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MORPHOMETRIC CHARACTERIZATION AND BODY MEASURMENT CORRELATIONS IN LIPSKA PRAMENKA SHEEP

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Original scientific paper

Abstract: Lipska sheep is an autochthonous Serbian population from the group of Pramenka (Zapfel) sheep, bred in the area around Smederevo, Požarevac and Mladenovac. The average weight of male animals (BW) is 95 kg and 62 kg of female animals. Other measurements of female animals are: wither height (WH) 74 cm, body length (BL) 78 cm, chest width (CW) 23 cm, chest depth (CD) 40 cm and hearth girth (HG) 91 cm. In the last sixty years, adult female animals gained in average ten kilograms of weight. Other linear measures also increased. The increase is a consequence of better animal management, especially improved diet. Since the reduction of the size of the population, larger animals have dominated, which probably caused the change in the genetic constitution of the breed. Female animals of Lipska sheep are higher compared to the animals of other fifteen Pramenka breeds with the exception of Istrian sheep. The area from which Lipska sheep originates has better soil and richer vegetation compared to the areas of other Pramenka breeds. Partial correlations corrected for weight between individual linear measures were positive, with values between 0.196 and 0.814. Most correlations range between 0.30 and 0.55. Body measurements were studied with ANOVA on females only. The effect of flock influenced all linear measures with the exception of CW, whereas the age of animals had no effect on BL, CW and CD. The increase of individual linear measures was 0.5 to 2.1 mm per kg of BW. The increase of most body measurements from the first to the fifth year was statistically significant ($P<0.05$).

Key words: Pramenka, Lipska sheep, endangered breeds, linear measurements

Introduction

Pramenka or Zapfel is a group of sheep breeds widely used in the western part of the Balkan Peninsula. One of them is a Serbian autochthonous Lipska

sheep. *Mitrović (1925)* considers that Lipska sheep developed from the long-tailed Celtic sheep and Arkali sheep. It was introduced through various migrations of people on the Balkans. It is named after the village Lipe located near Smederevo. Today, the main breeding area of Lipska sheep is the territory between the towns of Smederevo, Požarevac and Mladenovac. The reported population size is between 2000 to 3500 animals *(DAD-IS, 2022)*.

The endangered status of this population was defined/determined in accordance with the Rulebook on the List of genetic resources of domestic animals, the method of preserving the genetic resources of domestic animals, as well as on the List of autochthonous breeds of domestic animals and endangered autochthonous breeds (2017). Bearing in mind the mentioned criteria, Lipska sheep belongs to the "potentially endangered" category. The strategy of preserving autochthonous populations in general, as a basic prerequisite for conservation, implies the determination of the morphometric profile (*Ružić-Muslić et al., 2021*), which was the subject of research by numerous authors: *Mioč et al. (1998), Ćinkulov et al. (2003), Georgoudis and Ligda (2006), Szobolevski (2006), Stojanović (2006), Kompan (2006), Antunović et al. (2013), Važić et al.(2017a), Pihler et al. (2020).*

Morphometric characterization is of multiple importance since it represents one of the most important approaches when describing the population, it shows the morphological structure, the ability of the animal to grow and develop (*Attach et al., 2004*), it is a reflection of the breed standard (*Verma et al., 2016*), and it also represents guidelines for further breeding selection work (*Kumar et al., 2017*). The fact that the mentioned population has been less studied in our country in recent decades, and inaccurate and outdated literature data, indicates that the observation of this Pramenka is urgent.

The aim of the work is the morphometric characterization of the Lipska Pramenka as well as the determination of correlations between body measurements, in the function of its preservation and sustainable cultivation.

Material and Methods

The population is used for meat, milk and wool production. Milk yield is quite good, while the production of meat and wool is only moderate. Body characteristics of the breed and its productivity are not well recorded. The available records are inconsistent. In general, animals have a light and small head *(Milojić, 1952)*. Head profile in ewes is slightly bulging, while in rams the bulging is more pronounced. The head is covered with black, short hair. Ewes are almost always without horns. The horns of rams are strong, spirally twisted and triangular, transversely striated and of bright yellow colour. The ears are of medium size, laying horizontally and covered, same as the head, with black, short hair. The neck

is of medium length and strong. The withers are weak and the top line rises slightly towards the rear. The pelvis is slightly lowered. The rump is long. The legs are quite long too, they have proper posture and strong bones and they are covered with dark hair. Hooves are solid, usually yellow, in some individuals they are dark brown. The abdomen is properly developed. Lower abdominal line usually does not come from the plane of the sternum. It is well covered with wool. Udder is well developed. Lipska sheep belongs to the group of long-tailed Pramenka breeds. The tail is cylindrical in shape and reaching over the ankles.

Soil and vegetation in the area is rich. Flocks are small, between 5 and 20 animals, mostly kept on pasture. Only in short periods of the production cycle (before lambing and during lactation) they are fed with cereal supplement.

The measurement of the exterior of the Lipska sheep was carried out in the villages Umčari, Vlaška and Koraćica near Belgrade.The total of 257 ewes aged 1 to 4 years and 11 rams aged 2 to 4 years were measured. The following measures were taken: body weight (BW), hearth girth (HG), withers height (WH), body length (BL), chest width (CW), and chest depth (CD). Measures were taken using the measuring stick.

Since only 11 measurements on male animals were done compared to 257 measurements on female animals, all further evaluation was done only for female animals. The following linear statistical model was used:

$$Y_{ijk} = \mu + A_i + F_j + b_1(W_{ijk} - \overline{W}) + b_2(R_{ijk} - \overline{R}) + e_{ijk}$$

where: Y_{ijk} is dependent variable (BW, HG, WH, BL, CW, CD), μ is the mean value, A_i is the effect of age i in years, F_j is the effect of the flock j, $b_1(W_{ijk} - \overline{W})$ is the regression of weight (in case of Y_{ijk} = BW birth weight, in other cases actual body weight (BW)) dependent variables, $b_2(R_{ijk} - \overline{R})$ is the regression of year of birth and e_{ijk} is the residual for measurement ijk. The Pearson correlations were calculated between BW and other body measurements. Other correlations are partial with BW as control variable. Statistical analysis was done with package R ver. 3.0.0 (5).

Results and Discussion

Body measurements

BW of adult ewes was 61.95 ± 6.05 kg, and males 95.00 ± 7.03 kg (Table 1). BW of males is high, which is probably due to the fact that only a small number of selected animals have remained in the population. According to official data *(Stojanović, 2006)*, BW is 65 kg for adult male and 60 kg for adult female animals.

The estimation of females' weight is correct; however, the official figures for BW of male animals are probably underestimated. In genetically related Svrljig sheep, according to the same source *(Stojanović, 2006)*, BW is somewhat lower: 50 kg for rams and only 42 kg for ewes. BW of Svrljig rams is probably also underestimated. The historical sources *(Milojić, 1952)* state that the weight of Lipska ewes is 50.7 kg, and 66.3 kg of rams. According to *Pavlovich (1937)*, the average weight of Lipska sheep is 60 kg. The average body weight of Sjenica ewes *(Nikolić, 1952)* is 52.2 kg and 69.63 kg in rams. It can be established that the modern Lipska sheep is slightly heavier than it was fifty years ago. BW has increased by 10 kg or 20 percent. Svrljig breed, according to the available data, has not changed. The reduction of the population was significantly greater in Lipska sheep compared to Svrljig sheep. Only the largest animals remained in the population. Furthermore, Lipska sheep is nowadays better fed than half a century ago, while the Svrljig sheep is kept almost under the same conditions.

Table 1. Parameters of descriptive statistics for body measurements by gender

	Male			Female		
Variable	avg.±SD	min.	max.	avg.±SD	min.	max.
BW	95.00±7.03	85.0	107.0	61.95±6.05	42.0	73.0
WH	84.00±4.73	80.0	94.0	74.46±4.49	64.0	91.0
BL	91.64±4.61	85.0	99.0	78.05±4.79	67.0	91.0
CW	31.45±3.11	28.0	38.0	23.49±3.07	19.0	84.0
CD	47.82±3.03	45.0	53.0	39.76±2.76	34.0	49.0
HG	122.27±5.88	112.0	128.0	91.05±6.57	79.5	114.0

WH in females was 74.46 ± 4.49 and in rams 84.00 ± 4.73 cm. Lipska sheep is the largest among Pramenka breeds. According to *Stojanović (2006)*, WH is 65 and 60 cm in male and female animals, respectively, and is, according to our results, underestimated. In previous studies, the WH was 66.3 cm *(Mitrović, 1926)* and 67 cm *(Pavlovich, 1937)*. BL of ewes was 78.05 ± 4.79 and 91.64 ± 4.61 cm in rams. Historically, BL found on Lipska sheep was 69.4 *(Mitrović, 1925)* and 81.23 cm *(Pavlovich, 1937)*. The first one is significantly lower, while the second is similar to our results. There are no recent data of BL of other Pramenka breeds. The comparison is therefore impossible. CW of ewes is 23.49 ± 3.07 and 31.45 ± 3.11 cm of rams. According to previous studies, CW of Lipska ewes was 23.67 cm *(Milojić, 1952)*, which is nearly identical to our result. According to the same source, CW of rams was 24.56 cm. This is significantly less than in our study. CW according to *Pavlovich (1937)* is 24.05 cm, which is again close to our values. It can be concluded that CW has not changed. CD in female animals was 39.76 ±

2.76 and 47.82 ± 3.03 cm in rams. The comparable CD of ewes was 29.86 *(Milojić, 1952)* and 33.23 *(Pavlovich, 1937)*, and of rams 32.60 cm *(Milojić, 1952)*. It is evident that CW of modern Lipska sheep is greater than CW of earlier Lipska sheep. HG of females is 91.05 ± 6.57 cm and of males 122.27 ± 5.88 cm. Comparable data were not found in literature. The body of Lipska sheep has a square shape. The height of withers is nearly equal to the length of the body. The weight has increased during the last decades. Reduction of population size probably changed its genetic structure. Improved animal nutrition has also contributed to the increase of body measures.

Table 2. Body Weight (BW) and Wither Height (WH) for females of different Zapfel breeds

Breed	Country	BW	WH	Author
Kefallinias	Greece	42	60	*Georgoudis and Ligda, 2006*
Sfakia	Greece	39	58	*Georgoudis and Ligda, 2006*
Mytilini (Lesvos)	Greece	48	64	*Georgoudis and Ligda, 2006*
Boutsiko (Orino)	Greece	38	54	*Georgoudis and Ligda, 2006*
Cikta	Hungary	38	44	*Szobolevski, 2006*
Hortabagyi Racka	Hungary	40	50	*Szobolevski, 2006*
Gyimesi Racka	Hungary	58	55	*Szobolevski, 2006*
Pirot	Serbia	46	60	*Stojanović, 2006*
Bardoka	Serbia	48	60	*Stojanović, 2006*
Svrljig	Serbia	40	65	*Stojanović, 2006*
Krivovir sheep	Serbia	38		*Stojanović, 2006*
Bela Krajina sheep	Slovenia	48	60	*Kompan, 2006*
Istrian sheep	Croatia-Slovenia-Italy	65	74	*EFABIS, 2013*
Lika	Croatia	47	61	*EFABIS, 2013*
Dalmatian sheep	Croatia	37	56	*EFABIS, 2013*
Kupres sheep	Bosnia	46	69	*EFABIS, 2013*

The only source of data on the physical characteristics of autochthonous sheep populations are organizations that deal with their preservation. The data, which can be obtained there, is mainly for BW and WH. Table 2 presents the data for BW and WH of adult female animals of different Pramenka breeds. Lipska sheep (61.95 kg) is, following Istrian sheep (65 kg), the largest in Pramenka group. Similar BW can be observed in animals of Gyimesi Racka breed (58 kg). Weight of other breeds from Pramenka group ranges from 37 to 48 kg. Pramenka breeds originate from the Karstic terrain with poor pasture. Only Hungarian Pramenka sheep and Lipska sheep come from the area with rich vegetation, so it is possible that the breeders selected larger breeding animals. WH of Lipska breed females is 74.46 cm (Table 1) and it is equal to the WH of Istrian sheep. WH of all other

breeds (Table 2) is below 70 cm. While the average weight of Gyimesi Racke is almost equal to the weight of Lipska sheep, its height is quite low - only 55 cm. This suggests the animals of small frame, with developed soft tissues, while the larger weight of Lipska sheep is a consequence of a larger frame of sheep. The rough frame of the animals allows appropriate adaptation to poor breeding conditions.

Correlations between body measurements

Table 3 shows the correlation coefficients between the individual body measurements of female animals. Correlation coefficients between BW and other body measurements were calculated by Pearson method. The others are partial correlation coefficients with BW as a correction variable.

Table 3. Correlations between body measurements in females

	WH	BL	CW	CD	HG
BW	0.323	0.342	0.233	0.484	0.540
WH		0.814	0.301	0.503	0.454
BL			0.361	0.562	0.479
CW				0.318	0.196
CD					0.691

As seen, all correlations are positive. The highest is the correlation coefficient between WH and BL (0.814). Proportion between the height and the length of the animal is constant and independent of the size of adult animals. Correlation is higher than in Texel (0.31), Suffolk (0.37) and Bleu du Maine (0.44) breeds *(Janssens and Vandepitte, 2004)*, as well as in Menz breed (0.69) *(Gizaw et al., 2008)* and in Yankasa breed (0.76) *(Afolayan et al., 2006)*. The correlation between CD and HG is high (0.691). Correlation between CW and HG is low (0.196). The chest volume depends on the depth and not the width. Chest width is poorly correlated with other body measures; the highest correlation is 0.361. Correlation of BW with CD is 0.483, with HG 0.540, and with CW 0.233. Correlations between BW and CD and BW and HG *(Janssens and Vandepitte, 2004)*, in the following breeds are: Bleu du Maine (0.57, 0.69), Suffolk (0.63, 0.74) and Texel (0.56, 0.67). They are slightly higher than in our study. Correlations found in Menz breed *(Gizaw et al., 2008)* are 0.77 between BW and HG, 0.292 between GT and CW, and 0.255 between GT and HG. All other correlations are in the range from 0.3 to 0.5. These values are of medium size and similar to correlations between the same body measures in breeds Bleu du Maine, Suffolk and Texel *(Jansens and Vandepitte, 2004)*. It can be concluded that the mature females of Lipska breed are of similar shape, regardless of size, and that the

correlations between the same linear measurements are similar to the linear correlations in the literature. Parts of the body, regardless of the size of the individual animals, are of the same proportion. Only CW is relatively poorly correlated with other body measurements.

The effect of age, flock, animal weight (birth or body) and year of birth on body size

Table 4 presents the results of analysis of variance (ANOVA). Model explains the increase of all linear measures between ages one and four years as statistically significant. The coefficients of determination are: 0.6302 for HG, 0.6165 for BW and 0.4003 for CD. Other coefficients of determination, 0.2008 for WH, 0.1795 for BL, and especially 0.0823 for CW, are low. The model poorly explains the last three linear measurements

Table 4. Analysis of variance according to the linear model

	Model			Effect (P-value)			
	F-value	P-value	R^2	age (A_i)	flock (F_j)	$b_1(W_{ijk} - \overline{W})$	$b_2(R_{ijk} - \overline{R})$
BW	46.060	<0.00001	0.6165	<0.00001	<0.00001	0.20540	0.27060
WH	9.634	<0.00001	0.2008	0.00063	0.00095	0.01819	0.80130
BL	8.393	<0.00001	0.1795	0.11657	0.01132	0.00003	0.33929
CW	3.439	<0.00001	0.0823	0.75985	0.11194	0.00067	0.79901
CD	25.600	<0.00001	0.4003	0.98675	0.00063	<0.00001	<0.00001
HG	65.360	<0.00001	0.6302	0.00295	0.00105	<0.00001	<0.00001

The influence of all four effects on HG is statistically significant ($P<0.05$). The age of animal (A_i), the effect of flock (F_j) and the weight of the animal are significantly influenced by WH. CD is influenced by flock (F_j), weight and year of birth of the animal. BW is affected by age (A_i) and flock (F_j), while BL is influenced by flock (F_j) and weight of animals. CW is influenced only by weight.

Regression coefficients between the measured values and body weight (BW) were positive and statistically significant (Table 5, $P<0.05$). The increases of body size were small, from around one (WH, CW, CD) to around two millimetres (BL, HG) per kg of BW or birth weight, but statistically significant ($P<0.05$). Regression coefficients between the year of birth and body measures were negative. Animals born in later years, were probably not of mature size, so the influence can be considered as a correction of linear measure of the body for its age, although the age of the animals was included as an independent effect in the model (A_i).

Table 5. Regression coefficients and their standard errors according to the linear model

	W[1]	R[2]
BW	-0.423±0.333	-0.250±0.227
WH	0.099±0.042*	-0.054±0.215
BL	0.193±0.046***	-0.223±0.234
CW	0.102±0.030***	-0.039±0.153
CD	0.137±0.023***	-0.834±0.116***
HG	0.231±0.042***	-2.732±0.217***

[1]Body weight; [2]Birth year (Significance levels: * 0.05>P >=0.01; ** 0.01>P >=0.001;*** 0.001>P)

Conclusions

Lipska sheep is one of the Serbian native breeds from the group of Pramenka breeds (Zapfel), which is native in the area near Smederevo, Požarevac and Mladenovac, where the residuals of the breed can be found. Mature ewes of the breed, compared to 16 breeds of Pramenka group from Greece, Croatia, Hungary and Slovenia, were of the largest size following Istrian Pramenka.

Recently measured animals are much larger than the animals measured 60 years ago or more.

Correlations between linear body measurements of female animals were positive and quite high. It can be determined that the mature female animals of Lipska breed were of similar shape irrespective of their size. Their body was square like. The individual parts of the body were of the same proportion, regardless of the size of each animal. An exception was found in CW, where the correlation with other body measures was relatively low.

The effect of the flock showed an impact on all linear body measures with the exception of CW. That means that the animals in the same flock were uniform. Animal's age in years affected BW, WH and HG, but not BL, CW and CD. The frame of animals in the period of one to five years of age remained unchanged. The change in BW and HG is related to the increase in soft tissues (muscle and fat). For each kg of body weight, the linear body measures increased by half to two millimetres. The increase was small, but statistically significant ($P<0.05$).

Based on the existing results it can be concluded that the Lipska sheep is among the largest breeds in the Pramenka group. The size of animals has increased during the last half of the century. Recently measured animals are not phenotypically identical to the animals of the same breed fifty years ago, or more. Determined differences between modern and historical animals indicate the need of new researches on the remains of endangered breeds. Phenotypic changes can be

expected in those animals as well. New phenotypic studies on the remains of such breeds will help to define the standards for their future breeding.

Morfometrijska karakterizacija i korelacija telesnih mera Lipske pramenke

Dragana Ružić-Muslić, Bogdan Cekić, Ivan Ćosić, Nevena Maksimović, Violeta Caro-Petrović, Nikola Delić, Nemanja Lečić

Rezime

Lipska ovca je autohtona populacija iz grupe pramenki (Zapfel) koja se gaji u okolini Smedereva, Požarevca i Mladenovca. Prosečna telesna masa odraslih muških životinja iznosi 95 kg, a ženskih 62 kg. Eksterijerne mere ženskih životinja iznose: visina grebena (WH) 74 cm, dužina tela (BL) 78 cm, širina grudi (CW) 23 cm, dubina grudi (CD) 40 cm i obim grudi (HG) 91 cm. U poslednjih šezdeset godina, telesna masa odraslih ženskih životinja su u proseku povećala za oko deset kg, što je posledica izmenjenog menadžmenta, pre svega poboljšane ishrane. Nakon redukcije veličine populacije, dominantne su veće životinje, što je verovatno uslovilo promenu genetske konstitucije populacije.Područje na kome je nastala lipska ovca karakteriše plodnije zemljište i bogatija vegetacija u poređenju sa područjima iz kojih dolaze druge populacije pramenki. Korelacijski koeficijenti i uticaji stada, starosti životinje u godinama, godine rođenja i težine kod rođenja odnosno težine izmerene životinje, WH, BL, CW, CD i HG su ocenjeni samo na ženskim životinjama, obzirom da je bilo obavljeno jedanaest merenja na pet ovnova. Parcijalne korelacije sa korekcijom na telesnu težinu između pojedinačnih linearnih mera su pozitivne sa vrednostima između 0,196 i 0,814. Najviše korelacija nalaze se u intervalu od 0,30 do 0,55. Efekat stado uticao je na sve linearne mere sa izuzetkom CW dok starost životinja nije uticala na BL, CW i CD. Porast pojedinačnih linernih mera iznosio je za 0,5 do 2,1 mm na kg BW. Porast većine telesnih mera od prve do četvrte godine bio je statistički značajan ($P<0,05$).
Ključne reči: Zapfel, Lipska ovca, ugrožena rasa, linearne mere

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INFLUENCE OF SOME FACTORS ON FERTILITY AND WEIGHT IF SHEEP AND BODY WEIGHT DEVELOPMENT OF LAMBS

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Original scientific paper

Abstract: The research included a total of 921 lambs, 474 ewes and 8 sjenicke sheep rams from 4 different farms. The aim of the research was to examine the influence of the farm, cultivation technology and the influence of rams within the farm on production indicators. Statistical analysis showed that the body weight of lambs at birth ranged from 3.37 to 4.03 kg (average 3.68 kg), at 30 days from 11.51 to 12.41 kg (average 12.07 kg) and from 90 days from 27.82 to 29 kg (average 28.65 kg). A statistically highly significant influence of the farm was determined ($P<0.01$) on the body weight of lambs in all three control periods, as well as on the body weight of sheep and on the fertility of sheep. The influence of the ram on the body weight of the lambs at birth was statistically very significant within the farm ($P<0.01$). When it comes to the percentage share of birth type by farm, farm 1 had the most singletons (59.13%) both within the farm and in comparison between other farms, while there were fewer twins (40.87%), and triplets were not identified. On the other farms, the percentage of twins was the highest, and triplets were also present, while on farm 4 there were also quadruplets, lambs born as quadruplets (3.28%).

Key words: ram, the farm, reproductive performances, lambs

Introduction

Both genetic and non-genetic (environmental) factors in farm animals affect reproductive traits. These factors can be categorized as factors relating to the animal's environment, related to its genotype. The effect of the ram is an important factor affecting the fertility of sheep, the timely introduction of a ram into a group of sheep promotes the detection of ewes in estrus in time, which aims to reduce the number of infertility in sheep *(Adjibode et al., 2017)*. Productivity of sheep is determined by the fertility of the herd, and the success of sheep production mostly

depends on it *(García-Chávez et al., 2020)*. Economically important traits on which the success of production also depends are the body weight of lambs measured at different age stages *(Petrović et al., 2012).* The average across breed weight at breeding had a positive effect on fertility and prolificacy *(Gaskins et al., 2005).* The number of reared lambs per ewe is a very good indicator of production success, which is influenced by both genetic and environmental factors *(Adjibode et al., 2017).* Numerous authors state that the degree of mortality, the vitality of the lambs, as well as the final weight (weighing weight) depends on the weight of the lambs at birth. *(Cloete et al., 2001; Zapasnikiene, 2002; Berhan and Arendonk, 2006; Petrovic et al., 2009; Bancheva et al., 2022).*

Ewe productivity and growth of lambs from birth to weaning are indicators of flock profitability *(Garcia-Chavez et al., 2020).* A larger number of lambs obtained per sheep has a positive effect on the production of lamb meat, which is what sheep production is mainly based on *(Assan, 2020).*

In general, a well-balanced meal improves the productivity of animals, this factor not only depends on whether the male and female sheep will be in breeding condition, but this factor also has a significant impact on the health of the animals, because only healthy animals can express their potential.

The aim of the research was to determine the fertility results of sheep and the movement of body mass of lambs in the period from birth to weaning, depending on the influence of the farm and the ram.

Material and Methods

The examination of the reproductive and production characteristics of the parent flocks of the Sienica sheep was carried out on four private farms in the area of the Kolubar district.The research included a total of 921 lambs, 474 ewes and 8 barnyard sheep rams.

The number of heads per farm was: 128 adult sheep, 230 lambs and 2 rams (farm no. 1), 113 adult sheep, 217 lambs and 2 rams (farm no. 2), 123 adult sheep, 230 lambs and 2 rams (farm no.3) and 110 adult sheep, 244 lambs and 2 rams (farm no.4).

Heads were mated and lambed in the period from 2017 to 2018. During the research, the influence of the farm on the body mass of sheep and on the mass of lambs during the lactation period was analyzed.

The influence of the ram was measured through the mass of lambs at birth.

Statistical analysis of the obtained experimental data was performed using the statistical package Statistica for windows 7 (Stat. Soft. Inc.). The equality of variances of the analyzed treatments was tested using Levene's test. The influence of the farm (F) on the fertility and body weight of sheep and the weight of lambs from birth to weaning was investigated using the variance analysis method (one-

factor analysis). Also, the influence of the father on body weight of lambs at birth was tested within each farm using the analysis of variance method. Differences between the mean values of the investigated treatments were analyzed using Fisher's LSD test, T-test and HSD test. All analyzes were performed at a significance level of 0.05 and 0.01, and the obtained results are presented as means ± standard deviation ($\bar{X}$ ±SD*).*

Results and Discussion

Table 1 shows the determined values of body weight and fertility of sheep on the investigated farms.

Table 1. Influence of the farm on body weight (BW) and fertility of sheep

	N	BW sheep $\bar{X} \pm SD$	CV (%)	N	Fertility $\bar{X} \pm SD$	CV (%)
The farm 1	182	$66.18^{C} \pm 3.65$	5.52	182	$1.25^{cB} \pm 0.44$	35.20
The farm 2	162	$66.03^{C} \pm 4.52$	5.88	162	$1.41^{bA} \pm 0.54$	38.30
The farm 3	144	$76.81^{A} \pm 2.62$	3.97	144	$1.51^{abA} \pm 0.59$	39.07
The farm 4	155	$69.18^{B} \pm 3.19$	4.61	155	$1.57^{aA} \pm 0.66$	42.04

a,b,c - means marked with lowercase letters are statistically significantly different at the 0.05 level
A,B,C - means marked with lowercase letters are statistically significantly different at the 0.01 level.

From the results shown in Table 1, it can be seen that the body weight of the female gilts varied significantly across the observed farms (P<0.01).

The highest average body weight of 76.81 kg was determined in the sheeps on farm 2, while sheeps from farm 3 had the lowest body weight, which was 66.03 kg on average. Fertility of ewes expressed through litter size, i.e. the average number of lambs per ewe, was also significantly influenced by the farm (P<0.01).

Females from farms 3 and 4 where estrus synchronization was applied had more litters (1.51 and 1.57 respectively) compared to females from farms 1 and 2 (1.25 and 1.41) that were mated naturally, without the use of exogenous hormones to induce estrus. Ewes of different farms have different management practices and this may have an impact on fertility after AI *(Santolaria et al., 2011).* Ewes that are well fed have a higher body weight, and the potential to give birth to lambs with a higher initial body weight *(Koritiaki et al., 2013).*

Table 2 shows the average mean values with their standard deviation for the three measured characteristics, the body weight of the lambs at birth, at the age of 30 days and at the age of 90 days, depending on the farm.

Table 2. The influence of the farm on the body weight (BW) of lambs from birth to 90 days of age

	N	BW of lambs at birth $\overline{X} \pm$ SD	CV (%)	N	BW lambs at 30 days $\overline{X} \pm$ SD	CV (%)	N	BW lambs at 90 days $\overline{X} \pm$ SD	CV (%)
The farm 1	230	$3.64^{B} \pm 0.61$	16.76	230	$12.18^{B} \pm 0.98$	8.05	230	$28.85^{A} \pm 1.81$	6.27
The farm 2	217	$3.37^{C} \pm 0.61$	18.10	217	$11.51^{C} \pm 0.71$	6.17	217	$27.82^{B} \pm 1.86$	6.69
The farm 3	230	$4.03^{A} \pm 0.66$	16.38	230	$12.41^{A} \pm 0.97$	7.82	230	$29.00^{A} \pm 2.01$	6.93
The farm 4	244	$3.68^{B} \pm 0.70$	19.02	244	$12.12^{B} \pm 1.07$	8.83	244	$28.85^{A} \pm 2.06$	7.14

A,B,C - means marked with lowercase letters are statistically significantly different at the 0.01 level

From the attached results shown in Table 2 , the effect of farm on lamb body mass in all three control measurements was higly significant (P<0.01). The highest values of body weight of lambs were found on farm No. 3, and they averaged 4.03 kg at birth, 12.41 kg at the age of 30 days and 29 kg at the age of 90 days, while the lowest average body weights were lambs on farm No. 2 (3.37 kg, 11.51 kg and 27.82 kg respectively).

The influence of ram on body weight of lambs at birth within farms is shown in table 3.

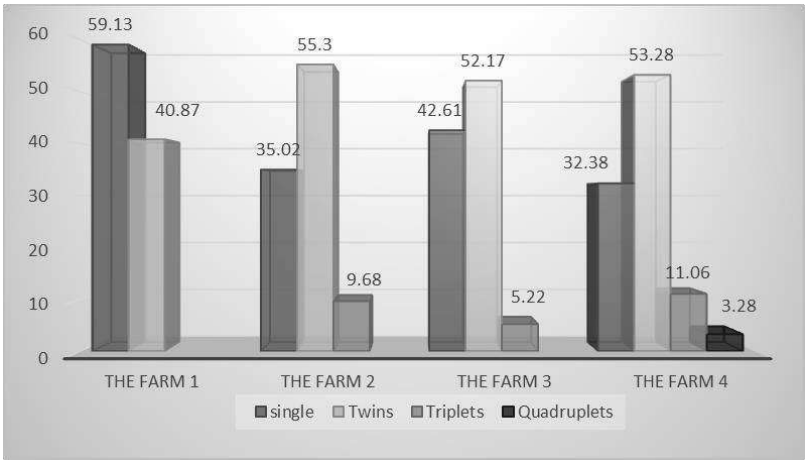
Table 3. Influence of ram on body weight (BW) of lambs at birth

	Ram (tattoo number)	N	BW of lambs at birth $\overline{X} \pm$ SD	CV (%)
The farm 1	190	81	$3.32^{A} \pm 0.50$	15.06
	7263	149	$3.82^{B} \pm 0.59$	15.45
The farm 2	4335	109	$3.48^{A} \pm 0.52$	14.94
	6198	108	$3.84^{B} \pm 0.64$	16.67
The farm 3	727	124	$3.83^{A} \pm 0.61$	15.93
	4308	106	$3.42^{B} \pm 0.53$	15.50
The farm 4	3559	118	$3.40^{A} \pm 0.52$	15.29
	8718	126	$3.83^{B} \pm 0.61$	15.93

A,B - means marked with lowercase letters are statistically significantly different at the 0.01 level.

The highest average body weight of lambs at birth was found in the offspring of ram tattoo number 6198 on farm 2, while lambs of ram tattoo number 192 on farm 1 had the lowest body mass. The analysis showed statistical significance in the differences in lamb body weights between rams within farms (P<0.01). The results of our research agree with the results obtained by (*Sánchez-Davila et al., 2015)* in Saint Croix hair sheep informed the effect of ram was siginficant (P < 0.01) on litter size and birth weight of lambs.

Graph 1 shows the percentage share of single lambs, twins, triplets and quadruplets by farms.



Graph 1. Percentage share of singletons, twins, triplets and quadruplets by farms

From the results shown in graph 1, it can be seen that in the structure of lambs by type of birth on farm 1 there were the most single lambs (59.13%), both within the farm and in comparison with other farms, while there were fewer twins (40.87 %), and triplets have not been determined. On the other farms, the percentage of twins was the highest, and triplets were also present, while on farm 4 there were also quadruplets, i.e. lambs born as quadruplets (3.28%).

Conclusion

By researching the influence of the farm and the ram on the weight of the lambs in the lactation period (weight at birth, at the age of 30 and 90 days of age), the following results were obtained:

- The influence of the farm on the fertility and mass of the sheep was highly statistically significant ($P<0.01$).
- The effect of farm on lamb weight from birth to 90 days of age was highly statistically significant ($P<0.01$).
- The effect of ram on lamb birth weight was statistically highly significant ($P<0.01$) within each farm.

Successful sheep production in today's conditions requires knowledge of biological, technological, organizational and marketing factors. The application of

the most modern technological achievements and technology is the key to the manifestation of the maximum performance of quality genetics.

Uticaj nekih faktora na plodnost i masu ovaca i telesnu razvoj jagnjadi

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Rezime

Istraživanjem je obuhvaćeno ukupno 921 jagnje, 474 ovce i 8 sjeničkih ovnova sa 4 različite farme. Cilj istraživanja je bio da se ispita uticaj farme, tehnologije gajenja i uticaj ovnova u okviru farme na proizvodne pokazatelje. Statistička analiza je pokazala da se telesna masa jagnjadi pri rođenju kretala od 3,37 do 4,03 kg (prosečno 3,68 kg), na 30 dana od 11,51 do 12,41 kg (prosečno 12,07 kg) i od 90 dana od 27,82 do 29 kg (prosečno 3,68 kg)28. Utvrđen je statistički visoko značajan uticaj farme (P<0,01) na telesnu masu jagnjadi u sva tri kontrolna perioda, kao i na telesnu masu ovaca i na plodnost ovaca. Uticaj ovnova na telesnu masu jagnjadi na rođenju bio je statistički veoma značajan u okviru svake farme (P<0,01).. Kada je reč o procentualnom učešću tipa rođenja po farmama, farma 1 je imala najviše jedinaca (59,13%) kako u okviru farme tako i u poređenju sa ostalim farmama, dok je blizanaca bilo manje (40,87%), a trojke nisu identifikovane. Na ostalim farmama procenat blizanaca je bio veći a bilo je i trojki, dok su na farmi 4 bila i jagnjad rođena kao četvorke (3,28%).

Ključne reči: ovan, farma, reproduktivni pokazatelji, jagnjad

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THE EFFECT OF GENOTYPE, FARM AND SEX ON THE PRODUCTION TRAITS OF FATTENING PIGS OF PEDIGREE BREED GENOTYPES

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Original scientific paper

Abstract: The study of the production traits of 22 genotypes of fattening pigs was carried out on two pig farms (Farm A and Farm B) in Central Serbia, under the influence of the following factors: farm, genotype and sex of fattening pigs, and pre-slaughter weight. The characteristics of fattening animals included in the research are: warm carcass side growth (WCSG); bacon thickness - rump (FTR); bacon thickness - back (FTB); bacon thickness - rump + back (FTRB); meat yield – carcass sides (JUSKG) and meat yield in percentage (JUSPRO), as well as weight and ratio of French dressing in warm carcass sides (FDKG and FDPRO). Animals of both sexes were used in the trial (female non-castrated rats and surgically castrated males). Total of 1166 fattening animals were included in the trials. Statistical data processing was performed using the Harvey software package. All included factors in the used models show a highly statistically significant effect on the variation of fattening traits ($P<0.01$; $P<0.001$). Animals of genotype DxSL (44.97%) had the highest share of meat in carcass sides, and animals of genotype SL (44.63%) for the trait JUSPRO, while for the trait FDPRO the highest value was recorded for the genotype DXSL (54.45%). In our study, animals of the genotypes (HxD)x(WxD) and Dx(WxD) had the highest values for bacon thickness - 39.95 and 38.32 mm, respectively, which implies lower share of meat in the carcasses. By calculating the genetic and phenotypic correlations, we came to the conclusion that the phenotypic correlation of the carcass side traits was of different strength (from very weak to complete) and different sign, while the genetic correlations were stronger than the phenotypic, so the genetic correlations between the bacon thicknesses FTB and FTR were complete, and between meat yield and traits FTB and FTR complete and negative.

Key words: fatteners, genotype, sex, bacon thickness, meat yield, genetic and phenotypic correlations

Introduction

In the Republic of Serbia, the Rulebook on the Quality of Slaughtered Pigs and the Categorization of Porcine Meat is still in force and used for the evaluation of fattened pigs on the slaughter line (*Regulation "Official Gazette of the SFRY" No. 2 and 12 from 1985*). According to this Rulebook, the meatiness of pork sides is calculated as the sum of the total mass of muscle tissue without the meat of the abdominal-rib part and without the meat of the head. The meatiness of the pig carcass sides is determined at the slaughter line, and the weight of the warm carcass sides and the thickness of the fatty tissue on the back are measured. Adipose tissue with skin is measured on the back and where the smallest thickness of the bacon is in the middle of the back (intercostal space between the 13th and 15th dorsal vertebrae) and on the withers at the place where the muscle M. Gluteus medius grows into adipose tissue. The sum of these measurements represents the thickness of the fatty tissue on the back. Yield and the share of meat in pork carcass sides is obtained using the tables that are an integral part of the Rulebook. The French way of dressing of carcass sides implies separation of the bacon from the area of the abdomen, neck, loin and partially from the thigh/leg and shoulder. The ribs are cut about 10 cm from the spinal column and the legs in the carpal and tarsal joints. The layer of bacon that covers the meat should not exceed 0.5 cm *(Stamenković and Radovanović, 2004)*.

Pig and porcine meat production is conditioned by a large number of parameters. Initial indicators of the quality of pork sides are data on mass and conformation, amount, distribution and mutual relationship of muscle and fat tissue. Carcass and meat quality traits vary under the influence of genetic and environmental factors (breed, sires, rearing method, individual animal, age and weight of animals, sex, castration, nutrition, season, procedures before slaughter, during and after slaughter, etc.). The overall success in the field of genetics, selection, nutrition, reproduction and health care is also assessed by evaluating the quality of carcass sides. In order to achieve genetic improvement of pig quality, it is important to know the variability of production characteristics of quality breeding heads *(Radović et al., 2007*). The values for the traits fat thickness - back (FTB), fat thickness - rump (FTR), sum of fat thickness back and rump (FTBR), yield and share of meat in carcass sides (JUSKG and JUSPRO) obtained by this group of authors show that the examined traits of the progeny varied between the breeds of the sires, genotype and sex. Castrated males, compared to female animals, had on average thicker fat tissue in the middle of the back and rump (19.8

and 18.3 mm compared to 15.5 and 13.2 mm, respectively), lower yield (34.8 vs. 35.9 kg) and the share of meat in carcass sides (42.9 vs. 44.2%), respectively.

In the research of *Radović et al. (2003),* it is established that the genotype of fattening pigs did not influence the variation of the examined traits (age at slaughter, fat thickness - withers, middle of back, rump, back+rump, and percentage of meat in warm carcass sides), while the sires of the examined fatteners influenced the variation of all traits. One of the most important proofs of quality carcass sides is the meat content in carcass sides (*Radović I. et al., 2007).* A larger group of authors in their research show uniform results about the meat content in carcass sides, ranging from 41.71 to 43.32% (*Pušić and Petrović, 2004; Petrović et al., 2006a).*

Determining the meatiness of pig carcasses is important for both pig producers and meat processors, because the meatiness of pig carcasses significantly affects their market price. Pig carcass quality can be evaluated objectively using destructive and non-destructive methods or by using mathematical expressions specially constructed for this purpose *(Lukač et al., 2013).*

The economic efficiency of pig production depends on the duration of fattening, average daily gain, feed -conversion, slaughter efficiency, quality of carcass sides, etc. The breed plays an important role. The large white breed was one of the most common breeds of pigs on former public farms in our country, primarily because of outstanding production performance (*Kosovac, 2002*), while today the most common breed of pigs in the Republic of Serbia is the Landrace and Large White. The most important traits of the Large White breed are: fast growth, high meateness of the carcass sides, excellent feed conversion and very high quality meat *(Kosovac, 2002).* At the average length of the carcass side of 97.60 cm, the fat thickness (back and rump) is 26.3 and 35.8 mm, respectively. If the greater variability of some traits (thickness of bacon, weight of the pork chop), than the greater the possibility of their further improvement through selection.

Radović et al. (2013) have determined that the phenotypic correlations between: fat thickness - withers and fat thickness - back are strong and positive (r_p=0.638). The fat thickness - rump and the percentage of meat, that is, the fat thickness – the back and the percentage of meat, are very strong and negative (r_p= - 0.880 and - 0.895). Genetic correlations are stronger than phenotypic ones, so that the correlation between the fat thickness - the rump and the back is complete (r_g= 0.930), as well as between the fat thickness - the loin and the back and the meatiness is complete and negative (r_g= -0.979 and -0.982).

The aim of this study was to determine the impact of the farm, genotype and sex of fatteners and body weight at the end of fattening on the following traits: warm carcass side gain (WCSG), fat thickness of – the rump (FTR), fat thickness - the middle of the back (FTB), fat thickness - the rump + the back (FTRB), yield and meat content in carcass sides (JUSKG and JUSPRO), yield and share of French dressing in carcass sides (FDKG and FDPRO).

Materials and Methods

In this study, the production traits of fattening pigs were examined in two pig farms in the Republic of Serbia. The research included 1166 fattening animals of both sexes (female animal and castrated males), 22 genotypes. The trial included the following genotypes of the progeny: purebred Swedish Landrace (SL, n=70), Large white (LW, n=49), and Duroc (D, n=31), as well as crosses SL×LW (n=24), SL×D (n=14), SL×(SL×D×LW) (n=14), SL×(SL×D×D) (n=38), LW×SL (n=86), LW×(SL×LW) (n=71), LW×(SL×D) (n=126), LW×(SL×D×LW) (n= 22), LW×(SL×D×D) (n= 155), (H (Hampshire)×D)×(SL×LW) (n=38), (H×D) ×(SL×D) (n=12), (H×D) ×(SL×D×LW) (n=31), (H×D) ×(SL×D×D) (n= 78), D×SL (n=7), D×(SL×LW) (n=106), D×(SL×D) (n=11), D×LW (n=9), D×(SL×D×LW) (n=93), and D×(SL×D×D) (n=81). Fattening animals come from 29 sires. There were at least 7 progeny per sire.

The body weight of each fattening animal was measured at the end of the research, before pig slaughtering. After slaughter, the weight of the warm carcass sides and the weight of the French dressed warm carcas sides were measured. Fat tissue on the back, together with skin, was measured in the middle of the back (between the 13th and 15th lumbar vertebrae) and at the withers, where the *M. Gluteus medius* muscle grows the most into fat tissue. The sum of these measurements represents the thickness of the fat tissue - the back. The yield and content of meat in the carcass sides of pigs was determined using Tables 1 and 2, which are the integral part of the *Rulebook on the Quality of Slaughtered Pigs and Categorization of Pork ("Official Gazette of SFRY", 1985).*

The following traits were included in the research: warm carcass side gain (WCSG, g), fat thickness – the rump (FTR, mm), fat thickness - the back (FTB, mm), sum of fat thicknesses - the rump and the back (FTRL, mm), yield and the share of meat in cacrass sides (JUSKG, kg and JUSPRO, %), mass and shareion of French dressing of warm carcass sides (FRKG, kg and FRPRO, %). Data processing was performed by applying the appropriate computer program, i.e., by using the procedure of the least squares method (LSMLMW and MIXMDL - *Harvey, 1990*) in order to determine the significance ($P<0.05$) of systematic influences on the examined traits. The models included: genotype of fatteners, farm, sex. The examined traits were corrected/equalized to the average body weight at the end of fattening of 105.9 kg *(Gogić et al., 2019a).*

Two models were used for the analysis of the examined traits, namely:

Model 1.

$$Y_{ijkl} = \mu + G_i + F_j + P_k + b_1 (x_1 - \bar{x}_1) + \varepsilon_{ijkl}$$

where: Y_{ijkl} = observation, i.e. the manifestation of the trait of the *m*-th animal, of the *i*-th genotype, of the $_j$-th farm, and $_k$-th sex, μ = general population average, *G* = genotype of the animal, *F* = farm, *P* = sex, b_1 = linear regression influence of weight at the end of fattening , ε_{ijkl} = random error, *i* = subscript for genotype of the animal (*i* =1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18 .19.20.21.22), *j* = subscript for the farm (*j* =1.2), *k* = subscript for the sex (*k* =1.2), *l* = subscript for progeny.

The model applied to calculate genetic and phenotypic correlations is:

Model 2.
$Y_i = P_i + o_i + \varepsilon_i$

In Model 2, only the factors of sex of the fatteners (fixed) and sire (random) were included due to the limitations of the software package.
The Roemer-Orphal classification presented in the work of *Latinović (1996)* was used to determine the strength of the correlation between the tested traits in Table 1.

Table 1. Roemer-Orphal classification of the strength of correlation between traits *(Latinović, 1996)*

Range of correlation coefficients	The meaning of correlation
0.0-0.1	None
0.1-0.25	Very weak
0.25-0.4	Weak
0.4-0.5	Medium
0.5-0.75	Strong
0.75-0.9	Very strong
0.9-1.0	Complete

Results and Disscussion

All traits included in the trial were corrected to the same weight at the end of fattening (WEF) of 105.9 kg (also in work of *Gogić et al., 2019a*). The average values and standard deviations ($\bar{x} \pm$ SD) of the tested traits are shown in Table 2.

Table 2. Average values and variability of studied traits

TRAIT		$\overline{x}$ ± SD
WCSG	Warm carcass side gain, g	406.45±54.56
FTR	Fat thickness - rump, mm	15.94±4.99*
FTB	Fat thickness - back, mm	20.51±5.43
FTRL	Fat thickness - rump +back, mm	36.45±10.02*
JUSKG	Meat yield of carcass sides, kg	36.50±4.59
JUSPRO	Meat yield, %	43.30±1.68
FRKG	French dressing, kg	43.97±5.18
FRPRO	French dressing, %	52.27±3.32

* these two values were obtained in the work of *Gogić et al. (2019a),* using another model for examining the variability of the studied traits of fattening animals

The average value for the trait warm carcass side gain (WCSG) was 406.45 grams, thickness of fat tissue – the rump (FTR) was 15.94 mm, thickness of fat tissue – the back (FTB) 20.51 mm, thickness of total fat tissue – the rump and back (FTRL) 36.45 mm, yield of meat in carcass sides (JUSKG) 36.50 kg, share of meat in carcass sides (JUSPRO) 43.30%, yield of French dressing of carcass sides (FRKG) 43.97 kg and share of French dressing in carcass sides (FRPRO) 52.27%.

Compared to the results of *Sonesson et al. (1998)* we found lower values for body mass and much lower values for FTB compared to this study. The meat yield was 60.3%. In the study by *Gogić et al. (2014),* at a pre-slaughter body weight of 101 kg, the values for FTB, FTR, JUSKG and JUSPRO were respectively 17.22; 15.96; 35.39 and 43.61, whereby it can be concluded that the values are very similar in our research. While observed by genotypes for the Swedish Landrace breed, identical values were measured as in the work of *Gogić et al. (2014).*

Tables 3 and 4 show the influence of genotype of fatteners, farm and sex within Model 1 on the examined fatteners' traits. Observing the genotype of the fattener as a source of variation in traits, it can be seen that the animals of genotype 19 - D×LW had the highest values for the trait WCSG (405.57 g); animals of genotype 13 - (H×D)×(SL×D) have the highest values for the traits FTR and FTB (18.21 mm and 21.74 mm), and therefore also for the trait FTRL (39.95 mm); while the highest values for all four traits of yield and meat share were observed in animals of genotype 16 - D×SL (38.08 kg, 44.97%, 46.17 kg and 54.45%, respectively). Observing the farm as a source of variations in the investigated traits, it was determined that fattening animals raised on farm 1 had higher average values for WCSG (+35.21 g), FTR (+2.22 g) and FTB (+4.00 mm). The established differences in mean values for WCSG, FTR and FTB were statistically highly significant ($P<0.001$). Contrary to this, animals raised on farm 2 had more meat in carcass sides (37.28 vs. 36.44 kg or 44.23 vs. 43.20%). The established differences

of the mean values were highly significant. Taking sex of the animal as a source of variation in traits, it can be seen that female animals had lower fat thickness but higher meat yields in carcass sides, compared to castrated males. The body weight of the animal at the end of fattening (before slaughter) has a linear regression effect on the variation of all investigated traits ($P<0.001$). By observing the regression effect of body weight at the end of fattening on the tested traits, it can be seen that increasing the body weight at the end of fattening by 1 kg increases the values for all the tested traits, except for the values in percentages for the traits JUSPRO and FRPRO (negative sign).

Table 3. The effect of genotype, farm and sex of animals on studied fatteners' traits (LSM±S.E.)

Source of variation		WCSG[2)], g	FTR, mm	FTB, mm	FTRL, mm	JUSKG, kg	JUSPRO, %
Genotype	1[1)]	393.20±1.05	12.86±0.49	16.34±0.51	29.20±0.94	37.66±0.16	44.63±0.18
	2	402.15±1.76	14.98±0.83	19.19±0.86	34.17±1.58	36.71±0.27	43.58±0.30
	3	401.54±2.27	15.03±1.07	19.83±1.11	34.86±2.05	36.73±0.35	43.66±0.39
	4	393.47±2.28	15.27±1.07	17.89±1.11	33.15±2.05	37.08±0.35	43.93±0.39
	5	401.20±1.37	15.43±0.64	18.78±0.67	34.21±1.23	36.95±0.21	43.79±0.24
	6	396.33±0.94	14.53±0.44	18.80±0.46	33.33±0.84	36.95±0.14	43.84±0.16
	7	397.91±1.02	13.68±0.48	17.49±0.50	31.17±0.92	37.24±0.15	44.17±0.18
	8	396.61±0.77	16.33±0.36	20.10±0.37	36.42±0.69	36.40±0.12	43.20±0.13
	9	398.19±1.25	16.14±0.59	20.54±0.6	36.68±1.12	36.40±0.19	43.18±0.22
	10	394.41±1.80	14.03±0.85	18.40±0.88	32.43±1.61	37.15±0.27	44.06±0.31
	11	398.25±0.68	16.93±0.32	21.18±0.33	38.12±0.61	36.19±0.10	42.94±0.12
	12	396.38±1.41	14.61±0.66	19.00±0.69	33.61±0.27	36.99±0.21	43.89±0.24
	13	400.52±2.46	18.21±1.16	21.74±1.20	39.95±2.21	35.65±0.37	42.47±0.43
	14	397.21±1.55	12.84±0.73	17.18±0.76	30.02±1.40	37.49±0.24	44.46±0.27
	15	393.81±1.03	15.41±0.48	19.14±0.50	34.55±0.92	36.87±0.16	43.72±0.18
	16	394.52±3.21	11.68±1.51	15.23±1.57	26.91±2.89	38.08±0.49	44.97±0.56
	17	397.50±0.86	14.10±0.40	18.20±0.42	32.30±0.77	37.03±0.13	43.98±0.15
	18	396.81±2.56	16.83±1.21	21.48±1.25	38.32±2.30	36.17±0.39	42.91±0.44
	19	405.57±2.83	14.73±1.33	19.61±1.38	34.33±2.54	37.03±0.43	43.95±0.49
	20	395.40±1.56	14.73±0.73	19.55±0.76	34.28±1.40	36.91±0.24	43.72±0.27
	21	395.79±0.94	15.05±0.44	19.08±0.46	34.14±0.85	36.83±0.14	43.70±0.16
	22	400.08±0.94	16.38±0.44	21.15±0.46	37.53±0.85	36.31±0.14	43.07±0.16
Farm	1	415.19±0.40	16.10±0.19	21.08±0.19	37.18±0.36	36.44±0.06	43.20±0.07
	2	379.98±0.64	13.88±0.30	17.08±0.31	30.97±0.58	37.28±0.10	44.23±0.11
Sex	1[4)]	396.90±0.50	13.54±0.23	17.43±0.24	30.97±0.45	37.34±0.07	44.29±0.08
	2	398.27±0.46	16.44±0.22	20.74±0.22	37.18±0.41	36.37±0.07	43.15±0.08
BWEF (b)		3.718[3)***]	0.158***	0.169***	0.327***	0.331***	-0.015***

[1)] Genotype: 1-SL. 2-SL×LW. 3-SL×D. 4-SL×(SL×D×LW). 5-SL×(SL×D×D). 6-LW×SL. 7-LW×(SL×LW). 8-LW×(SL×D). 9-LW. 10-LW×(SL×D×LW). 11-LW×(SL×D×D). 12-(H×D)×(SL×LW). 13-(H×D)×(SL×D).14-(H×D)×(SL×D×LW).15-(H×D)×(SL×D×D). 16-D×SL. 17-D×(SL×LW). 18-D×(SL×D). 19-D×LW. 20-D. 21-D×(SL×D×LW). 22-D×(SL×D×D); [2)]

WCSG- warm carcass side gain; FTR- thickness of fat tissue – the rump; FTB- thickness of fat tissue – the back; FTRL- thickness of fat tissue – the rump +back; JUSKG-meat yield in carcass sides; JUSPRO-meat yield in percentage; BWEF- body weight at the end of fattening; [3] ***=P<0.001; [4] Sex 1 females; Sex2 castrated males

The values for the traits JUSKG and JUSPRO were slightly higher for the SL genotype, as well as SL×LW genotype, compared to the research by *Radović et al. (2007).*

Table 4. The effect of genotype, farm and sex of animals on studied fatteners' traits (LSM ±S.E.)

Source of variation		FRKG[2], kg	FRPRO, %
Genotype	1[1]	43.52±0.29	51.81±0.33
	2	44.07±0.48	52.48±0.56
	3	43.98±0.62	52.24±0.73
	4	43.13±0.62	51.09±0.73
	5	43.00±0.37	51.26±0.44
	6	43.58±0.26	51.82±0.30
	7	44.78±0.28	53.24±0.33
	8	43.14±0.21	51.35±0.25
	9	43.84±0.34	52.10±0.40
	10	43.90±0.49	52.24±0.57
	11	43.14±0.19	51.31±0.22
	12	45.05±0.38	53.57±0.45
	13	42.59±0.67	50.67±0.79
	14	44.35±0.42	52.66±0.50
	15	43.32±0.28	51.49±0.33
	16	46.17±0.88	54.45±1.03
	17	44.44±0.23	52.82±0.27
	18	44.93±0.70	53.24±0.82
	19	44.36±0.77	52.76±0.90
	20	43.90±0.42	52.17±0.50
	21	44.06±0.26	52.37±0.30
	22	43.89±0.26	52.20±0.30
Farm	1	44.53±0.11	52.90±0.13
	2	43.39±0.18	51.58±0.21
Sex	1[4]	44.90±0.13	53.38±0.16
	2	43.03±0.13	51.10±0.15
BWEF (b)		0.336[3]***	-0.091***

[1] Genotype: 1-SL. 2-SL×LW. 3-SL×D. 4-SL×(SL×D×LW). 5-SL×(SL×D×D). 6-LW×SL. 7-LW×(SL×LW). 8-LW×(SL×D). 9-LW. 10-LW×(SL×D×LW). 11-LW×(SL×D×D). 12-(H×D)×(SL×LW). 13-(H×D)×(SL×D).14-(H×D)×(SL×D×LW).15-(H×D)×(SL×D×D). 16-D×SL. 17-D×(SL×LW). 18-D×(SL×D). 19-D×LW. 20-D. 21-D×(SL×D×LW). 22-D×(SL×D×D); [2] FRKG-French dressing in kg; FRPRO-French dressing in percentages; BWEF-body weight at the end of fattening; [3] ***=P<0.001; Sex 1 females; Sex2 castrated males

In the presented model, female animals had thinner fat tissue but a higher yield and share of meat compared to castrated males, which is in agreement with the research of *Radović et al. (2007)* and *Gogić et al. (2014),* where the values for the share of meat are almost identical to the results presented in the work of these two groups of authors. Estimated meatiness on live female animals of the Swedish Landrace breed in the research of *Gogić et al. (2019b)* shows a value of 58.94% which is significantly higher compared to our work for the trait FRPRO measured according to the Rulebook.

Table 5 shows the levels of significance of the influences included in the models on the studied traits of fattening animals.

Table 5. Statistical significance (level of significance) of the influences included in the models on the studied traits of fattening animals

Source of variation (the influence)		WCSG[1)]	FTR	FTB	FTRL	JUSKG	JUSPRO	FRKG	FRPRO
Model 1	Genotype	***[2)]	***	***	***	***	***	***	***
	Farm	***	***	***	***	***	***	***	***
	Sex	**	***	***	***	***	***	***	***
	R^2	0.977	0.380	0.441	0.440	0.924	0.260	0.807	0.354

[1)]WCSG- warm carcass side gain; FTR- thickness of fat tissue – the rump; FTB- thickness of fat tissue – the back; FTRL- thickness of fat tissue – the rump +back; JUSKG-meat yield in carcass sides; JUSPRO-meat yield in percentage; FRKG-French dressing in kg; FRPRO-French dressing in percentages; R^2-coefficient of determination; [2)] **=P<0.01; ***=P<0.001

In the Model, the genotype of the fattening animals, the farm and the sex of the animals were included as sources of variation, and it was determined that all three factors have a statistically significant effect on the variation of all traits of the fatteners (P<0.01; P<0.001). The coefficient of determination R^2 showed that the effects included in the Model (fatteners' genotype, farm and sex) explained the variation of WCSG with 97.7%, the variation of FTR with 38.0%, the variation of FTB with 44.1%, the variation of FTRL with 44.0%, the variation of JUSKG with 92.4%, the variation of JUSPRO with 26.0%, FRKG variation with 80.7% and FRPRO variations with 35.4%. So, the variations of WCSG and JUSKG were mostly explained by factor effects, and the least the variation of JUSPRO.

The application of this model showed that the sex of animals had a statistically significant effect on the traits of FTB, FTR, FTRL, JUSKG and JUSPRO, which is in agreement with the research of *Petrović et al. (2006b).*

The sex and genotype of the fattening animals in the Model had a statistically significant effect on the variation of all the examined traits of the fattening animals, which is in agreement with the research of *Radović et al. (2007).*

Table 6 shows the genetic and phenotypic correlations, where genetic correlations are presented above the diagonal, while phenotypic correlations are presented below the diagonal.

Table 6. Genetic and phenotypic correlations

TRAITS	BWEF	WCSG	FTR	FTB	FTRL	JUSKG	JUSPRO	FRKG	FRPRO
BWEF		0.866**	0.567**	0.547**	0.563**	0.937**	-0.296**	0.886**	-0.013NS
WCSG	0.951**		0.689**	0.773**	0.748**	0.714**	-0.530**	0.898**	0.269**
FTR	0.446**	0.475**		0.944**	0.981**	0.254**	-0.930**	0.416**	-0.219**
FTB	0.457**	0.532**	0.827**		0.990**	0.232**	-0.934**	0.537**	0.084**
FTRL	0.472**	0.528**	0.952**	0.960**		0.245**	-0.945**	0.493**	-0.044NS
JUSKG	0.955**	0.886**	0.189**	0.203**	0.205**		0.056NS	0.837**	0.011NS
JUSPRO	-0.138**	-0.208**	-0.861**	-0.854**	-0.897**	0.161**		-0.246**	0.061*
FRKG	0.885**	0.874**	0.204**	0.260**	0.244**	0.903**	0.071*		0.452**
FRPRO	-0.348**	-0.266**	-0.541**	-0.458**	-0.520**	-0.218**	0.429**	0.125**	

The correlation coefficient for 5 and 1% certainty (d.f. =1000) is 0.062 and 0.081. Anything less than 0.062 is NS, between 0.062 and 0.081 is *, over 0.081 is **. NS=P>0.05; *=P<0.05; **=P<0.01;

In the observation of the phenotypic correlations in the research of *Sonesson et al. (1998)* a strong negative phenotypic correlation (r_P=-0.67) between fat tissue thickness and meatiness is presented, while in our research a negative phenotypic correlation between these two traits was found, with the difference that the correlation is very strong (r_p=-0.854). Meatiness shows a very strong genetic correlation with back fat thickness (r_g=-0.77), while in our research the genetic correlation is complete and also negative (rg=-0.934).

The phenotypic correlation of the traits of the carcass sides was of different strength (from very weak to complete) and signs, which is in agreement with the research of *Petrović et al. (2006b),* where this group of authors establishes a positive weak or very weak correlation between the warm carcass side gain and the fat thickness (FTB and FTR), while in our research the correlations were positive but stronger (strong correlation). A very weak, negative and statistically significant correlation was established between WCSG and JUSKG, i.e. WCSG and JUSPRO traits, so that a more intensive growth led to an increase in the thickness of fat tissue and a decrease in the amount or content of meat in warm carcass sides *(Petrović et al., 2006b),* while in our research, in the case of the WCSG:JUSKG correlation, a positive and very strong connection was established. The value of phenotypic correlation between FTB and FTR traits was positive and strong (+0.610), while in our research it was also positive but very strong (+0.827). A very strong and positive phenotypic correlation exists between FTB and FTR traits, in study by *Radović et al. (2013)* showing a positive and complete phenotypic correlation. Also, the same group of authors states a negative and complete correlation between fat tissue thickness and meat yield, while in our research the

values are negative and the correlations are very strong. Genetic correlations are stronger than phenotypic ones, so between FTB and FTR thickness of fat tissue they were complete and complete and negative between meat yield and FTB and FTR traits, which is in agreement with the research of *Radović et al. (2013).*

Conclusion

This experiment aimed to determine the variation of carcass sides quality traits of 22 genotypes originating from 29 boars. There were a total of 1166 fattening animals of both sexes (females and castrated males) from two farms. The influence of the fattenrs' genotype, farm and sex on the variation of the traits of the fattening animals was examined. Based on the results obtained from the experiment, we concluded the following: by applying this Model, it was determined that the genotype, sex of the fattening animal and the farm had a statistically very high influence on all the examined traits of the fattening animals. The genotypes DxSL (44.97%) had the highest share of meat in the carcass sides and genotype SL (44.63%) for the trait JUSPRO, while for the trait FRPRO the highest value was measured for the genotype DXSL (54.45%). In the research, the highest thickness of fat tissue had the genotypes (HxD)x(SLxD) and Dx(SLxD) - 39.95 and 38.32 mm, respectively, leading to lower share of meat in the carcasses. Genetic and phenotypic correlations ranged from very weak (for certain traits they do not even occur) to complete correlations, with different signs. For a more precise determination of the yield of meat in carcass sides, it is necessary to apply a combination of non-destructive and destructive methods. In other words, we should take into account the results obtained on live animals with the help of ultrasound devices for measuring fat tissue thickness and meatiness, as well as the results obtained at the slaughter line by applying the Rulebook and dissection of carcass sides.

Uticaj genotipa. farme i pola na proizvodne osobine tovljenika plemenitih genotipova svinja

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Rezime

Na dve farme svinja (farma A i farma B) u Centralnoj Srbiji sprovedeno je ispitivanje proizvodnih osobina 22 genotipa tovljenika pod uticajem sledećih faktora: farma. genotip i pol tovljenika. i masa na pre klanja. Osobine tovljenika koje su uključene u istraživanje su: prirast tople polutke (WCSG); debljina slanine na krstima (FTR); debljina slanine na leđima (FTB); debljina slanine krsta+leđa (FTRL); prinos mesa u polutkama (JUSKG) i prinos mesa u procentima (JUSPRO). U ogledu su korišćena oba pola (ženska nekastrirana grla i muška hirurški kastrirana grla). Obuhvaćeno je 1200 tovljenika ispitivanjima. Statistička obrada podataka je sprovedena korišćenjem kompjuterskog programa Harvey. Svi uključeni faktori u korišćenim modelima utiču visoko statistički značajno na variranje osobina tovljenika ($P<0.01$; $P<0.001$). Izračunavanjem genetskih i fenotipskih korelacija došlo se do zaključka da je fenotipska povezanost osobina polutki bila je različite jačine (od jako slabe do potpune) i predznaka. dok su genetske korelacije jače od fenotipskih tako da su potpune između debljina slanine FTB i FTR a potpune i negativne između prinosa mesa i osobina FTB i FTR.

Ključne reči: tovljenici, genotip, pol, debljina slanine, prinos mesa, genetske i fenotipske korelacije

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EFFECT OF USING VANILLA SWEET AROMA IN DIETS FOR WEANING PIGS

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Abstract: The trial was conducted on 108 piglets of crossbreeds Landrace x Large White. Aim of this study was to determine influence of this aroma on production parameters of growing pigs. Whole trial was in total 57 days, and it was split in three trial periods. Piglets were weaned on day 27, when the trial started. First trial period was 18 days (27-44 day), second was 28 days (45-72 day) and third was 11 days (73-83 day). The control groups received standard farm mixtures, and the trial groups had added aroma Vanilla Sweet in different concentrations (0.02; 0.04%, respectively). During the first period, there was statistical difference ($p<0.05$) in all three prameters between the groups. Feed intake (FI), average daily gain (ADG) and feed conversion (FCR) differed between C and T1 group. T1 had best ADG of 261.53 g/d and FCR of 1.89 g/g. In the second period statistical significance was noted in FI and ADG, between T1 and other two groups. T1 had lower FI and ADG, but better FCR compared to control group. In the final period second experimental group had the best results in both FI (1309.29 g/d) and ADG (696.43 g/d). And eventualy for whole trial T2 had better results in all three production parametars compared to other two trial groups. In general, obtained results showed that use of Vanilla sweet aroma can be recommended in the nutrition of weaned pigs. Further investigation should be conducted to determine the effect of this flavour on fatteners.

Key words: piglets, nutrition, flavour, rearing

Introduction

Pork meat consumption is one of the largest in the world in recent years. In Serbia in 2021, the swine population was approximately 2.9 million heads and the pork meat production is 428 thousand tons (*FAO, 2021*). The intensive genetic selection of sows has resulted in a greater number of live born piglets per litter but with bigger problems at birth weight and increased animals mortality and productive efficiency (*Quisirumbay Gaibor and Vílchez Perales, 2019*).

Taste of foods is the problem that comes even from the beginning of domestication of animals. Livestock food has its nutritive value and characteristic smell and it is determined by its quality, composition and type of feeds used. Good feed intake is quite essential for the proper and healthy development of animals. Animals always first will take tasteful food, and only in case of starvation they would consume food they do not like. Pigs have really well developed sense of taste and smell, so the major problems with feed intake almost always occur in younger categories, or with the changes of feed mixtures.

Weaning is a great challenge of the modern swine industry and adjusting correct mixtures to satisfy pig basic functions, growth performance and welfare, is of crucial importance (*Val-Laillet et al., 2016*). Weaning is also the stage of greatest stress for piglets with a consequent decrease in dietary intake and weight loss, due to exposure to factors such as separation from the mother, switch from liquid to solid diet, new social order within the group, change of facilities, health challenge, food competition and other factors (*Barba-Vidal et al., 2018; Escribano et al., 2019*). Previous studies found that prenatal and postnatal flavoring may reduce effects related to stress, influence piglets acceptance and stimulate food intake (*Mennella et al., 2001; Oostindjer et al., 2010*). Some researchers have reported that feeding sows and their piglets with plant based aroma extracts in feed can improve production and also easy the transition in the period of weaning (*Charal et al., 2016; Oostindjer et al., 2011*). For that reason, generally, an optimally functioning gastrointestinal tract is very important to the overall metabolism and performance of pigs of all productive stages (*Pluske et al., 2018*). It has also been investigated that flavors has a tendencies to reduce weight loss of the sows, weaned piglets, and also can increase their survival rate (*He et al., 2017*). Early exposure to some flavors may result in later preference for these flavors. Some investigations has evidenced that flavors has important role later in life and could positively affect the acceptance of food with similar flavor and, therefore, can be beneficial to all production parameters (*Blavi et al., 2016*). Aim of this study was to determine influence of this aroma on production parameters of growing pigs.

Materials and Methods

The trial was conducted on 108 piglets of crossbreeds Landrace x Large White. All piglets were split in three treatments: control (C) and 2 trial groups (T_1, T_2). Each treatment had three replications (pens) with 12 piglets per pen. Weaning took place on day 27, when the trial started. Piglets were held in same environmental conditions, with same temperature, humidity and lighting. Whole trial was in total 57 days, and it was split in three trial periods. First trial period was 18 days (27-44 day), second was 28 days (45-72 day) and third was 11 days (73-83 day). During the observed three periods, three mixtures have been used (Table 1).

The control groups received standard farm mixtures, and the trial groups had same mixtures with added aroma Vanilla Sweet in different concentrations (0.02; 0.04%, respectively). The feed additive (aroma) used in this trial was artificial vanilla flavor from Polar Bear, China.

Table 1. Ingredient and nutrient composition of mixtures used in experiment

	Mixture 1 Day 27-44			Mixture 2 Day 45-72			Mixture 3 Day 73-83		
Group	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
Ingridients g/kg									
Corn	514.5	514.3	514.1	586.8	586.6	586.4	629.6	629.4	629.2
Wheat flour	-	-	-	25.0	25.0	25.0	50.0	50.0	50.0
Sugar	30.0	30.0	30.0	-	-	-	-	-	-
Soybean meal	205.0	205.0	205.0	182.0	182.0	182.0	165.0	165.0	165.0
Sunflower meal	-	-	-	20.0	20.0	20.0	25.0	25.0	25.0
Ecofish meal	50.0	50.0	50.0	45.0	45.0	45.0	40.0	40.0	40.0
Extruded full-fat soybean semolina	120.0	120.0	120.0	100.0	100.0	100.0	50.0	50.0	50.0
Milk replacer	40.0	40.0	40.0	-	-	-	-	-	-
Calcium carbonate	14.0	14.0	14.0	16.0	16.0	16.0	15.0	15.0	15.0
Monocalcium phosphate	12.0	12.0	12.0	10.0	10.0	10.0	10.0	10.0	10.0
Sodium chloride	2.5	2.5	2.5	3.2	3.2	3.2	3.2	3.2	3.2
Premix*	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
L-lysine	-	-	-	-	-	-	0.2	0.2	0.2
Minazel**	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Aroma Vanilla Sweet***	-	0.2	0.4	-	0.2	0.4	-	0.2	0.4
Calculated nutrient composition, g/kg of feed****									
Crude protein	218.00			192.40			178.40		
Lysine	12.90			10.80			9.60		
Methionine	4.00			3.40			3.30		
Cysteine	3.40			3.20			3.10		
Threonine	8.60			7.50			6.90		
Tryptophan	2.50			2.20			2.00		
Crude fiber	45.90			42.60			37.10		
Crude fat	49.80			47.70			41.90		
Calcium	11.55			11.17			10.41		
Phosphorus	7.71			6.78			6.69		
DE content, MJ/kg	16.58			16.41			16.25		

*Added per kg diet: 15,000 IU Vitamin A, 1500 IU Vitamin D3, 40 IU Vitamin E, 1.0 mg Vitamin K3, 2.0 mg Vitamin B1, 4 mg Vitamin B2, 10 mg d-Pantothenic acid, 18 mg Niacin, 70 mg Biotin, 18 mg Vitamin C, 0.03 mg Vitamin B12, 4 mg Vitamin B6, 170 mg Fe: Fe(II) sulphate, 4 mg Cu: Cu(II) sulphate, 16 mg Zn: Zn(II) oxide, 50 mg Mn: Mn(II) oxide, 0.304mg KI, 0.3 mg Se: Se-selenite.

**Natural mycotoxin adsorbent.

*** Polar Bear, China, 100% Vanillin;

**** Difference between groups within one mixture is irrelevant

Piglets were fed *ad libitum.* Average daily feed intake (FI) was calculated by subtracting unconsumed feed at the end of trial from the pre weighed amount and splited by the days. Body mass were weighted at start and at the end of trial. Piglets were weighed at the beginning and at the end of the experiment and the average daily gain (ADG) were calculated with the following equation:

$$ADG = \frac{\text{Final weight - Initial weight}}{\text{Duration of the experiment (days)}}$$

Feed conversion (FCR) was also calculated:

$$FCR = \frac{\text{Daily feed intake}}{\text{Daily weight gain}}$$

All statistical analyses were performed using R-project software. For the purpose of production performance analysis one way ANOVA was used while the Tukey test served to determine the statistical significance of the differences between individual means values.

Results and Discussion

Production performances were shown in Table 2. During the first period, there was statistical difference ($p<0.05$) in all three prameters between the groups. Feed intake (FI), ADG and FCR differed between C and T_1 group. T_1 had best ADG of 261.53 g/d and FCR of 1.89 g/g. In the second period statistical significance was noted in FI and ADG, between T_1 and other two groups. T_1 had lower FI and ADG, but better FCR compared to control group. In the final period second experimental group had the best results in both FI (1309.29 g/d) and ADG (696.43 g/d). And eventualy for whole trial T_2 had better results in all three production parametars compared to other two trial groups. There was no mortalities in trial.

Table 2. Production performance (mean ± SE) of post-weaning piglets fed with (T_1 and T_2) or without (C) added aroma in the feed

	Treatments		
	C	T_1 0.02%	T_2 0.04%
First period (27-44d)			
FI, g/d	387.11±0.016^{b}	426.64±0.023^{ab}	494.29±0.026^{a}
ADG, g/d	171.29±0.018^{b}	212.26±0.018^{ab}	261.53±0.023^{a}
FCR, g/g	2.26±0.045^{b}	2.01±0.025^{ab}	1.89±0.005^{a}
Second period (45-72d)			
FI, g/d	955.63±0.011^{a}	739.06±0.026^{b}	869.39±0.009^{a}
ADG, g/d	461.66±0.024^{a}	367.69±0.045^{b}	477.69±0.036^{a}
FCR, g/g	2.07±0.031	2.01±0.068	1.82±0.099
Third period (72-83d)			
FI, g/d	1217.99±0.023^{b}	1219.05±0.087^{b}	1309.29±0.036^{a}
ADG, g/d	615.15±0.078^{b}	603.49±0.036^{b}	696.43±0.036^{a}
FCR, g/g	1.98±0.011	2.02±0.045	1.88±0.059
Whole trial (27-83d)			
FI, g/d	822.86±0.015^{a}	732.29±0.069^{b}	810.58±0.035^{a}
ADG, g/d	386.32±0.016^{b}	362.52±0.023^{b}	431.16±0.026^{a}
FCR, g/g	2.13±0.022	2.02±0.051	1.88±0.013
Mortality, %	-	-	-

SEM, Standard error of the means; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; $^{a, b, c}$ In a row, the least squares means with a different superscript differ significantly ($p<0.05$)

Low nutrient intake of piglets during the first few days after weaning is a serious problem which influences intestinal integrity and later performance (*Spreeuwenberg et al., 2001*). Adding certain flavours during lactation period could enhance diet acceptance in later life, can improve ingestion, and to improve adaptability to conditions after weaning (*Langendijk et al., 2007*).

In previous studies of the nutritive value of the aroma *Vanilla butter cream* used in nutrition of suckling piglets, results showed the increase of their weaning mass by 390 g per litter (*Saftić et al., 2003; Živković et al., 2003*). Similar studies also showed that the piglets fed the mixtures containing aromas were heavier by 3.5% (*Ilsley et al., 2002*) and 7.35% (*Piva et al., 1989*) compared to the animals fed with mixtures without supplemented flavours, which concludes with our study. In case of fatteners, aroma influenced the improvement of ADG (*Kwon et al., 2001*) and in case of Apple aroma improvement of ADG by 4.78%, FCR by 3.75%, level of utilization of crude proteins, slaughter yields and lower price for 1 kg of gain by 2.06% (*Saftić et al., 2005*).

Moreno-Santillán et al. (2022) observed that the use of aromas (banana and cinnamon) in the diet of weanling piglets did not improve the weight gain, feed intake, feed/gain ratio after one month of feeding. Similar results were found by other researchers (*Blavi et al., 2016; Wang et al., 2021*). In the study of *Wang et al. (2014)* not all flavours accomplished the purpose of increasing feed intake. Fruit-milk flavour had little effect on the performance of sows and piglets, unlike the fruit-milk-anise flavour that had positive effect on production performance.

Conclusion

The effects of use of Vanilla sweet aroma in the nutrition of weaned pigs were investigated. Obtained results showed that introduction of studied Vanilla sweet aroma in mixtures have positive effects in the following way:

- Better feed intake in trial compare to the control group
- Animals of the experimental groups fed diet containing Vanilla sweet aroma had better ADG than control groups.
- Investigated flavour showed no differences in regard to FCR between the groups for the whole trial.

In general, obtained results showed that use of Vanilla sweet aroma can be recommended in the nutrition of weaned pigs. Further investigation should be conducted to determine the effect of this flavour on fatteners.

Efekat korišćenja arome slatke vanile u ishrani prasadi nakon zalučenja

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Rezime

Ogled je sproveden na 108 prasadi meleza Landras x Veliki Jorkšir. Cilj ovog istraživanja je bio da se ispita uticaj arome slatke vanile na proizvodne parametre prasadi u odgoju. Ceo eksperiment je ukupno trajao 57 dana i bio je podeljen u tri perioda. Prasad su zalučena sa 27 dana, kada je i sam ogled počeo. Prvi period je trajao 18 dana (27-44 dan), drugi 28 dana (45-72 dan) i treći 11 dana (73-83 dan). Kontrolne grupe su dobijale standardnu farmsku smešu, dok je u smešu za ogledne grupe dodavana aroma slatke vanile u različitim koncentracijama (0,02; 0,04%). Tokom prvog perioda postojala je statistička značajnost ($p<0,05$) za sva tri

proizvodna parametra između grupa. Unos hrane (FI), prosečan dnevni prirast (ADG) i konverzija (FCR) razlikovali su se između C i T1 grupe. T1 je imala najbolji ADG od 261,53 g/d i FCR od 1,89 g/g. U drugom periodu zabeležena je statistička značajnost kod FI i ADG, između T1 i druge dve grupe. T1 je imala niže FI i ADG, ali bolji FCR u poređenju sa kontrolnom grupom. U završnom periodu ogleda, druga eksperimentalna grupa je imala najbolje rezultate u FI (1309,29 g/d) i ADG (696,43 g/d). Kada se na kraju sagleda ceo ogled T2 grupa je imala najbolje rezultate za sva tri proizvodna parametra u poređenju sa druge dve ispitivane grupe. Generalno, dobijeni rezultati su pokazali da se upotreba arome slatke vanile može preporučiti u ishrani odbijenih prasadi. Trebalo bi sprovesti dalje istraživanje kako bi se utvrdio efekat ove arome i na tovljenike.

Ključne reči: prasad, ishrana, ukusi, zalučenje

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THE EFFECT OF SPACE ALLOWANCE IN THE CAGE AND FLOOR SYSTEMS ON FEATHER CONDITION AND EGG PRODUCTION

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Abstract: The egg production sector is in a transitional period with regard to the permitted housing systems, i.e. rearing in conventional cages under certain conditions to the complete ban of any form of cage system. The changes were caused by concern for the layer wellfare but with the expected effects on productivity as well. The aim of the research was to determine the effects of the floor space allowance in cage and non-cage housing systems on the feather score and egg production of laying hens of three ages, from the aspect of the regulated minimum and optimal space in the cage system (C) and the space provided in the extensive rearing system with hens in smaller groups in the facility (extensive indoor -EI). In order to determine the feather score, the body weight of the laying hens and the weight of the eggs, three groups of laying hens were formed: C4 (564 cm^2 per hen, cage system), C3 (751 cm^2 per hen, cage system) and EI (3000 cm^2 per hen, extensive indoor). The feather score and body weight of laying hens were determined in three ages of hens (30, 40, 50 weeks), by individual assessment and measurement of all hens in the experiment. The hen-day egg production and egg weight were determined in the same weeks of laying age. In addition to the expected decrease in feather score with the age of hens, results indicated a significant interaction between age and space allowance per hen. Observed by individual body parts, as well as based on the overall feather score, the space allowance per hen exhibited a full, cumulative effect at 50 weeks of age. Based on the space allowance, it was possible to rank the overall feather score, with the laying hens with the most space having the best feather score. The effect of the space allowance on the body weight of the laying hens was manifested through the space available on the feeder, which resulted in the lowest ($p<0.01$) body weight

values recorded in laying hens of the C4 group. Egg production was not significantly influenced by the space allowance per hen ($p=0.069$), but a connection between egg production and the housing system can be concluded. The average egg weight, in addition to the known effect of layer age, was the lowest ($p<0.01$) in the group with the least space allowance per hen.

Key words: laying hen, cage, non-cage systems, feather score, egg production

Introduction

Feather condition is an indicator of the health status of laying hens and is one of the parameters used to assess welfare. To some extent, feather damage and wear is a normal process related to the age of the layer. Feather damage occurs as a consequence of feather pecking, an abnormal behavior with a prevalence in the flock between 24 and 94 % *(Mens et al., 2020).*

Previous studies suggest that feather pecking is a multifactorial problem with a genetic basis. Individual selection for high egg production led to changes in behavior patterns and the occurrence of severe feather pecking (SFP), which leads to cannibalism *(Nicol et al., 2013).* In the studies of *Campe et al. (2018)* and *Ozenturk et al. (2022),* differences in feather condition between genotypes of laying hens are related to feather colour. The level of stress and state of fear in the flock has been linked to feather pecking based on the finding of lower corticosterone levels in second-generation hens selected for low mortality due to feather pecking, compared to non-selected hens (*Rodenburg et al., 2013*). Also, the authors indicate the importance of environmental conditions, which, by establishing an interaction effect with the genotype, can lead to certain deviations in the mentioned association. Many factors that exert effects on the welfare of laying hens, causing stress, influence the occurrence of feather pecking *(Mens et al., 2020).*

The effect of the housing system on the condition of the feathers can be manifested through the space allowance and the enrichment of the space. In cage systems, the cage material plays a significant role in the condition of the feathers, and it can increase the wear and damage of the feathers by abrasion *(Widowski et al., 2017)*. In non-cage systems, exposure of birds to feather pecking in large groups is increased. Also, the condition of feathers in non-cage rearing systems is affected by the way manure is managed, through air quality, as well as the type of floor: wire slatted floor or floor with litter (*Decina et al., 2019*). By comparing cage and non-cage housing systems, better feather condition of laying hens was determined in a floor system with litter *(Zorman Rojs et al., 2020; Pichova et al., 2016).* Similarly, in the free range system, a better condition of the feathers was determined compared to the laying hens in conventional and enriched cages (*Dikmen et al.,*

2016). However, there are different study results. *Petrik et al. (2015)* find no differences in feather scores in laying hens reared in conventional cages and floor systems. Similarly, no differences have been demonstrated in the study by *Khumput et al. (2019)* between hens in conventional and enriched cages. In the same study, stocking density in a cage system shows a greater effect on feather condition than cage type. The effects of stocking density on feather condition in non-caged systems are inconsistent, somewhat influenced by group size, and require further investigation *(Nicol et al., 2013; Liebers et al., 2019).*

Poor feathering makes thermoregulation difficult, increases energy needs and, in this sense, increases food consumption *(Sarica et al., 2008).* A high correlation between feathering of hens and food consumption, as well as higher egg production of hens with better feather condition, was confirmed in the study by *Glatz (2001),* while differences in egg weight were not determined.

Changes in the egg production sector related to housing systems are implied by the concern for the welfare of the laying hens. The tendency is to completely abandon cage systems in the EU as inhumane and undesirable. In Serbia, the egg production sector has been in a transition period towards the banning of conventional cages for the last decade. The currently valid legislation on animal welfare allows for laying hens to be reared in conventional cages under certain conditions, which, among others, refer to compliance with the minimum floor space allowance of 550 cm^2 per layer, excluding the feeding area.

Based on the above, the objective of the study was to determine the effects of the floor space allowance in caged and non-caged housing systems on the condition of feathers and the production of laying eggs at three ages, from the aspect of the regulated minimum and optimal space allowance in the cage system (C) and the space provided by extensive rearing of laying hens in smaller groups in the facility (extensive indoor - EI).

Material and Methods

The trial was carried out using Isa Brown laying hens, which at the age of 16 weeks were moved into a facility with conventional cages and into a facility with extensive indoor system that was divided into pens. The cage floor space allowance, per hen was 564 cm^2 and 751 cm^2, respectively, which was achieved by having 4 and 3 hens per cage, respectively. The hens had access to two nipple drinkers per cage and a feeding space length of 12.3 cm and 16.3 cm, respectively. The pen in the extensive indoor system provided floor space allowance of 3000 cm^2 per hen (3 hens/m^2), which allowed the hens considerable mobility within the box. Each box was equipped with two bell feeders, one round drinker and three nests for 20 hens. The pen floor was covered with chopped straw litter. The diet for laying hens was identical for hens in both rearing systems, with the same mixtures according to the hybrid manufacturer's recommendations for each stage of the

production cycle. Other technological norms (lighting, ventilation, temperature) were aligned with the needs of hybrids and were controlled in both facilities. During the production cycle, following parameters were recorded daily: number of eggs, feed consumption, mortality.

In order to determine the condition of the feathers, body weight of laying hens and weight of eggs, three groups of laying hens were formed: C4 (564 cm^2 per hen, cage system), C3 (751 cm^2 per hen, cage system) and EI (3000 cm^2 per hen, extensive indoor), with 3 repetitions (cage tier segment, i.e. pen), a total of 144 laying hens.

The condition of the feathers and the body weight of the laying hens were determined at three ages of hens (30, 40, 50 weeks), by individual assessment and measurement of all the hens in the trial. The hen-day egg production and egg weight were determined in the same weeks of laying age. During each week, three days in a row, all eggs laid within 24 hours were recorded, sampled and measured for each cage, pen, and layer group.

Feather score was determined by evaluating the feathers of five body parts (neck, breast, back, wings and tail). In addition, by summing up the scores, the total feather score was determined. A feather rating scale of 1 to 4 was applied, with a score of 1 indicating complete bare skin or skin with few feathers; 2 – a greater number of exposed places, more than 1/2 of the surface; 3 – a small part of the skin stripped (1/3) or feathers damaged (worn/deformed); 4 – complete feather coverage, undamaged or slightly worn feathers *(Sarica et al., 2008).*

Statistical data processing was performed using the STATISTICA software package (StatSoft Inc., 2012). A two-factorial analysis of variance of the effect of group and laying age on the feather score was applied. In addition, the effect of the group, that is, the floor space allowance, on the condition of the feathers was examined by a one-factor analysis of variance in each of the examined weeks of laying age. Hen-day egg production, body weight of laying hens and egg weight were analyzed by two-factor analysis of variance of the effect of group and laying age. The significance of the differences was assessed by LSD post hoc test. Data for feather score and hen-day egg production were transformed before statistical analysis in arcsine values.

Results

The results of the two-factor analysis of the variance of the effect of the space allowance per layer and their age on the condition of feathers are shown in table 1. A significant influence of both investigated factors, as well as their interaction on the overall feather score, was determined. It is observed that the overall feather score decreased with the age of laying hens and increased with the larger space allowance per layer.

The effect of the layer age showed the same, already mentioned, regularity of decreasing scores with a higher age of laying hens, as well as with regard to the feather score by individual regions of the body. In regard to the examined space allowances per layer, certain deviations were found regarding the influence on the condition of the feathers on the back and breast. The feather scores for the laying hens' back was not significantly influenced by the group, i.e. by the space allowance, nor by the interaction effect of the group and the layer age. The condition of the feathers on the breast was the best scored in the EI group, while the differences between the cage system were not significant regardless of the differences in the floor space allowance.

Table 1. Effects of space allowance and layer age on feather score (scoring scale 1-4)

Feather score, point		Neck	Breast	Back	Wings	Tail	Total
Experim. group	C4	3.10 ± 1.04^{c}	3.17 ± 0.89^{b}	3.47 ± 0.85^{ns}	3.37 ± 0.66^{c}	2.99 ± 1.02^{c}	16.10 ± 3.73^{c}
	C3	3.41 ± 0.87^{b}	3.33 ± 0.76^{b}	3.67 ± 0.64^{ns}	3.54 ± 0.53^{b}	3.33 ± 0.69^{b}	17.29 ± 2.82^{b}
	EI	4.00 ± 0.00^{a}	3.81 ± 0.51^{a}	3.62 ± 0.75^{ns}	3.97 ± 0.16^{a}	3.74 ± 0.46^{a}	19.14 ± 1.23^{a}
Age, week	30	4.00 ± 0.00^{a}	3.93 ± 0.29^{a}	3.97 ± 0.18^{a}	3.83 ± 0.38^{a}	3.89 ± 0.32^{a}	19.61 ± 0.66^{a}
	40	3.66 ± 0.64^{b}	3.45 ± 0.68^{b}	3.65 ± 0.70^{b}	3.64 ± 0.50^{b}	3.34 ± 0.67^{b}	17.75 ± 2.05^{b}
	50	2.82 ± 1.07^{c}	2.91 ± 0.89^{c}	3.13 ± 0.91^{c}	3.40 ± 0.68^{c}	2.82 ± 0.95^{c}	15.08 ± 3.63^{c}
Significance							
Group		***	***	ns	***	***	***
Age		***	***	***	***	***	***
Interaction		***	***	ns	***	***	***

C4-564 cm^2/hen, cage; C3-751 cm^2/hen, cage; EI-3000 cm^2/hen, extensive indoor
NS-non significance; **- $p<0.01$; ***-$p<0.001$; a, b, c- significant differences for the same row

In order to get a clearer view of the effect of space allowance, a one-factor analysis of the effect of space allowance on the feather score in three laying ages was performed (table 2). The obtained results showed that in 30-week-old hens, the floor space allowance had no significant influence on the overall feather score. At the age of 40 weeks, the differences in the overall feather scores were differentiated between the EI group, on the one hand, and the C3 and C4 groups, on the other hand. The overall feather score between the C3 and C4 groups did not differ. At the next age period (50 weeks), overall feather score was clearly differentiated between all three groups in relation to floor space allowance. Laying hens with the largest space allowance (EI) had statistically significantly highest total feather scores and it decreased with the reduction of available space in groups C3 and C4.

Looking at the feather scores by body regions, at 30 weeks of age, the effect of space allowance was manifested only on wing feather scores. A significant difference was found between the EI and C4 groups. At 40 weeks of age, feather scores differed significantly for all body regions, except the back

feather score, between space allowances in EI and C3, or EI and C4. Feathers on the neck were scored significantly worse in the C4 group compared to the C3 and EI groups. In other regions of the body, significant differences were found in the feather scores between laying hens housed in cages, regardless of space allowance per layer, and laying hens reared extensively indoors. According to the total feather score in the 50th week of laying age, the significance of differences in the feather scores by body parts was established between all three groups. The best condition of the feathers individually, in all body regions, was determined in the EI group, followed by C3 and the worst in the C4 group. The only deviation was in the feather score for the back, which did not differ significantly between laying hens in the EI and C3 groups, while laying hens in the C4 group had significantly worse feather score compared to both groups.

Table 2. Effect of space allowance on feather scores (scoring scale 1-4) of different body parts in laying hens at 30, 40 and 50 weeks of age

Feather score, point	Experimental group						p-value
	C4		C3		EI		
	Mean	SD	Mean	SD	Mean	SD	
30 week							
Neck	4.00	0.00	4.00	0.00	4.00	0.00	NS
Breast	3.98	0.16	3.90	0.30	3.90	0.38	NS
Back	4.00	0.00	3.98	0.15	3.92	0.27	NS
Wings	3.65^{b}	0.48	3.83^{ab}	0.38	4.00^{a}	0.00	***
Tail	3.83	0.38	3.88	0.33	3.95	0.22	NS
Total	19.45	0.68	19.60	0.70	19.78	0.58	NS
40 week							
Neck	3.30^{b}	0.79	3.69^{a}	0.60	4.00^{a}	0.00	***
Breast	3.20^{b}	0.72	3.31^{b}	0.64	3.87^{a}	0.47	***
Back	3.60	0.59	3.71	0.64	3.64	0.87	NS
Wings	3.53^{b}	0.55	3.45^{b}	0.50	3.97^{a}	0.16	***
Tail	3.10^{b}	0.81	3.29^{b}	0.55	3.64^{a}	0.49	***
Total	16.73^{b}	2.18	17.45^{b}	1.93	19.13^{a}	1.13	***
50 week							
Neck	2.00^{c}	0.75	2.55^{b}	0.86	4.00^{a}	0.00	***
Breast	2.33^{c}	0.69	2.79^{b}	0.78	3.66^{a}	0.63	***
Back	2.80^{b}	1.04	3.31^{a}	0.78	3.26^{ab}	0.83	**
Wings	2.95^{c}	0.71	3.33^{b}	0.57	3.95^{a}	0.23	***
Tail	2.05^{c}	0.88	2.83^{b}	0.70	3.61^{a}	0.55	***
Total	12.13^{c}	3.02	14.81^{b}	2.87	18.47^{a}	1.48	***

C4-564 cm^2/hen, cage; C3-751 cm^2/hen, cage; EI-3000 cm^2/hen, extensive indoor
NS-non significance; **- p<0.01; ***-p<0.001; a, b, c- significant differences for the same row

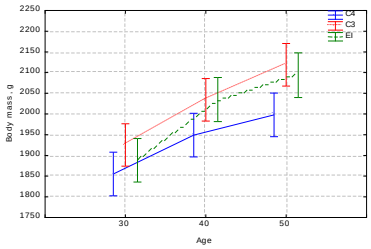

Figure 1. Body weight of laying hens, (g)

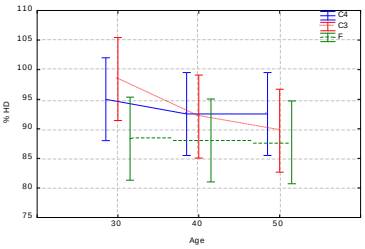

Figure 2. Hen-day egg production, %

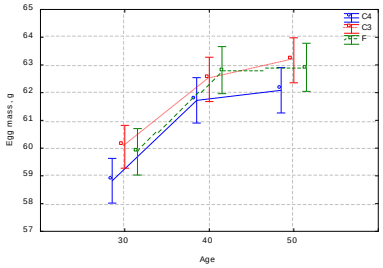

Figure 3. Average egg weight, g

The body weight of laying hens was influenced by the space allowance ($p<0.01$) and age of laying hens ($p<0.001$), without the interaction effect of these two factors ($p= 0.725$) (Fig.1). Laying hens with the least available space (C4) had the significantly lowest body weight (1934.1 g) compared to the C3 (2026.2 g) and EI (2003.9 g) groups.

Observed in relation to age of laying hens, differences between groups were confirmed at week 40, when laying hens in C4 had significantly lower body weight compared to C3 and EI groups, and subsequently, at week 50, when a difference was confirmed only between C3 and C4 groups. Although the laying hens in the C4 group met the minimum requirement in the available length of the feeder, the larger feeding space for layers in the C3 group resulted in higher body weight. In the extensive indoor system, the effect of a larger feeding area on body weight was reduced by greater mobility of laying hens.

The expected trend of decreasing hen-day egg production (% HD) with laying age was confirmed, which in the weeks 30, 40 and 50 was 93.92%, 90.87%, 89.97%, respectively. Due to the short time interval in which the trial was conducted in relation to the production cycle of laying hens (72-90 weeks) and therefore, the time required for a significant drop in laying capacity, the determined differences were not statistically significant. The effect of space allowance per

laying hen was not significant (p=0.069) for egg production. However, both groups of laying hens in cages had a higher hen-day egg production (93.3% and 93.4%), regardless of cage space allowance, compared to the extensive indoor group (88%). On the other hand, the drop in egg production in the observed period was the lowest in the EI group and the largest in the C3 group, which started with the highest laying capacity in the week 30 (Fig. 2).

Data on egg weight confirmed, as expected, an increase in egg weight with laying age (p=0.003). The average egg weight in the 30th week (59.59g) was significantly lower compared to the weeks 40 and 50 (62.72g and 62.33g). From the perspective of the space allowance, the egg weight in the C4 group was significantly lower ($p<0.01$) compared to the C3 and EI groups, which was not statistically different from each other. The interaction effect of the space allowance per laying hen and the age on the egg weight was not present (Fig. 3).

Discussion

Most studies confirm the results of our study on the decrease in overall feather score with the age of laying hens *(Petrik et al., 2015; Widowski et al., 2017; Liebers et al., 2019).* The greatest loss of feathers observed at the age of 50 weeks was on the neck and tail, which is partially in agreement with the findings of *Ozenturk et al. (2022)* who find the greatest loss of feathers at the end of the laying period on the back and tail. In a study by *Campe et al. (2018),* based on the overall feather score for the whole body, the effect of age on the condition of the feathers was confirmed, the feather condition worsened with the age of the laying hens, while the effect of age on the condition of the feathers of individual body parts was significant for the feather score for the head, breast and cloaca. Worse condition of feathers in the area of the back, tail and cloaca according to *Rodenburg et al. (2019)* arises as a consequence of feather pecking associated with a diverted form of foraging behavior, while the worse condition of feathers on the head and neck indicates the establishment of a social hierarchy which, according to *Mens et al. (2020)* represents normal behavior in contrast to the previous ones that are only seen in captive birds. *Yamak and Sarica (2012)* based on the established positive correlation between feather score and laying age, indicate the possibility of predicting the condition of feathers in older laying age based on the assessment performed in the earlier weeks of age.

The feather score was the best in the EI group with the largest space allowance per laying hen in the extensive indoor rearing system with litter. *Widowski et al. (2017)* report a dual effect of stocking density, based on the effect of space allowance and the effect of group size. Larger groups of laying hens represent a potential hazard due to greater exposure to layers "peckers" which, according to *Daigle et al. (2015),* when they develop this form of behaviour, about 5% constantly peck their feathers, while the percentage of victims is about 30.

Based on the results obtained in this study, it can be said that the effect of group size in EI was not present, and that the space allowance influenced the best feather scores overall and by body parts for laying hens in this group. A certain contribution to the condition of the feathers in the EI group was also made by the floor system with litter, which according to *Declina et al. (2019)* shows a lower prevalence of feather damage compared to wire and slatted flooring. Better feather condition in the floor system compared to the enriched cages and the aviary system is reported by *Zorman Rojs et al. (2020).* Similarly, in the enriched cages compared to the floor system with litter, there was more feather damage in the study by *Pichova et al. (2016),* while *Petrik et al. (2015)* find no differences in feather condition between conventional cage and floor systems.

If we compare the condition of the feathers of laying hens in the cages, the differences in the feather score were observed in older layers, where the larger space allowance in the cage resulted in a better condition of the feathers. The obtained results can be considered as a consequence of more available space on the feeder and less stress due to competition for food *(Ozenturk et al., 2022).* Similar results are reported by *Sarica et al. (2008)* comparing available cage spaces of 500; 667; 1000 or 2000 cm^2/laying hen. The feather score for individual parts of the body collectively gives an overall score of the condition of the feathers, while their analysis can identify the causes that lead to a worse condition of the feathers, which could remain hidden in the overall score *(Campe et al., 2018*). Accordingly, it is observed that the condition of the breast feathers in the cage system, regardless of the available space, is a consequence of frictional wear from the slatted material of the cage.

The problem of feather condition is mainly viewed from the aspect of behavior and welfare of laying hens, while the relationship between feather condition and production parameters has been significantly less researched. The results of our study indicate that the space allowance significantly affects the body weight of the laying hen and the average egg weight, while egg production is not significantly influenced by the space allowance. In a study by *Widowski et al. (2017),* the effect of cage stocking density on productivity parameters, i.e. hen-day egg production, egg weight and egg mass per laying hen, is completely absent. A higher body weight of laying hens in cages with a larger space allowance is determined by *Sarica et al. (2008),* but contrary to our findings, egg production is also higher in cages with lower stocking density, as well as egg weight. The rationale for the obtained results lies in more available food during the experimental period. *Glatz (2001)* states a high correlation between feed consumption and feathering of hens. In the same study, hens with worse feather condition have a 16% higher consumption compared to hens with better feathering. Also, egg production is higher in hens with better feathering, but there are no differences in egg weight. Food consumption is not presented in our study due to the expected large differences between cage and extensive indoor systems in terms

of mobility of laying hens and therefore energy needs. According to *Yamac and Sarica (2012),* the optimal laying age for assessing the condition of feathers is 40 weeks due to the established positive correlation with egg production in the weeks 50 and 60. In this way, it is possible to evaluate the profitability of the flock. This study shows a correlation between better feather condition, higher egg production and lower food consumption. *Fidan and Nazligul (2013)* link less available space on the feeder in a cage with a larger number of hens with a worse condition of the feathers. By comparing cage (conventional and enriched) and free range systems, *Dikmen et al. (2016)* show better feather scores for laying hens in the free range system. These hens have both a higher body weight at the end of production, as well as a higher egg production compared to cage systems which show no differences from each other, which is contrary to our results.

Conclusion

The results confirm the significant effect of space allowance and age on the condition of the layers' feathers. In addition to the expected decrease in feather score with the age of laying hens, the results indicate a significant interaction between age and space allowance per laying hen. Observed by individual body parts, as well as based on the overall feather score, space allowance per laying hen exhibited a full, cumulative effect at 50 weeks of age. Based on the space allowance for laying hens, it was possible to rank the overall feather score, with the laying hens with the most space having the best feather scores. The effect of space allowance on body weight was manifested through the available space on the feeder, which resulted in the lowest ($p<0.01$) body weights in the C4 group. Egg production was not significantly influenced by the space allowance per layer ($p=0.069$), but a connection between hen-day egg production and rearing system could be established. The average egg weight, in addition to the known effect of laying age, was the lowest ($p<0.01$) in the group with the least space allowance per laying hen.

Finally, the results of the study, based on the tested parameters of the welfare and productivity of laying hens, indicate that the rearing of laying hens in a cage system with 751 cm2 of available space per laying hen is most justified.

Efekti raspoloživog prostora u kaveznom i podnom sistemu na stanje perja i proizvodnju jaja

Zdenka Škrbić, Miloš Lukić, Veselin Petričević, Snežana Bogosavljević-Bošković, Simeon Rakonjac, Vladimir Dosković, Nataša Tolimir

Rezime

Sektor proizvodnje jaja se nalazi u tranzicionom periodu u pogledu dozvoljenih sistema gajenja, odnosno, od gajenja u konvencionalnim kavezima pod određenim uslovima do potpune zabrane bilo kakvog oblika kaveznog sistema. Promene su implicirane zabrinutošću za dobrobit nosilja ali sa očekivanim efektima i na produktivnost. Postavljeni cilj istraživanja je bio da se utvrde efekti raspoloživog podnog prostora u kaveznom i nekaveznom housing systems na stanje perja i proizvodnju jaja nosilja u tri starosti, sa aspekta propisanog minimalnog i optimalnog prostora u kaveznom sistemu (C) i prostora obezbeđenog ekstenzivnim gajenjem nosilja u manjim grupama u objektu (extensive indoor -EI). U cilju utvrđivanja stanja perja, telesne mase (body weight) nosilja i mase (weight) jaja, formirane su tri grupe nosilja: C4 (564 cm^2 po kokoši, kavezni sistem), C3 (751 cm^2 po kokoši, kavezni sistem) i EI (3000 cm^2 po kokoši, extensive indoor). Stanje perja i body weight nosilja su utvrđeni u tri starosti kokoši (30, 40, 50 nedelja), individualnim ocenjivanjem i merenjem svih kokoši u ogledu. Prosečna nosivost i masa jaja su utvrđeni u istim nedeljama starosti nosilja. Pored očekivanog smanjivanja ocene perja sa starošću nosilja, rezultati su ukazali na značajnu interakciju starosti i veličine dostupnog prostora po nosilji. Posmatrano po pojedinačnim delovima tela, kao i na osnovu zbirne ocene perja, raspoloživ prostor po nosilji je ispoljio potpuni, kumulativni efekat u 50. nedelji starosti. Na osnovu veličine dostupnog prostora za nosilje moguće je izvršiti rangiranje ukupne ocene perja, pri čemu nosilje sa najviše prostora su imale najbolje ocene perja. Efekat raspoloživog prostora na telesnu masu nosilja je ispoljen preko prostora dostupnog na hranilici, što je rezultiralo najmanjim ($p<0.01$) telesnim masama nosilja u C4 grupi. Proizvodnja jaja nije bila pod značajnim uticajem veličine prostora po nosilji ($p=0.069$) ali bi se mogla konstatovati povezanost između hen-day egg production i sistema gajenja. Prosečna masa jajeta je pored poznatog efekta starosti nosilja, bila najmanja ($p<0.01$) u grupi sa najmanjom veličinom dostupnog prostora po nosilji.

Ključne reči: kokoš nosilja, kavezi, nekavezni sistemi, ocena perja, proizvodnja jaja

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GASTROINTESTINAL PARASITE INFECTIONS IN SMALL RUMINANTS RELATIVE TO HOST SEX, AGE AND HUSBANDRY SYSTEM UNDER THE GUINEA SAVANNAH VEGETATION

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Abstract: Despite raising animals mostly as scavengers in the guinea savannah zone of Ghana, there is still scanty scientific information on the effects of this system on the health of these animals. A study was conducted to document factors influencing the prevalence rate of gastrointestinal (GIT) parasites and their loads in 500 small ruminants (250 each of sheep and goats). Prevalence rate of about 86% was recorded in small ruminants. Sheep, however, had higher ($P<0.05$) overall GIT parasite burden than goats. In sheep, significantly ($P<0.05$) more females harboured strongyles than males. Similarly, tapeworm and GIT parasites in general, were more prevalent ($P<0.05$) in younger than older sheep. Strongyle population was higher ($P<0.05$) in growers than adults, while *Eimeria* spp populations were higher ($P<0.05$) in lambs and adults than growers. The overall GIT parasite load, however, was higher ($P<0.05$) in lambs than all other age groups. In goats, *Eimeria* spp infections, coinfections of *Strongyloides* spp/*Eimeria* spp, tapeworm/*Emeria* spp, and overall GIT parasite load were much higher ($P<0.05$) in the extensive than the semi-intensive systems of management. The overall parasite population was also higher ($P<0.05$) in growers than adults and kids. Coinfections of tapeworm/ *Strongyloides* spp increased ($P<0.05$) with increasing age. The prevalence rate of GIT parasites in small ruminants is high. However, higher GIT parasite burden was found in sheep than goats. Younger small ruminants and goats kept in the extensive system of management are more prone to GIT parasite infestation than those kept in the semi-intensive system and older ones, respectively.

Key words: Scavenger, gastrointestinal parasite burden, sheep, goat, husbandry system

Introduction

The livestock sub-sector, including fisheries contributed over 700 million USD to Ghana's Gross Domestic Product (GDP) as of the year 2020 *(Statista, 2022)*. Notwithstanding such significant contribution by the sub-sector, meat importation still constitute a major effort towards bridging the widening national meat demand gap *(Asuming-Brempong and Nyantang 2003; Alexandratos and Bruinsma 2012)*. Small ruminants are raised by small-holder farmers mainly for monetary and socioeconomic benefits, such as hide, manure, insurance against crop failure, medium-term savings, means of diversifying investment, and to perform social and cultural functions *(Weyori et al., 2018)*. They have inherent advantages over cattle, including high prolificacy rate, high feed use-efficiency from course roughage, short gestation period, rapid growth rate, high tolerance to diseases and tannins. They are also marketabile within one season *(Terril, 1985; Lebbie, 2004; Peacock, 2005)*.

Despite all these benefits, GIT parasite infection remains the predominant factor affecting small ruminant productivity. Parasitism has a significantly negative impact on the sheep industry *(Mavrot et al., 2015)*. There is a relationship between the prevalence of GIT parasites and the agro-ecological conditions, including quality and quantity of pasture, grazing behaviour of the host, humidity and temperature *(Pal and Qayyum, 1993)*. Tropical countries have been cited as having more favourable ambient conditions for helminth spread, including malnutrition/undernutrition in the host organism *(Mbuh et al., 2008)* and poor environmental hygiene in the *hinterlands (Badran et al., 2012)*, hence the severe and persistent GIT parasites infections reported in these locations *(Mohanta et al., 2007; Zeryehun, 2012)*. This makes helminth diseases a leading challenge to tropical small ruminant production systems *(Kumsa et al., 2011)*. Small ruminants are clinically or sub-clinical infected by gastrointestinal helminths in developing countries. Helminths infection leads to a decline in reproductive and productive performance *(Zeryehun, 2012; Ayaz et al., 2013)* as a consequence of reduction in intake and or inefficient conversion of feed *(Kanyari et al., 2009)*. There is also ineffective utilization of imbibed nutrients, leading to stunted growth *(Terefe et al., 2012)*, and this is manifested as anaemia and even death at heavy loads *(Hassan et al., 2011)*. Additionally, helminths infection deteriorates the animal's defences against infection, rendering it susceptible to other opportunistic infections, and this may culminate into remarkable economic deprivation *(Garedaghi et al., 2011)*.

Despite the problems of gastrointestinal parasite in the tropics *(Zerychum, 2012)*, there is still a general paucity of information on their prevalence in small ruminants under some tropical climatic conditions, including the guinea savannah agro-ecological conditions. The farmers in the guinea savannah zones of Ghana raise their animals mostly extensively, serving as scavengers and grazing on

anything they find *(Adams and Ohene-Yankyera, 2015)*. This situation is likely to worsen the problem of gastrointestinal parasites in the animals, as quality and quantity of pasture have been reported to influence the prevalence of gastrointestinal parasites *(Pal and Qayyum, 1993)*. The objective of the present study, therefore, was to establish the prevalence and load of gastrointestinal parasites in small ruminants in the guinea savannah zone, and to establish if management system, host sex and age have effects on these phenomena in small ruminants.

Materials and Methods

Study area

The present work was carried out at Savelugu in the Savelugu/Nanton municipality of the Northern region (Ghana; latitude 9º 40' N and longitude 0º 49' W). The rainy season begins in April in an erratic pattern , and intensify with advancing season. The annal rainfall amounts raises from an average of 600 mm at the beginning of the season to 1 000 mm by the close of the season in October. Savelugu has an average temperature of 34°C. Minimum temperatures occur from December to February due to the North-East Trade winds *(Nyadzi, 2016)*. The district is located in the Savannah woodland, and could support commercial livestock rearing and cultivation of arable crops. The trees found in the area include *Vitellaria paradoxa* and *Parkia biglobosa*, which are drought resistant and are mostly of economic importance.

Experimental animals and management

Two hundred and fifty each of sheep and goats were randomly sampled and used for the study. In each species, 90 kids/lambs (3months old), and 80 each of growers (3-12 months) and adults (above 12 months) were involved. Similarly, in each species, 125 each of males and females were used. Also, 125 each were raised under the extensive and semi-intensive systems of management, respectively.

Both species (sheep and goats) under semi-intensive management system are housed in a pen made of wood and roofed with thatch. Animals are sent out for grazing at 7 am each morning and return at 5 pm. Their diets are supplemented with farms residues and mineral sources (e.g. salt lick). Animals are periodically vaccinated against *peste des petits* ruminants and breeding is controlled.

The sheep and goats under the extensive system have virtually no attention given them. Neither housing nor medication and supplementary feed are provided.

They are left to scavenge for food and water. Ages of the animals were estimated using their dentition *(Rahman and Hossain, 1997).*

Sample collection

Animals were adequately restrained, and faeces hygienically collected from the rectum by gloved hands. The faecal samples obtained were kept in an airtight, clean faecal sample vial, and transported to the laboratory for morphological analysis of helminths eggs. About 5-10 g of faeces were collected from each animal and were labelled with identification numbers, species, locality, sex and age of the animal. Samples were subsequently refrigerated at 4 °C until analysis at the laboratory *(Hayat and Akhtar, 2000)*. The University for Development Studies institutional Review board scrutinised and approved all procedures used.

Faecal analysis

Faecal samples were e xamined by standard direct and indirect parasitological techniques (flotation and sedimentation) *(Soulsby 1982; Hayat and Akhtar, 2000)*. About 3 g of the faecal sample was homogenised in 3 ml distilled water. The emulsion was centrifuged at 3000 rpm for 3 minutes. The supernatant was poured out leaving the sediment. Five millilitres NaCl solution was then added and centrifuged to enable the eggs float. A sample was then pipetted from the surface of the supernatant on to the McMaster counting chamber, and was examined under ×10 magnification.

Identification of helminths eggs and faecal ova counting technique were based on their characteristic morphological features *(Soulsby 1982; Rahman et al., 1996)*. Following faecal analysis, samples were preserved in 10% formalin for backup purposes.

Data analysis

The effects of species of animal, age, sex and management system on GIT parasites prevalence in Djallonke sheep and goats were determined using the chi square procedure. Data on worm load were scrutinised for homogeneity and normality of variance using the Shapiro-Wilk's W and Levene's tests, respectively. Variances were not homogenous, and therefore, the effects of the aforementioned factors on worm load were determined using Kruskal-Wallis test/ Mann Whitney U test. The level of significance for all comparisons was 5%.

Prevalence rate for a particular internal parasite was estimated as the percentage of the animals carrying the particular parasite to the total population of the animals inspected for the parasite during the study period (number of animals carrying the particular internal parasite/total population of animals inspected for the

internal parasite * 100) (CDC web Archive; https://www.cdc.gov /csels/ dsepd/ ss1978/lesson3/section2.html)

Results

Most (86%) of the small ruminants studied were infected with GIT parasites. No significant difference (P>0.05) was found between sheep and goats in the prevalence of GIT parasites. However, sheep was more heavily infested (P<0.05) with *Eimeria* spp and a multiple infection of *Eimeria* spp and *Strongyloides* spp than goats. Similarly overall GIT parasites load was higher (P<0.05) in sheep than goats (Table 1). *Strongylus* spp were more prevalent in female (80%) than in male (53%) sheep.

Table 1. Differences between sheep and goats in internal parasite load and prevalence

Eggs per gram of faeces (epg) (Median (Interquartile range) of the various species of GIT parasites in sheep and goats									
	N° Examined	**S. spp**	**Tpw**	**E. spp**	**SxT**	**SxE**	**TxE**	Overall	Prevalence rate (%)
Sheep	250	500 (200-800)	400 (200-850)	600 (325-1050)	1300 (335-1900)	1250 (950-1800)	1650 (1075-2225)	1800 (800-2900)	53
Goats	250	400 (200-500)	200 (200-400)	400 (200-600)	800 (700-800)	800 (600-900)	1000 (600-1000)	1400 (300-1800)	50
P-Value		0.08	0.065	0.034	0.141	<0.001	0.629	<0.001	0.274

S. spp: *Strongyloides* spp; **Tpw**: Tapeworm; **E. spp**: *Eimeria* spp; **SxT**: multiple infections of *Strongyloides* spp and tapeworm; SxE: multiple infections of *Strongyloides* spp and *Eimeria* spp; TxE: multiple infections of tapeworm and *Eimeria* spp.

No differences were, however, found between males and females in the prevalence rate of all other GIT parasites studied. Similarly, no difference was found between the semi-intensive and extensive management systems in the prevalence rate of all species of parasites studied. The overall prevalence rate did also not differ (P>0.05) between the two management systems. Tapeworms were more prevalent (P<0.05) in lambs (55%) than both growers (25%) and adults (15%). Also, the prevalence rate in growers was higher than in adults. Similarly, the overall prevalence of GIT parasites irrespective of species was highest (P<0.05) in lambs (100%) followed by growers and then adults. No differences were found among the various age groups in the prevalence rates of *Strongyloides* spp, *Eimeiria* spp, multiple infections with strongyles spp and tapeworm, and *Strongyloides* spp and *Eimeiria* spp. Similarly, incidence of all 3 parasites combined was not found in any sheep (table 2).

Table 2. Effects of sex, management and age on prevalence rate of GIT parasites in Djallonke sheep

					Prevalence rates (%) of the various species of GIT parasites														
Parameter	**N° examined**	**S. spp**	**X^2**	**P-value**	**Tpw**	**X^2**	**P-value**	**E. spp**	**X^2**	**P-value**	**SxT**	**X^2**	**P-value**	**SxE**	**X^2**	**P-value**	**overall**	**X^2**	**P-value**
Sex																			
Female	125	100(80)	3.675	0.028	31(25)	1.232	0.267	59(47.2)	0	1.000	21(17)	0.104	0.747	50(40)	0.293	0.588	108(86.4)	0	1.000
Male	125	65(52)			51(41)			59(47.2)			25(20)			38(30.4)			112(90)		
Management																			
Semi-intensive	125	78(62.4)	0.075	0.784	41(33)	0	1.000	47(37.6)	1.674	0.196	25(20)	0.104	0.747	34(27.2)	1.172	0.279	100(80)	2.588	0.108
Extensive	125	87(70)			41(33)			71(57)			21(17)			54(43.2)			120(96)		
Age group																			
Lambs	90	67(74.4)	1.950	0.377	50(55.6)	8.010	0.018	50(55.6)	0.937	0.626	30(33.3)	5.625	0.060	36(40)	0.440	0.803	90(100)	6.146	0.046
Growers	80	55(69)			20(25)			32(40)			14(17.5)			28(35)			71(89)		
Adults	80	43(54)			12(15)			36(45)			2(5)			24(30)			59(74)		

S. spp: *Strongyloides* spp; **Tpw**: Tapeworm; **E. spp**: *Eimeria* spp; **SxT**: multiple infections of *Strongyloides* spp and tapeworm; SxE: multiple infections of *Strongyloides* spp and *Eimeria* spp

Table 3. Effects of sex, management and age on GIT parasite load in Djallonke sheep

	Eggs per gram of faeces (epg) (Median (interquartile range)) of GIT parasite loads in sheep											
Parameter	**N° Examined**	**S. spp**	**P-value**	**Tpw**	**P-value**	**E. spp**	**P-value**	**SxT**	**P-value**	**SxE**	**P-value**	**overall**	**P-value**
Sex													
Male	125	500 (300-825)	0.608	700 (450-1100)	0.040	600 (400-950)	0.616	1600 (1150-2025)	0.668	1350 (1150-1850)	0.395	800 (400-1250)	0.080
Female	125	400 (200-725)		300 (175-525)		400 (250-1000)		1150 (850-1150)		1050 (775-1775)		500 (200-1100)	
Management													
Semi-intensive	125	400 (250-550)	0.045	600 (250-875)	0.563	400 (300-750)	0.423	1150 (775-1300)	0.198	1050 (700-1425)	0.153	600 (300-900)	0.564
Extensive	125	800 (200-1100)		400 (200-400)		700 (400-1100)		1900 (1075-2075)		1450 (1050-2025)		800 (400-1350)	
Age group													
Lambs	90	500 (500-750)^{ab}	0.016	800 (300-1000)^{a}	0.034	900 (500-1100)^{a}	0.015	1600 (1075-1900)	0.681	1300 (900-1900)	0.823	850 (400-1225)^{a}	0.043
Growers	80	550 (300-1175)^{a}		400 (400-500)^{b}		250 (125-400)^{b}		1300 (1000-1950)		1500 (950-1850)		550 (300-1225)^{b}	
Adults	80	300 (150-550)^{b}		100 (100-250)^{c}		700 (400-800)^{a}		800 (800-805)		1150 (1025-1350)		550(300-800)^{b}	

S. spp: *Strongyloides* spp; **Tpw**: Tapeworm; **E. spp**: *Eimeria* spp; **SxT**: multiple infections of *Strongyloides* spp and tapeworm; SxE: multiple infections of *Strongyloides* spp and *Eimeria* spp;

Median (interquartile range) within a column and group having no superscript in common are significantly different (P<0.05).

The effects of sex, management system and age on faecal egg count (FEC) in Djallonke sheep are shown in Table 3.

No differences (P>0.05) were found between males and females in the populations of the various GIT parasites studied except tape worm, whose count was much higher in males than females. Overall GIT parasites loads were also not different between the two sexes. Similarly, the two management systems did not differ (P>0.05) in the populations of *Eimeiria* spp, Tapeworm eggs and mixed infections. Strongyles egg count was, however, higher in sheep raised in the extensive system than those raised in the semi-intensive system. Strongyles population was also much higher (P<0.05) in growers than in adults. Even though strongyles egg count tended to be higher in lambs than in adults, the differences were not statistically significant (P>0.05). Tapeworm epg was significantly (P<0.05) higher in lambs than both growers and adults. Similarly, FEC for Tapeworm was higher (P<0.05) in growers than adults. Lambs harboured the most (P<0.05 *Eimeiria* spp, followed by adults and then growers. The difference in epg for *Eimeiria* spp between lambs and adults was not statistically significant (P>0.05). The overall worm load was higher (P<0.05) in lambs than all other age groups. The populations of combinations of either *Strongyloides* spp and tapeworm or strongyles and *Eimeiria* spp in individuals did not differ between various age groups.

The prevalence rates of the various species of worms in goats are shown in Table 4. No difference was noticed between males and females in the prevalence rates of any of the GIT parasites studied. The prevalence rates irrespective of species of worm involved were also similar (P>0.05) in both sexes. Similarly, goats under the two management systems were infected at the same rate by the various species of GIT parasites considered. Age of goats did not also influence (P>0.05) the prevalence of the various species of internal parasites, neither did it influence the overall prevalence rate of all GIT parasites.

Male and female goats were equally infested with all the species of GIT parasites studied (Table 5). Goats raised under the extensive system of management, however, were more heavily infested with *Eimeiria* spp, a combination of *Eimeiria* spp and *Strongyloides* spp or *Eimeiria* spp and tapeworms in multiple infections than those raised under the semi-intensive system. Also, overall parasite load was much higher (P<0.05) in extensively raised animals than semi-intensively raised animals (Table 5). Age had no influence (P>0.05) on populations of the various species of internal parasites that infested goats. However, epg in multiple infections of *Eimeiria* spp and tapeworm significantly (P<0.05) increased with increasing age. GIT parasite load irrespective of species of parasite involved was also much higher (P<0.05) in growers than both kids and adults (Table 5).

Table 4. Effects of sex, management and age on prevalence rate of GIT parasites in Djallonke goats

		Prevalence rate(%) of the various species of GIT parasites																	
parameter	**N° examined**	**S. spp**	**X^2**	**P-value**	**Tpw**	**X^2**	**P-value**	**E. spp**	**X^2**	**P-value**	**S xT**	**X^2**	**P-value**	**S x E**	**X^2**	**P-value**	**overall**	**X^2**	**P-value**
Sex																			
Female	125	63(50.4)	0.000	1.000	41(33)	0.328	0.567	103(82.4)	2.131	0.144	9(7.2)	0.000	1.000	54(43.2)	0.069	0.792	113(90.4)	1.080	0.299
Male	125	63(50.4)			29(23.2)			79(63.2)			13(10.4)			46(37)			96(77)		
Management																			
Semi-intensive	125	60(48)	0.070	0.796	29(23.2)	0.328	0.567	91(73)	0.000	1.000	9(7.2)	0.000	1.000	50(40)	0.000	1.000	100(80)	0.120	0.729
Extensive	125	66(53)			41(33)			91(73)			13(10.4)			50(40)			109(87.2)		
Age group																			
Kids	90	50(55)	0.400	0.819	22(24.4)	0.657	0.720	65(72.2)	0.170	0.918	9(10)	0.436	0.804	40(44.4)	0.417	0.812	77(85.6)	0.240	0.887
Growers	80	36(45)			28(35)			58(72.5)			5(6.3)			32(40)			64(80)		
Adults	80	40(50)			20(25)			59(74)			8(10)			28(35)			68(85)		

S. spp: *Strongyloides* spp; **Tpw**: Tapeworm; **E. spp**: *Eimeria* spp; **SxT**: multiple infections of *Strongyloides* spp and tapeworm; SxE: multiple infections of *Strongyloides* spp and *Eimeria* spp;

Table 5. Effects of sex, management and age on GIT parasite load in Djallonke goats

Egg per gram of faeces (epg) (Median (Interquartile range)) of the various species of GIT parasites in goats													
Parameter	**N° Examined**	**S. spp**	**p-value**	**Tpw**	**p-value**	**E. spp**	**p-value**	**SxE**	**P-value**	**TxE**	**P-value**	**overall**	**P-value**
Sex													
Male	125	400 (200-550)	0.389	200 (200-275)	0.212	400 (200-600)	0.858	800 (700-950)	0.065	800 (450-1000)	0.590	400 (200-800)	0.931
Female	125	300 (200-400)		400 (200-500)		400 (225-550		600 (400-825)		1000 (850-1000)		400 (200-700)	
Management													
Semi-intensive	125	250 (125-400)	0.294	200 (150-300)	0.516	300 (200-400)	0.015	600 (400-800)	0.048	650 (525-775)	0.042	400 (200-600) 0.026	0.001
Extensive	125	400 (275-525)		250 (200-400)		450 (300-775)		800 (700-975)		1000 (850-1000)		550 (300-800)	
Age group													
Kids	90	300 (200-400)	0.284	100 (100-200)	0.265	400 (200-500)	0.173	700 (600-800)	0.085	1000 (700-1100)^{a}	0.049	400 (200-725)^{b} 0.018	0.001
Growers	80	500 (400-700)		300 (200-350)		500 (250-800)		900 (800-1100)		650 (625-675)^{b}		600 (300-900)^{a}	
Adults	80	300 (200-400)		300 (200-400)		300 (225-400)		700 (400-800)		300 (10-350)^{c}		350 (200-600)^{b}	

S. spp: *Strongyloides* spp; **Tpw**: Tapeworm; **E. spp**: *Eimeria* spp; SxE: multiple infections of *Strongyloides* spp and *Eimeria* spp; TxE: multiple infections of tapeworm and *Eimeiria* spp

Median (interquartile range) within a column and group having no superscript in common are significantly different (P<0.05).

Discussion

The prevalence of GIT parasites of 86% observed among small ruminants in this study agrees with the report of *Emiru et al. (2013)* in Genchi district of Ethiopia. These were higher than the 56% reported by *Petros and Lakew (2014)* in Quarit (Ethiopia) and 56.77% reported by *Fayisa et al. (2020)* in North Western Ethiopia. Similar prevalence rates were found in sheep and goats in the present study. Even though sheep tended to have higher rate of prevalence than goats, the difference was not statistically significant. This is in line with the observation of *Getchew (1998)* in Mekele (Ethiopia). In contrast to these observations, other studies *(Arafa et al., 2007; Ibrahim et al., 2008)* reported higher prevalence in sheep compared to goats, and concluded that the former were more susceptible to GIT parasites infection than the latter. A possible reason for this observation is that goats derive about 60% of their diet from browsing tall forages, compared to sheep that graze closely to the ground. This predisposes sheep more to worm infestation than goats *(Walker, 1994)*. It is, therefore, not surprising that sheep had significantly higher overall GIT parasite load than goats in the present study. In both species, however, the GIT parasite burden fall within the very high intensity range, a median of 1800 epg in sheep and 1400 epg in goats. The implication is that small ruminants within the guinea Savannah vegetation are heavily infested with GIT parasites, even though sheep is at a higher risk than goats.

The insignificant influence of sex on prevalence rate of GIT parasites in both sheep and goats in the present study is similar to the reports of several studies *(Armour 1980; Getachew 1998; Fikru et al., 2006; Tefera et al., 2009).* In contrast to these findings, other studies *(Thrusfield, 2005; Bashir et al., 2012; Fayisa et al., 2020)* reported higher prevalence rates in females than males. The authors attributed their findings to the temporary loss of naturally acquired immunity to GIT parasites which occurs in females during the periparturient period *(Schoenian, 2012)*, as most of the ewes used were in periparturient state. In the present study, Strongyles were found to be more prevalent among female sheep than males. Similarly, *Ibrahim et al. (2014)* reported higher prevalence of *Paramphistomum* and *Haemonchus* in females than males.

Similar to the observation of the present study in sheep, *Fikru et al. (2006)* reported that young animals were more susceptible to GIT parasites infection than older animals. In both sheep and goats, higher overall epg were recorded in younger than older animals. Also, tapeworms were more prevalent in younger than older sheep. In this species, growers had also higher strongyle epg than any other age group. Similarly, lambs had more *Eimeria* spp than any other age group. These results may be linked to immunological maturity attained as the animals age, and increase in acquired immunity due to repeated exposure *(Chiejina, 1986)*. Contrary to these findings, other studies *(Fritsch et al., 1993; Waruiru et al., 2005)* reported

that sheep of all age groups were equally infected by GIT parasites. In goats, *Schoenian (2012)* reported that kids were more susceptible to coccids than any other age group. In contrast, the results of this study demonstrated that all age groups of goat were equally susceptible to all GIT parasite species studied, including coccids.

The higher GIT parasite loads recorded in extensively raised goats compared to semi-intensively raised goats in the present study is not surprising, as animals were largely left to fend for themselves in the extensive system, feeding on anything *(Ockling, 1987)*, and poor pasture conditions have been implicated in worm infestations in these animals *(Ockling, 1987)*. Similarly, *Notifor et al. (2013)* reported higher GIT parasite burdens in tethered and free ranging animals. The insignificant effect of management on parasite loads and prevalence in sheep reported in the present study is expected, since both extensive and semi intensively raised animals in the study location were raised on communal pasture during the study period. This period coincided with the end of the dry season when paddocks for the semi intensively raised sheep were mostly dried up, and could not support grazing. Unlike in sheep, goats under semi-intensive systems were heavily supplemented with browse, and may have ingested far less worm eggs compared to their extensively raised counterparts.

Conclusion

The prevalence rate and loads of GIT parasites are very high in small ruminants under guinea savannah conditions, but sheep was more heavily infested than goats. In goats, sex and management system had no influence on prevalence rate and population of any of the species of GIT parasites studied. Young goats, however, had higher parasite burden than older goats. In sheep, *Strongylus* spp were more prevalent in females than males. Also, younger sheep were more susceptible to tapeworms in particular, and GIT parasites in general than older sheep. Sheep kept under the extensive system of management are also more prone to worm infestation than those raised under the semi-intensive system. The practice of grazing animals of different ages and species together should be reduced in order to reduce cross infestations. Also, during rainy seasons where climatic factors are favourable for survival and development of parasitic stage of helminths, animals should not be sent out for grazing before sunrise. Animals, particularly sheep, must be heavily supplemented during the dry season in order to decrease the degree of exposure to parasite eggs.

Gastrointestinalne parazitske infekcije kod malih preživara različitog pola, uzrasta i sistema uzgoja u uslovima vegetacije gvinejske savane

Ibn Iddriss Abdul-Rahman, Paintsil Isaac Fuachie, Makija Joseph Tati

Rezime

Uprkos tome što se životinje uzgajaju uglavnom kao svaštojedi u zoni gvinejske savane u Gani, još uvek postoje oskudne naučne informacije o efektima ovog sistema na zdravlje ovih životinja. Sprovedeno je istraživanje kako bi se dokumentovali faktori koji utiču na stopu prevalencije gastrointestinalnih (GIT) parazita i njihovog opterećenja kod 500 malih preživara (po 250 ovaca i koza). Stopa prevalencije od oko 86% zabeležena je kod malih preživara. Ovce su, međutim, imale veće ($P<0,05$) ukupno opterećenje GIT parazitima od koza. Kod ovaca, značajno ($P<0,05$) više ženskih grla imalo je prisustvo oblih/valjkastih crva nego muška grla. Slično, pantljičara i paraziti GIT uopšte, bili su češći ($P<0,05$) kod mlađih od starijih ovaca. Populacija oblih/valjkastih crva je bila veća ($P<0,05$) kod grla u porastu nego kod odraslih, dok je populacija *Eimeria* spp bila veća ($P<0,05$) kod jagnjadi i odraslih grla, u poređenju sa jedinkama u porastu. Međutim, ukupno opterećenje GIT parazitima bilo je veće ($P<0,05$) kod jagnjadi nego u svim drugim starosnim grupama. Kod koza, infekcije *Eimeria* spp, koinfekcije *Strongyloides* spp/*Eimeria* spp, pantljičara/*Emeria* spp i ukupno opterećenje GIT parazitima bili su mnogo veći ($P<0,05$) u ekstenzivnim nego poluintenzivnim sistemima uzgoja. Ukupna populacija parazita je takođe bila viša ($P<0,05$) kod grla u porastu nego kod odraslih i jaradi. Koinfekcije pantljičare/ *Strongyloides* spp su se povećavale ($P<0,05$) sa povećanjem starosti. Stopa prevalencije GIT parazita kod malih preživara je visoka. Međutim, veće opterećenje GIT parazitima nađeno je kod ovaca nego kod koza. Mlađi mali preživari koji se drže u ekstenzivnom sistemu skloniji su infestaciji GIT parazitima od onih koji se drže u poluintenzivnom sistemu i starijih koza, respektivno.

Ključne reči: svaštojedi, opterećenje gastrointestinalnim parazitima, ovce, koze, sistem uzgoja

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Conflicts of interest
There is no conflict of interest to be declared.

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COMPARISON OF SOY PROTEIN CONCENTRATE AS AN ALTERNATIVE TO FISH MEAL IN COMMON CARP (*CYPRINUS CARPIO* L.) DIETS

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Original scientific paper

Abstract: The aim of this study was to replace fish meal (FM) with soy protein concentrate (SPC) in carp diets. During a carp feeding trial, the replacement of FM with SPC in four diets 100% replacement (SPC100); 50% replacement (SPC50); 25% replacement (SPC25), and; 0% replacement (SPC0) had no negative effects on the feed conversion ratio or the feed efficiency ratio of the live carp. However, significant differences in the specific growth rate and condition factor were found. The data obtained showed the four different carp diets led to differences in the chemical composition of the resultant carp meat. Between dietary treatments, significant differences were seen in the level of saturated fatty acids (FAs) in carp meat ($P < 0.05$). The levels of monounsaturated FAs and polyunsaturated FAs in carp meat differed significantly between dietary treatments ($P < 0.05$). Pearson's correlation coefficient indicates a statistically significant correlation between the FA composition of the diet and the resultant carp meat.
It is possible to replace up to 25% of the FM with SPC. Diets SPC25 and SPC0 had no significant FA composition and had amino acid balances that, more than the other diets studied, closely met the requirements of the carp.

Keywords: soy protein concentrate, fish meal, growth, amino acids and fatty acids composition

Introduction

Soy protein is one of the most common plant proteins that can replace fish meal (FM) in fish diets. Although soy products have been studied for a long time *(Tacon, 1994)* and are nowadays routinely used in fish feeds (*Hendricks, 2003; Brezas and Hardy, 2020*), recommendations for their incorporation in salmonid

diets vary (*Kaushik, 2008*). Soy protein concentrate (SPC) is promising for fish nutrition. Although SPC contains anti-nutritional factors (lectins, protease inhibitors, oligosaccharides etc.), these can be eliminated or deactivated (*Sealey et al., 2009; Day and Plascenia González, 2000*), and for some fish species, the solubility of protein was similar in fish diets with SPC and those with FM (*Day and Plascenia González, 2000; Kissil et al., 2000*). Several studies have shown that SPC diets can be used in fish nutrition. In sea bream diets, SPC can be used to replace FM but with a limit of 30% (*Kissil et al., 2000*). In turbot diets, up to 25% of the FM can be replaced with SPC (*Day and Plascenia González, 2000*). In rainbow trout diets, SPC caused a decrease in fish growth, but substitution of up to 50% of the FM was possible when diets with SPC were supplemented with amino acids (AAs) (*Mambrini et al., 1999*). However, studies have shown great variability when using soy products (soybean meal, full-fat soybeans, soya isolates and concentrates) in fish diets (*Welker et al., 2021*), and this variability is related to the processing technologies. Nonetheless, cyprinidae are considered to be more tolerant to soybean anti-nutritional factors (*Escaffre et al., 1997*) if synthetic AAs are utilized (*Lemme, 2011*). The whole-body AA profile of carp is not affected by the age of fish (*Kaushik and Seiliez, 2010*).

The aims of this study were to determine for common carp (*Cyprinus carpio* L.) whether a SPC diet without AA supplementation is suitable and how this diet influences the growth, AA and fatty acid (FA) composition of the carp meat.

Materials and Methods

Fish sampling

The study was conducted in the Laboratory of Fish Nutrition, Faculty of Agriculture, University of Belgrade, Serbia. Fish (n=26) with average weight of 9.55 g (the fish density was 2.069 kg m^{-3}) were stocked in each tank. Over a period of 90 feeding days, the 26 fish in each tank were given one of the four types of fish diet, each diet in three replicates (4 diets x 3 replicates = 12 tanks). In each tank, 3% of the ichthyomass was fed to the fish daily. Feed distribution was carried out continuously on a daily basis using automatic feeders (AGK Kronawitter GmbH, Germany). The ingredients of the experimental diets are presented in Table 1.

For calculation of growth parameters: body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF), the equations in *Shamna et al. (2017)* were utilized. At the end of the 90-day feeding trial, seven fish from each tank were chosen randomly and the fish were removed the dorsal side.

Table 1. Ingredients and chemical composition in experimental diets

Ingredients (%)	Diet 1 SPC100	Diet 2 SPC50	Diet 3 SPC25	Diet 4 SPC0
Fishmeal (FM)[1]	0	17.0	25.5	34.0
Soy protein concentrate (SPC)[2]	34.0	17.0	8.5	0
Soybean	28.3	28.3	28.3	28.3
Wheat	11.0	11.0	11.0	11.0
Soybean meal	10.0	10.0	10.0	10.0
Maize	10.0	10.0	10.0	10.0
Premix[3]	3.0	3.0	3.0	3.0
Monocalcium phosphate[4]	3.0	3.0	3.0	3.0
Chalk	0.7	0.7	0.7	0.7
Chemical composition (% on dry matter)				
Crude protein	41.83 ± 0.07^{a}	40.19 ± 0.66^{b}	40.44 ± 0.34^{ab}	39.54 ± 0.08^{b}
Crude fat	8.09 ± 0.11^{d}	10.25 ± 0.11^{c}	12.44 ± 0.10^{b}	14.11 ± 0.13^{a}
Crude ash	7.69 ± 0.01^{d}	8.93 ± 0.02^{c}	11.01 ± 0.02^{b}	13.05 ± 0.02^{a}
Fiber	2.52 ± 0.02^{a}	2.18 ± 0.02^{b}	1.67 ± 0.02^{c}	1.40 ± 0.02^{d}
NFE	39.87 ± 0.01^{a}	38.45 ± 0.79^{a}	34.44 ± 0.19^{b}	31.88 ± 0.05^{c}

Means in rows followed by different superscript letters are significantly different ($P < 0.05$); NFE – nitrogen free extract; number of samples n = 3

[1] Fishmeal contained 60% crude protein, 10% crude fat, 0.3 % crude fiber, 18% ash. Source of fishmeal was Apesabel Export S.A.C. Lima (Peru); [2]soy protein concentrate contained 65% crude protein, 0.3% crude fat, 4.5% crude fiber, 7.0% ash. SPC was sourced from Sojaprotein a.d. (Bečej, Serbia); [3]mineral vitamin mix contained (per kg of premix): potassium phosphate, 40 g; calcium phosphate, 5.5 g; magnesium sulfate, 6.1 g; sodium phosphate 2.5 g, vitamin A, 350 000 IU; vitamin D, 800 000 IU; vitamin E, 40 g; vitamin K, 15 g; vitamin B1, 20 g; vitamin B2, 15 g; vitamin B6, 20 g; vitamin B12, 10 mg; niacin, 40 g; pantothenic acid, 40 g; folic acid, 4 g; biotin, 400 mg; choline, 500 mg; inositol, 150 g (Veterinary Institute of Subotica, Serbia); [4] monocalcium phosphate (Veterinary Institute of Subotica, Serbia)

Chemical analysis

Analysis of the chemical composition of feed and carp meat was carried out using the following procedures: dry matter after drying in an oven at 105°C (*ISO 1442:1997, ISO 6496:1999*); ash by ashing in furnace at 550°C (*ISO 936:1998, ISO 5984:2002*); protein by Kjeldahl (N x 6.25) on a Kjeltec Auto 1030 analyzer (Manual Book, Tecator, Höganäs, Sweden); fat by petroleum ether extraction on a Soxhlet apparatus (*ISO 1443:1973, ISO 6492:1999*) and crude fiber using a standard method with intermediate filtration (*ISO 6865:2000*). Nitrogen-

free extract (NFE) was calculated by subtracting from 100 the percentages of moisture, crude protein, fat, crude fiber, and ash in the feed of carp. Chemical analyses of feed and carp meat were performed in triplicate.

Amino acid analysis

AAs of feed were analyzed according to the method in *Liu et al.* (1995). The AAs were analyzed by high pressure liquid chromatography (HPLC) (Waters, Milford, MA, USA) on a photodiode array detector (PDA) at 260 nm and fluorescence detector (FL) (all from Waters). An AccQ-Tag C-18 column (3.9 mm x150 mm x 4 μm) was used. Flow rate was 1.5 ml min^{-1}. The injected volume was 10 μL. Standard AAs were purchased from Supelco (Supelco, Bellefonte, USA). To control the HPLC system, data acquisition and data processing Empower Pro software was used.

Fatty acid analysis

The FA composition of feed and carp meat was determined by capillary gas chromatography, starting with accelerated solvent extraction (ASE) at 100°C at 10.3 MPa (Dionex, Sunnyvale, CA, USA). A solvent evaporator 500 (Sunnyvale, CA, USA) was used at 50°C until sample dryness was reached. Extracted lipids were dissolved in tert-butyl methyl ether. Furthermore, fatty acid methyl esters (FAMEs) were transesterificated using 0.25 M trimethylsulphonium hydroxide (TMSH) in methanol (*ISO 5509:2000*). FAMEs were determined by capillary gas chromatography on a Shimadzu 2010 gas chromatograph with a flame ionization detection (Kyoto, Japan) using a HP-88 capillary column (100 m × 0.25 mm × 0.20 μm, J&W Scientific, USA). The injector and detector temperatures were set at 250°C and 280ºC, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.33 mL min^{1}. The injector split ratio was set at 1:50. Temperature program for oven starting at 125ºC and ending 230ºC, was applied. The chromatographic peaks in the extracts were identified by comparing peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). Relative quantities were expressed as the weight percentage of the total content of FAs.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey-Kramer HSD test (JMP 10, SAS Institute, Inc.). Pearson's correlation with *t*-test was used to examine correlations between fish diet (SPC100, SPC50, SPC25 and SPC0) and the composition of resultant carp meat (carp meat 1-4), to evaluate the effects of dietary replacement of FM with SPC.

Results and Discussion

The effects of the four dietary regimes on growth parameters during the feeding trial are given in Table 2.

Table 2. The effects of dietary regimes on growth parameters of carp after the 90 day feeding trial (mean ± SD)

Diet	Diet 1 SPC100	Diet 2 SPC50	Diet 3 SPC25	Diet 4 SPC0
BWG[1]	196.71 ± 6.78^{NS}	199.68 ± 2.16^{NS}	183.73 ± 18.20^{NS}	168.25 ± 8.52^{NS}
SGR[2]	1.36 ± 0.02^{a}	1.13 ± 0.02^{b}	1.22 ± 0.06^{ab}	1.16 ± 0.07^{b}
FCR[3]	1.81 ± 0.11^{NS}	1.85 ± 0.12^{NS}	1.99 ± 0.14^{NS}	2.15 ± 0.11^{NS}
FER[4]	0.58 ± 0.04^{NS}	0.59 ± 0.02^{NS}	0.55 ± 0.04^{NS}	0.50 ± 0.03^{NS}
CF[5]	1.51 ± 0.03^{b}	1.61 ± 0.02^{ab}	1.60 ± 0.02^{ab}	1.63 ± 0.02^{a}

Means in rows followed by different superscript letters are significantly different ($P < 0.05$); NS – not significant; [1]BWG – body weight gain; [2]SGR – specific growth rate; [3]FCR – feed conversion ratio; [4]FER – feed efficiency ratio; [5]CF – condition factor; number of samples n = 6

Common carp grew well on diets with SPC. After the feeding trial, SPC had no negative effect on the BWG, FCR, or FER of the live carp. Some growth reduction was observed, although it was not statistically significant. The results were in agreement with those of another study (*Mambrini et al., 1999*). The SGR of carp on the SPC100 diet was 1.36 $g \cdot 100^{-1} \cdot day^{-1}$, while that of carp on the SPC0 diet (which contained FM but no SPC), was 1.16 $g \cdot 100^{-1} \cdot day^{-1}$. The values were statistically different ($P < 0.05$). The SGRs of carp fed on diets SPC50 and SPC25 were 1.13 $g \cdot 100^{-1} \cdot day^{-1}$ and 1.22 $g \cdot 100^{-1} \cdot day^{-1}$ respectively. These growth parameter results were in agreement with *Chen et al.* (*2019*). However, significant differences in the CF were found between carp fed on the SPC100 diet and those without any SPC in their diet (SPC0), so addition of FM to the diet led to the carp having better body condition at the end of the study.

The chemical composition of the experimental diets is given in Table 1.

Among the diets, the SPC100 diet had the highest protein content and the lowest crude fat content. Significant differences were observed, especially in regard to crude fat and ash contents, between the experimental diets, with FM having the highest crude fat content and ash content. Although the fiber content was highest in the SPC100 diet, as shown in Table 1, the SPC100 and SPC50 diets had larger fractions of nitrogen-free extract (NFE) than the SPC25 and SPC0 diets. The experimental diet in our study had similar chemical compositions to the diets fed to gilthead sea bream, cod, rainbow trout and common carp in other studies (*Francis et al., 2007; Palmegiano et al., 2006; Nasir et al., 2013*).

Table 3 presents the AA composition of the carp diets containing different levels of SPC.

Table 3. Amino acid composition (% dry weight) of the experimental carp diets

Amino acid	Diet 1 SPC100	Diet 2 SPC50	Diet 3 SPC25	Diet 4 SPC0
Cys + Met	4.8^{a}	4.3^{b}	3.9^{c}	3.6^{d}
Lys	3.5^{c}	3.6^{c}	6.1^{b}	7.7^{a}
Val	6.4^{a}	6.5^{a}	5.9^{b}	6.7^{a}
Leu	4.1^{c}	4.5^{b}	7.5^{a}	7.6^{a}
Ile	4.4^{c}	4.7^{bc}	4.8^{ab}	5.0^{a}
His	1.5^{c}	1.7^{bc}	1.9^{ab}	2.0^{a}
Arg	8.0^{a}	7.9^{a}	6.9^{b}	6.3^{c}
Thr	3.7^{NS}	3.6^{NS}	3.4^{NS}	3.6^{NS}
Phe + Tyr	9.2^{a}	8.9^{b}	7.6^{c}	6.6^{d}
Trp	4.2^{c}	4.4^{bc}	4.6^{ab}	4.8^{a}

Means in rows followed by different superscript letters are significantly different ($P < 0.05$); NS – not significant; number of samples n = 3

Both the SPC100 and SPC50 diets were deficient in lysine. The requirement of common carp for lysine is 5.7 % (*Hasan, 2000*). However, other AAs were within the described ranges suitable for carp. Both SPC25 and SPC0 had a balance of AAs that closely meets the requirement of these fish *(NRC, 2011).* The AA compositions of the fish diets were in agreement with another study (*Chen et al., 2019).*

Table 4 presents the chemical composition of carp meat from fish fed on diets containing different levels of SPC.

Table 4. Proximate composition of common carp meat from fish fed on diets containing various levels of SPC (mean ± SD)

Parameter	Carp meat 1, fish fed on SPC 100	Carp meat 2, fish fed on SPC 50	Carp meat 3, fish fed on SPC25	Carp meat 4, fish fed on SPC0
Protein, %	19.28 ± 0.23^{a}	17.80 ± 0.18^{b}	18.13 ± 0.28^{b}	17.80 ± 0.11^{b}
Moisture, %	74.30 ± 0.37^{NS}	71.80 ± 0.12^{NS}	73.14 ± 0.11^{NS}	73.06 ± 1.26^{NS}
Lipid, %	5.26 ± 0.10^{d}	8.46 ± 0.10^{a}	7.82 ± 0.11^{b}	7.72 ± 0.15^{c}
Ash, %	1.25 ± 0.02^{a}	1.12 ± 0.01^{b}	1.16 ± 0.02^{ab}	1.15 ± 0.02^{ab}

Means in rows followed by different superscript letters are significantly different ($P < 0.05$); NS – not significant; number of samples n = 6

The data show the different types of feed affected the chemical composition of the carp meat. Significant differences ($P < 0.05$) in protein content existed between carp meat 1 (SPC100) and carp meats 2, 3 and 4 (SPC50, SPC25, SPC0 respectively). However, the protein content was higher in our study than in a

previous study (*Barakat et al., 2007*). The differences in lipid content in the carp meat from fish fed the different diets were statistically significant ($P < 0.05$). Regarding lipid content, according to some published data *(Ćirković et al., 2011*), carp meat from dietary treatments 2 (SPC50), 3 (SPC25), and 4 (SPC0) can be considered fatty fish meat (with >8% fat content), while carp meat from dietary treatment 1 (SPC100) can be considered moderately fatty fish meat (with fat content of 4-8%) *(Mráz et al., 2012*). The lipid contents established in this study were similar to those reported in other studies (*Trenovszki et al., 2011*). The ash contents differed significantly ($P < 0.05$) in the carp meat from the different dietary treatments (*Honzlova et al., 2021*).

Table 5 presents the FA compositions of the experimental diets and the resultant carp meat from the fish fed on the different diets (SPC100 to SPC0).

The levels of saturated fatty acid (SFAs) in the carp meat were similar between dietary treatments with significant differences ($P < 0.05$). The levels of SFA was similar as in a previous study (*Barakat et al., 2007*). The level of monounsaturated fatty acids (MUFAs) was significantly higher in carp meat 1 (fed on SPC100) than in carp meat 2 (SPC50) ($P < 0.05$). The lowest MUFA levels were in carp meat 3 (SPC25) and carp meat 4 (SPC0) ($P < 0.05$). The PUFA levels were significantly higher in carp meats 2 (SPC50), 3 (SPC25), and 4 (SPC0) than in carp meat 1 (SPC100) ($P < 0.05$). As for the n-6 series of FAs among PUFAs, higher levels were measured in carp meat 2 (SPC50), 3 (SPC25), and 4 (SPC0), while carp meat 1 (SPC100) had the lowest levels. With respect to the n-3 series FAs, higher levels were present in carp meat 2 (SPC50), 3 (SPC25), and 4 (SPC0), with the lowest level in carp meat 1 (SPC100). The PUFA level has been reported to vary over a wide range and was similar in another study (*Ćirković et al., 2011*).

The n-6/n-3 ratio was 11.63 in carp meat 1 (SPC100), much higher than the 5.59-6.18 ratios in carp meat 2 (SPC50), 3 (SPC25), and 4 (SPC0). Also, different values of the n-3/n-6 ratio correlated with feed composition were observed by other investigators in freshwater fish such as carp (*Ćirković et al., 2011; Mráz et al., 2012; Trenovszki et al., 2011; Honzlova et al., 2021*).

Using correlation analysis of the FA composition of the four diets and resultant carp meat, we calculated Pearson's correlation coefficient between diet 4 (SPC0) and carp meat 4 (SPC0) and between diet 3 (SPC25) and carp meat 3 (SPC25) were r = 0.864 and r = 0.862, respectively. The *t*-test values were 10.38 and 10.30 (t_{crit} = 2.10) for pair 3 and pair 4, respectively, which means both correlation coefficients were statistically significant at a significance level of $P = 0.05$. The Pearson's correlation coefficient for diet 2 (SPC50) and carp meat 2 (SPC50) was r = 0.821, while that for diet 1 (SPC100) and carp meat 1 (SPC100) was r = 0.718.

Table 5. Fatty acid composition of experimental diets containing various levels of SPC (diets 1-4) and meat from common carp fed those diets (carp meat 1-4)

Fatty acids	Diet 1 SPC100	Diet 2 SPC50	Diet 3 SPC25	Diet 4 SPC0	Carp meat 1 fish fed on SPC100	Carp meat 2 fish fed on SPC50	Carp meat 3 fish fed on SPC25	Carp meat 4 fish fed on SPC0
C14:0	0.08^{c}	0.72^{b}	0.85^{a}	0.85^{a}	0.38^{y}	0.66^{x}	0.63^{x}	0.66^{x}
C15:0	nd	0.11^{b}	0.13^{a}	0.14^{a}	0.05^{y}	0.10^{x}	0.09^{x}	0.11^{x}
C16:0	10.51^{c}	13.01^{b}	13.90^{a}	14.09^{a}	14.19^{x}	14.64^{x}	15.02^{x}	14.94^{x}
C16:1	0.09^{b}	1.22^{a}	1.19^{a}	1.12^{a}	3.04^{y}	3.51^{x}	3.19^{y}	3.11^{y}
C17:0	0.08^{d}	0.26^{c}	0.45^{a}	0.49^{a}	0.10^{y}	0.21^{x}	0.23^{x}	0.24^{x}
C18:0	5.50^{a}	4.87^{a}	5.96^{a}	6.54^{a}	5.53^{x}	4.47^{x}	4.80^{x}	4.98^{x}
C18:1n-9	28.32^{a}	28.11^{a}	27.65^{b}	27.14^{b}	42.02^{x}	39.69^{x}	38.52^{y}	38.04^{y}
C18:2n-6	50.35^{a}	43.33^{b}	40.68^{c}	39.64^{c}	25.71^{y}	27.00^{x}	27.74^{x}	27.40^{x}
C20:0	0.40^{a}	0.39^{a}	0.40^{a}	0.38^{a}	0.16^{x}	0.13^{y}	0.13^{y}	0.13^{y}
C18:3n-6	nd	0.03^{b}	nd	0.07^{a}	0.74^{x}	0.38^{z}	0.42^{y}	0.45^{y}
C18:3n-3	4.64^{a}	4.81^{a}	3.99^{b}	4.02^{b}	1.53^{y}	2.09^{x}	2.22^{x}	2.24^{x}
C20:1	nd	nd	1.24^{b}	1.43^{a}	2.12^{y}	2.29^{y}	2.19^{y}	2.40^{x}
C20:2n-6	0.03^{a}	0.10^{a}	0.15^{a}	0.08^{a}	0.53^{x}	0.51^{xy}	0.49^{y}	0.44^{z}
C20:3n-6	nd	0.06^{c}	0.19^{a}	0.09^{b}	1.03^{x}	0.77^{y}	0.74^{y}	0.74^{y}
C20:3n-3	nd	0.27^{c}	1.21^{b}	1.55^{a}	nd	0.31^{y}	0.35^{x}	0.40^{x}
C20:4n-6	nd	0.23	nd	nd	1.99^{x}	0.99^{z}	1.07^{yz}	1.15^{y}
C20:5n-3	nd	0.85^{a}	0.59^{b}	0.59^{b}	0.07^{y}	0.34^{x}	0.33^{x}	0.35^{x}
C22:5n-3	nd	0.10^{b}	0.10^{b}	0.23^{a}	0.11^{y}	0.26^{x}	0.26^{x}	0.27^{x}
C22:6n-3	nd	1.53^{a}	1.31^{b}	1.53^{a}	0.71^{y}	1.63^{x}	1.81^{x}	1.93^{x}
SFA	16.56^{c}	19.36^{b}	21.69^{a}	22.50^{a}	20.41^{x}	20.22^{x}	20.86^{x}	21.07^{x}
MUFA	28.41^{c}	29.34^{b}	30.08^{a}	29.70^{a}	47.18^{x}	45.49^{y}	43.81^{z}	43.55^{z}
PUFA	55.03^{a}	51.07^{b}	48.23^{c}	47.80^{c}	30.42^{y}	33.30^{x}	34.27^{x}	34.23^{x}
n-6	50.38^{a}	43.52^{b}	41.02^{c}	39.88^{c}	28.01^{y}	28.67^{x}	29.32^{x}	29.04^{x}
n-3	4.64^{d}	7.56^{b}	7.21^{c}	7.93^{a}	2.41^{y}	4.64^{x}	4.95^{x}	5.20^{x}
n-6/n-3	10.85^{a}	5.76^{b}	5.69^{b}	5.03^{c}	11.63^{x}	6.18^{y}	5.92^{y}	5.59^{y}

All values are reported as mean; nd = not detected; $^{a, b, c,}$ Diet - Means followed by different superscript letters are significantly different ($P < 0.05$); $^{x, y, z}$ Carp meat- Means followed by different superscript letters are significantly different ($P < 0.05$); SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acid; number of samples for feed n= 3 for carp meat n = 6

The *t*-test values were 8.84 and 6.58 for pair 2 and pair 1, respectively, also indicating a statistically significant correlation between the FA composition of the diet and the FA composition of the resultant carp meat. Statistical analysis (ANOVA) showed there were no statistical differences in the FA composition of diet 3 (SPC25) and diet 4 (SPC0), and our results were in agreement with those obtained by other studies (*Nasir et al., 2013; Barakat et al., 2007; Ćirković et al., 2011; Mráz et al., 2012*).

Conclusion

There were small differences in the chemical composition of the fish diets, but they did not influence FCR and FER but they influence SGR and CF. Diets 3 (SPC25) and Diet 4 (SPC0) were very similar in regard to proximate and FA composition. The n-6/n-3 ratio was 5.59-5.92 in carp meat 3 (SPC25) and 4 (SPC0). These values are close to the dietary optimal n-6/n-3 ratio, which is between 1:1 and 4:1 and is desirable for reducing the risk of many diseases in humans. Therefore, we conclude that up to 25% of the FM in carp diet can be replaced with SPC, but we strongly caution against complete replacement because carp diet without FM would have an adverse effect on the FA composition of the resultant carp meat. Further studies are needed to evaluate the use of SPC in carp feed that is supplemented with synthetic AAs.

Poređenje koncentrata sojinih proteina kao alternativa ribljem brašnu u ishrani šarana (*Ciprinus carpio* L.)

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Rezime

Cilj ovog rada je bio da se ispita mogućnost zamene ribljeg brašna (RB) sa koncentratom sojinih proteina (SPK) u ishrani šarana. Tokom ogleda ishrana šarana po grupama bila je sa jednom od četiri hrane u kojoj je RB zamenjeno sa SPK: 100% (SPK100); 50% (SPK50); 25% (SPK25), odnosno, 0% (SPK0). Ishrana šarana sa četiri različite hrane nije imala negativan uticaj na konverziju hrane ili na koeficijent efikasnosti. Međutim, nađene su statistički značajne razlike u specifičnoj stopi rasta i kondicionom faktoru. Dobijeni podaci su pokazali da ishrana šarana sa četiri različite hrane dovode do razlika u hemijskom sastavu dobijenog mesa šarana. Uočene su značajne razlike u nivou zasićenih masnih kiselina (MK) u mesu šarana ($P < 0,05$). Nivoi mononezasićenih MK i polinezasićenih MK u mesu šarana značajno su se razlikovali između ishrane

šarana sa četiri različite hrane (P < 0,05). Pirsonov koeficijent korelacije ukazuje na statistički značajnu korelaciju između sastava MK u ishrani i mesa šarana. Moguće je zameniti do 25% RB sa SPK. Različite hrane za šarana sa SPK25 i SPK0 nisu imale značajno različit MK sastav i imale su balans aminokiselina koje su, više od drugih proučavanih hrana, u potpunosti ispunjavale zahteve šarana.

Ključne reči: koncentrat sojinih proteina, riblje brašno, rast, sastav aminokiselina i masnih kiselina

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QUALITY ASSESSMENT OF FRANKFURTERS PRODUCED FROM FRESH VS. FROZEN/THAWED GROUND BEEF

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Original scientific paper

Abstract: In order to evaluate the effect of raw meat's freezing/thawing process on meat products produced from them, frankfurter's quality was monitored. Fresh beef meat was grounded and separated into two lots – the first lot was immediately used in the preparation of frankfurters (G1F), and the other lot was frozen and stored at -18°C for 4 weeks, when it was used in the preparation of the second group of frankfurters (G2F). Physicochemical and technological properties were investigated on fresh (CG-FM) and thawed meat (G-F/TM) samples and the frankfurters made from these samples. Frozen storage significantly affected ($p<0.05$) moisture, TBA value, instrumental color parameters (*L*a*b**), WHC, total pigment, and total heme pigment in the raw meat samples; some frankfurters characteristics, such as moisture, L*(lightness), a* (redness) and b* (yellowness) were affected ($p<0.05$). However, the differences in the final products' process loss/cooking loss/frying loss and FRP were not significant. The sensory evaluation did not show any significance between the two groups of frankfurters It can be concluded that the frozen storage of minced beef meat for 4 weeks at -18°C and the consequential thawing process (at 4°C for 24h) does not significantly affect the overall acceptability of frankfurters prepared from them. However, effects on the marked changes in instrumental color are apparent.

Key words: frankfurter, ground beef, frozen storage, meat quality

Introduction

In the last 20 years, global meat consumption has increased by almost 60% (*Whitnall and Pitts, 2019*). Rising incomes and economic development in developing countries represent a significant driver of increased meat consumption,

accounting for around 85% of this rise. Although in most countries, beef represents considerably less than half of total meat consumption, beef consumption dynamics are changing rapidly due to the pace of economic development (*Smith et al., 2018; Whitnall and Pitts, 2019*). Growing demand has the global meat export industry blooming, and freezing plays a crucial role in ensuring the safety and quality of the meat products that are being supplied worldwide.

Although freezing and frozen storage represent an important preservation method, quality deterioration cannot be avoided since the freezing process can lead to a change in muscle foods' structural and chemical properties (*Jeong et al., 2011*). After microbiological degradation, the most significant changes that occur include the alterations in muscle fibers and their characteristics and oxidative processes, which affect not only lipids but also pigments and proteins of the meat. During these reactions, a sensory deterioration of the product can occur, which can cause consumers rejection of the meat and the final product produced from this meat (*Miller et al., 1980; Domínguez et al., 2019*).

Despite the fact that the meat color alone is usually a poor guide to choosing quality meat (due to varying in color among different meat cuts), most consumers will make a purchasing decision solely based on this trait and usually gravitate toward bright and red beef meat *(Young et al., 1999; Stanišić et al., 2012)*. However, the occurrence of deterioration of meat color has often been observed in thawed meat compared to fresh meat, and it is proven that prolonged frozen storage time does affect color change in meat *(Jeong et al., 2011; Stanišić et al., 2012*). The change in meat color occurs due to the drying during the freezing stage and denaturation of meat pigments due to the evaporation of water (sublimation) from frozen tissue and oxidation of meat pigments (*Josipović and Stanišić, 2022*). Myoglobin oxidation is a major contributor to color deterioration, as the oxidation of the bright red oxymyoglobin pigment leads to the formation of brown metmyoglobin, which is considered undesirable (*Johns et al.,* 1989). The proneness of beef meat to oxidative processes may result from its relatively high concentrations of unsaturated lipids, heme pigments, metal catalysts, and other oxidizing agents (*Johns et al., 1989; Jeong et al., 2011).* Also, damage to cell membranes caused by ice crystals formed during freezing and the subsequent release of pro-oxidants, especially the heme-iron, accelerate the further lipid oxidation by a number of heme compounds present in the meat *(Johns et al., 1989; Akhtar et al., 2013*). Lipid oxidation is closely linked to color changes in beef meat (*Akamittath et al., 1990).* There is also increasing evidence that indicates that lipid oxidation occurs primarily at the cellular membrane level (phospholipids) and then proceeds to spread to the triglyceride fraction. Thus, lipid oxidation is a problem occurring in both lean and fatty types of meat (*Thanonkaew et al., 2006; Akhtar et al., 2013*).

Authors *Gruić et al. (1993)* and *Petrović et al. (1993),* based on their previous research, stated that biochemical reactions still take place in meat stored

in temperatures higher than -70°C (temperatures below the eutectic point) since there is sufficient unfrozen water available remained for a period of time for such reactions to occur. In addition, a slower rate of the freezing process and higher temperatures define the size of the ice crystals formed outside the fibers (*Gruić et al., 1993)*. The growth of ice crystals causes distortion of tissue structure since, once removed, water from the fibers cannot be returned during the process of thawing and rebound to proteins (*Petrović et al., 1993; Jeong et al., 2011)*. The fraction of unfrozen water is also essential in the oxidation process since chemical reactions that occur during frozen storage initiate primary lipid oxidation (peroxidation) in the meat, which can lead to secondary lipid oxidation upon thawing (*Leygonie et al., 2012*), shown through TBA number. Freezing/thawing processes impact the content and moisture distribution in meat samples that could be, evaluated in several different ways, including the determination of total moisture content, water binding capacity, drip/thaw/cooking loss, etc. (*Leygonie et al., 2012*). Generally, there seems to be an agreement in the scientific literature on the notion that frozen storage and freezing/thawing processes all play an important role in a decrease in the water-holding capacity of meat (*Miller et al., 1980; Vieira et al., 2009; Kluth et al., 2021*). It has been reported by *Leygonie et al. (2012)* that the decrease in water-holding capacity is related to the modification and/or denaturation of the proteins, as well as the disruption of the muscle fiber structure.

Meat products are usually produced from frozen meat for various technological reasons, including the prevention of excessive heating during the manufacturing process (*Popp et al., 2013; Kluth et al., 2021*). Since the nutritive and physicochemical quality of frozen food is interlinked with the freezing/thawing processes, in order to provide consumers with a high-quality product prepared from frozen/thawed beef, our goal was to examine some of the possible effects involved in the freezing process concerning the quality of meat after thawing. Although there are numerous publications centered around the effects of the freezing/thawing process on muscle food quality, little has been reported concerning the relationship between the freeze-thaw cycle and the use of meat for processing. *Verma et al. (1985)* found that the use of mixed frozen meat (pork, beef, and mutton) in frankfurters negatively affected their texture, but that was no significant difference found in color or cooking loss. On the other hand, *Miller et al. (1980)* presented that frozen meat in frankfurters had an adverse effect on their texture, cooking loss, and sensory properties.

There are limited publications regarding quality characteristics and the functional changes that occur in the storage of frozen meat and the relation of these properties to changes within a meat product. This study offers the practical relevance of frankfurters prepared using fresh or frozen/thawed meat.

Material and Methods

Fresh beef meat (10 kg of a mixture of beef chuck muscles; 24h, post-mortem; storage temperature: 4°C) was grounded at 8 mm diameter (Laska W 130-H, Austria). Around 200 g of freshly grounded meat was used for proximate analysis, as well as the pH measurements, TBA measurement, water-holding capacity, total pigments, and objective color determination (readings of L*, a*, and b*), and the rest of the batch was separated into two groups. The first group (control group - freshly ground beef, CG-FM) was immediately used in frankfurter preparation (G1F). The second group (group-frozen/thawed meat, G-F/TM) was packed into 1 kg lots in moisture-proof plastic bags and left at -18°C for 4 weeks, after which it was thawed (4°C for 24h) and used for analysis (same as the freshly grounded meat) and preparation of the second group of frankfurters (G2F). The pork backfat used in formulations was obtained fresh (24h post-mortem) each time. Prior to mixing with the meat batter, pork backfat was grounded through an 8 mm plate (in the same manner as beef). Both series of frankfurters were made by the same formulation, as follows: 51.05% of grounded beef, 22.20% ice, 21.64% of pork backfat, 2.22% soy isolate, 1.66% commercially bought salt (99.5% NaCl + 0.5% $NaNO_2$), 0.67% polyphosphates, 0.55% commercially acquired spices. Ground beef, ice, salt, and condiments were blended for 8 minutes in the cutter (Seydelmann K60, Germany), and afterward, ground pork backfat was added and blended until the smooth batter was obtained. Meat emulsion was stuffed into 22 mm diameter collagen casings and manually paired (to approximately 70 g), measured and placed on the smoke sticks from the sausage hanging trolleys. Each group of frankfurters was spread onto six sticks and placed in the chamber for the smoking/cooking process as follows: 10 min drying at 50°C, 30 min smoking at 60°C and lastly, heating at 85°C (until the temperature in the center reached 72°C). Frankfurters were showered with ice-cold water and placed in a cooling chamber at 4°C for 48h before measuring and further analysis. Prior to analysis, frankfurters (four of both groups) were taken, collagen casings were removed, and samples were homogenized in a blender.

Proximate analysis – moisture (SRPS ISO 1442, 1998), ash (SRPS ISO 936:1999), protein (SRPS ISO 937, 1992; Gerhardt Vapodest 50S, Germany), and fat (SRPS ISO 1444, 1998; Gerhardt Multistat, Germany) were performed in meat and the final product. Hydroxyproline content (%) was done in frankfurters according to SRPS ISO 3496:2002 method (SPEKOL 1300, Analytik Jena, Germany), and collagen content (connective tissue content) was calculated by multiplying the hydroxyproline (%) result with factor 8. The relative content of connective tissue proteins to total protein content was then calculated by dividing the calculated collagen (%) value by the total protein (%) content in frankfurters multiplied by 100 (*Operta et al., 2012*). Levels of sodium chloride, nitrites, and

total phosphorus content were performed in frankfurter samples. NaCl content was obtained using the Volhard method (SRPS ISO 1841-1:1999), nitrite content was determined by SRPS ISO 2918:1999, and total phosphorus content by SRPS ISO 13730:1999 method. The pH value was obtained in raw meat as well as the frankfurters using a pH meter (model HI 83141, Hanna Instruments, USA), with a penetration electrode previously calibrated using standard buffer solutions (SRPS ISO 2917:2004). Analyses were performed in triplicate (per parameter) for each sample.

Thiobarbituric acid (TBA) was performed in raw meat samples (nine times total for each sample) following the procedure by *Buege and Aust (1978)* 2 ± 0.001 g of the homogenized sample was weighted into the cuvettes for centrifugation, and 10 mL of solution (0.375 g 2-thiobarbituric acid and 15 g TCA dissolved in 85 g 0.25 mol/dm^3 hydrochloric acid by stirring and heating in a water bath; freshly prepared each time) was added. The centrifuge tubes were sealed and immersed in a boiling water bath for 10 minutes to develop the color. The cuvettes were cooled under cold water and centrifuged for 10 minutes at g = 12. Afterward, the content of the cuvettes was filtered through a low-density filter paper (black strip) into a test tube. The intensity of the resulting red color was measured at 532 nm (SPEKOL 1300, Analytik Jena, Germany). The final value of TBA was calculated by multiplying the extinction (ε_{532}) by 2.77 and expressed as mg malonaldehyde/kg of the sample.

Instrumental color was determined by Chroma Meter CR-400 (Minolta, Japan) as described by *Stajić et al. (2014)*. The instrument was previously calibrated using a standard white surface (illumination D65, observer angle 2°, and aperture size 8 mm). Color values are presented in the CIE L* a* b* system (*CIE, 1976*), where factor L* indicates the lightness, a* corresponds to the relative proportion of red, and b* represents the yellowness of color of the samples. Measurements were performed in triplicate for each sample on non-overlapping areas, and their average value was used for statistical analysis.

The method of *Hornsey (1956)* was used to determine the total heme-iron concentration in raw meat groups (nine times per sample). Total pigments (mg/kg) were determined spectrophotometrically (SPEKOL 1300, Analytik Jena, Germany) by measuring absorbance at 640 nm, multiplying the extinction result with 680. Total heme-iron was calculated as total pigments (mg/kg) x 8.82/100 (*Von Seggern et al., 2005*).

Water holding capacity (WHC) in raw meat samples was conducted by *Grau and Hamm (1953),* and the value of WHC is expressed in cm^2 of the wet surface. Fluid release under pressure (FRP) of frankfurter samples was performed by the method suggested by *Stajić et al. (2020)*. Three frankfurters from each batch were taken, and samples were cut at 10±0.5 mm height, weighed, and compressed between two filter papers previously dried at 103ºC for 30 min and cooled in an exicator to room temperature for 5 min using the weight of 200±2 g. The samples

were then removed, and filter papers were measured. Fluid release under pressure (expressed as %) represents the amount of the released fluid relative to the initial sample weight.

Frankfurters were measured after stuffing the collagen casings and after the heat treatment in the smoking/cooking chamber. The process loss was determined as a weight difference between frankfurters before and after heat treatment (%).

Frying loss was determined based on the method described by *Fahimeh et al. (2019)*. Three frankfurters were selected and sliced 1 cm in thickness (in quadruplicate). Samples were then weighted and fried (in 250 mL borosilicate glass filled with 200 mL of oil). The oil temperature was maintained at 172 - 174°C. Frying was performed for 2 min, and samples were left to cool at room temperature (around 30 min) before weighing. The test was done in triplicate for each sample. Frying loss was calculated using the initial and final weights expressed in g/100 g, the initial sample weight.

The cooking loss was performed by the method suggested by *Amini et al. (2015)*. Three frankfurters were sliced at 3 mm thickness (in quadruplicate) and cooked in an oven (160°C for 2 min). The cooking loss was calculated by weighing the samples before and after the cooking process (expressed as g/100 g of the initial sample weight).

Sensory analysis was performed by the taste panel, which consisted of seven semi-trained evaluators on samples of frankfurters cut at approximately 3 cm piece after cooking in boiling water for 10 min. In sensory evaluation, the 5 points system was used: from 1-extremely unacceptable to 5-extremely acceptable to the following attributes: taste, smell, texture, juiciness, color, and general acceptability; rancidity scores were set to be given in reverse order – score of 5 was indicative of high present of rancid taste, while score 1 suggested that no rancid taste was found in the samples.

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

Results and Discussion

The first part of the experiment included the physicochemical and technological properties of raw ground beef chuck meat, shown in Table 1.

Table 1. Results on physicochemical meat quality parameters and technological properties of raw ground beef chuck meat before (CG-FM) and after freezing/thawing (G-F/TM) process

Parameter	CG-FM	G-F/TM	Significance
Physicochemical analysis			
Moisture (%)	68.94 ± 0.22	67.70 ± 0.30	*
Ash (%)	1.33 ± 0.20	1.45 ± 0.09	ns
Total fat (%)	12.46 ±0.36	12.73 ± 0.24	ns
Protein (%)	16.92 ± 0.21	17.11 ± 0.19	ns
pH	5.57 ± 0.01	5.56 ± 0.02	ns
TBA (mg malonaldehyde/kg meat)	0.21 ± 0.03	0.43 ± 0.03	*
Technological properties			
Instrumental color			
L*	45.68 ± 0.91	43.90 ± 1.66	*
a*	23.32 ± 1.40	19.79 ± 1.28	*
b*	10.30 ± 0.57	8.60 ± 0.60	*
WHC (cm^2)	11.52 ± 0.34	10.38 ± 0.40	*
Total pigment (mg/kg)	391.64 ± 34.85	267.20 ± 43.82	*
Total heme-iron (mg/kg)	34.54 ± 3.87	23.57 ± 3.07	*

TBA – 2-thiobarbituric acid; WHC – water holding capacity;
* $p<0.05$; ns: differences not significant;

Standard chemical analysis performed on fresh and frozen/thawed meat showed no significant difference except for the total moisture parameter ($p<0.05$). This marked change goes in hand with the fact that freezing and thawing processes are known to mainly influence the water fraction of meat and affect the amount of exudate through thaw loss and/or drip loss (*Akhtar et al., 2013; Met et al., 2013*). Loss of fluid as exudate represents a great quality concern within the meat processing industry (*Leygonie et al., 2012b*), as this occurrence results in the loss of water-holding capacity (WHC). The phenomenon in which ice crystal formation occurs during freezing in intracellular and extracellular space of myofibrillar fibers causes the puncturing of the cell membranes with subsequent leakage of moisture. This increase in solute concentration during the freezing process/prolonged frozen storage leads to protein denaturation, which additionally influences the water-binding property of meat. Both of these instances contribute to the loss in the ability of the meat to absorb fluid during thawing and retain fluid during the time of post-thawing (*Leygonie et al., 2011; Leygonie et al., 2012a; Leygonie et al., 2012b*). This is in agreement with the results obtained in our research. WHC of the frozen/thawed ground beef has decreased significantly ($p<0.05$). Our results also comply with the results obtained by *Miller et al. (1980)* and *Vieira et al. (2009)* who examined, among all, the water-holding capacity of beef meat during the frozen storage period of 30 days at -20°C.

The pH values of the minced beef in the present study do not show any significant differences between the fresh and frozen/thawed samples ($p>0.05$). The

results of the pH values varied throughout the papers. While *Kluth et al. (2021)* presented similar results obtained in fresh and frozen turkey meat, *Leygonie et al. (2011)* stated that the pH of meat that has been frozen and thawed tends to be lower than prior to freezing as a result of the loss of the fluid from the meat tissue due to an increase in the concentration of the solutes, which results in a decrease in the pH values. Contrary to this, *Verma et al. (1985)* found that the pH levels of all the meats examined (pork, beef, mutton) over a storage period of 52 weeks at -18°C increased significantly ($p<0.01$).

Various factors, such as heme concentration and oxidation status, influence the color of meat (*Kluth et al.,2021)*. The color of the muscle foods mainly comes from the intracellular heme protein myoglobin, with some contribution by hemoglobin, the blood pigment, which will account for 20–30% of the total pigment present (*Von Seggern et al., 2005*). Myoglobin has been identified in the exudate of meat that occurs after the freezing/thawing process, accounting in part for the change in color stability due to an increased susceptibility of myoglobin to autoxidation and subsequent loss of optimum color presentation (*Leygonie et al., 2012a*). Total pigment levels were observed, and a significant difference ($p<0.05$) compared to the control sample was found. Generally speaking, heme-iron content varies in different types of meat, with a high range of 26.20 - 75.60 % of its iron content, with red meat, such as beef, containing a higher heme iron content, and poultry meat which is on the lower scale of the spectrum. Since iron has 2 - 3 times higher bioavailability than non-heme iron (usually found in plant sources), it represents a very valuable micronutrient in battling iron deficiency (*Met et al., 2013*). Needless to say, changes in heme-iron levels portray important instances regarding the evaluation of meat quality. Heme-iron represents a water-soluble component that could be lost through thawing (and found in drip loss) (*Met et al., 2013*). Our research results cohere with this statement, with a decreased total heme iron value by a third, as same as a result of total pigment, which is similar to results obtained by *Met et al. (2013)* and *Jeong et al. (2011).* The variation in concentration of myoglobin and its oxidation state can affect color not only in fresh meat (whole muscle) but in processed meat applications, such as frankfurters, as well (*Von Seggern et al., 2005*). Frozen storage impacted the levels of surface color instrumentally determined as well. All of the parameters measured in frozen/thawed meat samples - L*, a*, and b* showed a significant decrease ($p<0.05$) in value compared to the control group. Our results agree with the research by *Jeong et al. (2011)* who noted a decrease in all parameters (L*, a*, and b*) after the freezing/thawing process in beef meat. Additionally, research by *Vieira et al. (2009)* showed the differences in the L* and b* parameters but not in the a* after 30 days of frozen storage. a* value showed a significant decline ($p<0.05$) only after 90 days of frozen storage but had a steady decreasing trend throughout the frozen storage. *Akamittath et al. (1990)* examined only the changes

in a*, since the consumer associate redness with the acceptability of red meat, and reported a decrease in the levels of a* after 30 days of frozen storage on the samples of beef round meat, while the values on L* were not shown. Still, the authors reported that during informal visual observation, the samples appeared darker with storage.

Changes in the color of the meat are closely linked with oxidative processes, as lipid oxidation results in the formation of pro-oxidation capable of reacting with oxymyoglobin, which leads to metmyoglobin formation (*Vieira et al., 2009; Leygonie et al., 2012a)*. In our research, the products of secondary lipid oxidation were measured using thiobarbituric acid (TBA). Results indicated an increase in TBA value ($p<0.05$), which provided evidence of oxidation during storage. *Keller and Kinsella (1973)* reported that TBA values act differently in relation to a different grade of meat – with an insignificant change in ground round and a progressive increase in ground chuck meat. Contrary to our research, *Akamittath et al. (1990)* did not find changes in TBA in beef meat during 30 days of frozen storage, opposing to increased values found in pork and turkey meat, and suggested that the pork and turkey rapid oxidation of pigments that occurred might have provided a pool of biological catalyst that initiated lipid oxidation in the relatively unsaturated fat system compared to beef. *Vieira et al*. (*2009)* stated that the TBA value of fresh meat was significantly lower than meat stored for 30 days at -20 °C with an increasing tendency over a frozen storage time. Obtained results indicate that frozen storage is not by definition sufficient to prevent oxidation from occurring, which adheres to remarks offered by *Petrović et al. (1993)* who stated that biochemical reactions still takes place in meat during frozen storage temperatures higher than -20 °C since sufficient unfrozen water remained available at these temperatures for such reactions to occur.

The second part of this experiment was composed of the physicochemical and technological properties of frankfurters are shown in Table 2.

Since freezing/thawing processes impact the technological and chemical properties of meats, it is fair to assume that, in return, these changes will affect certain quality characteristics of the product manufactured from them. Even though the same amount of water (ice form) was added to the formulation of both frankfurters, the total moisture in the final products differed. This result could potentially be explained by dissimilarity in the moisture of the building blocks themselves (fresh meat vs frozen/thawed meat). However, although *Lowry et al. (1982)* suggested that meat emulsions produced from frozen/thawed meat were less stable than those made from fresh meat, in terms of moisture, our results did not show significant differences in process loss, cooking loss, and/or frying loss. *Kluth et al. (2021)* suggest that the water release during the cooking process is mainly due to chemically bound water and melting fat. Our results are in alignment with the results offered by *Colmenero et al. (1995)* who concluded that the effect of the cooking loss of Bologna sausages was not altered by the freeze-

thawing process endured by the meat. Similarly, *Verma et al. (1985)* found no significant differences in terms of cooking loss in sausages prepared from minced meat stored for 52 weeks at -18°C.

Table 2. Results of physicochemical meat product quality parameters and technological properties of frankfurters prepared from fresh meat (G1F group) and freeze/thawed meat (G2F group)

Parameter	G1F	G2F	Significance
Physicochemical analysis			
Moisture (%)	61.31 ± 0.21	60.48 ± 0.33	*
Ash (%)	2.45 ± 0.03	2.56 ± 0.12	ns
Total fat (%)	20.92 ± 0.22	21.16 ± 0.37	ns
Protein (%)	14.84 ± 0.08	14.99 ± 0.21	ns
NaCl (%)	1.63 ± 0.07	1.69 ± 0.03	ns
Total phosphorus (mg/kg)	4.37 ± 0.04	3.95 ± 0.04	ns
Nitrites (mg/kg)	47.55 ± 0.23	49.11 ± 0.18	*
Hydroxyproline (%)	0.23 ± 0.01	0.30 ± 0.02	*
Collagen content (%)	1.86 ± 0.05	2.38 ± 0.12	*
Collagen-to-protein ratio	12.55	15.85	-
pH	6.14 ± 0.02	6.19 ± 0.03	ns
Technological properties			
Instrumental color			
L*	65.78 ± 0.79	64.41 ± 0.62	*
a*	15.61 ± 0.12	15.95 ± 0.15	*
b*	11.24 ± 0.08	10.80 ± 0.09	*
FRP (%)	4.53 ± 0.67	4.38 ± 0.83	ns
Process loss (%)	11.28 ± 0.51	11.76 ± 0.79	ns
Cooking loss (%)	12.74 ± 0.55	13.22 ± 0.31	ns
Frying loss (%)	13.98 ± 0.55	14.39 ± 0.49	ns

* $p<0.05$; ns: differences not significant;

Contrary to our results, *Miller et al. (1980)* demonstrated that cooking tests showed that frozen storage did affect cooking loss ($p<0.01$), and as frozen storage of meat was prolonged, the frankfurters produced lost their ability to retain moisture and fat, resulting in a higher cooking loss for the sausages made from frozen/thawed meat. *Smith (1987)* expressed that freezing causes protein insolubility and changes the myofibrillar microstructure - from filamentous to spherical, leading to a reduced water-holding capacity. However, our results did not adhere to this statement, as there was no statistical difference ($p>0.05$) found in expressible moisture (FRP %). Even though there was a significant change ($p<0.05$) found in the WHC parameter conducted in raw meat (CG-FM and GF/TM), it could possibly be assumed that different added components, such as soy isolate, which is being used as a water-holding-increasing agent, could affect these results (*Josipović and Stanišić, 2022*). In addition, the collagen content was found significantly higher ($p<0.05$) in the G2F group than in the G1F group, which

could indicate the possible compensation and cause a „false“ increase of the WHC found in the G2F group since the efficiency of collagen in retaining water in frankfurter type sausages is shown through several papers (*Prestes et al., 2012*; *Sousa et al., 2017)*. Differences ($p<0.05$) in hydroxyproline content and, consequently, collagen content could be explained by the nature of the meat cut selected for the purpose of this experiment. Beef chuck cut is composed of a vast number of muscles (27 of them) whose collagen content varies on a large scale (*Von Seggern et al., 2005*). However, the calculation of the ratio of collagen to total protein confirmed that both types of frankfurters adhere to the *Official Gazette of RS (2019).*

The results on instrumental color parameters (CIE L*a*b*) showed significant differences found in *L** (lightness), as well as the parameters a* (redness) and b* (yellowness) ($p<0.05$). According to *Kluth et al. (2021),* numerous different factors, such as heme concentration in the meats which frankfurters were made from and oxidation status, which increases during the thermic processes that frankfurters go through, influence the color of meat. It is, therefore, not surprising that there is a difference in relation to the way the frozen storage/thawing process influences the L* value. During the production process of frankfurters, salt containing sodium nitrite was added to the mixture for antimicrobial effect as well as for developing the characteristic reddish-brown color of the product. This color change occurs as a consequence of the nitrite reduction to nitric oxide (NO) and, consequently, the formation of nitroso-myoglobin, whose concentration is known to be increasing with the rise in the temperature during thermic treatment of the frankfurters (*Kluth et al., 2021; Bloukas et al., 1999; Josipović and Stanišić, 2022)*. Since there was a significant difference ($p<0.05$) found in physicochemical analysis on nitrites content, it could be assumed that this could potentially be the reason, apart from already discussed differences in raw meat analysis as the building blocks, for the shifts in the parameters indicative of redness (a*) and yellowness (b*). Similar to *Kluth et al. (2021)* results, and their research on turkey sausages produced from fresh and frozen/thawed turkey meat, we found that the close pH and color values of the ground beef meat samples (CG-FM and GF/TM) before processing, as well as the similar pH results of the frankfurters after production, are comparable.

The results of the sensory evaluation are shown in Figure 1. The taste panel could not determine any significant change with respect to the taste, smell, texture, juiciness, color, rancidity, and general acceptability of the frankfurters ($p>0.05$). These results are in agreement with results obtained by *Verma et al. (1985),* who observed a storage period of different meat groups for 52 weeks at -18°C prior to the characterization of sausages prepared from them. However, *Miller et al. (1980),* noted that meat and fat stored for 7 weeks at -17.8°C resulted in frankfurters being distinguishable ($p<0.01$) from controls, especially for the rancidity parameter, and these differences had that tendency which continued

throughout the course of storage, up until 31 weeks, when contrasts were notable to all of the panelists for all of the parameters observed (rancidity, texture, and flavor). Nonetheless, different results than the ones obtained in our experiment could lie in the formulation of sausages, as well as the experiment setting, as *Miller et al. (1980)* used and evaluated changes that occur in frozen storage not only in beef and pork meat but also, the pork fat. This could indicate that the higher rancidity scores were given in relation to oxidation that took place on a much larger scale than in our experiment (since we used fresh pork backfat in both formulations).

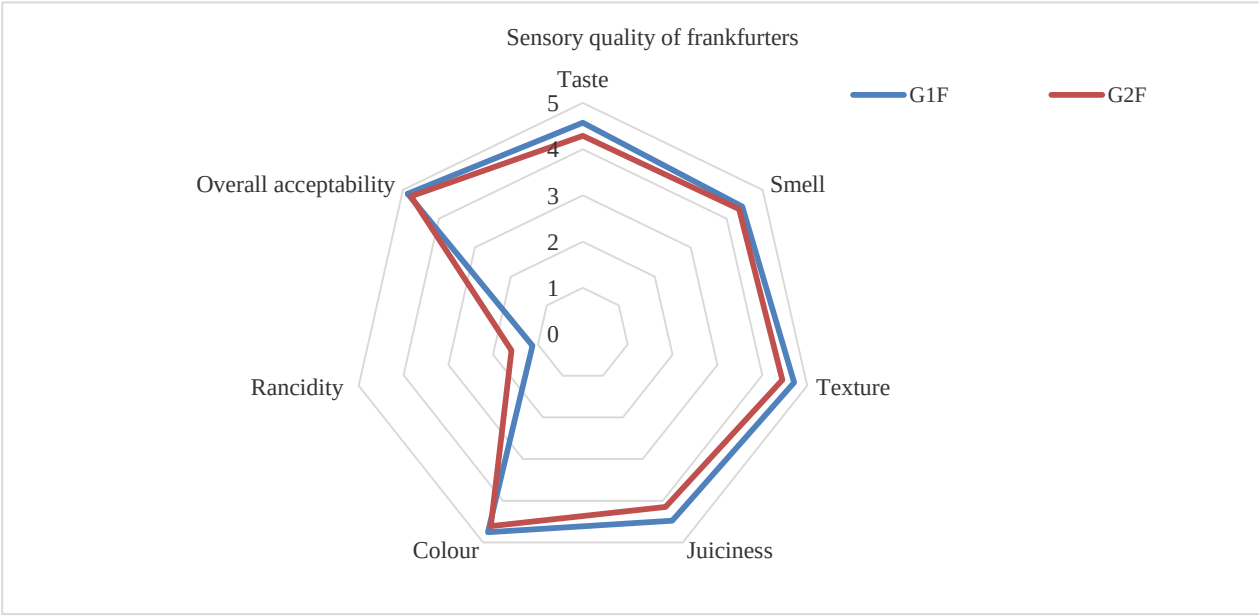

Figure 1. Spider plot for sensory quality of frankfurters

Since the results on technological parameters vary based on the frozen storage period and the freezing/thawing process, it could be assumed that 4 weeks in frozen storage is not a long enough period for severe changes that could greatly impact the final product, to occur. In conclusion, frozen storage of minced beef meat for 4 weeks at -18°C has small effects on the technological properties of frankfurters prepared from them.

Conclusion

Meat production that is of the highest quality and appealing to consumers' eyes is anticipated to translate into a revenue climb for meat producers and further stimulate the entire meat industry. The need to obtain meat freshness increases as global demand increases, and the distance between producer and buyer expands. Beef represents one of the meat products that are produced worldwide, and many studies have been conducted in relation to freezing rate, freezing storage duration, and, consequently, rates of the thawing process. However, not many studies have been conducted on the usage and impact of frozen raw materials on products, such as frankfurters, in terms of quality evaluation. This paper is of practical relevance for industries using frozen meat in the preparation of their products. Frozen storage of ground chuck beef meat for 4 weeks at -18°C has little to no effects on the technological and sensory properties of frankfurters. The thawed meat showed lower WHC and higher TBA values than the control group, and differences in color and total pigment contents were noticeable ($p<0.05$). On the other hand, WHC and process/cooking/frying loss performed on the frankfurters produced from frozen/thawed meat were comparable and showed no significant difference ($p>0.05$). However, even though the difference in color was present ($p<0.05$), based on the sensory evaluation, there were no significant color or any other (taste, smell, texture, juiciness, rancidity, and general acceptability) impairments noted by the panelists.

Procena kvaliteta viršli proizvedenih od svežeg i zamrznutog mlevenog govođeg mesa

Tamara Stamenić, Maja Petričević, Slađana Šobajić, Nikola Stanišić, Bogdan Cekić, Veselin Petričević, Nikola Delić

Rezime

U cilju procene uticaja procesa zamrzavanja/odmrzavanja sirovog mesa na mesne prerađevine proizvedene od njih, vršena je procena kvaliteta viršli. Sveže govođe meso je samleveno i podeljeno na dve partije – prva partija je odmah korišćena za pripremu viršli (G1F), a druga zamrznuta i čuvana na -18°C u period od 4 nedelje, kada je korišćena za pripremu druge grupe viršli (G2F). Fizičko-hemijska i tehnološka svojstva ispitivana su na uzorcima svežeg (CG-FM) i odmrznutog mesa (G-F/TM) , kao i viršli pripremljenih od ovih uzoraka. Skladištenje smrznutog mesa je značajno uticalo ($p < 0,05$) na vrednos ukupne vlage, kao i TBA vrednost i instrumentalno određene parametre boje (L*a*b*), SVV, ukupne pigmente i ukupne pigmente koji potiču od hema u uzorcima sirovog mesa. Kod određenih

parametara viršli, poput ukupne vlage, L* (svetloća), a* (udeo crvene boje) i b* (udeo žute boje) došlo je do značajne promene ($p < 0,05$). Međutim, razlike u kalu proizvodnje finalnih proizvoda/kalu kuvanja/kalu prženja i FRP-u nisu bile značajne. Senzorna procena nije pokazala nikakavu značajnu između dve grupe viršli ($p > 0,05$). Može se zaključiti da skladištenje smrznutog mesa mlevenog junećeg mesa u trajanju od 4 nedelje na -18°C i posledični proces odmrzavanja (na 4°C tokom 24h) ne utiču značajno na ukupnu prihvatljivost hrenovki pripremljenih od njih. Međutim, efekti promene u instrumentalnoj boji su očigledni.

Ključne reči: viršle, juneće mleveno meso, skladištenje smrznutog mesa, kvalitet mesa

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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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[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

Example 2

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

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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"Modern Trends in Livestock Production"
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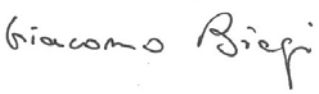

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