigs in studied population. We found 9 statistically

significant results: biggest loin area, weight of ham and overall muscularity was determined by genotype AA; highest fat content at 6-7th rib, at 10th rib, behind last rib and point Fat_1 was determined by genotype AG; highest fat content at last waist vertebra and point Fat_2 was determined by genotype AG, as well as genotype GG.

Conclusion

In studied pig population biggest overall muscularity, loin area and weight of ham was determined by genotype AA; higher fat content at 6-7th rib, 10th rib, at last waist vertebra, behind last rib and at both points measured with "Piglog" device was determined by both genotype AG and GG. In studied pig population, allele A is related to better animal muscularity, allele G – higher fat content.

Uticaj polimorfizma LIPE gena c.442 g> A na osobine trupa kod svinja

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Rezime

Hormon osetljiva lipaza je jedan od tri enzima koji su uključeni u proces lipolize i kodirana LIPE genomom. U ovom istraživanju smo ispitali uticaj polimorfizma LIPE gena c.442 G>A na osobine trupa kod hibridnih svinja. Genomska DNK je ekstrahovana korišćenjem Chelex-a, genotipovi određeni korišćenjem RFLP-PCR. Alele A je posmatran sa frekvencijom 0,738, alel G - 0,262. Najčešći genotip bio je AA, genotip GG je primećen sa nižom frekvencijom, genotip AG je bio najređi. Prilikom procenjivanja heterozigotnosti populacije, primećeno je da je zabeležena heterozigotnost bila samo 0,075, dok je očekivana heterozigotnost iznosila 0,387. U posmatranoj populaciji svinja, alel A je povezan sa boljom mišićavošću životinja, alelom G - sa većim sadržajem masti.

Ključne reči: LIPE gen, SNP, polimorfizam, c.442 G> A, osobine trupa

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Received 2 October 2017; accepted for publication 25 January 2018

Biotechnology in Animal Husbandry 34 (1), p 41-48 , 2018
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.5'64
https://doi.org/10.2298/BAH1801041T

COMPARISON OF THE CONTENT OF LEAN MEAT IN PIGS ON FARM AND SLAUGHTER LINE

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Original scientific paper

Abstract: Measurement of lean meat on slaughter line and formation of price on the basis significantly contribute to the overall improvement of the quality and profitability of production and distribution of pork. The content of lean meat on live pigs was measured on farm using ultrasound device PIGLOG 105. While in slaughterhouse, the content of lean meat measured using Fat-O-Meater (FOM), two-point method (TP) and partial dissection. 59.30% of lean meat in vivo was estimated by the apparatus PIGLOG-105 one day before slaughter. It is 0.91% more then partial dissection and when compared to FOM and TP it is more 4.86% and 4.02%. Great deviation between PIGLOG-105 on one side and FOM and TP on other side indicated some error, and then partial dissection solved this mystery. After this study, slaughterhouse constructed new formulas for FOM in pig carcass classification. Regarding that, slaughterhouses which used FOM or similar equipment for measuring percentage of lean meat, should control results of the equipment described in this study, minimum twice a year.

Keywords: pig carcass classification, meatiness, classification methods

Introduction

Determination of lean meat content on carcass is a procedure of crucial importance in modern production of pork around the world *(Petrović et al., 2009)*. Meatiness means the percentage of meat in pig carcasses *(Ukmar et al., 2008)*. On one side, the information of lean meat content is sent to further processing or sold as fresh meat, while on the other side, feedback sent to farmers regarding the meat quality shows results in breeding and selection of pigs *(Petrović et al., 2009; Vasilev et al., 2015)*. Measurement of lean meat on slaughter line and formation of

price on the basis significantly contribute to the overall improvement of the quality and profitability of production and distribution of pork *(Petrović et al., 2009; Jovanović et al., 2009)*.

Determination of lean meat content on carcass is measured by different electronic-optical devices, such as PIGLOG 105 (produced by SFK Technology, Denmark), Fat-O-Meater (FOM) (produced by Carometec, Denmark), and other methods like „two-points method" (TP), partial dissection, total dissection and others *(Krška et al., 2002; Bahelka et al., 2005; Pulkrabek et al., 2006).* Common characteristic of all electronic-optical devices are adapted to work in unfavorable microclimate conditions, such as on the farm and in the slaughterhouse, the devices are simple to use, and trained staff are using them easily *(Mörlein et al., 2005; Vitek et al., 2012).* The content of lean meat on carcass, regardless of device type s determined on the basis of thickness of the back fat tissue (measure on different places) and thickness of *M. longissimus dorsi (Dokmanović et al., 2013)*.

The aim of this study was to investigate the content of lean meat on carcass in slaughterhouse using three different methods (FOM, TP and partial dissection) and compare to results of content of lean meat on live farm pigs (PIGLOG 105).

Material and methods

The investigation was carried out between December 2015 and June 2016, and this experiment was performed on forty finishers. Pigs originated from a commercial farm which produced 40.000 finishers per year. In this study, pigs were chosen randomly, after that pigs were adequately tagged in order to follow traceability in the chain until the end of the measurement in the slaughterhouse. The Danish line genetics was presented on the farm (Landrace x Large White x Duroc), both sex (barrows, gilts), age 6 to 7 months, and weight 80 to 120 kg. The content of lean meat on live pigs was measured on the farm using ultrasound device PIGLOG 105, while in slaughterhouse, the content of lean meat was measured using FOM, TP and partial dissection.

Fat-O-Meater (FOM)

Optical device called Fat-O-Meter (FOM) was used for determining percentage of lean meat (%) and it is produced by Carometec, Denmark. The measurement of FOM was carried out on the slaughter line, 45 minutes from the moment of stunning and bleeding of animals at the latest. FOM operation was based on placing the probe on certain points of the carcass, between 12 and 13 ribs, 7 cm laterally from the dorsal line of cutting. Thus the penetration of the optical probe through subcutaneous fatty tissue and *M. longissimus dorsi* was performed. Results were shown on display: thickness of fatty tissue, thickness of muscle tissue,

the content of lean meat on carcass (% meatiness) and quality of carcass (S, E, U, R, O or P).

Ultrasound device PIGLOG 105

PIGLOG 105 is an ultrasound device, produced by SFK Technology, Denmark, which is used to measure content of lean meat on live animals. Measurement was performed on a farm 24 to 48 hours before sending animals to slaughter. This device works on the basis of input date of age and weight of animal, while probe is put on accurately determined places of animal body. Determining thickness of the bacon in the back part, measuring was performed between the 3rd and the 4th lumbar vertebrae from the last lumbar vertebrae, 7 cm of lateral from back line. While determining thickness of the bacon in back part and deep *M. longissimus dorsi*, measurement was performed between 3rd and 4th ribs from the back, the 7 cm of lateral from back line. On the basis of the measurement value, data about percentage of lean meat on farm were generated.

Partial dissection

According this method, carcass was cut up, by anatomically precisely defined scheme,on twelve parts, but only on four parts (ham, the shoulder, back-lumbars and abdominal-ribs part) further dissection was performed on muscle tissue, fat tissue and bones. On the basis of meat in these four areas, the most importnat part, with 75% of total meat of carcass and under the lumbar muscle of the carcass, calculated % of lean meat *(Walstra and Merkus, 1996).*

Two-point method (TP)

According to Rulebook *(„Sl. List SFRJ", br. 2/85, 12/85 i 24/86)*, fat tissue on back with skin was measured on the middle back, where bacon is the thinnest and lumbar part where *M. gluteus medius* is mostly grown in bacon. Thickness of *M. longissimus dorsi* was measured as the shortest connection of the cranial end of *M. gluteus medius* with the dorsal edge of the spinal canal. Measurement was performed by a ruler. On the basis of measured values and on the tables which are an integral part of this Rulebook, data about percentage of lean meat was provided.

Statistical analyses

The results were analyzed statistically, taking into consideration arithmetic means, standard deviations, coefficients of variation, and coefficients of simple correlation. Furthermore, the basic ANOVA model was performed using the LSD procedure. Also, results were analyzed by Pearson's correlation coefficient between methods used in the trial.

Results and Discussion

Forty years ago, some countries used the sonographic apparatus for carcass quality evaluation *(Miles and Fursey, 1974)*, while in Serbia there is still Rulebook on the Quality of Slaughtered Pigs and Pork Meat Categorization *(Sl. list SFRJ, 2/85)*. Even though in Serbia the grading of pig carcasses was not obligatorily performed based on the SEUROP system, slaughterhouses which measure the content of lean meat, use this classification. The carcasses are graded according to the content of lean meat and carcass weight. Farmers often did not believe the results of percentage of lean meat from slaughterhouse, especially when they received payments for live pigs, based on results from slaughter line. These results show how farmers can control percentage of lean meat on farms and compare with results from slaughterhouse. The content of lean meat is presented in Table 1, for each methods measure.

Table 1. Summary Statistics of lean meat content

	Count	*Average %*	*Standard deviation*	*Coeff. of variation %*	*Minimum %*	*Maximum %*	*Range*
PIGLOG-105	40	59.30	2.53205	4.26990	53.3	63.5	10.2
FOM	40	54.43	2.84827	5.23219	49.2	61.6	12.4
TP	40	55.28	4.48007	8.10469	42.1	60.8	18.7
Partial dissection	40	58.39	3.06825	5.25519	48.4	64.6	16.2
Total	160	56.85	3.87089	6.80895	42.1	64.6	22.5

By using the apparatus PIGLOG-105, 59.30% of lean meat in vivo was estimated one day before slaughter. It is 0.91% more then partial dissection and when compared to FOM and TP it is more 4.86% and 4.02%.

Table 2. The content of lean meat gained by various methods (Multiple Range Tests (95,0 percent LSD))

Contrast	*Sig.*	*Difference*	*+/- Limits*
PIGLOG-105% - FOM%	*	4.862	1.46507
PIGLOG-105% - TP %	*	4.022	1.46507
PIGLOG-105% - Partial dissection%		0.915	1.46507
FOM% - TP %		-0.840	1.46507
FOM% - Partial dissection%	*	-3.947	1.46507
TP% - Partial dissection%	*	-3.107	1.46507

Results of the content of lean meat that were measured using PIGLOG-105 were similar to results from partial dissection, and that shows the validity and reliability of this method *(Krška et al., 2002)*. Great deviation between PIGLOG-105 on one side and FOM and TP on other side indicated some error, and then partial dissection solved this mystery. In Table 2, there are results representing content of lean meat gained by various methods. Between PIGLOG-105 - partial dissection and FOM - TP were not significantly different, while between other methods there was a significant difference.

Table 3. The content of lean meat gained by various methods (Pearson's correlation coefficient)

	PIGLOG-105	FOM	Partial dissection	TP
PIGLOG-105	1			
FOM	0.618**	1		
Partial dissection	0.741**	0.562**	1	
TP	0.650**	0.623**	0.721**	1

** Correlation is significant at the level $p<0.01$ (2-tailed).

Differences in the content of lean meat gained by various methods is shown in Table 3. Among all methods treated by Pearson's correlation coefficient, PIGLOG-105, FOM, Partial dissection and TP, were significantly different.

Calibration of the fatometer was necessary and more reliability of staff who measure values for TP (*Bak et al., 2003*). Regardless of the method for measuring the content of lean meat before and after slaughter, results have to be the same, as it has already been described in previous research (*Borzuta, 1999; Ostrowski et al. 2000).*

Conclusion

After this study, slaughterhouse constructed new formulas for FOM in pig carcass classification. Regarding that, slaughterhouses, which used FOM or similar equipment for measuring percentage of lean meat, should control results of these equipment as described in this study, minimum twice a year. On the other hand, farmers should get feedback from slaughterhouse about the quality of their pigs, improve genetics, diet, conditions of keeping pigs, and check percentage of lean meat on farm, in order to avoid possible litigation and court case.

Poređenje mesnatosti svinja na farmi i liniji klanja

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Rezime

Merenje mesnatosti na liniji klanja i formiranje cene na osnovu mesnatosti doprinosi unapređenju kvaliteta i profitabilnosti proizvodnje i distribucije svinjskog mesa. Mesnatost kod živih svinja je merena na farmi korišćenjem ultrazvučnog aparata PIGLOG 105. U klanici, mesnatost je merena korišćenjem FOM, metode dve tačke i parcijalnom disekcijom. Kod živih svinja je izmerena mesnatost 59.30% jedan dan pre klanja. To je 0.91% veća vrednost nego što je dobijena parcijalnom disekcijom i 4.86% i 4.02% veća u poređenju sa FOM i metodom dve tačke. Velika razlika između vrednosti izmerenih PIGLOG-105 sa jedne strane i FOM i metodom dve tačke sa druge strane je ukazivala na neku grešku pri merenju i onda je parcijalna disekcija rešila ovu misteriju. Nakon ovog istraživanja, klanica je konstruisala novu formulu za FoM. Prema tome, klanice koje koriste FOM ili sličnu opremu za merenje mesnatosti treba da kontrolišu te uređaje kao što je opisano u ovom istraživanju, najmanje dva puta godišnje.

Ključne reči: klasifikacija polutki svinja, mesnatost, metode za klasifikaciju

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project “Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer” (TR 31034).

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Received 14 August 2017; accepted for publication 28 December 2017

Biotechnology in Animal Husbandry 34 (1), p 49-58 , 2018
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082.4'64
https://doi.org/10.2298/BAH1801049A

POTENTIAL OF *Telfairia occidentalis* LEAF EXTRACT AS BOAR SEMEN EXTENDER

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Original scientific paper

Abstract: The preservation of semen for Artificial insemination (AI) required the use of extender, which served as a buffer media to keep the sperm cell in good condition for fertilization. In this study, fluted pumpkin *Telfairia occidentalis* leaf extract (TOLE) potential as an unconventional boar semen extender was compared to Beltsville thawing solution (BTS), a conventional semen extender over a 48hr storage period. Aqueous solution of TOLE was prepared at three (3) concentration levels (25, 50 and 75%). Fresh ejaculates from boar were extended at (1:4) in TOLE solutions and BTS extender in a completely randomized design; treatments were replicated four (4) times. Extended semen was stored at 17^0C and evaluated for semen qualities which include pH, dead/live ratio (%) and sperm cell concentration at time intervals (0, 12, 24, 36 and 48) hrs. Extended semen was significantly ($P < 0.05$) affected by semen extenders and storage periods for pH, and dead/live ratio (%) but sperm cell concentration ($10^3/cm^3$) not ($P > 0.05$) affected. The pH values for BTS was significantly ($P < 0.05$) higher as compared to TOLE extenders. 75% TOLE extender had highest value (43.89%), and BTS had lowest value (12.15%) for dead/live ratio significantly ($P < 0.05$). The pH and dead/live ratio (%) values increased over storage time in all extender. The optimum performance of TOLE as compared to BTS extender was recorded at 50% concentration of TOLE extender. TOLE showed a potential as boar semen extender, but further studies are required to validate and improve its application.

Keywords: Semen extender, boar, pH, *Telfairia occidentalis*, beltsville thawing solution

Introduction

Swine farming in sub-Saharan Africa is faced with challenges which include nutrition, health management and breeding. The need to sustain existence

of good breeds has led to importation of pigs from other continents a procedure since replaced by artificial insemination (AI). An important aspect of AI that has facilitated its wide application is storage of semen for subsequent use.

Artificial insemination (AI) techniques enhance production rates and carcass homogeneity as well as the application of new management systems; hence have increased in the last decade. The advantage of AI is that the genetic potential of the best boar can be transferred to a large number of sows, leading to genetic improvement at a large scale (*Kaeoket et al., 2010*). An important aspect of AI that has facilitated its wide application is storage of semen for subsequent use. The seminal plasma supplies the necessary nutrients for the high metabolic demands of sperm transport through the female genital tract. In the ejaculate, this high metabolic activity can only be maintained over a limited period *(Rijsselaere et al., 2012)*. As temperature declines, the proportion of spermatozoa that maintain normal membrane integrity, ultrastructure and biochemical components decreases (*Johnson et al., 2006*).

The important traits for quantitative and qualitative evaluation of ejaculate include: volume of ejaculate, concentration of sperm, motility, percentage of abnormal spermatozoa, total number of spermatozoa and number of dead/live spermatozoa (*Savić et al., 2015, 2017*). Evaluation of sperm cell concentration, volume and percent of live spermatozoa is very important for the determination of maximal dilution of sperm suitable for artificial insemination or for a number of sows which can be inseminated (*Savić et al., 2017, Ivelina 2017*).

The fertilizing ability of stored semen is achieved by using a semen extender, which is a liquid diluent added to the semen (*Savić et al., 2017).* This ensures that the functional characteristics of the sperm cells are maintained such that a higher conception rate is achieved (*Gadea 200*3). Factors affecting the properties of an extender include pH, ionic strength, ions and osmotic pressure of the medium. Anti-microbial substances are also commonly included in diluents *(López Rodríguez et al., 2012*). A number of extenders have been developed which decrease the metabolic activity of spermatozoa using an environment high in CO_2 at ambient temperature *(Gadea 2003; Johnson et al., 2006; Boonkusol et al., 2010*). Presently, Beltsville-TS (BTS) is one of the most widely used semen extender for both short and long term storage. However, with the increase in AI practices, sub- Saharan African countries are faced with the challenges of semen extenders availability, due to the high cost through importation. Therefore, there is need to find an alternative diluent for short term storage of boar semen.

Telfairia occidentalis (*T. Occidentalis*) (family *cucurbitaceous*) is a tropical vine grown in West and Central Africa. It is a popular vegetable in Nigeria, commonly called “Ugwu” and highly reputed in traditional medicine *(Agatemor 2006; Fasuyi and Nonyerem 2007; Kayode and Kayode 2011*). The plant produces luxuriant edible green leaves, which are rich in minerals, *(Salman et al., 2008; Oboh et al., 2010*), and vitamins (*Kayode and Kayode 2011*). The leaf also contains

phytochemicals and has some antiplasmodial properties (*Salman et al., 2008; Oboh et al., 2010; Kayode and Kayode 2011*). Despite the need for affordable semen extenders in Nigeria and the proven rich nutritional profile of *T. Occidentalis*, studies on the usage of *T. Occidentalis* as a potential semen extender are lacking in Nigeria pig production industry. Therefore, the broad objective of this research is to investigate the potential of *T. Occidentalis* as a short-term extender for boar semen.

Materials and methods

Location of study

The study was conducted at the Piggery unit, Teaching and Research Farm of the University of Ibadan, Nigeria. All analyses and examinations were carried out at the University Physiology Laboratory.

Processing of aqueous Telfairia occidentalis leaf extracts (TOLE)

Fresh leaves were harvested from matured stems at onset of flower emergence. Leaves were rinsed off debris with distilled water. *T. Occidentalis* leaves (350 g) were homogenized with distilled water using a homogenizer and the homogenate filtered with a Whatman filter paper (*Oboh et al., 2010*). Proximate analyses content were determined using the standard protocols (AOAC, 1995). The filtrate was then concentrated to one tenth of its original volume and stored at 4^{o}C in a refrigerator using an airtight plastic jar. At the point of application as semen extender, a serial dilution was carried out to have 25%, 50% and 70% concentrations.

Experimental animal and semen collection

Four seven-month-old boars were housed in individual pens, under the same environmental conditions, and fed a standard commercial diet to meet their nutrient requirements. Prior to semen collection, males were trained to mount a dummy sow and semen was collected using the hand gloved method. Ejaculates were collected once a week over the entire 6 weeks' training period. On the 7^{th} week, ejaculates were collected using the gloved-hand technique (*Malo, 2010*) into a thermos pre-warmed to 37 ^{o}C; lined with a disposable plastic bag (IMV International Corp., Maple Grove, MN); and covered with a disposable milk filter (IMV International, Eden Prairie, MN) . All ejaculates were pooled together and the volume of semen was estimated by weighing the ejaculate.

Extended semen analysis

Each ejaculate was diluted (1: 4) semen to extender respectively at room temperature ($22^0C \pm 0.6^0C$). Evaluation of semen was carried out at time intervals (0, 12, 24, 36 and 48) hrs. The 0 hr evaluation was carried out at 10mins post semen addition to extenders. Dead/live (D/L) ratio was evaluated by using the eosin-nigrosin stain described by (*Łukaszewicz et* al., 2011). The pH was determined using the pH meter (Model pH 25) by dipping the pH electrode into the semen and the readings shown on digital scale were taken after 5mins. The concentration of sperm cells present in extended samples was determined by using the improved Neuber haemocytometer as described by (*Bonato* et al., 2014).

Table 1. Proximate composition (%) of *T. Occidentalis* leaves extract (TOLE) (w/v)

Parameters	Percentage (%)
Moisture	78
Crude protein	20
Crude fibre	13
Crude fat	8
Ash	10
Nitrogen free extracts	65

Statistical analysis

A two way analysis of variance (ANOVA) was performed using the fixed effect model. Bonferroni was used to test for the significance ($P<0.05$) of variance for all recorded and calculated data between different treatments, main effect of factors (semen extenders and storage period were considered) using model:

$$Yijk = \mu + Ti + Sj + eijk$$

Where Yij = Individual observation
μ = General mean
Ti = Fixed effect of semen extender (i = 1…..4)
Sj = Fixed effect of storage period (j = 0……48)
eijk = Expected error

Results and Discussion

The results of the proximate composition, as presented in Table 1, showed that aqueous extract of *T. Occidentalis* leaf meal has a potential protein and carbohydrate source comparable with other conventional plant protein and energy sources (*Salman* et al., 2008). The relatively rich nutrient profile of the leaf extract

is complemented with early age of the leaves as at the time of harvesting *(Fasuyi and Nonyerem 2007; Salman et al., 2008).*

Table 2. Composition of BTS extender used in this study

Composition	Volume (g/l)
Glucose	37.75
Sodium citrate	6.0
EDTA (ml/l)	1.25
Sodium bicarbonate	1.25
Potassium chloride	0.75
Penicillin	1.50

Where: EDTA- Ethylene diethyl tetra acetate

In Table 3, the effect of extenders on pH, dead/live (D/L) %, and sperm cell concentration ($10^3/cm^3$) were presented. The pH and D/L (%) parameters were significantly ($P < 0.05$) affected by semen extenders.

Table 3. Main Effect of semen extenders on extended boar semen

Parameters			
Treatments	pH	D/L ratio (%)	Sperm Conc. ($10^3/cm^3$)
BTS	7.78^{a}	10.15^{d}	250.48
25% TOLE	6.95^{b}	39.03^{ab}	252.10
50% TOLE	6.83^{c}	24.24^{c}	253.7
75% TOLE	6.30^{d}	43.89^{a}	250.20
SEM	0.02	0.80	1.52

^{abcd} Means along the same column with different superscripts are significantly ($P < 0.05$) different using Bonferroni as post hoc analysis

The pH of extended boar semen in BTS extender had the highest value (7.78) which was significantly ($P < 0.05$) different to the values obtained for TOLE extenders. A unique trend observed among the TOLE semen extenders was that pH decreases as inclusion level increase. However, 75% TOLE had the lowest pH value (6.30) which was significantly ($P < 0.05$) different to 50% (6.83) and 25% (6.95) inclusion levels. The D/L ratio (%) was significantly ($P < 0.05$) affected by semen extenders as shown in Table 3. As reported for D/L ratio (%), TOLE (75%) had the highest value (43.89%) which was significantly ($P < 0.05$) different as

compared to other extenders. BTS had value (10.15%), which was significantly lower ($P < 0.05$) as compared to TOLE extenders. The sperm cell concentration (10^3) was not affected by the semen extenders during storage.

As shown in table 4, pH decreased significantly ($P < 0.05$) over storage time in all TOLE extender treatments, while BTS showed an earlier increase in pH before a decrease. The dead/live ratio (%) increased significantly ($P < 0.05$) over storage time for all semen extenders. The sperm cell concentration (10^3) was not affected by storage period as reported in table 4.

Table 4. Effect of storage period on extended semen quality

pH	Period (Hours)					
Extender	0	12	24	36	48	SEM
BTS	7.20^{c}	9.00^{a}	8.93^{a}	8.93^{a}	8.60^{b}	0.02
25%TOLE	6.85^{d}	7.90^{c}	8.72^{a}	8.65^{a}	8.35^{ab}	0.05
50% TOLE	6.63^{d}	7.71^{c}	8.47^{a}	8.32^{a}	8.11^{ab}	0.11
75% TOLE	6.25^{c}	6.62^{bc}	6.74^{b}	6.90^{ab}	7.05^{a}	0.12
Dead/Live (%)						
BTS	6.12^{d}	10.83^{cd}	12.83^{c}	13.32^{b}	17.67^{a}	0.18
25% TOLE	16.62^{e}	25.39^{d}	43.58^{c}	52.52^{b}	60.04^{a}	0.13
50% TOLE	10.50^{de}	15.59^{d}	24.64^{c}	31.78^{b}	38.70^{a}	0.12
75% TOLE	18.16^{e}	28.07^{d}	47.45^{c}	55.78^{b}	67.04^{a}	0.15
Sperm cell conc. (10^3 /cm^3)						
BTS	252.01	252.05	251.10	249.11	248.15	1.18
25% TOLE	253.11	253.01	252.10	251.10	251.21	1.13
50% TOLE	255.21	255.13	253.15	253.01	252.03	1.12
75% TOLE	251.26	251.15	250.04	249.31	249.25	1.15

^{abcde} Means along the same column with different superscripts are significantly ($P < 0.05$) different using Bonferroni as post hoc analysis

The initial pH of TOLE was 6.20, BTS 7.52, while that of fresh boar ejaculate used in this study was 7.40. Therefore, the initial variation between the pH values of the extended semen in TOLE and BTS can be attributed to difference in initial pH of extenders. The high content of carbohydrate in the form of NFE may be responsible for the low pH in TOLE, while bicarbonate in BTS is responsible for the relatively alkaline pH. The earlier increase in pH of BTS extended semen can be attributed to instability of the extender. According to *Kaeoket et al. (2010),* the pH does not become stable from the start of dilution in water and that different extenders show a different pattern of pH change over time. In this study, the presence of a bicarbonate buffering system in the BTS extender explained earlier pH-rise. This increases the CO_2 in the media to reduce metabolic

activities of sperm cell during storage (*Vyt et al., 2004, 2007*). The results of pH in this study are consistent with study of *Kaeoket et al. (2010)* who reported that the pH values of the extended semen increase with increased storage time. As observed in this study, the presence of glucose in each extender caused a considerable reduction of intracellular pH during storage. This intracellular pH reduction obviously reduced sperm cell metabolism and increase sperm cells survive during storage (*Johnson et al., 2006*). However, high content of glucose in form of NFE found in TOLE might have resulted into intracellular acidosis, which caused sperm cell distortion and resulted into dead sperm cells with increase concentration (*Bonato et al., 2014*).

As shown in table 4, the values of D/L ratio (%) increased in all extenders over time. The quantity of living sperm cell is a function of dead/live ratio. The high values of dead/live ratio of about 50% recorded in TOLE extenders may be attributed to bacterial infection. In most cases, the testicular tissue and accessory glands of the boar are bacteria-free, and bacterial contamination of the ejaculate occurs during the semen collection process (*Martin Rillo et al., 1998*). BTS used in this study contained an antibiotic (penicillin), which inhibits bacteria proliferation. The extender component (glucose) and the temperature at which semen doses are stored (15-16ºC) promote the growth of most Gram negative bacteria such as *Escherichia coli, Salmonella spp* and *Pseudomonas spp* (*Gadea 2003*).

In extended semen, bacterial contamination leads to a series of alterations including reduced sperm motility, sperm agglutination, an increased proportion of altered acrosomes, dead sperm cells and pH lowering to acidic levels 5.7 - 6.4 (*Althouse et al., 2000*). However, the reduction in dead/live ratio for semen extender (TOLE 50%) as compared to other concentration can be attributed to presence of phytochemicals in the leaf extract in required proportion. TOLE was reported to show inhibitory effect to some microbes which include *Escherichia coli, Pseudomonas aeroginosa, Proteus spp and Salmonella typhii* (*Kayode and Kayode, 2011*). The high concentration of these phytochemicals in TOLE 75% may be toxic for the sperm cells and resulted to high dead/live ratio, while low concentration of phytochemicals at TOLE 25% can enhance bacteria proliferation and high dead/live ratio recorded as compared to TOLE 50%.

Sperm cell concentration was not significantly ($P<0.05$) affected by extender treatments. Although the value decreased slightly over time as shown in table 4. An equal concentration of sperm cell was randomly extended for each treatment at the onset of storage. The slight decrease in values of sperm cell over time may be attributed to dissolution of dead sperm cells in extended media (*Martin Rillo et. al., 1998*)

Conclusion

The BTS extender, a commercial short – term diluent used in this study performed better as compared to TOLE extenders. TOLE 50% concentration had the optimum performance within the TOLE concentrations. The study shows that TOLE has potential to serve as short – term semen extender, with best result in 50% dilution. However, further studies are required to effectively validate TOLE application as short term extender with increase in storage time and evaluation of other semen quality parameters.

Potencijal ekstrakta listova *Telfairia occidentalis* kao ekstendera semena nerastova

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Rezime

Čuvanje semena za veštačku inseminaciju (AI) zahteva korišćenje ekstendera, koji je služio kao puferski medijum za zaštitu i očuvanje ćelija sperme u dobrom stanju za oplodnju. U ovoj studiji, poređen je potencijal ekstrakta lista *Telfairia occidentalis* (TOLE) kao nekonvencionalnog ekstendera semena nerastova sa rastvorom za otapanje Beltsville (BTS), konvencionalnim ekstenderom semena tokom perioda od 48 časova skladištenja. Vodeni rastvor TOLE-a je pripremljen u tri (3) nivoa koncentracije (25, 50 i 75%). Sveži ejakulati nerastova su tretirani u (1: 4) TOLE rastvorima i BTS ekstenderu u potpuno slučajnom dizajnu; tretmani su ponovljeni četiri (4) puta. Tretirano seme je skladišteni na temperaturi od 17^0C i ocenjivano je njegov kvalitet odn. pH, odnos mrtvih/živih (%) i koncentracija spermatozoida u vremenskim intervalima (0, 12, 24, 36 i 48h). Tretirano seme je bilo značajno ($P<0,05$) pod uticajem ekstendera semena i perioda skladištenja za pH, a procenat/udeo mrtvih/živih (%), ali i koncentracija spermatoznih ćelija (103) nisu bile pod uticajem faktora ($P>0.05$). Vrednosti pH za BTS su značajno ($P <0.05$) veće u poređenju sa TOLE ekstenderima. TOLE ekstender u koncentraciji od 75% je imao najveću vrednost (43,89%), a BTS je imao najmanju vrednost (12,15%) za odnos mrtvih/živi šti je statistički signifikantno ($P <0,05$). Vrednosti pH i odnos mrtvih/živih (%) povećane su tokom vremena čuvanja u svim ekstenderima. Optimalne performanse TOLE-a u poređenju sa BTS ekstendorom zabeležene su u koncentraciji TOLE-a od 50%. TOLE je pokazao potencijal kao ekstender semena nerastova, ali su potrebne dalje studije za validaciju i poboljšanje njegove primene.

Ključne reči: ekstender semena, nerast, pH, *Telfairia occidentalis*, rastvor za otapanje Beltsville

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Received 14 August 2017; accepted for publication 25 November 2017

Biotechnology in Animal Husbandry 34 (1), p 59-68 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 575.21'62
https://doi.org/10.2298/BAH1801059P

PHENOTYPE CORRELATIONS OF LINEAR ASSESSMENT SCORES OF TYPE AND PRODUCTION TRAITS OF SIMMENTAL COWS

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Original scientific paper

Abstract: Determining the degree of correlation between two or more traits depends to a large extent on their manifestation. The knowledge of genetic and phenotypic correlations between body characteristics of the animal and product characteristics can help define the breeding goal, but also to define and harmonize the assessment criteria. Although the phenotypic and genetic correlations between the traits of body development and type and the milk yield show different degrees of variation, they should be taken into account in the final assessment of the breeding value of the animal so that the selection programs are more comprehesively defined. The examination of phenotypic correlations of linear assessment scores of the type, milk and fertility traits was performed on a total of 303 cows of the Simental breed in the first three lactations. The examination of phenotypic correlations included the following milk performance properties in the first three standard lactations: milk yield, milk fat content, milk fat yield, yield of 4% corrected milk; also fertility traits: age at current calving and service period in each lactation; while the linear type scoring included a group of traits: type or frame, muscularity, fundament, udder.

Key words: correlation, milk performance, fertility, type traits, Simmental breed.

Introduction

The main goal of the breeding and selection work is to create new generations that will be superior to the previous ones in terms of their production results and show greater productive effects in the production of milk and meat. For these reasons, it is necessary in the selection work to know the breeding value of parental couples, as well as the degree of heredity and the correlation of important traits (*Pantelić 2006*).

Phenotypic correlation of milk and fertility traits is very important when it is desirable to perform comparative selection for several properties, and even more important for indirect selection in conditions where some properties can not be directly promoted. At the same time, opportunities for increasing the success of selection by making early selection conclusions and decisions are created (*Pantelić et al., 2007*). In these studies, the age of calving was in a negative phenotypic correlation with all production indicators: milk yield -0.023, % of milk fat -0.005, quantity of milk fat -0.023, and production of 4% FCM -0,023. The mutual phenotypic correlation of age at calving and service period was slightly positive 0.047. The service period was also in a negative correlation with the milk yield traits, except for the percentage of milk fat 0.001, as well as the duration of lactation 0.329.

Hermas et al. (1987) have found coefficients of phenotypic correlations between milk properties in standard lactation and some fertility properties. Phenotypic correlations had the following values: milk yield - service period 0.19, milk yield - age at calving -0.01, milk fat, kg - service period 0.17, milk fat, kg - age at calving 0.06, milk fat, % - service period -0.03, and milk fat, % -age at calving 0.07.

Moore et al. (1991) provide data on the values of the coefficients of the phenotypic correlations between production of milk and milk fat in standard lactation with age at calving of 0.20 and 0.21, respectively.

Stojić (1996) has calculated the coefficients of phenotypic correlations between milk properties in standard lactation and age at calving (AC) and service period (SP). Correlation coefficient values were: milk yield - AC 0.034; milk yield - SP 0.095; milk fat, % - AC 0.034; milk fat, % - SP -0,032; milk fat, kg - AC 0.045; milk fat, kg - SP 0.072; yield of 4% FCM - AC 0.042; yield of 4% FCM - SP 0.085.

Marković (1999) has noted the values of phenotypic correlations between milk properties. The results of phenotypic correlations ranged from -0.35 between milk yield and milk fat content up to 0.96 between milk yield and 4% FCM.

Linear type properties are the basis of all modern classification systems, and represent the basis of all systems for describing dairy cows. Linear classification is based on measures/measurement of individual characteristics of the type instead of giving opinions. It describes the degree of presence of a trait, and not desirability (*Pantelić et al., 2011*).

Body development and type are very important indicators of cow's production capabilities, their ability to consume sufficient amounts of food, give high-quality milk, reduce energy consumption in production and prolong production, giving more number of offspring.

Živanović (2002) has examined the variability of the linearly assessed type traits and milk yield of the primiparous Black and White heifers on a sample of 2,976 cows. Negative genetic correlations between milk properties and linearly

assessed type traits have ranged from -0.241 to -0.856, for a large number of investigated properties. Positive genetic correlations have ranged from 0.544 to 0.744.

Analyzing the production and body traits of Holstein cows, *Koenen and Groen (1998)* have established the strength of genetic and phenotypic correlations between the type and conformation properties. The values of the coefficients of the phenotypic correlations have ranged from -0.28 (the depth of the udder and the depth of the body) and 0.95 (body wight and pelvic height), and genetic correlation from -0.49 (body depth and udder depth) to 0.77 (body weight and chest girth).

By examining the phenotypic and genetic correlations of the milk yield traits and the type of bull dams of the Holstein Friesian breed, *Pantelić et al. (2012)* have found negative phenotypic correlations between milk production and type traits. Phenotypic correlations have ranged from -0.12 (position of the rear legs, the side view) to -0.01 (pelvic height and body depth), and positive from 0.03 (position of the rear teats) to 0.23 (central ligament). Phenotypic correlations between the percentage of milk fat and the type traits have ranged from -0.08 (position of the front teats) to 0.14 (pelvic height).

Material and method

The examination of phenotypic correlations of linear assessment of the type, milk and fertility characteristics was performed on a total of 303 cows of the Simental breed in the first three lactations. The examined throats were cultivated in different individual farms, but can be said mainly in very similar conditions of keeping and eating. The cows were kept in large numbers in stables with a connected holding system, on long and medium-sized bays with straw. The diet was based on hay and seedling alfalfa, less common grass, silage the whole corn plant and mainly concentrated concentrates. The husband was machine-made, mostly in buckets, and milk was stored in lacto-frys until delivery. Productivity control was carried out by the princes of the AT4 control of productivity by the breeding organizations, in which the measurement of the milk is done only during the morning or only during the evening moon on the control day (alternative method), but using their results must be mathematically corrected to the reference method.

The examination of phenotypic correlations of linear type scores, milk and fertility traits was performed on a total of 303 cows of the Simental breed in the first three lactations. The examined animals were reared on different individual farms, but mainly in very similar conditions of housing and nutrition. The cows were kept mainly in stables with a tied system, on long and medium-sized beds with straw. The diet was based on hay and alfalfa silage, to lesser extent grass was used, whole maize plant silage and mixtures of concentrates. The milking was

carried out using the machine, mostly into buckets, and milk was stored in lacto - freeze storage until delivery. Productivity control was carried out by the principles of the AT4 control of productivity by the breeding organizations, the milk quantity was measured only during the morning or only during the evening milking in the test day (alternative method), but the results obtained in this way must be mathematically corrected to the reference method.

Linear assessment of the type and body development of the examined cows of the Simental breed was carried out according to the established criteria, individually or during the animal evaluation, with an immediate insight into the appearance and condition of the animals. Each trait is judged individually using wide range of grades from 1 to 9, whereby the qualities in the evaluation are grouped as follows: frame, muscularity, fundament and udder. In such a way, a greater accuracy is achieved both of individual scores and of the overall external appearance. Although a linear score does not describe the desirability of a feature, grade 9 will be either the most desirable or least desirable of two possible extremes.

The examination of phenotypic correlations included the following milk performance traits in the first three standard lactations:

- Milk yield, kg;
- Milk fat content, %;
- Milk fat yield, kg;
- Yield of 4% fat corrected milk, kg.

In addition to the milk performance traits for each cow, the age at current calving and the service period in each lactation were determined, while the linear type score included a group of traits:

- Type or frame;
- Muscularity;
- Fundament;
- Udder.

Phenotypic correlation between milk performance traits, fertility traits and linear type scores was tested using the linear correlation method, and it was discussed based on the Roemer-Orphal classification.

Phenotypic correlations were calculated according to the formula:

$$rp_{xy} = \frac{Cov_{Pxy}}{\sqrt{\sigma^2_{Px} \times \sigma^2_{Py}}}$$

The symbols have the following meanings:
rPxy = coefficient of phenotypic correlation between traits *x* and *y*
CovPxy = phenotypic covariance between traits *x* and *y*
σ^2Px = phenotypic variance of trait *x*
σ^2Py = phenotypic variance of trait *y*

Results and Discussion

Phenotypic correlations are determined both by genetic and external factors. If the environmental conditions in related animals were identical, then the phenotypic value of the correlations would be equal to the genetic one. However, as there are no identical conditions in the practical cattle breeding, the values between these correlations are also different. If the external environment conditions are more stable or less variable, the degree of correlation between the phenotypes of the animals would be greater *(Petrović and Pantelić, 2015*).

Table 1. Coefficients of phenotypic correlations (r_{xy}) between the studied indicators of milk performance traits, fertility and type in the first lactation.

Indicators	A	B	C	D	E	F	G	H	I
Milk yield, kg **(A)**	\								
Milk fat content, % **(B)**	0.14*	\						* - P<0.05	
Milk fat yield, kg **(C)**	0.98*	0.32*	\						
Yield of 4%FCM, kg **(D)**	0.99*	0.25*	1.00	\					
Age at calving, days **(E)**	0.23*	0.10	0.24*	0.24	\				
Service period, days **(F)**	0.03	-0.02	0.03	0.03	0.04	\			
Frame **(G)**	0.27*	0.14*	0.28*	0.28*	0.15*	0.05	\		
Muscularity **(H)**	0.29*	0.18*	0.31*	0.30*	0.22*	0.06	0.71*	\	
Fundament **(I)**	0.28*	0.15*	0.30*	0.29*	0.17*	-0.01	0.66*	0.80*	\
Udder **(J)**	0.30*	0.15*	0.32*	0.32*	0.20*	0.01	0.66*	0.78*	0.76*

Table 2. Coefficients of phenotypic correlations (r_{xy}) between the studied indicators of milk performance traits, fertility and type in the second lactation.

Indicators	A	B	C	D	E	F	G	H	I
Milk yield, kg **(A)**	\								
Milk fat content, % **(B)**	0.03	\						* - P<0.05	
Milk fat yield, kg **(C)**	0.98*	0.21*	\						
Yield of 4%FCM, kg **(D)**	0.99*	0.14*	1.00*	\					
Age at calving, days **(E)**	0.19*	0.06	0.20*	0.20*	\				
Service period, days **(F)**	0.06	-0.06	0.05	0.05	0.03	\			
Frame **(G)**	0.32*	0.01	0.32*	0.32*	0.16*	0.09	\		
Muscularity **(H)**	0.26*	-0.06	0.25*	0.25*	0.21*	0.13*	0.71*	\	
Fundament **(I)**	0.25*	-0.02	0.24*	0.24*	0.14*	0.13*	0.66*	0.80*	\
Udder **(J)**	0.31*	-0.01	0.30*	0.30*	0.17*	0.09	0.66*	0.78*	0.76*

The coefficients of phenotypic correlations (r_{xy}) between the studied parameters of milk performance traits, fertility and type in the first three standard lactations are shown in Tables 1-3.

Table 3. Coefficients of phenotypic correlations (r_{xy}) between the studied indicators of milk performance traits, fertility and type in the third lactation.

Indicators	A	B	C	D	E	F	G	H	I
Milk yield, kg **(A)**	\								
Milk fat content, % **(B)**	-0.08	\						* - P<0.05	
Milk fat yield, kg **(C)**	0.98*	0.13*	\						
Yield of 4%FCM, kg **(D)**	0.99*	0.04	1.00*	\					
Age at calving, days **(E)**	0.14*	-0.10	0.12*	0.13*	\				
Service period, days **(F)**	0.13*	0.03	0.13*	0.13*	0.10	\			
Frame **(G)**	0.29*	-0.11	0.26*	0.28*	0.18*	0.10	\		
Muscularity **(H)**	0.24*	-0.07	0.22*	0.23*	0.24*	0.04	0.71*	\	
Fundament **(I)**	0.22*	-0.08	0.20*	0.21*	0.18*	0.08	0.66*	0.80*	\
Udder **(J)**	0.26*	-0.02	0.26*	0.26*	0.18*	0.02	0.66*	0.78*	0.76*

Mutual positive and full phenotypic correlation between the yields of milk, milk fat and 4% fat corrected milk was established in all three lactations with the coefficient of correlation ranging from 0.98 to 0.99. Low and mainly positive correlation was established in indicators milk yield and milk fat content (0.13, 0.21, 0.32).

Low and generally positive phenotypic correlation was recorded in all three lactations, both for milk performance and fertility indicators, but also for fertility and type indicators. The coefficient of phenotypic correlations ranged from 0.03 between the milk yield and the duration of the service period in the first lactation (Table 1) to 0.24 between the yield of 4% FCM and the age at first calving.

The phenotypic correlation of the indicators milk yield and the average type score was moderate and generally positive. The interval of variation of the coefficient of correlation between milk yield and the properties of the type were in the range from 0.22 for milk yield and fundament score (Table 3) to 0.32 milk yield and score for body frame (Table 2). Weak and mainly negative correlation was established between the properties of the type and the milk fat content for cows in the second and third lactation. The coefficient of phenotypic correlation ranged from -0.01 milk content and udder score (Table 2) to -0.11 milk fat content and frame score (Table 3).

A negative phenotypic correlation between milk production and percentage of milk fat, and a positive between the yield of milk and the quantity of milk fat, or 4% of FCM in their researches, was found by a number of authors: *Moore et al. (1991)* and *Markovic (1999).* Negative phenotypic correlations between age at calving and milk production in their researches were established by *Hermas et al. (1987)* and *Pantelić et al (2007).* Unlike them *Moore et al. (1991)* and *Stojić (1996),* provide data on the positive values of the coefficient of phenotypic correlation of the above mentioned properties.

Determination of the degree of association/correlation of two or more traits depends to a large extent on their expression. The knowledge of genetic and phenotypic correlations between body properties of animals and production performance traits can help define the breeding goal, but also to define and harmonize the assessment criteria. Although the phenotypic and genetic correlations between the body development traits and the type and the milk yield show different degrees of variation, they should be taken into account in the final assessment of the breeding value of the animal so that the selection programs become more comprehensive.

Conclusion

The knowledge of the phenotypic association of milk and fertility traits with linear type scores is very important in the selection work, where, in addition to high production, good health is expected as well as the long productive life of the animals in the herd.

In this study, the positive and full phenotypic correlation between the yields of milk, milk fat and 4% fat corrected milk was established in all three lactations, as well as the low and mostly positive correlation between the indicators of milk yield and the milk fat content. Low and generally positive phenotypic correlation was recorded in all three lactations and for milk and fertility indicators, but also for fertility and type indicators. Phenotypic correlation between the milk yield indicator and average type score was medium strong and generally positive for the yield traits and weak and mostly negative between the type properties and the milk fat content.

The phenotypic correlation of the examined properties shows that when selecting both breeding heifers and semen for the fertilization of cows and heifers, the type traits must be taken into account, as this, in addition to the selection based on milk performance, shall contribute to the realization of higher milk production and faster genetic improvement of our population.

Fenotipske korelacije linearnih ocena tipa i proizvodnih osobina krava simentalske rase

Vlada Pantelić, Dragan Nikšić, Nevena Maksimović, Dušica Ostojić-Andrić, Marina Lazarević, Miloš Marinković, Nenad Mićić

Rezime

Poznavanje fenotipske povezanosti osobina mlečnosti i plodnosti, sa linearnim ocenama tipa je veoma značajno u selekcijskom radu, gde se pored visoke proizvodnje, očekuje dobro zdravlje kao i dug produktivni život grla u stadu.
U ovom istraživanju medjusobna pozitivna i potpuna fenotipska povezanost prinosa mleka, mlečne masti i 4% mast korigovanog mleka ustanovljena je u sve tri laktacije, kao i niska i uglavnom pozitivna povezanost pokazatelja prinosa i sadržaja mlečne masti. Niska i uglavnom pozitivna fenotipska povezanost utanovljena je u sve tri laktacije i za pokazatelje mlečnosti i plodnosti, ali i za pokazatelje plodnosti i tipa. Fenotipska povezanost pokazatelja mlečnosti i prosečnih ocena tipa je bila srednje jaka i uglavnom pozitivna za osobine prinosa i slaba i uglavnom negativna izmedju osobina tipa i sadržaja mlečne masti.

Fenotipska povezanost ispitivanih osobina pokazuje da se prilikom odabira kako priplodnih junica, tako i semena za oplodnju krava i junica, mora povesti računa i o tipskim karakteristikama, jer će to pored odabira po mlečnosti doprineti ostvarenju veće proizvodnje mleka i bržem genetskom unapredjenju naše populacije.

Ključne reči: korelacije, mlečnost, plodnost, osobine tipa, simentalska rasa.

Acknowledgment

This research is part of the Project EVB: TR-31053 financial supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received 9 January 2018; accepted for publication 18 March 2018

Biotechnology in Animal Husbandry 34 (1), p 69-81 , 2018
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'63
https://doi.org/10.2298/BAH1801069S

EFFECT OF SUPPLEMENTATION WITH INORGANIC AND ORGANIC SELENIUM ON SPERM QUALITY AND QUANTITY IN NORTH-EAST BULGARIAN MERINO RAMS

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Original scientific paper

Abstract: Selenium is a trace element, which stimulates antioxidant defenses and improves reproductive functions in human and animals, under the form of selenoproteins. The objective of the study was to evaluate the effect of selenium, supplemeted as inorganic or organic form in the diet of stud rams, on some of their semen parameters. The experiment was performed with 15 clinically healthy rams from North East Bulgarian merino breed. The animals were divided in three groups (5 per group). The rams from first experimental group (G1) received a diet with supplementation of 4,0mg sodium selenite (Na_2SeO_3) per animal per day, while the animals of the second experimental group (G2) obtained diet with 1.83g L-selenomethionine (Sel-Plex, Alltech, USA) per animal per day. Eventually, each animal from the G1 and G2 received 1.83g selenium per day. The control group (GC) received a diet without supplementation of selenium. The principal composition of the diet in each group was the same. The ejaculates were obtained via artificial vagina. The evaluated parameters were volume and pH of the ejaculates and motility, concentration and *in vitro* survivability of the spermatozoa at 39˚C for 360 min.

It was found that the supplementation of ram studs diet either with inorganic and organic selenium led to increase in the volume of the

ejaculates, motility and survivability of the spermatozoa. The pH of the freshly obtained semen was not affected by selenium treatment.

Key words: ram, selenium, diet, ejaculate, sperm quality

Introduction

The different products which can be obtained from sheep, namely meat, milk, wool and furs are the reason why the sheep breeding is one of the major sectors of animal husbandry worldwide. The development of this sector implies more intensive use of reproductive techniques in order to obtain animals with better productive traits. Some of the most popular techniques used to improve the fertility in sheep are artificial insemination and cryopreservation of semen. Central role in their successful application plays the quality of ram semen. There are many factors which influence the semen quality and feeding is one of them. The composition of the ram diet can improve or worsen the quality and quantity of their ejaculates subsequently their spermatozoa *(Kheradmand et al., 2006; Brown, 2004).*

Selenium (Se) is one of the microelements with important biochemical functions. It is a component of the enzyme glutathione peroxidase that protects the cells from accumulation of peroxide oxidation products *(Surai, 2002).* Under the form of selenoprotein it stimulates antioxidant protection and promotes reproductive activity. Low sperm Se content is associated with abnormal sperm morphology and motility in humans and several animal species *(Saaranen et al., 1989; Marzec-Wróblewska et al., 2012).* The addition of various selenium containing compounds has led to increased growth of young animals and improved productivity and health status *(Dimanov et al., 1982; Profirov et al., 1981; Dimanov et al., 1992).* Applied individually or in combinations with other additives like vitamins (A, E, D) or other microelements (i.e. Co, Zn, etc.) Se was reported to have positive effect on reproductive performance, including semen parameters, of different animal species and humans (*Sikka et al., 1995 ;Scott et al., 1998, Kendall et al., 2002; Surai, 2002; Zubair et al., 2015).* Recent data showed that Se supplementation could be useful even in improvement of the quality of dog semen with lowered fertility (*Domosławska et al., 2015*). *El-Sheshtawy et al. (2014)* reported that administration of Se increase both sperm cell concentration and percentage of alive sperms and decrease sperm abnormalities and acrosomal damage in

Baladi goat bucks. *Marin-Guzman et. al. (1997)* found that selenium accelerates the maturation of spermatozoa in the epididymis and reduces the amount of sperm with cytoplasmic droplets.

The form of Se (organic or inorganic) was also found as important factor for the outcome of the supplementation. *Lopez et al.* (2010) showed that addition of organic Se to the regular rations of boars lead to increased sperm concentration compared to inorganic Se but reduced some motility parameters and resistance to oxidative stress. Specifically in Sanjabi rams organic selenium (alone and in combinations with zinc) was recently reported to improve semen characteristics (*Ghorbani et al., 2018*). On the other hand inorganic selenium was found to decrease the percentage of sperm defects but without direct influence on ram sperm volume, total motility, concentration and membrane integrity in Brasil rams (*Piagentini et al., 2017*).

The above mention data along with the insufficient knowledge about the effect of Se on the reproductive performance of local Bulgarian sheep breeds were major clues to test the effect of selenium, supplemeted as inorganic or organic form in the diet of stud rams from North East Bulgarian merino breed (NEBM) focusing on the quality and quantity of the obtained semen.

Materials and methods

Experimental animals and diet

The experiment was performed with 15 rams from North East Bulgarian merino breed - Shumen type in The Experimental Station of Agriculture – Targovishte, Bulgaria. The animals were divided into three groups of five – a control (GC) and two experimental (G1 with addition of inorganic selenium and G2 with organic selenium supplementation. Each group consisted of rams aged 3.5 to 6.5 years of age and 90 to 110 kg of body weight. All rams were clinically healthy, without external and internal parasites and grown according to generally accepted standards for animal welfare (*Council Directive 98/58/EC*).

Throughout the experimental period the daily rations consisted of quality feed providing 100-110 g of protein digestible in the intestines, as 50% of the energy was supplied by concentrated feed.

Semen assessment

Evaluation of the ejaculates was performed at the Laboratory for artificial insemination in the Experimental station of agriculture, Targovishte, Bulgaria. The following parameters were assessed: volume of the ejaculate (in ml) - with a graduated pipette, accurate to 0.01ml; sperm motility (in %) – under microscope (Carl Zeiss, Jena, Germany) at magnification of 400 x, by a trained technician; pH of semen by pH meter (Denver Instruments, USA); sperm count in 1 ml – by using of Thoma counting chamber.

The *in vitro* survivability of the spermatozoa was assessed as follows: The ejaculates were diluted in semen extender 6A in ratio 1:3 and incubated in thermostat at 39 ˚C for 360 minutes. Since only the spermatozoa which are alive possess the ability to move, we use that as indicator for sperm survivability. Evaluation of the sperm motility was performed on 10^{th} and 360^{th} minute of incubation by the method described above.

Design of the experiment

The groups were formed 21 days prior the experiment, so that rams can be adapted to the same rearing conditions.

The study was divided into two periods - adaptive and experimental. The adaptive period continues for 21 days, during which the groups were formed, and the animals were allowed to accustom to the same living conditions. During this period the daily rations were not supplemented with selenium. In the experimental period, rams from first experimental group (G1) received a diet with supplementation of 4,0mg sodium selenite (Na_2SeO_3) per animal per day, while the animals of the second experimental group (G2) obtained diet with 1.83g L-selenomethionine (Sel-Plex, Alltech, USA) per animal per day. Eventually, each animal from the G1 and G2 received 1.83g selenium per day. The control group (GC) received a diet without supplementation of selenium. The experimental period was divided into two sub periods – 1^{st} sub period - from the 1^{st} day to the 30^{th} day of selenium addition; 2^{nd} sub period - from the 30^{th} day to the 45^{th} day of selenium addition.

The semen collection in the adaptive and experimental periods was performed once a week by artificial vagina from a trained technician. After obtaining the semen was transferred to the laboratory and processed as it was described in *SEMEN ASSESSMENT*.

Statistical analysis

The statistical analyses were performed by software R.2.8.1. Values are presented as mean ± standard deviation. The effect of the different type of selenium supplementation on semen parameters was assessed for every period by multiple comparisons between treatment groups using Student-Newman-Keuls method (SNK). For all statistical procedures performed, p values < 0.05 were considered significant.

Results and discussion

One of the important parameters which affect the semen quality is pH *(Semkov et al., 1989; Zhou et al., 2015).* The results of pH measurement of the different groups are presented at *Table 1*. It was found that the average pH of the ram ejaculates of both the control group and the experimental groups throughout the entire period of the study was in close range from 6.46 to 6.60. *Bartoov et al.(1980)* found that optimal pH for the normal function of ram semen mitochondria is in the range of 6.0 to 6.5 and as it is well known, the mitochondria are responsible for many of the sperm functions including their motility and subsequently fertilizing ability *(Piomboni et al.,2011).*

Table 1. Effect of Se supplementation on pH in ram ejaculates

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD	mean± SD	mean± SD
Adaptation	6.53±0.07a	6.6±0.03a	6.46±0.04a
1st experimental sub period	6.46±0.03a	6.44±0.04a	6.47±0.03a
2nd experimental sub period	6.42±0.03a	6.39±0.03a	6.39±0.06a

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

All pH values of the GC and the experimental groups, at the adaptation and experimental period were at the normal range for the North East Bulgarian merino breed. The results indicate that the addition of

organic and inorganic selenium in the rations of the rams did not lead to a significant change in the pH value of the ejaculates. Even more at the end of the second experimental period the pH of the Se treated groups were lower (6.39) compared to the GC (6.42). In experiment conducted with rams under heat stress in sub-tropical environment of Egypt was found that supplementation of inorganic Se (sodium selenate) in their diet leads to significant decrease of semen pH, percentages of dead spermatozoa, sperm abnormalities and acrosomal damage and increase in sperm motility, sperm-cell concentration as well as improvement of other physiological parameters *(Marai et al., 2009*).

In the adaptive period the mean ejaculate volume in GC and in G2 was equal – 1.07 ml, while in the G3 was a little bit higher- 1.19, but without statistical significance (*Table 2*).

Table 2 Effect of Se supplementation on ram ejaculate volume.

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD (ml)	mean± SD (ml	mean± SD (ml)
Adaptation	1.07±0.07a	1.19±0.07a	1.07±0.06a
1st experimental sub period	1.03±0.05a	1.27±0.06b	1.22±0.06b
2nd experimental sub period	1.10±0.05a	1.46±0.09b	1.33±0.14b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

After the addition of inorganic and organic Se to the diet of the animals from the experimental groups (G1 and G2), the volume of their ejaculates become significantly higher ($p<0.05$) in comparison to the control group.

It is interesting to note that while the average increase in the volume of the ejaculate in the two experimental groups (in absolute values) was the same at the end of the experimental period (about 0.26ml), the effect of organic selenium addition was more pronounced at the 1st experimental sub period compared to the inorganic Se supplementation. The obtained results suggest that the addition of both organic and inorganic Se to the main diet

increase the ejaculate volume in rams. The effect was more rapid with organic selenium.

Similarly to us *Mahmoud et al., (2013)* found significant increase in ejaculate volume in rams after application of combination of Se and vitamin E. In experiment with Barbari bucks, the supplementation of their diet with combination of zinc and selenium also lead to significant increase of ejaculate volume *(Kumar et al.,2014).* On the other hand *El-Sheshtawy et al.,(2014),* didn't found significant difference in the ejaculate volume of bucks injected with Se, vitamin E or combination of both compared to non-treated group.

Another important factor for determining the quality of semen material in farm animals is sperm survivability at 39°C for 360 min. At *Table 4* are presented the data from the sperm resistance at 39°C in the supplemented with Se and non-supplemented groups. During the adaptation period, the average survival rate of the ram spermatozoa at the 10^{th} and the 360^{th} min of incubation in GC and G1 are similar. In G2 the survival rate is significantly higher than in GC and G1, at both measurements.

During the entire experimental period, the groups which received Se supplemented diet shows significantly higher sperm survival rate in comparison to the control group, both at the 10^{th} and at the 360^{th} minute of incubation at 39°C. The results in the first experimental sub period (from the 1^{st} to the 30^{th} day of Se supplementation) are similar in G1 and G2, but in the second experimental sub period (from the 30^{th} to the 45^{th} day of Se supplementation), the animals which were supplemented with organic selenium, displayed a significantly higher survival rate (45%) after 360 min of incubation at 39°C, in comparison to those which were supplemented with inorganic selenium (33%).

Table 3. Effect of Se supplementation on ram sperm motility

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD (%)	mean± SD (%)	mean± SD (%)
Adaptation	48.61±4.33a	47.63±3.52a	64.38±3.29b
1^{st} experimental sub period	56.33±3.18a	67.43±0.60b	68.33±0.91b
2^{nd} experimental sub period	53.5±4.87a	71.5±0.54b	70±1.62b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

The presented results demonstrated that the additional intake of inorganic and organic selenium has a positive effect on the survival of sperm cells during incubation for 360 min at 39˚C.

The effect of selenium supplementation on sperm motility is presented in *Table 3*. The average percent of motile spermatozoa in GC during the adaptation and trial period varies within narrow limits and ranges from 48.61% to 56.33%.

There is a statistically significant difference between the percent of motile spermatozoa in G1 (47.63%) and G2 (64.38%) in the adaptive period, which eventually disappeared after the addition of inorganic or organic selenium to the ram's diet. In both experimental sub periods the percent of motile spermatozoa is significantly higher in the groups with inorganic (67.43% for the 1st and 71.5% for the 2nd) and organic (68.33% for the 1st and 70% for the 2nd) Se supplementation, compared to the group without Se supplementation (56.33% for the 1st and 53.5% for the 2nd). This result suggests that Se supplementation as inorganic or organic form has positive effect on sperm motility.

Table 4. Effect of Se supplementation on ram sperm survivability at 39˚C for 360min

Periods	Groups					
	Control (GC)		Inorganic selenium suppl. (G1)		Organic selenium suppl. (G2)	
	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)
Adaptation	48.33±4.33b	5.28 ±2.00e	46.39 ±4.33b	5.94 ±2.00e	61.88 ±4.33a	16.14 ±3.33d
1st experimental sub period	57.25±6.21a	4.0 ±0.92e	65.4 ±1.00a	12.32 ±2.43d	65.5 ±1.20a	16.30 ±2.43d
2nd experimental sub period	53.0±3.15a	3.8 ±0.61e	71.5 ±0.96a	33.0 ±2.38c	70.5 ±1.12a	45.00 ±2.38b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

Sperm motility is accepted as a major factor for assessment of semen quality and fertilization ability *(David et al., 2015),* which means that Se supplementation to the NEBM ram's diet may have a beneficial effect on the reproductive performance of the animals.

In experiment conducted by *Piagentini et al. (2017)* was found that Se supplementation to ram's diet significantly improve the morphology, but not the motility of the sperm. The difference between the results of *Piagentini et al.(2017)* and those obtained in our research can be contributed to different factors like the different climate (subtropical in their case, moderate in ours), breed differences or the way of semen collection (we use artificial vagina and they used electroejeculation). On the other hand there is a lot of data which shows that Se alone or in combination with Zn or vitamin E improves not only the morphology but also the sperm motility in rams (*Kendall et al., 2000; Marai et al., 2009; Ghorbani et al., 2018*). Moreover it was found that the injective application of selenium alone or in combination with vitamin E increase the sperm motility in bucks (*El-Sheshtawy et al., 2014),* and rams *(Mahmoud et al., 2013).* None the less it was found that addition of selenium to semen of ram, water buffaloes and human has positive effect on sperm parameters after freezing and thawing procedures *(Seremak et al., 1999; Dorostkar et al., 2012; Rezaeian et al., 2016).* In experiments with boars was found that addition of selenium improves the sperm quality including motility and fertilization rate *(Marin-Guzman et. al., 1997).* The same authors also stated that the insufficiency of Se in boar's diet is even more detrimental for semen quality than the insufficiency of vit E *(Marin-Guzman et. al., 1997).*

Conclusion

The addition of selenium as organic or inorganic form to the diet of the North East Bulgarian merino rams has positive influence on the ejaculate volume, sperm motility and sperm survival rate after incubation at 39˚C for 360 min, without negative effect on pH of the ejaculates.

The represented data combined with the findings of the other studies on that topic, suggested that selenium supplementation in the main diet can be used for improving the reproductive traits of ram studs.

Uticaj dodavanja neorganskog i organskog selena na kvalitet i količinu sperme ovnova severoistočne bugarske merino rase

Rossen Stefanov, Mihail Chervenkov, Georgi Anev, Nevena Maksimović, Madlena Andreeva, Teodora Ivanova, Aleksandar Milovanović

Rezime

Selen je element u tragovima, koji stimuliše antioksidantske odbrane i poboljšava reproduktivne funkcije kod ljudi i životinja, u obliku selenoproteina. Cilj studije je bio da se proceni efekat selena, dopunjenog u neorganskom ili organskom oblik u ishrani ovnova, na neke parametre semena. Eksperiment je obavljen sa 15 klinički zdravih ovnova severnoistočne bugarske merino rase. Životinje su podeljene u tri grupe (5 po grupi). Ovnovi prve eksperimentalne grupe (G1) dobijali su obrok sa dodatkom 4,0 mg natrijum selenita (Na_2SeO_3) po grlu dnevno, dok su životinje druge eksperimentalne grupe (G2) hranjene obrokom sa 1,83 g L-selenometionina (Sel- Plek, Alltech, SAD) po grlu dnevno. Na kraju, svaka životinja iz G1 i G2 dobijala je 1,83g selena dnevno. Kontrolna grupa (GC) dobila je obrok bez dodatka selena. Glavni sastav obroka u svakoj grupi bio je isti. Ejakulati su dobijeni preko veštačke vagine. Ocenjivani su sledeći parametri: volumen i pH ejakulata i pokretljivosti, koncentracija i preživljavanje in vitro spermatozoida na 39°C tokom 360 min.

Utvrđeno je da dodatak obroku neorganskog i organskog selena doveo do povećanja zapremine ejakulata, pokretljivosti i preživljavanja spermatozoida. Na sveže dobijeno seme nije uticao tretman sa selenom.

Ključne reči: ovan, selen, ishrana, ejakulat, kvalitet sperme

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Received 15 June 2017; accepted for publication 12 December 2017Received 9 January 2018; accepted for publication 18 March 2018

Biotechnology in Animal Husbandry 34 (1), p 83-93 , 2018
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.312:637.5'65
https://doi.org/10.2298/BAH1801083S

EFFECTS OF INTENSITY OF LIGHT AND STOCKING DENSITY ON BROILER BODY WEIGHT AND YIELD OF VALUABLE CARCASS PARTS

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Original scientific paper

Abstract: In order to determine the effect of intensity of light and stocking density, as well as the interaction of light intensity x stocking density on body weight and individual carcass traits, an experiment was performed on 1200 broilers of the Ross 308 genotype. The investigated factors were the intensity of light (LI): 20 lx (K) ; 150 lx (O) and stocking density (SD): 10 broilers/m^2 (A); 13 broilers/m^2 (B); 15 broilers/m^2 (C), in 4 repetitions. The light source was incandescent bulbs of adequate intensity and a light program 16L: 4D: 2L: 2D was applied. Broiler body weight was controlled on 11th, 21st, 35th and 42nd day, by individual measurement of all chickens in the trial. The average sample containing 12 chickens per treatment with equal gender representation (total of 72 broilers) was used to study the slaughter quality of carcasses based on the parameters of absolute and relative yield of more valuable carcass parts (breast, thighs and drumsticks) and meat in the more valuable parts of the carcass. The effect of light with different intensity on the body weight differed depending on the broiler rearing phase. The interaction effect of the intensity of light and stocking density on the body weight of broilers was confirmed in all stages of growing up to the age of 42 days. A higher intensity of light (150 lx) showed the potential to alleviate the negative effects of higher stocking density (15 broilers/m^2) on the final body weight of the broiler. The carcass traits were not significantly affected by the intensity of the light, while the stocking density, as well as the intensity of the light x stocking density did influence the yield of whole breast and the yield of breast meat.

Key words: light intensity, stocking density, broiler, body weight, carcass characteristics

Introduction

In commercial production conditions, broiler activity has been reduced to a minimum in order to achieve higher growth and more efficient use of food. In such conditions, the problem of endangered welfare and health of broilers occurs, and efforts are made to establish the control of their physical activity by certain environmental factors. The environmental factors that exert the effect on the physical activity of broilers are light and stocking density (*Estevez, 2007; Kristensen et al., 2006b, Deep et al., 2010b*). In addition to the duration of photoperiod and colour, an important aspect of the effect of light is based on its intensity. The intensity of illumination should enable broilers to smoothly navigate the area and find feeders and waterers. The high-intensity light is preferred in the starter phase of broiler rearing due to the favourable effect on the development of locomotor apparatus and the prevention of heart problems (*Classen, 1996*). The use of low-intensity light is based on the effect of reducing the physical activity of broilers (*Downs et al., 2006*) and the expected benefits in terms of gain and quality of the carcass. However, the results of the study of the effect of light of varying intensity on production performance and slaughter characteristics are inconsistent (*Olanrewaju et al., 2011; Lien et al., 2007, 2008; McKee et al., 2009*). Low-intensity light leads to welfare specific problems associated with the incidence of ulcerative foot-pad lesions, eye size disorders, and various eye sight defects (*Deep et al., 2010; Blatchford et al., 2009*). According to *Alvino et al. (2009),* the high-intensity light is desirable for the expression of certain patterns of behaviour.

The stocking density was studied in numerous researches dealing with the welfare aspect of broilers. A better development of the locomotor apparatus and a better condition of legs under conditions of lower stocking density are confirmed, partly as a result of greater physical activity of broilers (*Sanotra et al., 2002*). Also, the stocking density has a confirmed significant impact on the body weight and quality of the broiler carcass (*Dozier et al., 2006; Škrbić et al., 2008*). Stocking density is a factor in rearing that establishes interaction effects with other rearing factors, such as a light program. It has been shown that certain intermittent light programs can mitigate the negative consequences of a higher stocking density on the gain and carcass conformation (*Škrbić et al., 2011*).

The aim of the conducted research was to determine the effect of light intensity, stocking density and their interaction effect on the gain of broilers, yield and quality of valuable parts of the carcass.

Materials and Methods

The trial was performed on 1200 broilers of Ross 308 genotype in the period up to 42 days of age. The design of the experiment was dual-factor by random block system, with 6 treatments and 4 repetitions. The investigated factors were following: the intensity of light (LI): 20 lx (K); 150 lx (O) and stocking density (SD): 10 broilers/m^2 (A); 13 broilers/m^2 (B); 15 broilers/m^2 (C). Broilers were reareded according to the principles of standard technology of fattening in the floor system. The nutrition was *ad libitum* and consisting of complete mixtures based on maize and soybean. The content of energy and protein in the four-phase diet program was 3000 Kcal/kg, 21.0% SP; 3100 Kcal/kg, 19.2% SP; 3110 Kcal/kg, 19.0% SP, respectively, i.e. 3170 Kcal/kg and 17.3% SP. The duration of the photoperiod was 23 hours till 8th day, followed by 18 hours, with the applied light program 16L: 4D: 2L: 2D. The light source was incandescent bulbs of adequate intensity. Light intensity control was carried out by an illuminometer (Testo 540) at the level of the broiler eye in 3 positions, at right angle *(Lewis and Morris, 2006).* The body weight of chickens was controlled on 11th, 21st, 35th and 42nd day, by measuring individualy all chickens in the trial. The average sample containing 12 chickens per treatment with equal gender representation (total of 72 broilers) was used to study the slaughter quality of carcasses based on the parameters of absolute and relative yield of more valuable carcass parts (breast, thighs and drumsticks) and meat in the more valuable parts of the carcass. The cutting of chilled carcasses and the separation of the breast, drumstick and thighwas done in accordance with Commission Regulation (EC) No 543/2008. After determining of their weight, their yields relative to the pre-slaughter body weight were calculated. Weight and share of the breast meat (*m. Pectoralis major* and *m. Pectoralis minor*), drumstick and thigh meat in the pre-slaughter body weight were obtained after the dissection, i.e. separation of muscle tissue from the skin and bones.
Data was analyzed by ANOVA followed by LSD post hoc test using StatSoft software (STATISTICA 8, 2007).

Results

Data on the effect of light intensity, stocking density and their interactions on the body weight of the broilers during the fattening period are shown in Table 1. A significant influence of the intensity of light on the broiler body weight at the 11th day of age was determined and a highly significant impact of the intensity of light on the body weight of broilersat the 42nd day of age. However, the character of the effect determined was opposite. At the initial fattening stage, the chickens of O group showed higher values for body weight (light intensity 150 lx), while in the

last phase, the chickens in the K group (light intensity 20 lx) had significantly higher body weight values. The stocking density showed a significant effect on the body weight of broilers in all control measurements. In the first two control measurements, the highest body weight of chickens was in the C group (15 broilers/ m^2) and the differences between A (10 broilers/m^2) and B (13 broilers/m^2) groups were not statistically significant. After this period, broilers in group A had a more intensive gain of body weight compared to groups B and C, which resulted in significantly higher ($p<0.01$) body weight after 42 days.

The interaction effect of intensity of light and stocking density on body weight was statistically confirmed in all control periods. The lowest body weight was recorded in broilers of OB group as a result of the interaction of light intensity and stocking density of 13 broilers/m^2 in all growth periods of broilers. However, the interaction effect of high-intensity light (O) and higher stocking density (15 broilers/m^2) resulted in the highest broiler body weight values at all ages except for the 42nd day.

Table 1. Effects of intensity of light and stocking density on the body weight of broilers in individual phases of fattening

		Body weight, g / Age, day			
		11	21	35	42
Light intensity (LI)	K	262.0 ± 1.7 ^{b}	725.2 ± 5.3	1659.2 ± 11.5	2302.5 ± 15.0 ^{A}
	O	267.2 ± 1.8 ^{a}	721.7 ± 5.6	1643.2 ± 11.3	2228.6 ± 15.2 ^{B}
Stocking density (SD)	A	257.3 ± 2.3 ^{B}	715.6 ± 7.6 ^{B}	1656.9 ± 16.2 ^{ab}	2330.4 ± 21.8 ^{A}
	B	254.7 ± 2.0 ^{B}	696.0 ± 6.6 ^{B}	1621.6 ± 14.0 ^{b}	2218.7 ± 19.1 ^{B}
	C	277.9 ± 2.0 ^{A}	751.4 ± 5.8 ^{A}	1672.0 ± 12.4 ^{a}	2262.1 ± 15.8 ^{B}
LI x SD	KA	253.0 ± 3.3 ^{C}	707.4 ± 11.2 ^{BC}	1668.6 ± 24.6 ^{A}	2359.5 ± 32.1 ^{A}
	KB	260.3 ± 2.8 ^{BC}	721.5 ± 8.7 ^{B}	1667.7 ± 19.1 ^{A}	2328.5 ± 24.3 ^{AB}
	KC	269.4 ± 2.8 ^{B}	740.2 ± 8.1 ^{AB}	1645.7 ± 17.6 ^{AB}	2242.5 ± 22.7 ^{B}
	OA	261.7 ± 3.2 ^{BC}	724.0 ± 10.3 ^{AB}	1645.2 ±21.2 ^{AB}	2300.1 ± 29.2 ^{AB}
	OB	249.1 ± 2.9 ^{C}	670.8 ± 9.7 ^{C}	1576.2 ± 20.1 ^{B}	2110.0 ± 27.4 ^{C}
	OC	286.8 ± 2.9 ^{A}	762.5 ± 8.3 ^{A}	1698.4 ± 17.3 ^{A}	2281.8 ± 21.8 ^{AB}
Significance					
LI		*	ns	ns	**
SD		**	**	*	**
LI x SD		**	**	**	**

The influence of the different light intensity and stocking density of broilers on the absolute and relative yield of the breast, drumstick and thighis shown in Table 2. The yields of the breast, drumstick and thigh were not significantly affected by the intensity of light. The stocking density showed a significant effect on the breast weight ($p<0.05$) and breast share ($p <0.01$), while yields of drumstick and thigh were not significantly affected by this factor. Absolute and relative breast yield in the C treatment was significantly higher in

relation to B treatment and without statistical confirmation of the difference compared to treatment A. The analysis of the interaction of light intensity and stocking density showed significantly the lowest relative breast yield in the treatment OB compared to other treatments, except KA treatment.

Table 2. Effects of intensity of light and stocking density on the yield of more valuable parts of the carcass

		Breast		Drumstick		Thighs	
		g	%	g	%	g	%
Light intensity (LI)	K	492.15±77.43	22.36±1.82	225.09±31.25	10.23±0.54	260.17±36.91	11.82±0.61
	O	485.14±67.35	22.25±7.71	222.19±26.18	10.21±0.55	257.64±29.28	11.83±0.55
Stocking density (SD)	A	497.38±72.40^{ab}	22.33±1.85^{ab}	224.53±29.95	10.08±0.45	263.69±36.50	11.83±0.56
	B	458.19±66.35^{b}	21.55±1.73^{b}	220.92±26.42	10.41±0.50	252.93±31.56	11.91±0.66
	C	510.36±69.86^{a}	23.03±1.41^{a}	225.47±30.48	10.17±0.54	260.08±31.49	11.75±0.51
LI x SD	KA	492.25±85.82	21.89±2.00^{ab}	227.44±34.83	10.12±0.42	268.27±42.89	11.93±0.63
	KB	485.89±75.56	22.31±1.88^{a}	224.89±30.09	10.34±0.70	259.68±36.84	11.93±0.79
	KC	498.31±76.94	22.87±1.58^{a}	222.94±31.26	10.24±0.49	252.57±31.68	11.61±0.29
	OA	502.51±59.49	22.77±1.64^{a}	221.63±25.38	10.03±0.49	259.12±30.01	11.73±0.49
	OB	430.49±42.69	20.80±1.23^{b}	216.95±22.78	10.47±0.50	246.19±25.04	11.89±0.54
	OC	522.41±63.00	23.19±1.26^{a}	227.99±30.83	10.11±0.60	267.60±30.76	11.89±0.64
Significance							
LI		ns	ns	ns	ns	ns	ns
SD		*	**	ns	ns	ns	ns
LI x SD		ns	*	ns	ns	ns	ns

The intensity of light and stocking density showed a similar effect on the yields of the muscle tissue of the breast, drumstick and thigh (Table 3), as well as on the yields of whole breast, drumstick and thigh. The light intensities tested did not have a significant effect on the yield of breast, drumstick and thigh, as opposed to the studied stocking densities that influenced the yield of breast meat. The difference in the effect of stocking density on the yield of whole breast is at the probability level of the identified differences ($p<0.05$).

Table 3. Effects of intensity of light and stocking density on meat yield of more valuable parts of the carcass

		Breast meat		Drumstick meat		Thigh meat	
		g	%	g	%	g	%
Light intensity (LI)	K	374.09±63.49	16.99±1.71	146.16±22.11	6.64±0.45	189.32±30.16	8.59±0.58
	O	365.26±56.37	16.75±1.61	142.33±16.93	6.54±0.38	186.08±23.29	8.55±0.58
Stocking density (SD)	A	377.85±64.58^{a}	16.93±1.81^{ab}	145.22±20.82	6.51±0.38	190.92±29.04	8.55±0.48
	B	344.68±54.93^{b}	16.21±1.63^{b}	141.71±18.62	6.67±0.46	181.73±26.13	8.55±0.67
	C	386.50±53.15^{a}	17.461.31^{a}	145.80±20.04	6.58±0.39	190.45±25.21	8.60±0.59
LI x SD	KA	374.45±75.91	16.61±1.57^{ab}	147.06±24.81	6.54±0.42	194.72±34.76	8.64±0.52
	KB	370.21±61.75	17.00±1.10^{a}	146.34±22.17	6.72±0.55	189.00±30.53	8.67±0.73
	KC	377.63±56.74	17.36±1.31^{a}	145.07±21.13	6.66±0.38	184.22±26.34	8.46±0.50
	OA	381.25±54.16	17.25±2.05^{a}	143.38±16.82	6.49±0.36	187.12±22.87	8.46±0.45
	OB	319.15±32.76	15.43±1.73^{b}	137.07±13.66	6.62±0.38	174.47±19.52	8.43±0.62
	OC	395.38±50.15	17.56±1.35^{a}	146.53±19.81	6.50±0.40	196.67±23.47	8.75±0.65
Significance							
LI		ns	ns	ns	ns	ns	ns
SD		*	*	ns	ns	ns	ns
LI x SD		ns	*	ns	ns	ns	ns

Discussion

A study of the effect of light of varying intensity showed the opposite effect of high-intensity light on the gain of body weight in the initial and final phases of broiler rearing. In the period of up to 11 days, light intensity of 150 lx showed positively influence on the gain of body weight of the broiler, but as a result of the opposite effect in the subsequent phases, the final weight of broilers was significantly higher in treatment with an intensity of 20 lx. The presented results are partially in line with the results of the research of *Kristensen et al. (2006a)*, which confirm the gain of body weight of the broiler under light intensity of 5.4 to 6.45 lx, and a decrease in body weight as a result of the light intensity of 107.6 to 124.7 lx. The effect of light is reflected on the physical activity of the chickens (*Blatchford et al., 2009*) and, consequently, the body weight gain. The higher physical activity of the chickens under the influence of high-intensity light in the starter phase acted positively on the development of the bone-muscle system (*Classen, 1996*), which can explain the beneficial effect on the body weight. The transient effect of light intensity is stated by *Downs et al. (2006),* with a low-intensity of light inducing higher body weight of females at an early age but up to 56 days of age differences in body weight were not significant. *McKee et al. (2009)* have established a higher body weight of broilers grown at a lower light intensity of up to 51 days of age. The non-significant effect of light intensity on the body weight of broilers was determined by *Olanreway et al. (2010, 2011), Deep et al. (2010a). Kristensen et al. (2006b)* have found that light intensity increases activity of chickens only in treatment that provided alternating light intensity of 5 and 100

lx in 16 hours of photoperiod. The inconsistency of the results obtained on the effect of the light intensity on the body weight of broilers can be explained by different research conditions in terms of the investigated levels of light intensity, the duration of the photoperiod and the applied light program, the duration of the fattening period, the nutrition, etc.

According to *Commission Regulation (EC) No 543/2008*, the applied stocking densities in the trial (10, 13 and 15 broilers/m^2) did not exceed the limit for the extensive rearing system in the facility, suggesting that the conditions for rearing broilers in the trial were "friendly" and in line with the recommendations for broiler welfare preservation. In that sense, it was possible to expect the absence of differences in terms of significant effects on the gain, i.e. body weight of broilers. However, the results of the experiments confirmed that even in conditions of application of stocking density recommended for the preservation of the welfare of broilers, in the extensive rearing system in the facility, there is a significant effect on the body weight. In previous studies, the intensification of the effects of higher stocking density in the last stages of broiler fattening was determined (*Škrbić et al., 2009; Škrbić et al., 2011*), as opposed to the initial stages, when the body warmth, produced as a result of intense metabolism, can be used for growth (*Dozier et al., 2005*), as confirmed by the results of this study. The confirmed interaction effect of light intensity and stocking density is in line with previous research (*Škrbić et al., 2011; Škrbić et al., 2012*) which pointed to the significance of the interaction effect of light with biological and environmental factors on production performance and the welfare of broilers.

The carcass traits were not significantly affected by the intensity of light in the presented study. There are indications of an increase in absolute and relative yields of whole breasts, drumsticks and thighs, as well as breast, drumstick and thigh meat in treatments with lower intensity of light. The positive effect of weaker light (15 FC vs 0.5 FC) on absolute values of breast, file and tender yields, is reported by *Lien et al., (2008*). However, by comparing light intensity 1 FC vs. 0.1 FC *Lien et al. (2007)* have determined the negative effect of reducing the intensity of light on breast yield, primarily on the tender. The relative yield of the drumsticks and thighs decreased linearly with an increase in the intensity of light from 1 to 40 lx in research by *Deep et al. (2010a).* These results indicate that the effect of light intensity on the share of more valuable carcass parts depends to a large extent on the level of applied intensities. *Downs et al. (2006)* link low illumination intensity with the effect of substitution of the part of the breast with the share of the wings and legs as a result of compensatory weight gain which leads to the delay of progressive maturation in the growth process.

Researches of the stocking density on the carcass traits indicate a lower file yield (*Dozier et al., 2006*) and yield of whole breast (*Škrbić et al., 2011*) in treatments with higher stocking densities, which is confirmed by the results of the present study, despite significant differences in the applied stocking densities

between these studies. The intensity of light in interaction with other factors has been the subject of several studies with different effects on the production, slaughter and physiological parameters of broilers (*Lien et al., 2007; Olanrewaju et al., 2008, 2010, 2014).* Our research has shown a significant influence of interaction of light intensity x stocking density on breast size and share of breast meat. A similar effect is reported by *Lien et al. (2007)* as a result of the interaction between the light intensity x duration of the photoperiod. These results indicate that the intensity of light can be a significant factor in interaction with other rearing factors despite the absence of its main effect.

Conclusion

Based on the results of the presented research, it can be concluded that the effect of light of different intensity on the body weight of broilers differed depending on the growing stage (fattening). The interaction effect of the intensity of light and stocking density on the body weight of broilers was confirmed at all phases of fattening until the age of 42 days. A higher intensity of light (150 lx) has the potential to alleviate the negative effects of greater stocking density (15 broilers/m^2) on the final body weight of the broiler.

The carcass characteristics were not significantly affected by the intensity of the light, while the main effect of stocking density was determined for the whole breast yield and the yield of breast meat. The interaction of light intensity and higher stocking density in the treatment of OC resulted in the highest relative yield of whole breast, i.e. breast meat.

Acknowledgment

This research is part of the Project EVB: TR-31033 financially supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Efekti intenziteta svetlosti i gustine naseljenosti na telesnu masu i prinose vrednijih delova trupa brojlera

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Rezime

U cilju utvrđivanja efekta intenziteta svetlosti i gustine naseljenosti, kao i interakcije intenzitet svetlosti x gustina naseljenosti na telesnu masu i pojedine karakteristike trupa, sproveden je ogled na 1200 brojlera genotipa Ross 308. Ispitivani faktori su intenzitet svetlosti (LI): 20 lx (K); 150 lx (O) i gustina naseljenosti (SD): 10 grlo/m^2 (A); 13 grlo/m^2 (B); 15 grlo/m^2 (C), u 4 ponavljanja. Izvor svetlosti su bile incandescent bulbs odgovarajućeg intenziteta i primenjen je svetlosni program 16L:4D:2L:2D. Telesna masa pilića je kontrolisana 11., 21., 35. i 42. dana, pojedinačnim merenjem svih pilića u ogledu. Na prosečnom uzorku od 12 pilića po tretmanu sa podjednakom zastupljenošću polova (ukupno 72 brojlera) izvršeno je ispitivanje klaničnog kvaliteta trupa na osnovu parametara apsolutnog i relativnog prinosa vrednijih delova trupa (grudi, bataci i karabataci) i mesa u vrednijim delovima trupa. Efekat svetlosti različitog intenziteta na telesnu masu se razlikovao u zavisnosti od faze gajenja brojlera. Interakcijski efekat intenziteta svetlosti i gustine naseljenosti na telesnu masu brojlera je potvrđen u svim fazama gajenja do starosti 42 dana. Veći intenzitet svetla (150 lx) je pokazao potencijal da ublaži negativne efekte većih gustina naseljenosti (15 grlo/m^2) na završnu telesnu masu brojlera. Karakteristike trupa nisu bile pod značajnim glavnim efektom intenziteta svetlosti dok je gustina naseljenosti, kao i interakcija intenzitet svetlosti x gustina naseljenosti, uticala na prinos celih grudi i prinos mesa grudi.

Ključne reči: intednzitet svetlosti, gustina naseljenosti, brojleri, telesna masa, kvalitet trupa

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Received 12 February 2018; accepted for publication 27 March 2018

Biotechnology in Animal Husbandry 34 (1), p 95-105 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.55'712
https://doi.org/10.2298/BAH1801095J

EVALUATION OF MEAT QUALITY OF WEANED RABBITS ADMINISTERED DIFFERENT CONCENTRATIONS OF PROBIOTIC STRAIN (*Saccharomyces boulardii*)

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Original scientific paper

Abstract: The meat quality and sensory properties of weaned rabbits administered different concentrations/cell count of probiotic *Saccharomyces boulardii (*flora norm) were investigated. A total of 36 mixed breeds of rabbits were randomly divided to four treatments with three replicates and three rabbits per replicate. The weaned rabbits were fed the same diet. Treatments T_1, T_2, T_3 and T_4 had zero (0) concentration/cell count of probiotic, 1ml each of 160 $x10^6$ it is%1.6 x 10^8 cfu/ml concentration of probiotic, 1 ml each of 80 $x10^6$cfu/ml =8.0 x 10^7 cfu/ml of probiotic and 1 ml each of 40 x 10^6cfu/ml concentration of probiotic, respectively. The administration of the probiotic was done once every 14 days. Results obtained showed that there was no significant difference in moisture and crude protein content of meat from rabbits while ether extract differed significantly ($P<0.05$). The physical properties, cooking yield, cooking loss and water holding capacity were not significantly ($P>0.05$) influenced by concentrations of probiotics while pH and thermal shortening were significantly ($P<0.05$) influenced. All the sensory parameters measured were significantly ($P<0.05$) different. It was found that oral administration of probiotic *Saccharomyces boulardii (*flora norm) at 4 mg/ml 80 x 10^6cfu/ml concentration improved meat qualities and overall acceptability of rabbit meat.

Key words: Meat quality, rabbits, administered, probiotic

Introduction

The anticipated increase in the world's population is to have a severe consequence on food intake especially animal protein intake. According to

Delgade et al. (2001), the yearly requirement for animal protein in the third world is projected to increase from 11million tons in 1997 to 213 million tons in 2020. This requirement for meat as well as the financial difficulty experienced by the people in the third world is motivating larger attention to rapid growing animals as well as short production interval likes rabbits (*Aduku and Olukosi, 1990*). The demand for safe and quality meat in the market has considerably increased. The producers are eager to use natural and nonchemical supplements which positively affect the animal health, increase productivity and improve meat.

The word probiotic was in 1953 introduced firstly by Kollath (*Isolauri et al., 2002)*. Several meanings have been written for the word "probiotic". The most generally recognized one is "live microorganisms which, if added in sufficient quantity, promote health on the host" (*FAO, 2003*). Application of probiotics can lead in increase of the carcass output and water holding capacity and decrease the meat hardness (*Ceslovas et al., 2005*). In animal nutrition, microorganisms used as probiotic were linked with a proven efficacy on the gut microflora. Administration of probiotic in feed significantly improves the feed intake, feed conversion ratio, daily weight gain and total body weight in pig, chicken, sheep, goat, cattle and equines (*Samli et al., 2007*). Several probiotic strains have been utilized for fermented sausages such as lactic acid producing bacteria, mainly *Lactobacillus, Pediococus* and *Streptococcus* (*Hammes and Knauf, 1994*). In many countries of the world, particularly in Europe, the use of antibiotics in animal food is now banned as a result of residues in meat and meat products, and increase in bacteria resistance in human population. As a result of this, coupled with the increased pressure by consumers and agencies of government to decrease and even eliminate the usage of antibiotics in food producing animals, the usage of antibiotics as growth promoting agent has been banned. This action now created the need to find an alternative for the maintenance of health and production in livestock. This has led to the concept of using probiotics to replace antibiotics (*Fuller, 1997*). Therefore, the aim of this study was to evaluate meat quality of weaned rabbits administered different cell count of strain *Saccharomyces boulardii* (flora norm)**.**

Materials methods

The experiment was conducted at the rabbitry section of the Teaching and Research Farm of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology Minna, Niger State, Nigeria. Minna lies between the Latitude 9° 31 and 9^{o} 45 North, and Longitude 6°31 and 6^{o} 45, East of the equator *(Usman, 2011*).

Source of probiotic and preparation

Sa*ccharomyces boulardii* (flora norm**)** used for the experiment was procured from Prisma Pharmaceutical Limited Jubilee House, Merrion Avenue, Stanmore, and Middlesex, U.K. It is a product of Bharat Biotech International Limited, Genome Valley, Shameerpet, and Hyderabad, India. Serial dilution

methods were used to obtain the required inclusion rates for the probiotic in 1 ml of the mixture as described by Donev *et al.* (2008). Description of the dose. Serial dilution and bacterial count
1 (one) sachet of Saccharomyces boulardii contains 250mg and 5 (five) billion colony forming units (cfu) of *Saccharomyces boulardii*. Four levels were used; 0, 8 mg/ml, 4 mg/ml and 2 mg/ml respectively.Therefore, 8mg/ml = 8/250 x 5,000,000,000 = 160, 000,000 cfu/ml (160 x 106cfu/ml), 4mg/ml = 4/250 x 5,000,000,000 = 80,000,000 cfu/ml (80 x 106cfu/ml) and 2mg/ml = 2/250 x 5,000,000,000 = 40,000,000cfu/ml(40x106cfu/ml). After the preparation of probiotic concentration, clean syringe (10 ml) was used to administer the concentration orally.

Experimental rabbits and their management

The weaned rabbits were bought from the National Veterinary Research Institute (N.V.R.I) Vom, Plateau State, Nigeria. The rabbits were mixed breeds. They were fed with concentrates, forages and clean drinking water *ad-libitum.* The concentrate were pelletized grower mash (Vital feeds, Grand Cereals and Oil Mills Limited, Jos, Nigeria). The forages used were *Tridax*, *Stylosanthes* and cabbage. A day after their arrival, they were given Ivomectin at 0.3 ml subcutaneously against both ecto and endo-parasites. Completely randomized design (CRD) was used for the research. The experiment consisted of four treatments; each treatment had three replicates with each replicate having three rabbits. Treatment one (T_1) represented zero level of probiotic, treatment two (T_2) represented 8 mg/ml (160 x10^6cfu/ml) of probiotic, treatment three (T_3) represented 4 mg\ml (80 x10^6cfu/ml) of probiotic while treatment four (T_4) represented 2 mg/ml (40 x10^6cfu/ml) of probiotic respectively. The probiotic was administered orally using a syringe at 1ml per rabbit once every two weeks. The experiment lasted for 8 weeks (56 days).

Table 1. Doses of probiotic *Saccharomyces boulardii* orally administered to weaning rabbits.

Treatment	**Concentration (ml)**
T1	Control (no probiotic)
T2	8mg/ml (160 x 10^6cfu/ml)
T3	4mg/ml (80x10^6cfu/ml)
T4	2mg/ml (40 x 10^6cfu/ml)

The meat quality characteristics of rabbits were determined according to the method of *Awosanya (1989).* The slaughtering method was approved by the authorities of the Federal University of Technology Minna. After the conclusion of the growth experiment, 12 rabbits were randomly selected from the four treatments, one rabbit from each replicate. Their final live weights were determined and recorded, then fasted overnight by allowing the animals' access to water only. They were then slaughtered using a knife by means of cutting through the jugular vein and carotid artery around the atlas bone. The rabbits were suspended with the head facing down-ward for 20 minutes to ensure complete bleeding. The changes in the shrunk weight and the weight after bleeding were taken as the blood loss. The rabbits were dressed by complete removal of their hairs, skins (pelts); and were cut at the atlanto-occipital joint. The rear legs were cut at the junction linking the tibia calcaneus while the front legs were severed close to the carpal area and the tail (near the base) removed, and their weights taken separately to accomplish evisceration. The meat quality characteristics were determined.

pH of rabbit meat

The pH of meat samples were determined according to the method of *Marchiori and deFelicio (2003)* using a pH meter (Model 191, Knick, Berlin Germany). A 10 g sample of meat was homogenized in 90 ml distilled water using a blending machine (model 242, Nakai, Japan) at speed 5. The pH meter was standardized using buffers 4 and 7, after which the pH reading of the meat samples were taken.

Water holding capacity (WHC)

The water holding capacity of meat samples were determined using the method described by *Kauffman et al. (1992).* A section of the meat from the chunk and shank were cut, weighed and kept in a container. Water holding capacity was carried out by cutting a portion of meat weighing approximately 10 g. The sample was pressed using a screw jack until all the free water was expelled. The meat sample was then removed, unwrapped and re-weighed. The difference in weight of meat sample represents the weight of expelled fluid and expressed as a percentage of the initial sample weight and recorded as water holding capacity of the meat.

Cooking yield and cooking loss

The cooking yield and cooking loss were determined using the method described by *Kauffman et al. (1992).* A portion of the chuck and Shank were selected for broiling. Broiling was done in an open gas oven. The racks were covered with perforated aluminum foils for ease of drainage; the oven was preheated for 5minutes, before loading samples, which was boiled to a temperature of 72^{o}C as measured with a skewer meat thermometer. The sample was allowed to

cool to room temperature, excess fluid was mopped up with paper serviette and their weights were taken and recorded. The difference between the pre-cooked weight and post weight was the cooking loss. While the cooking yield was calculated as cooked weight/thawed weight x100.

Sensory Evaluation of Meat from the Hind Limb of Rabbits

Lean meats from the hind limb from each treatment group were used to evaluate the sensory attributes. Various cuts of the meat were made into bite sizes and boiled in water without salt at 80 ^{0}C for 10 minutes. The meat samples were left to cool to room temperature and then served in coded plates to a 20-member panelist comprising of staff and students of Federal University of Technology Minna, Nigeria who are familiar with rabbit meat. The order of presentation of the samples to the panelist was randomized. The Panelists were instructed to evaluate the meat for appearance, taste, juiciness, chewiness, texture, aroma and overall acceptability on a 9-point Hedonic scale (where 1= dislike extremely and 9=like extremely). Panelist were served with cold water after each evaluation of the meat sample to rinse their mouth to avoid carryover effect during sensory evaluation.

Proximate Composition

The moisture, crude protein and ether extract content of meat samples from the hind limb were determined according to AOAC (2000) method.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and differences among means were compared Duncan's Multiple Range Test at 5% probability level. All computations were made by statistical software SPSS (version 6).

Results

The meat quality of weaned rabbits orally administered different doses of probiotic (*Saccharomyces boulardii)* is presented in Table 2.The table revealed that the cooking yield, cooking loss and the water holding capacity of the rabbit meat were not significantly ($P>0.05$) influenced by the count cell of probiotic (*Saccharomyces boulardii).* However, the pH values and the thermal shortening of the rabbit meat were significantly ($P<0.05$) influenced by the different cells count of probiotic (*Saccharomyces boulardii).* The pH of rabbit meat in T_1 (6.79) and T_3 (6.75) are similar but significantly ($P<0.05$) higher than those of T_2 (6.63) being the least. Furthermore, the outcome revealed that the thermal shortening was significantly ($P<0.05$) higher in T_1 (5.42%), and the least value for thermal shortening was obtained in T_3 (1.70 %). The proximate composition of the meat of

rabbits orally administered probiotic (*Saccharomyces boulardii)* is shown in Table 3. The result revealed that different cells count of probiotic (*Saccharomyces boulardii*) orally administered to the weaned rabbits did not significantly (P>0.05) influenced the moisture and crude protein content of the rabbit meat. However, the ether extract of the rabbit meat was significantly (P<0.05) influenced by the different cells count of the probiotic. The result showed that ether extract was significantly (P<0.05) higher in meat of weaned rabbit in T_3 (9.51%), but statistically (P>0.05) similar to those in T_4 (8.49%), while the lowest ether extract was obtained in meat sample of the weaned rabbit in T_1 (6.31%). The sensory evaluation of rabbit meat orally administered probiotic *Saccharomyces boulardii* is shown in Table 4. All sensory items studied were significantly (P<0.05) influenced by various cell counts of probiotic *Saccharomyces boulardii* orally administered to the weaning rabbit. The result showed that all the parameters measured (colour, tenderness, juiciness, flavour and over all acceptability) had a similar trend. The meat from rabbits orally administered probiotic (80×10^6cfu/ml=8.0×10^7) in T_3 had the highest sensory scores compared to all other treatments which are similar.

Table 2. Meat quality characteristics of rabbits orally administered *Saccharomyces boulardii* (flora norm)

Parameter	T1	T2	T3	T4	SEM	LS
pH	6.79^a	6.63^d	6.75^a	6.72^{ab}	0.02	*
Thermal shortening (%)	5.42^d	2.15^c	1.70^b	2.77^a	2.48	*
Cooking yield (%)	71.70	73.93	72.57	74.13	0.98	N/S
Cooking loss (%)	28.30	26.07	27.42	25.87	0.98	N/S
Water holding capacity (%)	25.49	25.45	21.84	23.84	0.78	N/S

a,b,c: Mean denoted by different superscript are significantly differing (P < 0.05)

T_1 = 0 ml of probiotic *Saccharomyces boulardii*

T2 = 1ml of 160 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally

T3 = 1ml of 80 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally

T4 = 1ml of 40 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally

SEM = Standard error of mean

LS = Level of significant

N/S = Not significant

Table 3. Proximate composition of rabbit meat orally administered *Saccharomyces boulardi* (flora norm)

Parameter (%)	T1	T2	T3	T4	SEM	LS
Moisture content	67.00	67.49	65.1	66.49	0.48	N/S
Crude protein	28.30	27.85	30.80	28.00	0.58	N/S
Ether extract	6.31^{c}	7.05^{bc}	9.51^{a}	8.49^{ab}	0.42	*

a,b,c: Means denoted by various superscript are significantly different ($P < 0.05$)
T 1 = 0 ml of probiotic *Saccharomyces boulardii*
T2 = 1ml of 160 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered oral
T3 = 1ml of 80 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally
T4 = 1ml of 40 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally
SEM = Standard error of mean
LS = Level of significance
N/S = Not significant

Table 4. Sensory properties of rabbit meat orally administered *Saccharomyces boulardii* (flora norm)

Parameter	T1	T2	T3	T4	SEM	LS
Colour	5.75^{b}	6.25^{b}	7.10^{a}	5.90^{b}	0.13	*
Tenderness	6.00^{b}	6.05^{b}	7.60^{a}	6.15^{b}	0.13	*
Juiciness	6.10^{b}	6.21^{b}	7.80^{a}	6.20^{b}	0.15	*
Flavour	6.25^{b}	6.30^{b}	7.80^{a}	6.20^{b}	0.13	*
Overall acceptability	6.25^{b}	6.60^{b}	8.10^{a}	6.65^{b}	0.14	*

a,b: Mean denoted by various superscript are significantly differing ($P < 0.05$)
T1 = 0 ml of probiotic *Saccharomyces boulardii*
T2 = 1ml of 160 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally
T3 = 1ml of 80 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally
T4 = 1ml of 40 x 10^6 cfu/ml of probiotic *Saccharomyces boulardii* administered orally
SEM = Standard error of mean
LS = Level of significant

Discussion

The non-significant differences in water holding capacity, cooking yield and cooking loss among various inclusion rates of *Saccharomyces boulardii* is in line with the result of *Pelicano et al. (2003).* The authors reported no difference in water holding capacity and cooking loss among different levels of probiotic tested. *Contreras – Castillo et al. (2008)* reported that lower water holding capacity is an indication of nutrient loss in the exudates, resulting in a tough and less tender meat. The authors also reported a significant difference in pH. The result was in agreement with the findings of *Bonai et al. (2008).* The authors in a study used a different doses of probiotic *Bacillus cereus vartoyoi* reported pH values of 6.3 - 6.8 of the rabbit meat which falls within the pH values obtained in our study.

The values of moisture content and crude protein observed in the current study falls within the values (63.6 – 76.8% moisture, 20.38 – 29% crude protein and 0.33 – 14.6% ether extracts reported by *Pla et al. (1996).* The significant effect on ether extract could have been due to manipulation in nutrition for domesticated rabbit as reported by *Olorunsanya et al. (1999)* and *Jiya (2012).* The authors observed that the hare rabbits solely survive on herbs in the wild, which could have been responsible for the higher percentage of fats normally observed with the domesticated rabbits. The highest scores for colour, tenderness, juiciness, flavour and overall acceptability in the result of rabbit meat administered 1ml of 80 x 10^6cfu/ml of probiotic (*Saccharomyces boulardii*) agrees with the reports of *Savković et al. (2005).* The authors reported improvement for juiciness, as well as the tenderness in the sensory attributes of meat administered probiotic - supplemented diets.

Conclusion

Oral administration of probiotic strain *Saccharomyces boulardii (*flora norm) at 80 x 10^6cfu/ml concentration/ cell counts improved meat qualities and overall acceptability of rabbit meat.

Evaluacija kvaliteta mesa zalučenih zečeva pod uticajem različitih koncentracija probiotskog soja (*Saccharomices boulardii)*

Elisha Zhiri Jiya, Chiemela Enyinnaya Chinma, Ahmed Sanusi

Rezime

U ovom radu je ispitivan kvalitet mesa, kao i senzorna svojstva odbijenih zečeva pod uticajem različitih koncentracija/broja ćelija probiotika *Saccharomices boulardii* (standarda flore). Ukupno 36 grla mešanih rasa zečeva je podeljeno nasumično u četiri tretmana sa tri ponavljanja i tri zeca po ponavljanju. Odbijeni zečevi su hranjeni istim obrokom. Tretmani T_1, T_2, T_3 i T_4 su imali nulu (0) koncentraciju broj ćelija probiotika, po 1 ml od 160 x10^6 što je % 1.6 x 10^8 cfu/ml koncentracija probiotika, po 1 ml od 80 x10^6cfu/ml =8.0 x 10^7 cfu/ml probiotika i po 1 ml svaki od 40 x 10^6cfu/ml koncentracije probiotika, respektivno. Probiotik je davan životinjama svakih 14 dana. Dobijeni rezultati pokazuju da nije postojala značajna razlika u sadržaju vlage i sirovog proteina u mesu zečeva dok se ekstrakt

etra značajno razlikovao (P <0,05). Fizičke osobine, prinos kuvanja, kalo kuvanja i kapacitet zadržavanja vode nisu bili značajno (P> 0,05) pod uticajem koncentracija probiotika, dok su pH i termalni tretman bili pod značajnim uticajem (P <0,05). Svi mereni senzorni parametri bili su signifikantno (P<0,05) različiti. Utvrđeno je da je oralno davanje probiotika *Saccharomices boulardii* (standard flore) koncentracije 4 mg/ml 80 x 10^6cfu/ml poboljšalo kvalitet mesa i ukupnu prihvatljivost mesa.

Ključne reči: kvalitet mesa, zečevi, davanje, probiotik

Acknowledgement

The authors are grateful to the management of the federal University of Technology for granting the permission to carry out the studies.

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Received 2 October 2017; accepted for publication 13 January 2018

Biotechnology in Animal Husbandry 34 (1), p 107-117 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.513.2
https://doi.org/10.2298/BAH1801107J

OMISSIONS IN THE DISINFECTION OF A CRAFT-SLAUGHTERHOUSE

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Original scientific paper

Abstract: The aim of this study was to establish the difference in the total number of bacteria present on some surfaces after disinfection was performed either by a professional or a layman employed at the slaughterhouse. Based upon the obtained results it can be concluded that there were omissions in the disinfection procedure. The study material consisted of wet and dry swabs taken during a five week period, before and after disinfection was performed either by a professional or a laymen. The following surfaces were sampled: meat carving knife, meat hooks, floor of the stunning area, and corridor floor. The procedure for wet swabs was carried out in accordance with the standard ISO 18593 method. The number of bacteria was estimated from each sample with the standard ISO 4833 method. Disinfection was performed with a 0.02% chlorine solution; the exposition time was 30 min. According to the obtained results it can be concluded that after disinfection was carried out by a professional- veterinarian, or by a layman, all surfaces which were previously treated correctly (mechanical cleaning and sanitary washing), and disinfected measured a significant decrease in the number of total bacteria (log cfu/cm2). The results for the total number of bacteria obtained after disinfection of the stunt area indicate on possible omissions as the number of bacteria did not decrease.

Key words: disinfection, omissions, craft slaughterhouse

Introduction

Implementation of good hygiene practice in slaughterhouses and procedures based on hazard analysis and critical control point (*Hazard Analysis Critical Control Points*) principles are essential to prevent microbial carcass contamination in order to ensure meat safety (*Lindblad and Berking, 2013*). The food business operators (FBOs) have the primary responsibility of ensuring food safety. Adequate meat hygiene is the result of the implementation of conditions and procedures based on HACCP principles. The predetermined conditions are crucial for the implementation of HACCP principles, and should be accomplished prior to HACCP. The main goal is to avoid the possibility that a low risk hazard evolves into a high risk food hazard. In addition to all other requirements, the pre-requisites include sanitation (cleaning, washing and disinfection) aimed at preventing possible sources of contamination, as well as reducing the total number of bacteria to the lowest possible extent (*Bunčić, 2009*).

Disinfection includes daily and constant disinfection of the equipment, utensils, desktops, as well as sanitary facilities. A daily and conscientious disinfection routine is needed in order to avoid the microbial contamination of the carcasses as different microorganisms are introduced into the slaughterhouse in large numbers on a daily basis. Primary microbial contamination can occur in the pen or stable, as the animal comes into close contact with feces. A further source of contamination can be the transport vehicle that has not been properly disinfected (*Rostagno and Callaway, 2012; Mannion et al., 2008*). Lairage can be a major source of contamination as it is the place where a large number of animals with different epizootiological status are gathered. Thereon, the microorganisms can be transferred from the skin onto the animal carcasses subsequently produced (*Small et al., 2006*). *De Busser et al. (2011*) indicate that the lairage area is a primary source of *Salmonella* in slaughter pigs and that carcass contamination originates from the environment rather than from the pig (inner contamination). Contamination of the carcasses is possible by contamination with gastrointestinal contents, or during the slaughter process it may occur as a result of direct or indirect contact with contaminated tools and equipment, personnel clothing and shoes, hands, floors, sewage outlets, air or water (CVPH, 2001; *Eustace et al., 2007; Gun et al., 2003*). *Haileselassie at al. (2013)* carried out a study in order to assess the food safety and practices in meat handling, and to determine the microbial load and pathogenic microorganisms present in the meat. He established that the microbial profile was higher compared to standards set by *WHO* as the result of inadequate sanitation as in the abattoir there was no hot water, nor sterilizing and cooling facilities. *Boughton et al. (2007)* and *Small et al. (2007)* reported that routine cleaning and washing of the lairage with cold water are not sufficient for the removal of pathogenic microorganisms. Total aerobic viable counts and Enterobacteriaceae (mean levels) from the samples was critical to

surfaces in contact with meat (splitting equipment) and indicated an inadequate application of good manufacturing and hygiene practices during slaughtering and sanitization (*Piras at al., 2014*). *Haileselassie et al. (2013)* reported that among bacterial contaminants of meat isolated in a study carried out at the municipality abattoir and butcher shops the predominant organisms included *E. coli, S. aureus* and *B. cereus*. The higher rate of contamination of meat with these bacteria is an indication of a deplorable state of hygienic and sanitary practices employed starting from slaughtering, transportation, butcher shops and processing. Pig carcass contamination can result from the intestinal carriage of Salmonella in the pig itself, but also from contact with other surfaces at the slaughterhouse (*Botteldoorn et al., 2003*). Hygiene varies between abattoirs and can have an important impact on carcass contamination (*McDowell et al., 2007*).

One of the food safety key elements is adequate disinfection. The aim of our research was to determine the efficiency of disinfection in a craft-slaughterhouse by determining the number of bacteria on a surface prior to and after disinfection was carried out either by a non-professional employed at the abattoir or a professional i.e. veterinarian.

Material and methods

Testing of the disinfection efficacy performed by a non-professional and a professional was done under field (abattoir) and laboratory conditions. The material used in this study consisted of wet-dry swabs taken prior to and after disinfection of the determined surfaces. Surfaces (carving knife, meat hook, stunting pen floor, corridor floor) treated by the veterinarian were samples every week (1st week- Monday; 2nd week- Tuesday; 3rd week – Wednesday; 4th week- Thursday; 5th week- Friday). Swabs were taken from the same surfaces after disinfection has been performed by a non-professional (1st week- Tuesday; 2nd week- Wednesday; 3rd week- Thursday; 4th week- Friday; 5th week- Monday). The procedure was performed according to the standard method SRPS ISO 18593 (*Microbiology of food and animal feeding stuffs − Horizontal methods for sampling techniques from surfaces using contact plates and swabs*). The total number of present bacteria was estimated by the standard SRPS ISO 4833 -1:2014 method *(Microbiology of food and animal feeding stuffs − Horizontal method for the enumeration of microorganisms − Colony-count technique at 30°C)* at the Department of Food Hygiene, Veterinary Institute, Banja Luka. Disinfection of the slaughterhouse was carried out with a chlorine preparation (sodium dichlorisocyanurate dihydrate) at a concentration of 0.02% and an exposure time of 30 minutes. The interpretation of the results was based on the limit values in the assessment of the hygiene of equipment, tools and work surfaces, as disclosed in Commission Decision 471/2001 / EC.

Basic data processing was performed using variation statistical methods, and testing the differences between experimental groups was done by means of t-test. The significance of the differences was determined at significance levels of 5% and 1%. The results obtained are tabulated. Statistical processing of the obtained results was done with the statistical package PrismaPad 4.00.

Results

The difference in the total number of bacteria (log CFU / cm2) on the examined surfaces in the craft-slaughterhouse before disinfection was carried out by the unskilled employee of the slaughterhouse and the expert i.e. the veterinarian was not statistically significant (p> 0.05).

The results of the total number of bacteria (log CFU / cm2) obtained after disinfection of a knife used for meat processing carried out by the unprofessional and the veterinarian are shown in Table 1.

Table 1. Total numbe of bacteria on the carving knife after disinfection *(log cfu/cm2)*

Week	Unprofessional person		Professional person-veterinarian	
	$\overline{X}$ ± Sd	CV%	$\overline{X}$ ± Sd	CV%
1.	1.87 ±0.55^{x}	29.34	1.11 ± 0.15^{x}	13.83
2.	2.00±0.96^{y}	48.02	0.94± 0.15^{y}	16.13
3.	1.99 ±0.85^{z}	42.70	0.93±0.14^{z}	14.64
4.	1.83 ±0.60^{q}	32.73	0.87^{y}±0.12^{q}	13.32
5.	2.13± 0.41^{w}	19.18	0^{w}	-

Statistically significant differences are shown by the same letters p< 0.01 x, y, z, q, w; p< 0.05 a, b, c; ns- not significant

From the obtained results, it can be seen that the decrease in the total number of bacteria (log CFU / cm2) was significant (p <0.01) after expert disinfection during all V experimental weeks versus the total number of bacteria identified on the knife after disinfection performed by an unskilled person.

The results of the total number of bacteria on the hooks after disinfection carried out by the unprofessional face of the slaughterhouse and the expert veterinarian can be seen in Table 2.

Table 2. Total numbe of bacteria on the meat hooks after disinfection (log cfu/cm2)

Week	Unprofessional person		Professional person-veterinarian	
	$\overline{X}$ ± Sd	CV%	$\overline{X}$ ± Sd	CV%
1.	2.34±0.17^{x}	7.11	1.63±0.42^{x}	25.79
2.	2.31±0.18^{y}	7.93	1.07±0.08^{y}	7.65
3.	1.54±0.43^{z}	27.72	1.05±0.14^{z}	13.75
4.	1.84±0.51^{q}	27.59	1.25±0.49^{q}	39.60
5.	2.60±0.47^{w}	18.14	0.90±0.00^{w}	0.00

Statistically significant differences are shown by the same letters p< 0.01 x, y, z, q, w; p< 0.05 a, b, c; ns-not significant

By analyzing the obtained results, we have determined a significantly lower (p <0.01) total number of bacteria (log CFU / cm2) during all V experimental weeks after disinfection of the hook was carried out by the veterinarian, compared to the number of bacteria when disinfection was carried out by the unprofessional person.

Table 3 shows the results of the total number of bacteria on the floor of the box for stunning after disinfection was carried out by the responsible person of the slaughterhouse and the expert veterinarian.

Table 3. Total number of bacteria on the stunning pen floor after disinfection (log cfu/cm2)

Week	Unprofessional person		Professional person-veterinarian	
	$\overline{X}$ ± Sd	CV%	$\overline{X}$ ± Sd	CV%
1.	2.34±2.10	35.52	2.10±0.14	6.73
2.	2.45±0.51	20.68	2.40 ±0.24	9.99
3.	2.76±0.51	18.41	2.91±0.79	27.06
4.	3.19 ±0.71	22.29	2.61 ±0.45	17.17
5.	3.24 ±0.58	17.86	2.93 ±0.23	7.91

Statistically significant differences are shown by the same letters p< 0.01 x, y, z, q, w; p< 0.05 a, b, c; ns- not significant

Results of the total number of bacteria on the floor of the corridor after disinfection was carried out by the unskilled and the professional-veterinarian are shown in Table 4.

Table 4. Total number of bacteria on the corridor floor after disinfection (log cfu/cm2)

Week	Unprofessional person		Professional person-veterinarian	
	$\overline{X}$ ± Sd	CV%	$\overline{X}$ ± Sd	CV%
1.	4.36 ± 0.10^{x}	6.48	2.58 ± 0.12^{x}	4.53
2.	3.29 ± 0.47^{y}	14.22	2.48 ± 0.13^{y}	5.25
3.	3.16 ± 0.57^{a}	18.14	2.64 ± 0.33^{a}	12.33
4.	3.16 ± 0.57^{ns}	18.14	2.65 ± 0.45^{ns}	17.17
5.	3.49 ± 0.37^{z}	10.57	2.44 ± 0.22^{z}	8.94

Statistically significant differences are shown by the same letters $p< 0.01$ x, y, z, q, w; $p< 0.05$ a, b, c; ns- not significant

During the first and second week after disinfection was carried out, we determined a very significantly lower ($p <0.01$) and significantly lower ($p <0.05$) total number of bacteria after disinfection was done by the veterinarian compared to the total number of bacteria after disinfection by an unskilled person ($p <0.01$). In the fourth week, there were no differences, while in the fifth week the differences were significant ($p <0.01$) because the total number of bacteria after disinfection was carried out by the veterinarian was lower compared to the values obtained by the responsible person at the slaughterhouse.

Discussion

Proper disinfection and rinsing of the disinfected surfaces are integral parts of every operation and every stage of the production process in the slaughter industry, as well as one of the important elements of food safety.

The results of the total number of bacteria (log CFU / cm2) obtained after disinfection of the hook and meat knife indicate that the professional veterinarian properly carried out all the disinfection phases, as well as the disinfection itself, since the total number of bacteria was significantly lower ($p <0.01$) during the course of all five weeks, in relation to the number of bacteria after disinfection by an unprofessional person. Properly conducted disinfection and

replacement of knives during work are very important because studies have shown that the most common way of meat contamination is with dirty hands and dirty tools (*Haileselassie et al., 2013; Piras at al., 2014; Abdalla et al., 2009; Svobodová et al., 2012; Gun et al., 2003*). In the slaughterhouses the knives are washed in a traditional manner by rinsing with water at a temperature of 20˚-40˚C, followed by a brief immersion in a bath (sterilizer) in which the water temperature is below 82˚C (EC Regulation 853/2004). *Eustace et al. (2007)* found microorganisms on 20 knives out of the 230 (8.7%) tested in the slaughterhouse after such a traditional method of washing knives and short dipping (sterilizer). The British Meat Producers Association (BMPA) indicated that, in laboratory trials, alternative procedures such as knife immersion in water at temperatures of 72 ° C / 15s and 75 ° C / 10s led to 3-4 log10 reductions in *E.coli*. It suggests that different procedures may be effective at different points of the process and suggests a 3 log10 reduction in E. coli as a performance standard for disinfection of meat knives (ACM / 817).

After disinfection of the stunning box floor during the five weeks of the trial we did not detect a significant reduction in the total number of bacteria, thus indicating that the disinfection was not well performed by both contractors. Reduction of the total number of bacteria on the corridor floor during 4 experimental weeks was described only after disinfection was done by the professional. The unqualified person used cold water for sanitary washing, so the sanitation was not efficient in the disinfection phase. Problems arising from unprofessionally conducted disinfection lie in the ignorance of the very measure, their ineffective implementation, and the inadequate education of the workers or direct executors of these jobs (*Naglić and Hajsig, 2005; Haileselassie et al., 2013*).

The first phase of disinfection is mechanical cleaning by which from the surface 25-50% microorganisms can be removed. The next stage is sanitary washing, which removes the residue of impurities and organic matter that weaken the power of the disinfectant. Sanitary washings should be done with hot water and under pressure (*Jankovic et al., 2017, Veljić and Rajković, 2012*). When the water temperature is 50^0C with the addition of surfactants or detergents, a high number of microorganisms (90% and more) can be removed from the surfaces (FAO). The use of disinfectants on surfaces where all the preceding disinfection phases were not well implemented can hardly produce results, because the disinfectant will not be able to penetrate the microorganisms. There is very little data in the literature on the effectiveness of the disinfection conducted on the floor of the stunning box and the corridor. *Swanenburg et al. (2001)* collected samples by swabbing floor and wall surfaces and collecting the residing fluids on the floor throughout the lairage. In 70 to 90% of the samples Salmonella was isolated when pigs were present. The usual cleaning and disinfection reduced the level of contamination with Salmonella to 25% positive samples, whereas improved cleaning and disinfection reduced this level to 10% positive samples. It is concluded that the usual cleaning and

disinfection of the lairage were not sufficient to eliminate this risk, whereas an improved procedure for cleaning and disinfection was still unsatisfactory. In the literature, most authors point to the fact that a significant reduction in the level of microorganisms in the slaughterhouses can be achieved only when effective sanitary washing with hot water is carried out prior to disinfection (*Piras at al., 2014; McDowell et al., 2007; Rajkowski et al., 1998).*

Conclusion

Studies have shown that after disinfection was done by an unprofessional person or by an expert veterinarian, on the examined surfaces mechanical cleaning and sanitary washing, as well as disinfection have been properly carried out, the total number of bacteria (log / CFU cm2) decreased significantly. The obtained results of the total number of bacteria after disinfection of the floor of the stunning box indicate failure in the implementation of disinfection because the number of bacteria has not significantly decreased ($p > 0.05$). It is necessary to improve procedures that precede disinfection, which among other things include the obligatory use of hot water for washing of the surfaces to be disinfected.

Propusti u sprovođenju dezinfekcije u zanatskoj klanici

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Rezime

Cilj rada je bio da se na osnovu dobijenih rezultata utvrdi da li postoje razlike u ukupnom broju bakterija na određenim površinama posle dezinfekcije stučnog i nestručnog lica zanatske klanice i da se na osnovu toga zaključi da li su postojali propusti u sprovođenju dezinfekcije. Materijal za istraživanja su bili uzorci vlažno-suvih briseva uzetih tokom V nedelja, pre i posle dezinfekcije nestručnog i stručnog lica i to sa: noža za obradu mesa, kuka, poda boksa za omamljivanje i poda koridora. Postupak uzimanja vlažno-suvog brisa je urađen prema standardnoj metodi ISO 18593. Iz uzetih uzoraka određen je ukupan broj bakterija standardnom metodom ISO 4833.Dezinfekcija je vršena sa 0.02% hlornim preparatom pri vremenu ekspozicije od 30 min. Na osnovu podataka dobijenih ovim istraživanjem utvrđeno je da je posle dezinfekcije nestručnog lica zanatske klanice i dezinfekcije stručnog lica-veterinara na ispitivanim površinama na kojima su pravilno sprovedene faze dezinfekcije (mehaničko čišćenje i sanitarno

pranje) i dezinfekcija, došlo do značajnog smanjenja ukupnog broja bakterija (log cfu/cm2). Dobijeni rezultati ukupnog broja bakterija posle dezinfekcije poda boksa za omamljivanje ukazuju na propuste u sprovođenju dezinfekcije jer se broj bakterija nije značajno smanjio ($p>0.05$).

Ključne reči: dezinfekcija, propusti, zanatske klanice

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Received 28 November 2017; accepted for publication 8 February 2018

Biotechnology in Animal Husbandry 34 (1), p 119-126 , 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.234
https://doi.org/10.2298/BAH1801119L

MILK YIELD PREDICTION BETWEEN BUNAJI HOLSTEIN FRIESIAN CROSS BRED AND HOLSTEIN FRISIAN COWS USING LINEAR, CUBIC AND QUADRATIC MODELS

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Communication

Abstract: The aim of this study was to compare three different models (Linear, cubic and quadratic) to find best model for predicting milk yield. Data originated from the monthly milk yields records of 251 Bunaji Holstein Friesian crossed and Holstein Friesian cows from 2010 to 2015. The daily milk yield data were regressed against time (day of lactation) for individual cow, using the procedure of SAS, (2002). The resulting polynomial regression coefficients (linear, quadratic and cubic) were then subjected to variance of analysis. All models provided an acceptable level of accuracy in predicting milk yield for Bunaji Holstein Friesian crossed and Holstein Friesian cows, but cubic model is observed to be the most suitable with (R^2) values of (0.659, 0.582, 0.810 and 0.621) followed by quadratic model (0.447, 0.516, 0.614 and 0.605) while linear model has the least R^2 values (0.02, 0.496, 0.548 and 0.309) in all the study farms.

Keywords: Bunaji, Holstein Friesian, polynomial regression coefficients, milk yield, Nigeria.

Introduction

In Nigeria, cattle provides more than 90% of the total annual domestic milk output (*Walshe et al., , 1991)* with the White Fulani or 'Bunaji' breed recognized as the principal producer (*Adeneye, 1989*). Unfortunately, the domestic output of about 407,000 metric tons of milk (*Olaloku, 1999*) from an estimated 14 million cattle (*RIM, 1992*) can hardly satisfy the dairy demands of an ever-

increasing population of Nigerians (*Ibeawuchi et al., 2000*). It is documented that milk yield increases from calving to the peak production, which is attained between 20 and 70 days post-partum, there after decreases smoothly until the end of lactation (*Scott et al., 1996; Val-Arreola et al., 2004*). Knowledge of milk yield in dairy cattle is important for decision making on herd management and selection strategies, and it is also a key element in determining optimum strategies for insemination and replacement of dairy cows *(Olori et al., 1999; Koçak and Ekiz, 2008).* It has been acknowledged that milk yield is influenced by environmental factors such as the herd, year of calving, parity, age and season of calving (*Wood, 1967; Tekerli et al., 2000*). Most of mathematical functions proposed to fit lactation patterns in dairy cattle are mainly aimed at describing the phenomenon. Their basic assumption is that lactation is characterized by a continuous and deterministic component with an increasing phase till a maximum followed by a decreasing slope (*Macciotta et al., 2011*). Early models paid more attention to the deterministic component of the lactation pattern, being essentially aimed at describing average lactation curves of homogeneous groups of animals for management purposes (*Tekerli et al., 2000*). An efficient model was therefore required to unravel the general frame of the process from environmental perturbations and to predict milk yield with good accuracy. No comprehensive study has been carried out concerning milk yield prediction in Bunaji Holstein Friesian and Nigerian Holstein-Friesian dairy cows. Therefore, this study was aimed at comparing the goodness of fit of three models (linear, cubic and quadratic) to predict milk yield of Bunaji Holstein-Friesian and Holstein-Friesian cows in North Central Nigeria using field data from dairy farms.

Materials and methods

The data used for this study originated from the monthly milk yields records of 251 cows of Bunaji (White Fulani) crossed with Holstein Friesian cow and exotic Holstein Friesian cow from 4 dairy farms. The data were collected from 2010 to 2015 and the animals were kept under semi -intensive system with natural and established pasture for grazing. The study farms include National Veterinary Resource Institute Vom and Agric Services and Training Centre in Jos, Farm Fresh Jos (Plateau State) and Nagari farm Keffi (Nasarawa State). Plateau State is situated in the tropical zone, has a near temperate climate with an average temperature of between 18 and 22 °C while the geographical coordinates of Jos are 9.8965^0N and 8.8583^0E. Harmattan winds cause the coldest weather between December and February. The warmest temperatures usually occur in the dry season months of March and April. The mean annual rainfall varies 131.75 cm (52 in) in the southern part to 146 cm (57 in) on the Plateau (Blench 1999). The highest rainfall is recorded during the wet season months of July and August. Nasarawa

State lies within the guinea Savannah region and has tropical climate with moderate rainfall (annual mean rainfall of 1311:75cm) (52 in) with average annual temperature of 28.4 °C (Blench 1999). Nasarawa state is made up of plain lands and hills measuring up to 300ft above the sea level at some points. Keffi town is Local Government Area in Nasarawa State and its headquarters is Keffi and the geographical coordinates are 8.8471^0N and 7.8776^0E. Economic and technical data from two commercial private farms (Farm fresh, Jos and Nagari farm, Keffi) and two government farms (National Veterinary Research Institute, Vom and Agricultural Services and Training Centre, Jos.) were used for the study.

Experimental Procedure

Data used for the study were extracted from records kept for Bunaji (White Fulani) Holstein Friesian cross bred cows and exotic Holstein Friesian cows from 2010 to 2015. The raw data entered into the computer (MS EXEL environment) were average number of cow per farm, number of cows in milk, milk yield (kg/cow in flock/305 days).

Statistical Analysis

Predicting milk yield

The daily milk yield data were regressed against time (day of lactation) for individual cow, using the procedure of *SAS, (2002).* The resulting polynomial regression coefficients (linear, quadratic and cubic) were then subjected to variance analysis (*Allen et al., 1983, Morris 1999*), using GLM procedure of SAS, (2002). The following models equation of polynomial regression was fitted for expressing the regression of daily milk yield against time:

$Y= b_0 + b_1X + e$ 1 (linear model) *equation (i)*

$Y= b_0 + b_1 X + b_2 X^2 + e$ 2 (quadratic model) *equation (ii)*

$Y= b_0 + b_1 X + b_2 X^2 + b_3 X^3 + e$ 3 (cubic model) *equation (iii)*

Y = Milk yield
b_0 = the intercept
X = independent variables (Day of lactation)
b_1, b_2 and b_3 = regression coefficients
e = random error.

The full regression model of the measured milk yield over lactation period (days) was defined as:

$Y = a + b_1X^1 + b_2X^2 + b_3X^3$
where,
Y = dependent or endogenous variable (Milk yield)
a = intercept
b 's = regression coefficients
X's = independent or exogenous variables (lactation period)

Results and Discussion

Milk yield prediction over a period of time is presented in the Tables. The coefficient of determination (R^2) values from the tables for the three models in all the farms were all highly significant (P<0.01). However, the cubic regression model has the highest (R^2) values of (0.659, 0.582, 0.810 and 0.621) followed by quadratic model (0.447, 0.516, 0.614 and 0.605) while linear model has the least R^2 values (0.02, 0.496, 0.548 and 0.309) in all the farms.

Table 1. Prediction of milk yield on day of collection using linear, quadratic and cubic models at ASTC Govt. Farm, Jos

Model	Equation	R^2	Adjusted R^2	Significance
Linear	MY= 15.622 – 0.002D	0.02	0.02	**
Quadratic	MY= 8.381 + 0.038D – 0.00003613D^2	0.447	0.446	**
Cubic	MY= 2.355 + 0.103D + 0.000D^2 + 0.0000000913D^3	0.659	0.658	**

MY= milk yield; D= day; R^2= coefficient of determination. ** Significant at P<0.01.

Table 2. Prediction of milk yield on day of collection using linear, quadratic and cubic models at Nagari Farm, Keffi

Model	Equation	R^2	Adjusted R^2	Significance
Linear	MY= 2.995 + 0.004D	0.496	0.495	**
Quadratic	MY= 3.376 + 0.001D + 0.000003994D^2	0.516	0.515	**
Cubic	MY= 4.181– 0.012D + 0.00004609 D^2 – 0.00000003713D^3	0.582	0.580	**

MY= milk yield; D= day; R^2= coefficient of determination. ** Significant at P<0.01.

Table 3. Prediction of milk yield on day of collection using linear, quadratic and cubic models at Farm Fresh, Jos, Plateau State

Model	Equation	R^2	Adjusted R^2	Significance
Linear	MY= 11.067 + 0.031D	0.548	0.545	**
Quadratic	MY= 10.016 + 0.073D + 0.000D^2	0.614	0.609	**
Cubic	MY= 12.193 – 0.099D + 0.003D^2 – 0.00001265D^3	0.810	0.806	**

MY= milk yield; D= day; R^2= coefficient of determination. ** Significant at P<0.01.

Table 4. Prediction of milk yield on day of collection using linear, quadratic and cubic models at National Veterinary Research Institute, Vom, Jos

Model	Equation	R^2	Adjusted R^2	Significance
Linear	MY= 5.220 – 0.013	0.315	0.309	**
Quadratic	MY= 4.318 + 0.035D + 0.000D^2	0.605	0.598	**
Cubic	MY= 4.571 + 0.009D + 0.000D^2 –0.000003609D^3	0.621	0.610	**

MY= milk yield; D= day; R^2= coefficient of determination. ** Significant at P<0.01.

It can be said that the models were adequate for predicting milk year over a period of time but cubic model was the best predictor of milk yield, on day of collection compared to quadratic and linear regression models. *Birol-Dag et al, (2006)* reported that estimated total milk yield according to the cubic model was very close to total milk yield calculated by the Fleischmann method. However, differences between models were not statistically significant. The findings of this study is also in line with the report of (*Cankaya et al., 2014*) who noted that cubic regression model gave best values when comparing with other models for the first lactation curve of jersey cows. *Yakubu et al., (2013)* also reported that cubic model appeared to produce better goodness of fit when traits are considered singly. The result also agreed with the report of *Druet et al., (2003*) and *Silvestre et al., (2006)* who used the Wood models and cubic spline regression models for modeling lactation curves. Also, *White et al., ,(1999), Sahin and Efe, (2010); Nicolo et al., (2010); Koncagul and Yazgan, (2011); Geha et al., (2011)* used cubic spline regression for modeling of lactation curves of dairy cattle and all reported that cubic model produce a better fit when compared to other models.

Conclusion

All the three models could be used in predicting milk yield over time but cubic regression model gave a best fit in predicting of milk yield, on day of collection compared to quadratic and linear regression models.

Predviđanje prinosa mleka meleza bunaji holštajn - frizijskih i holštajn-frizijskih krava koristeći linearne, kubične i kvadratne modele

Mohammed Gambo Liman, Gwaza Deeve Sylvester, Bala Dogara

Rezime

Cilj ove studije bio je upoređivanje tri različita modela (linearni, kubični i kvadratni) kako bi se pronašli najbolji modeli za predviđanje prinosa mleka. Podaci potiču iz mesečne evidencije prinosa mleka 251 krava meleza Bunaji i holštajn-frizijske rase i holštajn-frizijskih krava od 2010. do 2015. godine. Dnevni podaci o prinosu mleka su se regresirani prema vremenu (dan laktacije) za pojedinačnu kravu, koristeći proceduru *SAS (2002).* Rezultujući polinomski regresioni koeficijenti (linearni, kvadratni i kubični) potom su podvrgnuti varijansi analize. Svi modeli daju prihvatljiv nivo preciznosti u predviđanju prinosa mleka za meleze Bunaji i holštajn-frizijske rase i holštajn-frizijske krave, ali je kubni model najprikladniji sa (R^2) vrednostima (0.659, 0.582, 0.810 i 0.621), a zatim kvadratni model (0.447, 0.516, 0.614 i 0.605), dok linearni model ima najmanje vrednosti R^2 (0.02, 0.496, 0.548 i 0.309) na svim ispitivanim farmama.

Ključne reči: Bunaji, holštajn-frizijska rasa, polinomski regresioni koeficijenti, prinos mleka, Nigerija.

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Received 30 January 2018; accepted for publication 23 March 2018

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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

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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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ŠKRBIĆ Z., LUKIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., PETRIČEVIĆ V., DOSKOVIĆ V., STANOJKOVIĆ A. (2015): Importance of farm management in reducing broilers skin lesions. Proceedings of the 4th International Congress "New Perspectives and Challenges of Sustainable Livestock Production", October 7 – 9, Belgrade, 145-158.

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