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THE EVALUATION OF BREEDING VALUE OF SIMMENTAL BULLS FOR MILK PERFORMANCE IN SERBIA

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Original scientific paper

Abstract: The basis for selection work is knowledge of the quality of bull sires used for conception, as well as how the major traits are passed on to the progeny. BLUP method (Best Linear Unbiased Prediction) is the basis of the most favourable solution for evaluation of additive gene value in cattle production, and it is implemented in various variants depending on the structure of data used. This research included 2.121 Simmental first cavers under control, with lactations completed within one year. All first calvers were located on holdings of individual agricultural producers on the territory of the Republic of Serbia. Evaluation of the bull breeding value for lactation duration, milk production, milk fat yield, yield of 4% FCM and percentage of milk fat, was carried out by using the mixed model (BLUP), the calculation included random effect of bull sire and fixed effect of the region, year and season of calving. In this study, bull sires which had in two or three regions over 20 daughters – first calvers of Simmental breed. Number of first calving heifers ranged from 22 to 215 animals per bull sire. By using BLUP method in evaluation of breeding value of bulls in terms of yield of milk, milk fat, content of milk fat and 4% FCMI and by ranking, results were obtained showing superiority and inferiority of breeding males.

Key words: Simmental breed, bulls, breeding value, milk performance

Introduction

Simmental breed of cattle is predominant in Central Serbia, where the total cattle population is approx. 819 000 heads, of which approx. 538.000 are cows and pregnant heifers. Active population of Simmental breed in Republic of Serbia consists of animals recorded in the main livestock/cattle registry (approx. 75.000 animals or 14%). Breeding goal for Simmental breed is to achieve maximum genetic potentials in economically relevant traits, in accordance with economical

effects of genetic improvement achieved by this breed in developed world countries.

In evaluation of breeding value for milk traits/performance, especially of bulls in progeny testing, in Serbia until recently the CC method – Contemporary comparison was used, as well as selection index, and in last years, the Best Linear Unbiased Prediction is used (BLUP). According to *Sullivan and Schaeffer (1994)* the Best Linear Unbiased Prediction (BLUP) has become world standard in evaluation of the genetic value.

Vidović et al. (1993) evaluated the breeding value of Simmental bulls based on their progeny using both BLUP and CC method. Results obtained by authors indicated that the evaluation of the bulls' breeding value and their ranks, obtained by BLUP-1 and BLUP-2 methods, were similar to evaluation and rank obtained by CC method.

The effect of selection in population depends, among other things, on the accuracy of the evaluation of additive value of the parent genotype, i.e. breeding value of bull sires, as concluded by *Đurđević and Vidović (1994)*. The authors evaluated the breeding value using BLUP and CC methods. Correlation of the breeding value rank showed that there was no complete concordance between these two methods.

Miščević (1995) obtained in his research results according which the correlation coefficient of the rank showed that there was significantly high dependence between BLUP and CC methods. However, the method of Best Linear Unbiased Parameters, due to inclusion of fixed effects, gave more precise results and more reliable evaluation of the breeding value of male breeding animals.

Stojić (1996) concluded that application of correction factors contributes to the accuracy of the evaluation of the breeding value in the way that more important factors with certain direction of their action are brought to the same level, and also because of increase of repeatability.

Evaluation of the genotype additive value, i.e. accuracy of its evaluation (bull breeding value) is directly associated with selection effect/success in cattle population *(Petrović et al. 1997)*. By using mixed model, random effect of bull sire on milk yield, yield of milk fat, content of milk fat and 4% FCM and forming of bull rank based on breeding value, results were obtained showing significant superiority or inferiority if male breeding animals.

One of the methods for evaluation of breeding value of cows and bulls is »test-day model«. Advantage of this method is in the correction of the effect of the environment factors on individual cow performance, as stated by *Freeman (1998)*. The »test-day« model is most often used for several traits, primarily milk yield, yield of milk fat, proteins and somatic cell count in milk. »Test-day« model enables genetic evaluation for additional traits, such as persistence, milk yield in specific part of lactation, etc.

Swalve (1998) pointed out that the »test-day« model, i.e. data collected on the day of testing are used for analysis instead of concluded lactations calculated based on certain daily controls. Conventional system of data collection is an expensive and slow process, and it is necessary to wait for lactations to be put in order before proceeding with the evaluation of genetic value. By application of »test-day« model the attempt is made to calculate the effect of genetic and paragenetic factors immediately and directly when this effect is exhibited, i.e. on the day of testing.

Method officially used by INTERBULL today is multiple trait model using data from many countries, which was developed by *Schaeffer and Zhang (1993)*. This method is based on the hypothesis that the genetic correlation for certain traits between countries is less than one, i.e. that there is interaction between genotype and environment. In this way the milk performance is evaluated, also milk fat and protein yield.

Materials and Methods

This research included 2.121 Simmental first calvers under control, with lactations completed within one year. All first calvers were located on holdings of individual agricultural producers on the territory of the Republic of Serbia. In selection of model for evaluation of the bull breeding value, it is important to evaluate as accurately as possible, and eliminate the effects of many systematic factors of the environment, and to establish the heritability value for traits included in the selection process. In immediate processing and analysis of data base the following mixed model of least squares was used *(Harvey 1990):*

1. Mixed model for evaluation of bull breeding value for milk performance traits (BLUP):

$$Y_{ijklm} = \mu + B_i + R_j + G_k + S_l + e_{ijklm}$$

Y_{ijklm}= expression of the trait in *m* cow, daughter of bull-sire *i*, which produced in *j* region, and calved in year *k* and *l* season

μ = general average
B_i = random effect of *i* bull sire
R_j = fixed effect of *j* region
G_k = fixed effect of *k* calving year
S_l = fixed effect of *l* calving season
e_{ijklm} = random error

The evaluation of the breeding value of bulls for following traits: duration of lactation, milk yield, milk fat yield, yield of 4% FCM and content of milk fat, was calculated using mixed model (BLUP), in which equation also random effect of bull sire and fixed effect of region, year and season of calving were included. Bull sires with over daughters – first calvers of Simmental breed in two or three regions, were tested. Number of first calvers ranged from 22 to 215 per bull sire, and total of 2.121 daughters were tested.

Results and Discussion

Breeding values and bull rank for milk performance traits in standard lactation, evaluated based on production of their daughters in all regions are presented in Table 1.

Depending on the structure of linear model, which usually contains combination of several effects on traits, absolute BLUP solutions are obtained which are used for ranking of the selection candidates. Equations/formulas contain effects with fixed (region, year, season) and random classification (sires). Model can also include information about relatives, genetic trend in the population, matrix of kinship, genetic groups or their combination. BLUP is the best linear method because it is the least biased method in evaluation of genetic and paragenetic factors, as concluded by *Latinović et al. (1997).*

The best ranked bull in regard to milk yield was HB 1197 whose production deviated by 357,62 kg, followed by HB 1206 with deviation of 329,60 kg, and on the third place was HB 1334 with deviation of 291,11 kg. The lowest ranked bull was HB 1260 which deviated by -666,69 kg and was ranked on 37th place. In front of him was the bull HB 973 with breeding value of -532,61 kg ranked 36th. At 35th place in regard to milk yield was the bull HB 1039, with deviation of -340,92 kg.

Obtained results indicate that the best production was realized by foreign bulls that are on loan in our country or whose semen had been imported for planned insemination of female breeding animals of domestic Simmental population. Some of these bull sires have in their genetic basis certain share of Red Holstein or Montbeliard genes. The lowest ranked bulls were domestic bulls.

In regard to the content of milk fat, the best ranked bull was HB 920 (BV 0,08), followed by HB 921 (BV 0,06), and on the third place the bull HB 1319 (BV 0,06). The lowest ranked bull for the percentage of milk fat was HB 1039 (BV -0,09), in front of him, ranked 36 was the bull HB 1189 (PV -0,07), and ranked 35th the bull HB 877(PV -0,05).

Table 1. Superiority (BV) and bull rank for milk performance traits in standard lactation

HB bull	No. of daughters	DL, days		MY, kg		CMF, %		MFY, kg		4%FCM, kg	
		BV	Rank	BV	Rank	BV	Rank	BV	Rank	BV	Rank
671	63	-2.85	22	-181.47	32	0.03	9	-6.02	30	-162.82	32
788	30	-4.71	29	-209.29	33	0.02	10	-7.39	33	-194.64	33
877	27	-7.17	33	152.55	8	-0.05	35	3.80	14	118.06	13
895	34	-4.75	30	-6.75	20	0.00	21	-0.60	21	-11.61	20
920	46	-3.17	23	-81.97	27	0.08	1	-0.10	20	-34.34	23
921	62	6.06	9	123.44	12	0.06	2	7.12	6	156.15	7
926	42	-7.38	34	97.08	15	-0.04	33	2.24	18	72.52	17
973	24	15.20	1	-532.61	36	0.05	6	-19.09	36	-499.28	36
975	63	3.93	11	55.38	18	0.00	20	2.09	19	53.53	18
978	215	-1.32	19	76.64	17	0.01	13	3.10	16	77.15	16
980	97	-3.19	24	165.91	5	-0.02	28	5.74	8	152.46	8
1000	55	5.19	10	160.88	6	-0.02	29	5.52	9	147.09	9
1032	46	12.37	2	-63.47	26	0.00	19	-2.39	26	-61.11	26
1039	38	8.86	3	-340.92	35	-0.09	37	-16.48	35	-383.53	35
1053	49	7.16	6	201.98	4	0.01	15	8.20	5	203.80	4
1066	67	1.07	13	-13.51	22	-0.03	31	-1.73	24	-31.41	22
1102	60	-0.86	18	7.62	19	0.06	5	2.67	17	43.13	19
1108	89	-3.94	25	-7.04	21	-0.02	24	-0.86	22	-15.75	21
1111	27	-1.34	20	144.35	9	0.03	8	6.89	7	160.94	6
1121	77	8.40	4	-101.72	29	0.01	17	-3.47	27	-92.67	28
1122	34	-4.03	26	115.94	13	0.01	16	4.78	11	118.10	12
1133	36	-4.22	27	-219.39	34	0.03	7	-7.40	34	-198.81	34
1143	69	-6.28	32	88.78	16	0.00	18	3.57	15	89.06	15
1144	29	6.89	7	-153.65	31	0.06	4	-3.60	28	-115.54	29
1151	60	-0.08	17	-53.82	25	-0.05	34	-3.86	29	-79.45	27
1166	32	8.14	5	-30.40	23	-0.02	25	-1.82	25	-39.57	25
1177	30	-2.78	21	112.68	14	0.01	14	4.77	12	116.62	14
1178	77	1.00	14	141.17	10	-0.02	26	4.71	13	127.09	11
1185	22	-8.27	36	-137.77	30	-0.04	32	-6.58	32	-153.92	31
1189	39	-7.82	35	-83.53	28	-0.07	36	-6.17	31	-125.95	30
1197	38	2.28	12	357.62	1	-0.02	27	13.16	2	340.51	1
1198	126	-4.37	28	137.16	11	-0.01	23	4.96	10	129.22	10
1206	69	-5.80	31	329.60	2	0.01	12	13.37	1	332.33	2
1260	54	-9.67	37	-666.69	37	0.01	11	-25.78	37	-653.39	37
1287	110	6.40	8	-30.57	24	-0.01	22	-1.51	23	-34.85	24
1319	48	0.48	16	154.68	7	0.06	3	8.47	4	189.03	5
1334	37	0.59	15	291.11	3	-0.03	30	9.69	3	261.84	3

Similar to the previous case, the best ranked bulls were imported ones, however, bulls who are brothers after their father from the famous Horror line, took the first two places. The lowest ranked bull for percentage of milk fat - HB 1039 was ranked exceptionally low also in regard to milk yield - 35th place. Austria has managed by application of strict selection in 2004 to reach the average content of milk fat of 4,21% for entire population of Simmental cattle in 212.563 concluded lactations *(Zoufaly et al. 2005)*.

Bulls with good results in regard to milk yield also were superior in regard to milk fat yield but with changed mutual order. So the best ranked bull was HB 1206 (13,37 kg), followed by the bull HB 1197 (13,16 kg), and the bull HB 1344 (9,69 kg).

However, in regard to the lowest ranked bulls for milk fat yield, the situation is identical to results obtained for yield of milk. The lowest ranked bull was HB 1260 with deviation of -25,78 kg and at the 37th place. In front of him at the 36th place is the bull HB 973 with deviation of -19,09. At the 35th place in regard to milk fat yield was the bull HB 1039, with negative breeding value of -16,48 kg.

If milk yield is calculated as 4% FCM, identical rank of bulls is obtained, however deviation values are slightly higher and lower. The best ranked bull was HB 1197 whose production deviated by 340,51 kg, followed by HB 1206 with breeding value of 332,33 kg, and bull HB 1334 with deviation of 261,84 kg. The lowest production was recorded in daughters of the bull HB 1260 with values deviating by -653,39 kg at 37th place. In front of him was the bull HB 973 with deviation of -499,28. At 35th place in regard to yield of 4% FCM was the bull HB 1039, with breeding value of -383,56.

For duration of lactation, the best ranked bull sire was HB 973 (15,20 days) who had the lowest results for milk performance traits. At the last place, 37th, was the bull HB 1260 (-9,67 days) who was the lowest ranked bull for all tested production traits.

The following authors studied also the evaluation of the bull breeding value using the BLUP method, their ranking, and also their effect on milk performance traits and fertility traits: *Latinović et al. (1997), Vidović et al. (1993), Đurđević and Vidović (1994), Sullivan and Schaeffer (1994), Petrović et al. (1997), Miščević (1995), Stojić (1996), Freeman (1998), Swalve (1998), Marković (1999), Panić and Vidović (2006), Trivunović (2006)*.

Production of milk and milk fat is limited to only one gender, therefore potential capacity of breeding males for these traits is established based on production results of mothers, sisters, daughters. Knowledge of the quality of bulls and how certain traits are transmitted to the progeny is central issue of breeding programs. Application of artificial insemination and long term semen storing

results in decrease of number of bulls in populations, therefore their individual effect on the selection results is considerably greater.

Conclusion

Basic requirement of the modern cattle production is improvement and enhancement of the genetic capacities of animals for production of milk, meat and calves. In application of high quality breeding males with proven genetic potential will contribute to improvement of production and reproduction traits of the cattle population in Serbia.

In this process, the evaluation of the parents' breeding value is one of the priorities. Application of linear methods in evaluation of breeding value contributes to significant improvement of the evaluation from the aspect of inclusion of more available information.

BLUP is one of the leading methods used to achieve progress in population genetics, since it is possible to include own results and production results of individual animals. By combining the methodology of mixed model, method of least squares and selection index method, BLUP is applied world wide as standard.

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Ocena priplodne vrednosti bikova simentalske rase za osobine mlečnosti u Srbiji

V. Pantelić, M. Plavšić, S. Trivunović, S. Aleksić, Lj. Sretenović, D. Ostojić-Andrić, D. Nikšić

Rezime

Osnovu za svaki selekcijski rad predstavlja poznavanje kvaliteta bikova očeva koji se koriste za oplodnju, kao i način prenošenja važnijih svojstava na potomstvo. U osnovi najpovoljnijeg rešavanja problema ocene aditivne genotipske vrednosti osobina u govedarstvu leži BLUP metod (Best Linear Unbiased Prediction), koji se primenjuje u raznim varijantama zavisno od strukture podataka.

Ovim istraživanjem je obuhvaćena 2.121 kontrolisana prvotelka simentalske rase, sa laktacijama zaključenim u toku jedne godine. Sve prvotelke su

se nalazile na imanjima individualnih poljoprivrednih proizvođača na području Republike Srbije. Ocena priplodne vrednosti bikova za osobine trajanje laktacije, proizvodnju mleka, mlečne masti, 4% MKM i procenat mlečne masti, izračunata je pomoću mešovitog modela (BLUP), u čiju jednačinu je uključen slučajni uticaj bika-oca i fiksni uticaj regiona, godine i sezone telenja. Ispitani su bikovi-očevi koji u dva ili tri regiona imaju 20 i više kćeri prvotelki simentalske rase. Broj prvotelki je bio od 22 do 215 po biku-ocu. Korišćenjem BLUP metoda za ocenu priplodne vrednosti bikova na prinos mleka, mlečne masti, saržaja mlečne masti i 4% MKM i formiranjem ranga dobijeni su rezultati koji pokazuju znatnu superiornost ili inferiornost priplodnjaka.

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THE EFFECT OF CROSSING OF DOMESTIC SIMMENTAL BREED AND FRENCH FATTENING BREEDS ON CONFORMATION AND FAT COVER OF BEEF CARCASSES

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Original scientific paper

Abstract: Evaluation of conformation and fat cover of beef carcasses has great importance in modern systems of carcass quality evaluation. In this way, adequate price is achieved for every classified carcass side according to market demand. In this paper, the effect of experimental crossing of Domestic Simmental breed with Charolais and Limousine breed (N=96) on conformation and fat cover was investigated. Evaluation was carried out on the whole carcass and partially for certain parts of carcass according to special evaluation pattern/form (1-5). In regard to evaluation of carcass conformation, statistically significant differences ($p<0,01$) were established between crosses and Domestic Simmental breed. Crosses with Charolais achieved the highest score for conformation (3,94), the highest score for round conformation (3,77) and shoulder (4,06). Considerably more fat tissue on outside of the carcass was determined in Domestic Simmental breed (3,44) compared to crosses with Charolais (3,27). Presence of fat in pelvic cavity was more expressed in Charolais (3,34) and Limousine crosses (3,28), with better score for covering of kidneys (3,50 and 3,53) compared to Domestic breed (3,17).

Key words: fattening breeds, crossing, carcass, conformation, fat cover

Introduction

Evaluation of conformation and cover of beef carcasses with fat is very important in modern systems of the evaluation of carcass quality (e.g. SEUROP classification, USDA Beef grade, etc). In this way, adequate score or each graded carcass side is realized according to the demands on the market. Importance of application of said grades is also in achievement of genetic progress in population of fattening cattle, since breeders are stimulated to rear animals from which explicit

meatiness and equal cover of carcass can be expected in case of moderate to medium presence of fat.

Correct evaluation of conformation can result in indirect obtaining of information about the development of high quality muscles sections on the carcass (round, loin, back), as well as meat : bone ratio. In practice, the opinion is generally accepted that roundness and width of carcass is formed by musculature, whereas in the length of carcass bones participate predominantly. Significance of the evaluation of carcass cover with fat reflects in the effect of this trait on technological and nutritive meat properties. Namely, subcutaneous fat tissue isolates the carcass and slows down post-mortal cooling, mitigating the effect of so called »cold-shortening« and improves the meat tenderness. On the other hand, high correlation between cover of carcass with fat and content of intra muscular fat is present *(May et al., 1992)* so in spite of positive effect on cooling loss and meat tenderness, high amounts of exterior fat on animal carcass cannot be considered desirable.

In research by *Aleksić et al. (1999), Chambaz et al. (2003)* and *Dhuyvetter et al. (1985)*, genotype is pointed out as factor of significant importance on improvement of conformation and cover of carcass with fat. Industrial crossing is fast and efficient way, by way of heterosis and complementarity, improve said properties in progeny of F1 generation *(Ostojić-Andrić et al., 2008).* According to studies by *Marshall (1994),* heterosis for carcass cover with fat was 10,1%.

In this research the effects of experimental crossing of domestic Simmental cattle and Charolais and Limousine breed cattle on conformation and beef carcass cover with fat.

Materials and Methods

For research purposes, total of 96 fattening cattle were divided into three genotype groups (G1-G3) with 32 heads in each one: G1-young cattle of domestic Simmental breed (DS) as control group; G2-crosses of F1 generation of DS x Limousine and G3-crosses of F1 generation of DS × Charolais. Young male cattle at 155-165 age of days and 175-220 kg body weight were included in experiment. They were housed in free system, fed concentrated feed, hay and maize silage *ad libitum* (Table 1) to the certain degree of fattening and approximate body mass of 600 kg.

The average duration of fattening was 295 days. At the end of fattening all heads were slaughtered in experimental abattoir of the Institute for Animal Husbandry, Belgrade-Zemun. Evaluation of conformation and fat cover was done on beef carcasses without skin (no trimming), head, lower leg parts, tail and internal organs, 24 h subsequent to cooling (0-4°C).

Table 1. Feeding regime durring the fattening

Feeds	Body weight, kg		
	to 220	from 220 to 300	over 300
Concentrate mixture composition, %			
Dry corn	48.6	65.7	---
Corn grain silage	---	---	60
Wheat middlings	15	10	15.3
Sunflower meal	20	16.2	21
Soyabean meal	13	5	---
Di-Calciumphosphate	0.3	0.1	0.2
Calciumcarbonate	1.6	1.5	1.5
Salt	0.5	0.5	1
Premix	1	1	1
Mean amount, kg/day	3	5	9
Mean amount, kg/100kg body weight	1.5	1.9	1.5
Whole plant maize silage			
Mean amount, kg/day	5	10	15
Mean amount, kg/100kg body weight	2.5	3	2.5
Medium quality hay			
Mean amount, kg/day	1	2	0
Mean amount, kg/100kg body weight	0.5	0.75	0

Evaluation of carcasses in sense of conformation and fat cover was done visually according to determined linear scale scores (Scheme 1) by professional board in order to avoid the subjectivity of the score. Carcass conformation was evaluated partially through individual score of the development of round, central carcass part (back, loin and pelvis) and shoulder part, and subsequently, these scores were presented through total conformation score. Fat carcass cover was scored based on layer on carcass and depots in thoracic and pelvic cavity. Scale used for scoring conformation and fat carcass cover is presented in.

Scheme 1. Linear evaluation of fat cover and conformation score

Carcass parts	***Conformation scores***				
	5 excellent	*4 very good*	*3 good*	*2 medium*	*1 poor*
Profiles	Convex to super convex	Convex in whole	Straight in whole	Straight to concave	Concave to very concave
Round	Very round	Round	Well developed	Insufficiently developed	Poorly developed
Back	Wide and very full	Wide and full	Full	Insufficiently full	Narrow with visible bones
Shoulder strip	Very round	Round	Well developed	Average to almost flat	Flat with visible bones
Interior round section	Distinctly above symphysis	Above symphysis	Poorly round	Almost flat profile	Flat profile
Pelvic section	Very round	Round	Poorly round	Almost flat profile	Flat profile
Carcass parts	***Carcass fat cover score***				
	1 low	*2 slight*	*3 medium*	*4 high*	*5 excessive*
Carcass	Insignificant or poor	Poor, meat mainly visible	Round and withers, meat is almost totally covered	Good cover, Except round and withers	Fully covered
Round	Insignificant or poor	Poor, meat mainly visible	Poorly covered	Fat sutures distinct	Almost totally covered, sutures are not visible
Chest cavity	No fat	Rib muscles clearly visible	Small fat depots, and rib muscles clearly visible	More distinct fat depots, rib muscles permeated with fat	Large fat depots, rib muscles strongly permeated with fat
Pelvic cavity	No fat	Small amounts of fat	Medium presence of fat	Larger amounts of fat	Distinct presence of fat
Kidneys	Insignificantly covered	Poorly covered	About 2/3 covered	Over 2/3 covered	Fully covered

All data were developed statistically by using program *Statistica for Windows*, Computer program manual. *StatSoft.Inc.(2007)*, Tulsa, OK. Significance of mean differences was estimated by Student's t- test.

Results and Discussion

In Table 2. the results of the effect of crossing domestic Simmental with French fattening breeds on conformation and fat cover were presented. In regard to conformation score of carcasses statistically significant differences were

demonstrated (p<0,01) between crosses and domestic Simmental cattle, whereas between the crosses genotypes no statistical significance of differences was established (Graph 1).

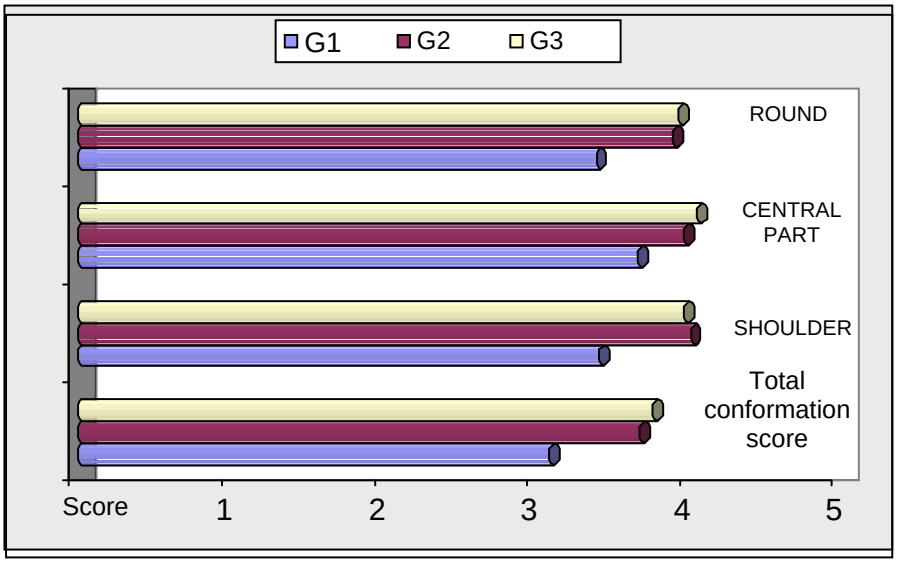
Table 2. Effect of crossing of domestic Simmental cattle with Charolais and Limousine on conformation and fat cover

Traits	Genotype			F	T test		
	G1	G2	G3		G1/G2	G1/G3	G2/G3
Final body weight, kg	579	590	621	**	ns	**	*
Carcass weight , kg	324.1	360.0	372.6	**	**	**	ns
Dressing percentage, %	55.7	59.8	59.9	**	**	**	ns
Total conformation score (1-5)	3.40	3.90	3.94	**	**	**	ns
External fat cover (1-5)	3.72	3.63	3.55	**	ns	**	ns
Internal fat cover (1-5)	3.23	3.21	3.27	ns	ns	ns	ns
Total fat cover (1-5)	3.47	3.42	3.41	ns	ns	ns	ns

* denominates statistically significant differences at the level of P<0.05; ** at the level of P<0.01; ns-at the level of P>0.05

Crosses with Charolais realized the highest conformation score (3,94), with the highest scores for round conformation (3,77) and shoulder (4,06), whereas crosses with Limousine had in average slightly more developed central carcass part (4,02), but without any statistically significant differences. These results are in concordance with research results obtained by *Aleksić et al. (1999)* where positive deviations of crosses with Charolais (+0,13) and Limousine (+0,03) to general average (4,65) were determined in conformation evaluation, whereas domestic breed demonstrated negative deviation of -0,16.

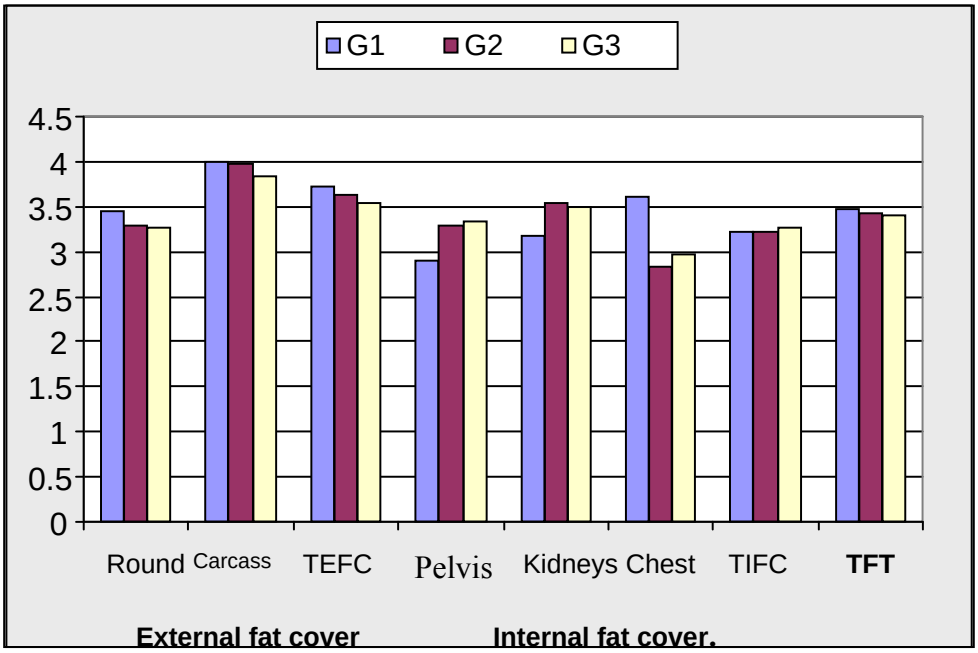
In research by *Chambaz et al. (2003),* carcasses obtained from Simmental cattle demonstrated the worst conformation compared to Charolais and Limousine cattle carcasses which were significantly heavier. These authors established no significant effect of genotype on thickness of subcutaneous tissue and cover of carcass with fat.



Graph 1. Differences between genotypes in carcass conformation

In regard to total cover of external carcass domestic Simmental cattle has significantly more fat only compared to crosses with Charolais. The most expressive differences, significant at the level of $p<0,01$, were present in case of cover of rounds with fat, where crosses with Charolais and Limousine were scored 3,27 and 3,30, and domestic Simmental cattle 3,44. However, it can be concluded that all three genotype groups had desirable cover of rounds with fat from the aspect of assessment of carcass quality.

In regard to evaluation of internal fat cover, final analysis of the sum of individual scores showed that differences between three genotypes had no statistical significance. However, results of partial scores of cover of chest and pelvic cavities with fat, as well as kidneys, showed significant differences. So the highest presence of fat on the chest cavity was recorded in heads of domestic Simmental cattle (3,61) ad the lowest in crosses with Limousine (2,83) with statistically significant differences at the level of $p<0,01$. Differences between genotypes in fat cover are presented in Graph 2.



TEFC-Total external fat cover; TIFC-Total internal fat cover; TFT-Total fat cover

Graph 2. Differences between genotypes in fat cover

Presence of fat in pelvic cavity was more distinct in crosses with Charolais (3,34) and Limousine (3,28), where also the cover of kidneys with fat was better (3,50 and 3,53) compared to domestic breed (3,17). These significances were also significant at the level of $p<0,01$.

Results of this research are in concordance with results obtained by *Aleksić et al. (1999)* where carcass cover with fat established in domestic breed was also more distinct compared to crosses of F1 generation with statistical significance of differences at the level of $p<0,05$. Deviation from the average (4,5) for this trait was negative for crosses with Charolais and Limousine (-0,05 and for domestic breed - 0,06.

Dhuyvetter et al. (1985) established slightly lower presence of internal and external fat in crosses with Charolais compared to crosses with Limousine. This corresponds to differences in values of scores for internal and external carcass cover of said genotypes obtained in this experiment.

Conclusion

Based on previously presented results it can be concluded that crossing of domestic Simmental breed with French fattening breeds resulted in significant improvement in conformation in F1 crosses, especially in regard to high quality sections (round, back and loin).

Fat layers on outside carcass were significantly greater in domestic Simmental cattle compared to crosses with Charolais. Despite the aforementioned, positive effects of fat cover on the carcass quality, this can not be considered desirable from the standpoint of fat content in carcass and the meat given the high correlation of these two properties.

In regard to total internal fat cover of carcass, no statistically significant differences were established between genotypes. However, it can be concluded that distribution of fat tissue in depots between domestic breed and crosses varied. Also, whereas in case of domestic Simmental cattle fat is deposited in chest cavity, in crosses this occurred in pelvic cavity, which resulted in better cover of kidneys and better grading of the carcass quality.

Acknowledgment

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Uticaj ukrštanja domaće simentalske rase i francuskih tovnih rasa na konformaciju i prekirvenost junećih trupova lojem

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Rezime

Ocena konformacije i prekrivenosti junećih trupova lojem ima veliki značaj u savremenim sistemima ocene kvaliteta trupova. Na ovaj način postiže se odgovarajuća cena za svaku klasiranu polutku prema zahtevima potrošača. U ovom radu ispitivan je uticaj eksperimentalnog ukrštanja domaće simentalske rase sa šarole i limuzin rasom (N=96) na konformaciju i prekrivenost junećih trupova lojem. Ispitivanje je obuhvatilo ocenu pomenutih osobina na celim trupovima kao i parcijalno na određenim delovima trupova a prema odgovarajućoj linearnoj skali ocene (1-5). U odnosu na ocenu konformacije trupova utvrđene su statistički

značajne razlike ($p<0,01$) između meleza i domaće simentalske rase. Melezi Šarolea postigli su najbolju ocenu konformacije trupova (3,94), konformacije buta (3,77) i plećki (4,06). Značajno više masnog tkiva na spoljašnjem delu trupa utvrđeno je kod domaće simentalske rase.(3,44) u poređenju sa melezima Šarolea (3,27). Prisustvo masti u karličnoj šupljini bilo je izraženije kod Šarole (3,34) i Limuzin meleza (3,28), sa boljom ocenom prekrivenosti bubrega (3,50 and 3,53) u odnosu na domaću simentalsku rasu (3,17).

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EVALUATION OF STEROID HORMONES ANABOLIC USE IN CATTLE IN CROATIA

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Original scientific paper

Abstract: Natural sex hormones are part of the endocrine system and are found in animal biological material. On analysis of residual substances with anabolic effect and detection of their abuse, it is necessary to know the physiological levels of these hormones to be able to differentiate physiological concentrations from the illegal use of anabolics. The hormone concentrations exceeding the physiological ones, found on monitoring for illegal substance use, would point to the abuse of these substances for anabolic purpose. In the present study, concentrations of the natural hormones 17β-estradiol, progesterone and testosterone were determined in bovine plasma according to animal age and sex. Natural hormone concentrations were determined using quantitative validated ELISA methods in plasma samples from cattle of different breed composition collected at several farms in Croatia during the 2006-2009 period. Methods validation showed good mean recovery and repeatability (approx. 75-87%), demonstrating the methods efficiency in determination of 17β-estradiol, progesterone and testosterone level in cattle plasma, respectively. The level of sex hormones was statistically significantly higher in yearling plasma as compared with calf plasma ($P<0.05$). The highest levels of 17β-estradiol (0.03±0.01 ng/mL) and progesterone (4.87±1.63 ng/mL) were recorded in female yearlings, and of testosterone (9.44±5.47 ng/mL) in male yearlings. Results showed the steroid hormone levels to vary with animal age and sex, indicated that illegal use of anabolic substances could not be suspected in none of the study animals.

Key words: steroid sex hormones, anabolics, physiological levels, plasma, bovine

Introduction

The sex hormones 17β-estradiol, progesterone and testosterone are steroid molecules involved in endocrine regulation of growth in humans and animals. They

are synthesized in sex glands and act through specific gene activation. Besides influencing the development of sex characteristics, testosterone also influences protein synthesis, 17β-estradiol has a major role in protein deposition, and progesterone exerts antagonistic action to estrogen hormones *(Griffin and Wilson, 1998; Meyer, 2001)*. These very properties provoke their illegal use in fattening animals for anabolic purpose.

The physiological levels of sex hormones in animal plasma vary according to animal species, categories, sex and age *(Heitzman, 1994)*. Their body concentration is influenced by sexual maturity of the animal, presence of hormone in the diet, and overall rearing conditions *(Scippo et al., 1993; Schilt et al., 1996)*. The occurrence of estrus and thus the level of sex hormones can be influenced by dozens of plants found in animal feed, i.e. by the estrogenic effect of their constituents such as isoflavones, resorcyclic acid lactones, coumestans, etc. *(Barnes, 2010)*. Considering all these factors, it appears quite difficult to definitely determine the standard physiological levels of particular sex hormones in each animal category.

Steroid sex hormones are part of the endocrine system and are found in physiological ranges in animal biologic material. Therefore, their mere presence in animal blood need not always be taken as a proof of illegal anabolic use. The physiological presence and variation of these hormones according to age, sex and many other factors make identification of abuse of these substance for anabolic purpose still problematic *(Le Bizec et al., 2009)*. On determination of these hormone concentrations in biologic material, all data of animals and history data should also be taken in consideration when assessing the hormone levels as physiological or associated with substance abuse.

In the past, estradiol, testosterone, progesterone and synthetic steroids were used as growth promoters in the form of various implants, tablets with estradiol or a combination of estradiol and testosterone *(Annon., 1998; Annon., 2005)*. However, ban has been placed on their use for anabolic purpose because of their adverse effects on human and animal health *(Council Directive 1996/22/EC; Council Directive 2003/74/EC; Stephany, 2010)*. Therapeutic use of these substances has also been restricted (disorders of reproduction and pregnancy), while the possible accumulation of their residues in animal products and adverse effects on human health are prevented by strictly professional drug administration *(Lone, 1997; FAO/WHO, 2000)*. Therapeutic administration of hormones and their effects on productivity have been investigated for years in numerous studies *(Kesler et al., 1981; El-Zarkouny and Stevenson, 2004; Colazo et al., 2007; Alnimer and Husein, 2007)*. In Croatia, the use of sex hormones is currently allowed in veterinary practice, exclusively for therapeutic purpose, in accordance with the Directive on Placing a Ban on the Use of Certain Beta-Agonists and Substances with Hormonal and Thyrostatic Effects on Farm Animals *(Official*

Gazette of the Republic of Croatia, 2008) and application should be properly documented.

The administration of sex hormones as anabolics to farm animals results in meat with a higher proportion of muscle tissue and lower proportion of adipose tissue, i.e. meat of better organoleptic properties *(Lone,1997; Deshpande, 2002)*. The anabolic effect is obtained through direct and indirect mechanisms of action resulting in enhanced nitrogen retention and increased protein synthesis, i.e. animal growth *(Van Der Wal and Berende, 1983; Meyer, 2001)*. The efficiency of animal growth promotion depends on the animal breed, age, reproductive status, and route of hormone administration *(Michel and Baulieu, 1980)*; a growth gain by up to 20% can thus be achieved *(Meyer, 2001)*. Of natural hormones, estrogens in the form of 17β-estradiol or estradiol-benzoate have been most widely used. Progesterone, testosterone and some synthetic hormones have generally been used in combination with estrogens *(Andersson and Skakkebaek, 1999)*. According to literature data, some other substances (insulin-like growth factors) exert a synergistic effect on gonadotropic hormones *(Lucy, 1999)*.

On the analysis of anabolic substance residues, the ability of demonstrating the presence of a particular substance in animal biologic material by the analytical method employed is used as a criterion for the respective substance abuse in meat production. Samples of the liver, kidney, fat and muscle at the slaughterhouse, and urine, feces and hair *(Cacciatore et al., 2009; Duffy et al., 2009; Divari et al., 2010)*, and serum *(Scalas et al., 2007)* in live animals are most frequently used to determine anabolic substance residues.

The aim of the present study was to determine the levels of sex hormones (17β-estradiol, progesterone and testosterone) in cattle plasma of various sex and age using validated ELISA methods, to get an insight into the hormonal levels that might indicate to the illegal use of steroid hormones on farm animals in this region.

Materials and Methods

Plasma samples. Natural hormone concentrations were determined in plasma samples from 40 male animals (20 calves and 20 yearlings) and 40 female animals (20 calves and 20 yearlings) collected at several farms in Croatia during the 2006-2009 period. Animal blood was sampled into EDTA tubes and centrifuged for 10 min at 2000 rpm. Upon complete plasma separation, it was transferred to tubes by a micropipette and stored at -20 ºC until analysis. Plasma samples were divided according to sex (male and female) and age (calves and yearlings) and the concentrations of 17β-estradiol, progesterone and testosterone were determined in all samples.

Sample purification. Plasma purification was done by the liquid-liquid extraction. Five mL of ether mixture (tertiary butylmethyl ether/petrol ether 30/70

v/v) were added to 1 mL of the sample and left in a shaker for 20 min at room temperature. Then the content was frozen at -25 ºC for 60 min, the ether supernatants were decanted and evaporized on a vacuum vaporizer at 60 ºC (Laborota 4001-Efficient, Heildolph). Hormone residues were then dissolved in 0.5 mL (progesterone and 17β-estradiol) or 1 mL (testosterone) of the buffer for sample dilution. The content was vigorously shaken for 1 min and warmed in water bath (Haake SWB25, Thermo) at 37 ºC for 5 min. The last step was repeated two more times. The supernatants were used on ELISA analysis.

Hormone analysis. Hormone concentrations were determined by use of the commercial ELISA kits (Immunolab GmbH, Kassel, Germany), according to the manufacturer's instructions. Standard solutions of 17β-estradiol, progesterone and testosterone (six concentration levels) and prepared solutions of plasma samples were instilled in the microwells. Then solutions of the dissolved enzyme conjugate (peroxidase), substrate (tetramethylbenzidine -TMB) and antibodies were added, with microplate incubation at room temperature in the dark. The microwells were washed in phases with the use of ELISA washer (ELx50, Bio-Tek Instruments, USA). The reaction was stopped by the addition of stop solution (0.5 M sulphuric acid) and absorbance was measured on an ELISA reader at a wavelength of 450 nm (ELx800TM, Bio-Tek Instruments, USA). Upon plotting the calibration curve, the plasma hormone concentration was determined by use of the R-Biopharm Ridasoft Win software. Results were expressed in ng/mL (ppb) taking the plasma dilution factors into account. In order to assess differences of hormone concentrations in plasma of different sex and age categories of cattle ANOVA was performed. When ANOVA assumptions were not meet, after testing for data normality and homoscedasticity by Shapiro-Wilk W test, non-parametric Spearman correlation was used. Statistical analyses were performed using Stata 10.0 (StataCopr. 2005. Stata Statistical Software: Release 10.0, College Station, TX). Statistical significance was tested at the level of $P<0.05$.

Results

Validation results of quantitative ELISA methods include determination of the recovery, repeatability and detection capability (CCβ) of the test methods. Values of the validation parameters on determination of 17β-estradiol, progesterone and testosterone by ELISA method in plasma of different animal categories are presented in Table 1. The mean hormone concentrations (mean ± SD) according to animal sex are shown in Table 2. Hormone values according to animal age and sex are graphically presented in Figures 1-3.

Table 1. Mean values of the validation parameters of ELISA methods

Analite	Validation parameter		
	Recovery (n=18)	Repeatability (n=54)	CCβ (n=20)
17β-estradiol	84.5 %	86.3 %	0.02 ng/mL
Progesterone	80.3 %	78.9 %	0.09 ng/mL
Testosterone	75.6 %	82.3 %	0.11 ng/mL

Table 2. Natural sex hormone concentrations in bovine plasma according to animal sex

Animal sex	Number of samples	Hormone concentrations (ng/mL)					
		17β-Estradiol		Testosterone		Progesterone	
		min	max	min	max	min	max
Male	40	0.025	0.038	1.283	16.502	0.076	0.145
Female	40	0.026	0.043	0.145	0.518	0.225	7.402

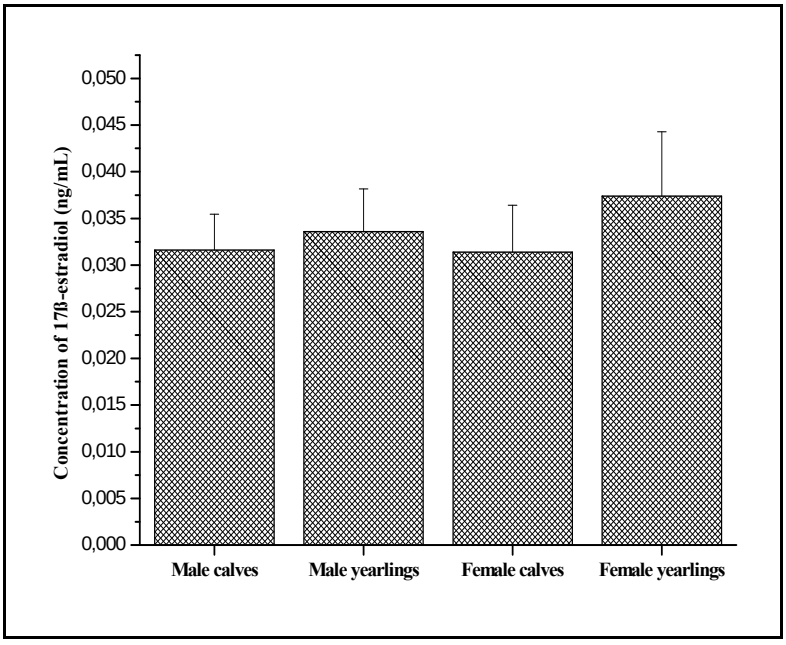

Figure 1. Concentration of 17β-estradiol (mean ± SD) in bovine plasma.

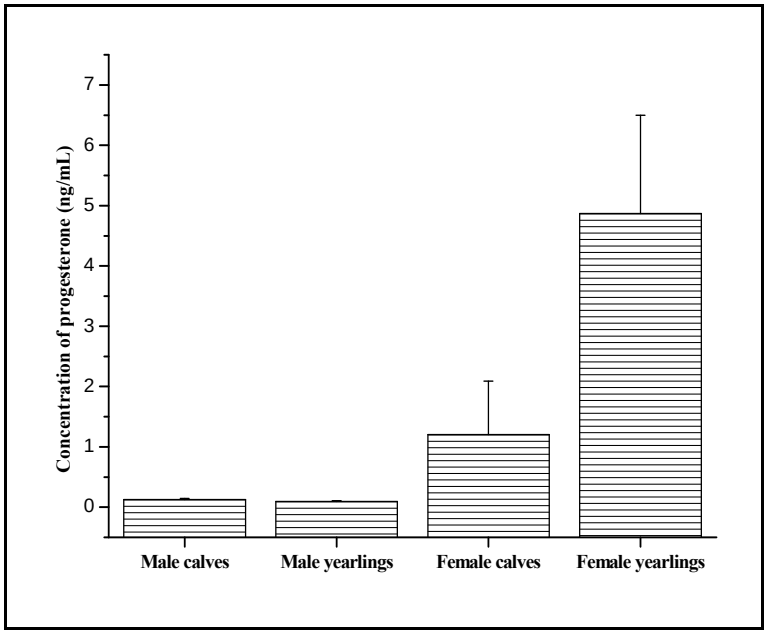

Figure 2. Concentration of progesterone (mean ± SD) in bovine plasma.

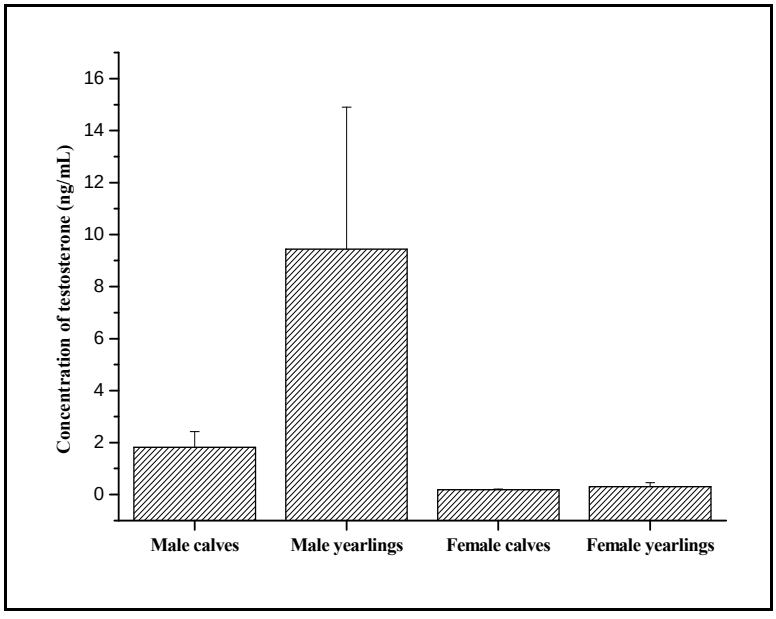

Figure 3. Concentration of testosterone (mean ± SD) in bovine plasma.

Results shown significant difference in testosterone and progesterone levels in plasma between male calves and male yearlings (P<0.05). In females, significant differences between calves and yearlings were found for progesterone levels (P<0.05). When comparing to animals sex of the same age categories (male calves with female calves and male yearlings with female yearlings) significantly differences were found in plasma testosterone and progesterone levels (P<0.05).

Discussion

Endogenous sex hormones are synthesized in the gonads, adrenal gland and placenta, and bind to protein plasma to be transported to target organs. By binding to specific receptors in particular tissues, these hormones cause numerous physiological effects in humans *(Lone, 1997)* and animals *(Berisha et al., 2002)*. Residual sex hormones taken with food of animal origin can cause the same physiological activity in humans as endogenous hormones. The influence on the physiological processes in the body depends on the amount taken relative to their natural level *(Andersson and Skakkebaek, 1999)*. Toxicological studies have shown that chronic animal exposure to anabolic doses of sex hormones induces mutagenic and carcinogenic lesions of sex organs *(Zimmerman, 1998; Delatour and Parisch, 1986)*.

Besides natural origin, residual sex hormones may also derive from various feed additives used for therapeutic and prophylactic purpose and can significantly increase the level of productivity. The effect of endogenous hormones in animals is potentiated by the administration of estrogens, gestagens and androgens in therapeutic or anabolic doses. Unprofessional use of veterinary drugs for the treatment of reproductive disorders can also cause accumulation of residues in animal tissues. Therefore, the dosage, route of administration and withdrawal period between the last dose and slaughter or using milk as food in humans are strictly regulated to eliminate the risk of residues in food of animal origin and adverse effects on human health. Natural gonadotropin and synthetic gonadotropin-releasing factor products used in the management of anestrus, and progesterone products and their synthetic analogs (gestagens) for estrus synchronization are currently most common veterinary medicine products on the Croatian market.

In the present study, concentrations of the natural hormones 17β-estradiol, progesterone and testosterone were determined in bovine plasma by use of previously validated ELISA methods. Methods validation resulted in mean recoveries ranging from 75.6% to 84.5%, repeatability from 78.9% to 86.3%, and detection capability (CCβ) ranging from 0.02 ng/mL to 0.11 ng/mL (Table 1). Acceptability of the validation parameter results and thus the appropriateness of the analytical methods for determination of hormone concentrations in animal plasma were demonstrated by comparison with the validation criteria given in *Commission*

Decision 2002/657/EC. The described ELISA methods should be used in monitoring of steroid hormones abuse as an anabolics in meat production, with a confirmation method required in case of non-compliant sample.

Determined hormone levels in bovine plasma vary greatly according to animal sex (Table 2). As expected, determination of sex hormone levels according to animal age showed statistically significantly higher levels in yearlings as compared with calves ($P<0.05$). The highest levels of 17β-estradiol (0.03±0.01 ng/mL) and progesterone (4.87±1.63 ng/mL) were recorded in female yearlings, and of testosterone (9.44±5.47 ng/mL) in male yearlings (Fig. 1-3), which is consistent with physiological values for that categories.

In most EU countries, studies of anabolic effects and abuse control mostly refer to 17β-estradiol because of its pronounced anabolic activity and growth increase, in cattle and sheep in particular (*Meyer, 2001*). Previous studies found plasma to be the most reliable matrix to discriminate between physiological concentrations and elevated hormone levels due to the administration of natural anabolics, with very low limits of detection for 17β-estradiol and testosterone *(Arts et al., 1991; Scippo et al., 1994)*. Literature data show the concentration of progesterone in bovine plasma to range from 0.2 to 8 ng/mL, and from 8 to 12 ng/mL in pregnancy *(EMEA, 1999)*. In the present study, progesterone concentration was 0.10-7.40 ng/mL, which is consistent with data reported from other studies for non-pregnant cattle. In a study by *Shafie et al. (1982)*, progesterone levels according to phases of sex cycle ranged from less than 0.1 ng/mL at the beginning of the cycle, reached a peak of 5.5±1.4 ng/mL during the luteal phase, then decreased abruptly to less than 1 ng/mL. The concentration of 17β-estradiol reached peak level of 0.02 ng/mL during the follicular phase of the cycle. The same study found the levels of 17β-estradiol and progesterone to show seasonal variation as well. However, little data have been published on the levels of various sex hormone metabolites, and this information would be of great importance knowing that some of them are also biologically active in the body *(Andersson and Skakkebaek, 1999)*.

The levels of 17β-estradiol and testosterone in calf plasma, which require due measures to be taken for suspect abuse are defined according to animal age and sex by the *Council Directive 1996/22/EC*. The borderline plasma level of 17β-estradiol demanding due measures has been set at 0.04 ng/mL in both male and female calves to obviate the possibility of a great number of false-positive results. The concentration of 17β-estradiol ranging from 0.1 to 1 ng/mL is only measured in plasma obtained from pregnant cows or illegally treated animals. In male and female calves, the allowed level of progesterone is 0.1 ng/mL and 0.4 ng/mL, respectively. The testosterone level characteristic of female yearlings is <0.5 ng/mL, whereas in male yearlings it may range from 10 to 30 ng/mL, depending on

animal age. The hormone concentrations exceeding those mentioned above point to abuse of these substances as anabolics.
In comparison with physiological values reported in the literature, the results obtained in the present study and data on study animals indicated that illegal use of anabolic substances could not be suspected in none of the studied animals.

As data on plasma hormones concentrations have not yet been precisely determined, are quite inadequate for different animal species and categories, and depend on numerous factors, additional studies are definitely necessary. Future studies should be focused on the hormone pattern of the population because these results would contribute to better identification of the possible abuse. Evaluation of the analytical results on hormone concentrations should always consider all the known factors that may potentially influence the result interpretation and apply some of the corroborative analytical methods if necessary. Furthermore, scientific concepts on the effects of some substances are modified and their potential undesired effects are discovered on a daily basis. Therefore, besides determination of the physiological levels of 17β-estradiol, progesterone and testosterone in particular cattle categories, the fate of other as yet uninvestigated substances that may exert a similar anabolic effect should also be monitored.

Ocena anaboličkog korišćenja steroidnih hormona kod goveda u državi Hrvatskoj

J. Pleadin, S. Terzić, N. Perši, A. Vulić

Rezime

Prirodni polni hormoni su deo endokrinog sistema i nalaze se u biološkom materijalu životinja. U analizama rezidua supstanci sa anaboličkim efektom, radi otkrivanje njihove zloupotrebe, potrebno je znati fiziološke nivoe ovih hormona kako bi mogle da se razlikuju fiziološke koncentracije od nezakonite upotrebe anabolika. Koncentracije hormona koje prevazilaze fiziološki nivo, otkrivene prilikom nezakonitog korišćenja anabolika, ukazuju na zloupotrebu ovih supstanci za anaboličke svrhe. U ovom radu, koncentracije prirodnih hormona, 17β-estradiola, progesterona i testosterona merene su u plazmi goveda različite starosti i pola.

Koncentracije prirodnog hormona utvrđene su pomoću kvantitativne ELISA metode u uzorcima plazme iz goveda raznih rasa sa nekoliko farmi u Hrvatskoj tokom 2006-2009. godine.

Korišćene metode su pokazale efikasnost (oko 75-87%), za određivanje koncentracija 17β-estradiola, progesterona i testosterona u plazmi goveda.

Koncentacije polnih hormona bile su statistički značajno više u plazmi jednogodišnjih grla u odnosu na plazmu sa teladi ($p < 0,05$). Najviši nivo 17β-estradiola (0,03 ± 0,01 ng / ml) i progesterona (4,87 ± 1,63 ng / mL) zabeležen je u plazmi ženskih jednogodišnjih grla, a testosterona (9.44 ± 5,47 ng / mL) u plazmi jednogodišnjih muških grla goveda. Rezultati su pokazali da koncentracije steroidnih hormona variraju u zavisnosti od starosti i pola životinja, ukazujući da sumnja o ilegalnoj upotrebi anaboličkih supstanci nije mogla biti postavljena ni kod jedne od ispitivanih životinja.

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EFFICIENCY OF BOVINE IGF-I GENE IN THE IMPROVEMENT OF MILK PRODUCTIVITY USING MARKER-ASSISTED SELECTION (MAS)

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Original scientific paper

Abstract: Because of insulin-like growth factor-I (IGF-I) gene plays an important regulatory function in milk secretion in cattle, IGF1 gene is potential quantitative trait locus and genetic marker (i.e, SNP) associated with milk production trait in cattle. Consequently, marker-assisted selection (MAS) will be useful to increase and accelerate the rate of genetic improvement on milk productivity. In this study, 48 female Holstein cattle reared under Egyptian conditions were selected based on their milk productivity and DNA from blood was extracted to amplify 249-bp of the gene encoding IGF-I. According to the breeding value, PCR products of IGF-I gene (249-bp) were sequenced only in the 15 highest and lowest milk productivity animals (GenBank accession numbers from gb|HQ183710| to gb|HQ183724|, sequentially). The result indicated that two single nucleotide polymorphisms (SNP's) at two different positions were observed in one of the highest milk productivity animals. Where, all 15 animals have adenine (A) and cytosine (C) bases at the positions 33 and 63, respectively, except, one animal (GenBank Acc. No. gb|HQ183711|) has thymine (T) and guanine (G) bases at the same positions (33 and 63, respectively). Thus, this finding can be used as marker-assisted selection (MAS) for high milk productivity in Holstein cattle.

Keywords: Holstein cattle, IGF-I gene, milk production, DNA sequencing

Introduction

Most traits of economic importance in farm animals are quantitative, in another words, are influenced by many genes and by environmental factors *(Zhang et al., 1998)*. For example, milk production trait is quantitative in nature. The

observed phenotype of this trait is the combined results of the action of large numbers of polygenes or quantitative trait loci (QTL) and environmental factors.

Marker assisted selection (MAS) is used for indirect selection of a genetic determinant of a trait of interest (milk productivity). The development of molecular genetic markers (AFLP's, RFLP's, SSCP's and SNP's) for genes (i.e. GH, Prl or IGF1) associated with quantitative productive traits in cattle will be the objective of this study for improvement of quantitative milk production trait using marker-assisted selection (MAS).

The growth hormone (GH)/insulin-like growth factor (IGF) system plays a critical endocrine role controlling nutrient metabolism in dairy cattle. In liver, growth hormone receptor (GHR) and IGF-1 are dynamically regulated by lactation and energy balance *(Rhoads et al., 2008)*. Insulin-like growth factor-I (IGF-I) gene plays an important regulatory function in milk secretion in cattle. Hence, the IGF-I gene is potential quantitative trait locus and genetic marker (RFLP's and SNP's) associated with milk production trait in cattle. Consequently, marker-assisted selection (MAS) will be useful to increase and accelerate the rate of genetic improvement on milk production trait *(Mackinnon and Georges, 1998; Reinecke et al., 2005; Reißmann et al., 2006)*.

Materials and Methods

Animals. Forty-eight female Holstein cattle reared under Egyptian conditions were chosen according to milk productivity (from the highest to the lowest milk production). Blood samples from these animals were collected by Jugular vein puncture into tubes containing an anticoagulant disodium EDTA. The samples were stored at -20 until needed for DNA extraction.

DNA extraction. From the 48 blood samples, DNA extraction was carried out according to *Sharma et al. (2000)* as follows: 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added to 100 µl thawed blood. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform-isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of double distillated water (ddH_2O).

PCR Amplification of IGF-I gene. A segment (249-bp) of IGF-I gene in 48 female Holstein cattle was amplified with the use of primer sequence *(Ge et al., 2001)*: 5'-ATTACAAAGCTGCCTGCCCC-3' (forward) and 5'-ACCTTACCCGTATGAAAGGAATATACGT-3' (reverse). PCR was performed in a reaction volume of 25 µl using 25 ng of genomic DNA of each sample, 25

pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ µl Taq DNA polymerase (Bioron, Germany). Thermal cycling (Autorisierter Thermocycler and Mastercycler Gradient) was carried out by initial denaturation at 94°C for 4 min, followed by 34 cycles each at 94°C for 1 min, annealing temperature at 62°C for 1 min, polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min., then the samples were held at 4°C. The amplified DNA fragments were separated on 2-3% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Statistical analysis. In 48 female Holstein cattle, the actual milk yield was corrected or adjusted for 305-day lactation length, age at first calving (AFC) and milking frequency (2x) using the equations described by Schmidt and Vanvleck (1974). Breeding value (BV) was calculated to rank animals according to their excellence in milk production using the equations described by *Falconer and Mackay (1996)*. BV= X□ + h^2 (X - X□), where: BV= breeding value, X□= average milk yield of the herd, h^2 = heritability for milk production trait (0.25) and X= corrected milk for animal.

Sequencing and analysis of the IGF-I gene. DNA sequencing for a fragment (249-bp) of the IGF-I gene (5' noncoding region) was performed according to *Sanger et al. (1977)* using 3130xl Genetic Analyzer (Applied Biosystems-Hitachi, Japan) at Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt. Where, sequencing was carried out for the noncoding region (294-bp) of the IGF-I gene in 15 female Holstein cattle (the highest and lowest milk productivity). Consequently, fifteen different sequence submissions were submitted to NCBI GenBank database for getting the accession numbers. Using ClustalW (1.8), sequence alignment was compared with IGF-I genes that are available in the GenBank database (http://www.ncbi.nlm.nih.gov).

Results and Discussion

Genomic DNA from 48 female Holstein cattle (highest and lowest milk productivity) was extracted to amplify IGF-I gene. PCR amplification of the gene encoding IGF-I gene yielded 249-bp in length in all 48 selected animals (Figure 1). PCR products of the gene encoding IGF-I (249-bp) in 15 female Holstein cattle (ordered from high to low milk productivity) were sequenced and read. Consequently, DNA nucleotide sequences were submitted to the GenBank and the recorded accession numbers were as shown in Table 1. To demonstrate the sequence alignment of IGF-I gene (249-bp) among the 15 female Holstein cattle under study (selected and ordered according to the highest and the lowest milk

productivity), sequence alignment was carried out using ClustalW program (Figure 2).

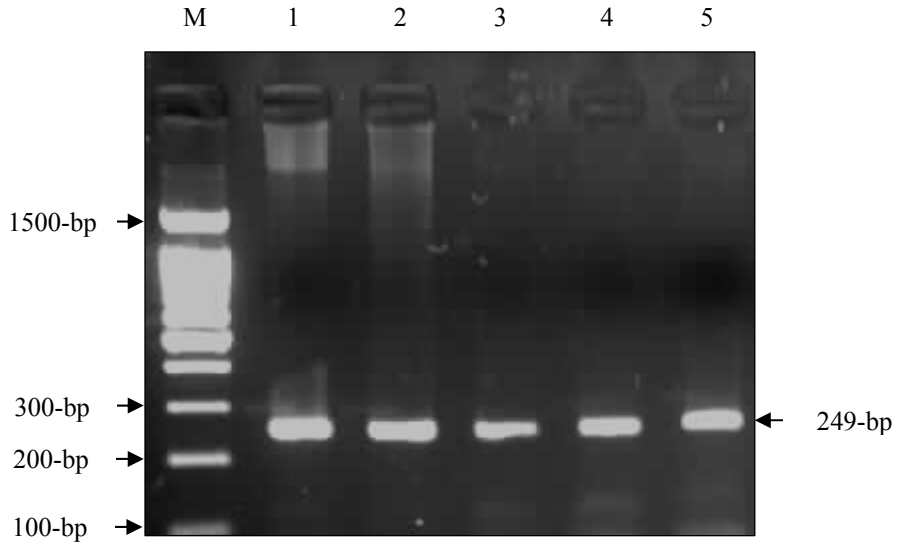

Figure 1. PCR products generated by the IGF-I gene primer. Where, lane M is DNA marker and lanes 1-5 are female Holstein cattle (as an example).

Cattle represent the most important part of animal husbandry in the most countries in the world and the genetic improvement of milk production in this farm animal is of economic importance, especially in development countries which have not arrived yet to the self-sufficiency. Components of the growth hormone (GH)/IGF system play an important role in the metabolic transition that favors high milk production after calving *(Lucy et al. 2001)*. For improvement milk production trait in cattle using marker-assisted selection (MAS), development of molecular genetic marker (SNP) for IGF-I gene was the objective of the present study.

Consequently, these two SNP's markers in bovine IGF-I gene may be useful in the genetic improvement of milk production trait in Holstein dairy cattle in general and in particular which reared under Egyptian conditions. Before leaving this part, it's important to note that animal number 1 is the only animal has two SNP's (T/G - 33/63), while all the other 14 animals have the same nucleotide sequence at the same positions (A/C - 33/63) including the highest milk productivity animal (animal number 2, BV = 11569). Because of there is no a big difference in milk productivity between animal number 1 (BV = 11553) and animal number 2 (BV = 11569), we are highly motivated for prediction to select the high

milk productivity animals using this experimental finding (marker-assisted selection).

Table 1. The animal numbers and their accession numbers.

Serial no.	*Animal no.*	*Accession no.*
1	*2*	*gb|HQ183710|*
2	*1*	*gb|HQ183711|*
3	*4*	*gb|HQ183712|*
4	*9*	*gb|HQ183713|*
5	*8*	*gb|HQ183714|*
6	*6*	*gb|HQ183715|*
7	*16*	*gb|HQ183716|*
8	*3*	*gb|HQ183717|*
9	*43*	*gb|HQ183718|*
10	*30*	*gb|HQ183719|*
11	*36*	*gb|HQ183720|*
12	*46*	*gb|HQ183721|*
13	*38*	*gb|HQ183722|*
14	*45*	*gb|HQ183723|*
15	*48*	*gb|HQ183724|*

In 48 female Holstein cattle reared under Egyptian conditions, 249-bp of IGF-I gene was amplified and only in 15 animals (selected according to the highest and the lowest milk productivity) was sequenced. Table 2 shows the nucleotide sequence variation among these 15 animals in 249-bp of IGF-I gene, which was only in 12 nucleotides. As indicated, two single nucleotide polymorphisms (SNP)

at two different positions were found in one of the two highest milk productivity animals (animal number 1, BV = 11553). Where, all the 15 animals have adenine base (A) at the position 33 except animal number 1 has thymine base (T) at the same position (33). Also all the 15 animals have cytosine base (C) at the position 63 except animal number 1 has guanine base (G) at the same position (63), see Figure 2. In contrast, we could not identify any SNP in the other 14 highest and lowest milk productivity animals in these 12 nucleotide sequence variations.

Thus, the present experiment showed that animals with T (SNP1) and G (SNP2) nucleotide sequence (33 and 63 positions) for the IGF-I gene can be used as marker-assisted selection (MAS) to select for high milk production trait.

In previous related two studies to link between IGF-I gene polymorphisms and milk production trait, *Siadkowsk et al. (2006)* indicated effect of polymorphism in IGF1 gene on milk production trait in Polish Holstein-Friesian cattle. Where, restriction analysis of PCR-RFLP-SnaBI of the IGF1 gene (249-bp) showed three genotypes AA (223- and 26-bp), AB (249-, 26- and 223-bp) and BB (undigested band, 249-bp). Cows carrying AB genotype yielded more FCM, VCM milk, more milk fat and more milk protein than those AA and BB genotypes. Also, *Mehmannavaz et al. (2010)* studied IGF1 gene (249-bp) polymorphism and milk production trait in Iranian bulls. Digestion of the 249-bp PCR product with SnaB1 restriction enzyme (C-T substitution creates a SnaB1 restriction site) yielded three genotypes TT (223- and 26-bp), TC (223-, 26-, 249-bp) and CC (249-bp). Results revealed that bulls with genotype TC had higher estimated breeding values of milk and fat yield compared to CC and TT genotypes.

Table 2. Nucleotide sequence variation among 15 animals ordered from high to low milk productivity.

Animal no.	*Breeding value (BV)*	*Nucleotide sequence variation*											
2	*11569*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*T*	*A*	*C*	*C*
1	*11553*	*C*	*T*	*-*	*C*	*G*	*T*	*G*	*T*	*A*	*T*	*A*	*C*
4	*11396*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
9	*11392*	*C*	*T*	*A*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
8	*11390*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*T*	*A*	*C*	*C*
6	*11366*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*T*	*A*	*C*	*C*
16	*11321*	*C*	*T*	*A*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
3	*11312*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
43	*10348*	*-*	*T*	*C*	*G*	*T*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
30	*10345*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*A*	*C*	*C*
36	*10337*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*A*	*C*	*-*
46	*10316*	*C*	*T*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
38	*10279*	*C*	*-*	*A*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
45	*10249*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
48	*10036*	*-*	*T*	*C*	*G*	*T*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
Nucleotide number		*18*	*22*	*23*	*24*	*25*	*33*	*63*	*175*	*191*	*192*	*193*	*194*

A is for adenine base, C is for cytosine base, G is for guanine base, T is for thymine base and - is absent base.

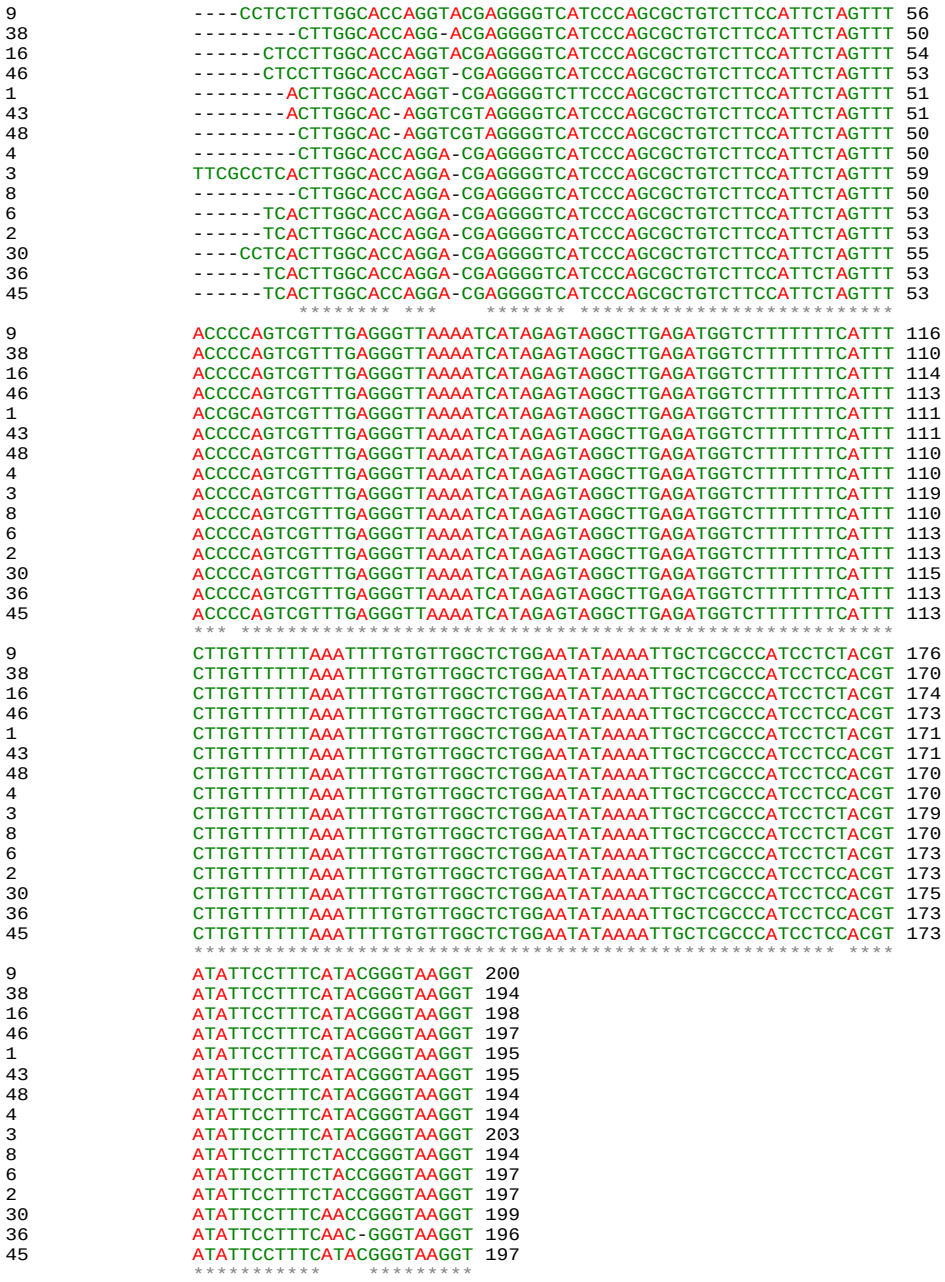

Figure 2. DNA sequence alignment of IGF-I gene (249-bp) among the 15 female Holstein cattle (9, 38, 16, 46, 1, 43, 48, 4, 3, 8, 6, 2, 30, 36 and 45). The asterisks represent the similarity.

Conclusion

In current study, PCR products of IGF-I gene (249-bp) were sequenced in 15 highest and lowest milk productivity animals (GenBank accession numbers from gb|HQ183710| to gb|HQ183724|, sequentially). Two single nucleotide polymorphisms (SNP's) at two different positions were found in one of the highest milk productivity animals. However, 14 animals have adenine (A) and cytosine (C) bases at the positions 33 and 63, respectively. While, animal number 1 (GenBank Acc. No. gb|HQ183711|) has thymine (T) and guanine (G) bases at the same positions (33 and 63, respectively). This finding can be used as a genetic marker associated with milk production trait. Hence, it could be used as marker-assisted selection (MAS) for high milk productivity selection in Holstein cattle.

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Efikasnost IGF-I gena goveda u poboljšanju produktivnosti proizvodnje mleka korišćenjem marker asistirane selekcije (MAS)

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Rezime

Zbog faktora porasta –I sličnog insulinu (IGF-I) gen ima važnu regulatornu funkciju u izlučivanju mleka kod goveda, IGF1 gen je potencijalni lokus kvantitativne osobine i genetski marker (npr. SNP) koji ima veze sa proizvodnjom mleka goveda. Zbog toga, marker-asistirana selekcija (MAS) će biti korisna za povećanje i ubrzanje genetskog napretka u proizvodnji mleka i produktivnosti. U ovom istraživanju, 48 ženskih grla rase holštajn odgajanih u uslovima u Egiptu su odabrana na bazi mlečnosti i DNK iz krvi je ekstrahovan da bi se amplificirao 249-bp gena koji šifrira IGF-I. Prema priplodnoj vrednosti, PCR proizvodi IGF-I gena (249-bp) su sekvencirani kod 15 grla sa najnižom i najvišom mlečnošću (GenBank pridruženi brojevi od gb|HQ183710| do gb|HQ183724|, redom). Rezultat ukazuje da dva pojedinačna nukleotidna polimorfizma (SNP's) na dve različite pozicije su

registrovani kod jedne od životinja sa najvećom proizvodnjom mleka. Takođe, svih 15 grla je imalo adenin (A) i citozin (C) baze na pozicijama 33 i 63, respektivno, osim jednog grla (GenBank Acc. No. gb|HQ183711|) koje je imalo timin (T) i guanin (G) baze na istim pozicijama (33 i 63, respektivno). S toga, ovi rezultati se mogu iskoristiti kao marker asistirana selekcija (MAS) za visoku proizvodnju mleka kod holštajn grla.

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THE EFFECT OF GENOTYPE ON PRODUCTION AND SLAUGHTER PROPERTIES OF BROILER CHICKENS

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Original scientific paper

Abstract: Objective of the research was to investigate the effect of genotype on production and slaughter properties of broiler chickens. The usual technology of broiler production was implemented, therefore fattening lasted 42 days. Trial included total of 2070 broiler chickens of following hybrids: Cobb 500, Ross 308 and Hubbard Classic. Chickens of genotypes Cobb 500 and Ross 308 realized significantly higher average body masses compared to Hubbard chickens. The highest mortality rate in the trial was recorded in Hubbard genotype. Chickens Cobb 500 and Ross 308 had the same feed conversion and statistically insignificant differences in production indices, whereas the values of these parameters in Hubbard chickens were significantly less favourable. The breast depth as carcass conformation measure showed no statistically significant differences between genotypes. Significantly higher values of breast angle were established in chickens of Cobb 500 compared to other two genotypes, whereas the highest values of thigh girth and the metatarsus length were established in Ross 308 chickens. Better slaughter results were obtained in carcasses of Cobb 500 genotype, chickens of both genders. Share of abdominal fat in percentage was similar in all genotypes, so the genotype had no significant effect on variation of this trait.

Key words: broiler chickens, genotype, production results and slaughter properties.

Introduction

Test of broiler chickens is common selection measure carried out all over the world. The first broiler test in our country was carried out in 1967, *Ejdupović et al. (1967),* in order to compare production properties of domestic and foreign proveniences. Since 1991, there is no domestic poultry selection. Testing of different genotypes today is done in order to compare production and slaughter properties of foreign proveniences of fattening chickens. Identification of genetically superior hybrids is of great importance for production.

For production of poultry meat in our country various hybrids are used, among which the following are predominant: Cobb 500, Ross 308, Hybro and Hubbard Classic. Comparison of their production performances in our rearing conditions is very important and interesting.

Slaughter/carcass properties of different genotypes are always relevant subject of research. Quality of processed carcasses of broiler chickens can be evaluated from several aspects. Slaughter yields and presence of abdominal fat in carcass are major elements which determine the quality of processed carcasses of broiler chickens. Numerous factors influence these, as well as other elements of the quality of broiler meat. Of biological factors, genotype and gender have the greatest influence on the carcass quality, *Bošković-Bogosavljević et al. (2004)*.

Objective of this research was to determine the production and slaughter/carcass properties of broiler chickens of different genotypes which are present on our market.

Materials and Methods

Trial was carried out during March and April 2010, in Experimental centre of Institute for Animal Husbandry. As trial material, 690 one day old broiler chickens of every genotype - Cobb 500, Ross 308 and Hubbard Classic were selected. Chickens of every studied genotype, i.e. provenience were housed in six boxes according to random block system, so that the effect of potential differences in the environment would be reduced to the minimum. Chickens were fed 4 mixtures during the trial. Composition of mixtures is presented in Table 1. Feeding was ad libitum, mixture composition was equal for all proveniences.

Mortality and consumption of feed were monitored per box, so that in the statistical processing of data unit of observation was the box. At the end of fattening (42 days) body masses of all chickens were measured.

Based on data on body mass, feed conversion and mortality, the value of production index was calculated according to formula:

$$\text{Production index} = \frac{\text{Body mass (kg)} \times \text{vitality(\%)} \times 100}{\text{Duration of fattening (days)} \times \text{feed conversion (kg/kg)}}$$

Table 1. Composition of mixtures used in the trial

Components	Pre-starter (1-10.Day) %	Starter (11-20.Day) %	Grower (21-32.Day) %	Finisher (33-42.Day) %
Corn	52.0	53.5	54.0	57.6
Soybean meal (44% Cp)	20.0	30.0	29.0	27.0
Extruded fullfat soybean	18.0	-	-	-
Sunflower meal (33% Cp)	-	3.0	4.0	4.0
Fish meal	4.0	4.0	2.0	-
Soybean oil	2.0	5.5	7.0	7.0
Limestone	1.2	1.0	1.4	1.6
Monocalcium Phosphate	1.4	1.6	1.2	1.4
Salt	0.2	0.2	0.2	0.2
Mikozel	0.2	0.2	0.2	0.2
Premixture	1.0	1.0	1.0	1.0
Chemical composition and nutritional value (%)				
Energy (Kcal)	2974	3014	3080	3100
Crude proteins	22.30	21.10	19.70	17.80
Crude fat	6.80	8.40	9.60	9.70
Ca	0.95	0.95	0.90	0.90
P Total	0.76	0.74	0.70	0.70
P Available	0.44	0.41	0.35	0.34

Subsequent to fattening, by method of random sample, 10 male and 10 female chickens of each genotype were selected, and after 12 h starvation, measured and slaughtered. After slaughtering, carcasses were processed, and warm and cooled carcasses measured according to *Rulebook on quality of poultry meat (1981)*. In this way the following carcasses were obtained:

- "conventional processing" – carcasses with head, neck, bottom leg parts and edible internal organs;
- "ready to roast" – carcasses with lungs and kidneys, heart, liver, stomach, spleen and neck;
- "ready to grill" – carcasses with lungs and kidneys, without heart, liver, stomach, spleen and neck.

In processing of carcass, abdominal fat was separated, i.e. fatty tissue which is not connected to the carcass. Obtained masses of carcass and abdominal fat were put in relation to pre-slaughter body mass of chickens. In this way, yields " conventional processing ", " ready to roast " and " ready to grill", were obtained, as well as share of abdominal fat in carcass.

Conformation measures were determined according to method by *Pavlovski and Mašić (1983)*:

- Metatarsus length was measured, using calliper, from the most prominent distal area of the foot (opposite the third finger) and area of tibia-metatarsal joint on metatarsus.
- Keel length was measured, using calliper, between final keel points.
- Breast depth indicates the curvature of the breast, measured also by calliper, between the cranial part of the keel and dorsal area between the first thoracic vertebrae.
- Thigh girth was measured, by using measuring tape, and it was expressed in mm indicating the widest part of the thigh.
- Breast angle is considered to be the most important conformation measure. It is the indicator of the development of musculature and its curvature. Breast angle is measured by protractor vertically in relation to the line of the back.

Index values of carcass conformation measures of tested broiler genotypes were obtained as relation between the pre-slaughter body masses of broiler chickens and adequate absolute values obtained for conformation measures.

Statistical processing of obtained data was done by using the software package "STATISTICA". Variance analysis was used as well as F-test to establish the presence of statistically significant differences. Tuckey test was used for determination of statistical significance of differences between mean values.

Results and Discussion

In Table 2, indicators of production traits of broiler chickens of three different genotypes are presented. Hubbard broilers had significantly lower body mass on 42nd day, compared to broilers of genotypes Cobb 500 and Ross 308. Significant differences in body masses between different genotypes of broiler chickens were determined in studies of *Hopić et al. (1995)* and *Vračar et al. (1996)*. Results on average body masses obtained in our research are higher compared to mentioned studies as well as results obtained by *Yalcin et al. (1996)* and *Farran et al. (1995)*.

Feed conversion in chickens of genotypes Cobb 500 and Ross 308 was significantly better compared to Hubbard chickens, and lower compared to research by *Petrović et al. (2002)* and *Tolimir et al. (2002)* and in concordance with the research of *Hopić et al. (1996)*. Mortality during fattening in case of Hubbard genotype was most expressed, significantly lower mortality rate was recorded for other two genotypes. Our results are in accordance with results

obtained by *Hopić et al. (1996)*. Values of production index for Cobb 500 chickens and Ross 308 differ insignificantly, but these values are significantly better than values obtained for Hubbard chickens, and are result of lower mortality, better feed conversion and higher body masses at the end of trial. Values of production indexes established by this research were higher compared to data stated by *Hopić et al. (1996), Petrović et al. (2002)* and *Vračar et al. (1996).*

By comparison of production results obtained in our research with the results of broiler chickens realized 15 years ago, the selection and genetics progress in poultry production as the most industrialized branch of livestock production, is evident.

Table 2. Production traits of tested broiler genotypes

Genotype		Body mass 42.day, g $\bar{x}$ ± Sd	Mortality, %	Feed conversion, kg	P.I.
Cobb 500	♂ ♀	2658.35±270.81 2278.33 ± 258.93	2.6	1.83 ^{A}	310.47 ^{A}
	♂ + ♀	2449.17± 324.90^{A}			
Hubbard	♂ ♀	2549.14 ± 346.63 2113.19 ± 304.75	9.28	2.11 ^{B}	234.43 ^{B}
	♂ + ♀	2289.64± 386.90^{B}			
Ross 308	♂ ♀	2746.47±273.73 2283.50 ± 263.22	3.19	1.83 ^{A}	309.85 ^{A}
	♂ + ♀	2462.85± 349.68^{A}			

Values are presented as $\bar{x}$ ± Sd

* A-B Average values in each column without common designations are significantly different at the level of 1%

In Table 3, absolute values of carcass properties are presented. Statistically, significantly lower pre-slaughter body masses were recorded in chickens of both genders of Hubbard provenience, in comparison to Cobb 500 and Ross 308 chickens. Significantly lower absolute values of carcass properties of Hubbard chickens compared to other two genotypes in regard to masses of conventionally processed carcasses, masses of carcass „ready to roast“ and „ready to grill“ are consequences of significantly lower pre-slaughter masses of this hybrid. Established differences between genotypes Cobb 500 and Ross 308 for pre-slaughter body mass were not statistically significant, which can be concluded also for carcass masses „conventional processing“, „ready to roast“ and „ready to grill“.

Table 3. Absolute values of broiler slaughter/carcass properties of tested genotypes

Genotype	Gender	Pre-slaughter body mass, g	Mass "conventional processing", g	Mass "ready to roast", g	Mass "ready to grill", g
Hubbard	♂	2567.0±252.1	2138.7±227.1	1993.8±232.9	1769.0±196.8
	♀	2356.0±231.2	1959.2±171.0	1823.8±152.8	1623.5±145.4
	♂ + ♀	2461.5±259.1^{Bb}	2048.9±216.3^{B}	1908.8±210.6^{B}	1696.3±184.2^{B}
Cobb 500	♂	2915.0±202.4	2451.5±161.5	2273.5±157.7	2045.0±145.9
	♀	2440.0±171.2	2059.7±143.8	1924.4±137.1	1717.9±139.3
	♂ + ♀	2676.5±304.4^{Aa}	2255.6±250.1^{A}	2098.9±229.7^{A}	1878.5±217.8^{A}
Ross 308	♂	2910.0±183.8	2437.9±142.3	2262.9±140.1	2040.7±127.1
	♀	2390.0±165.9	1996.6±128.2	1867.6±118.2	1665.9±101.2
	♂ + ♀	2650.0±316.6^{ABa}	2217.2±262.0^{A}	2065.2±238.8^{A}	1853.3±222.4^{A}
Assessment of significance					
Genotype		p<0.01	p<0.01	p<0.01	p<0.01

Values are presented as $\bar{x}$ ± Sd

* A-B Average values in each column without common designations are significantly different at the level of 1%

* a-b Average values in each column without common designations are significantly different at the level of 5%

Content and share of abdominal fat are presented in Table 4. The lowest share of abdominal fat was recorded in male birds of Hubbard provenience, and the highest in female chickens of Cobb 500 provenience. Differences between trial birds of different proveniences in share of abdominal fat were not statistically significant, so it can be said that shares of fat in the carcass were not under the influence of genotype, which is in accordance with results of *Hopić et al. (1999), Hopić et al. (2000)* and *Bošković-Bogosavljević et al. (2004)*. Deviations which occurred can be attributed to differences in pre-slaughter body mass.

In Table 5, carcass yields ("conventional processing", "ready to roast" and "ready to grill") in broiler chickens of investigated genotypes, are presented. Based on data presented in table it can be concluded that chickens of genotype Cobb 500 had higher values for all three studied yields compared to broiler chickens of genotypes Ross 308 and Hubbard. Analysis of expressed differences from the aspect of the effect of genotype, significant differences between these three hybrids were established only for yield "ready to grill".

Table 4. Content and share of abdominal fat in carcass

Genotype	Gender	Content of abdominal fat, g	Share of abdominal fat, % BM
Hubbard	♂	24.4±9.5	1.0±0.4
	♀	26.7±10.9	1.1±0.5
	♂ + ♀	25.5±10.0[b]	1.0±0.4
Cobb 500	♂	32.2±8.8	1.1±0.3
	♀	34.4±5.7	1.4±0.3
	♂ + ♀	33.3±7.3[a]	1.3±0.3
Ross 308	♂	35.2±9.1	1.2±0.3
	♀	32.6±7.7	1.4±0.4
	♂ + ♀	33.9±8.3[a]	1.3±0.3
Assessment of significance			
Genotype		P<0.05	p=0.06

Values are presented as $\bar{x}$ ± Sd

* a-b Average values in each column without common designations are significantly different at the level of 5%

Females of Cobb 500 provenience had values for all three investigated yields compared to males of the same hybrid, but also compared to females and males of other two hybrids.

Table 5. Relative values of broiler slaughter/carcass traits of tested genotypes

Genotype	Gender	Yield "conventional processing", %	Yield "ready to roast", %	Yield "ready to grill", %
Hubbard	♂	83.3±1.9	77.6±3.5	68.8±1.3
	♀	83.3±2.4	77.5±2.6	69.0±2.5
	♂ + ♀	83.3±2.1	77.6±3.0	68.9±2.0[b]
Cobb 500	♂	84.1±1.6	78.0±1.4	70.2±1.4
	♀	84.4±1.3	78.9±1.4	70.4±1.7
	♂ + ♀	84.3±1.4	78.4±1.4	70.3±1.5[a]
Ross 308	♂	83.8±0.9	77.8±1.5	70.1±1.5
	♀	83.6±1.1	78.2±0.9	69.7±1.3
	♂ + ♀	83.7±1.0	78.0±1.2	69.9±1.4 [ab]
Assessment of significance				
Genotype		p=0.14	p=0.41	p<0.05

Values are presented as $\bar{x}$ ± Sd

* a-b Average values in each column without common designations are significantly different at the level of 5%

Values of carcass traits established in this research are slightly lower than data stated by *Bošković-Bogosavljević et al. (2004), Vračar et al. (1997)* and *Tolimir et al. (2002)* and similar to results obtained by *Pavlovski et al. (1987), Hopić et al. (1995)* and *Petrović et al. (2002)*.

In Table 6 and 7, absolute and index values of carcass conformation are presented. Chickens of Ross 308 hybrid had significantly longer metatarsus compared to Hubbard chickens, and significantly higher value of thigh girth, which contributed to better carcass conformation. Statistically, significantly higher value of breast angle characterized processed carcasses of Cobb 500 genotype compared to Hubbard. Significant differences in metatarsus length and breast angle in chickens of different genotypes were also established in research by *Hopić et al. (1999)* and *Hopić et al. (2000)*. Established differences between genotypes for absolute values of keel length and breast depth were not statistically significant. Significantly lower index values of Hubbard chickens in regard to metatarsus length and keel length, as well as thigh girth indicated poorer carcass conformation properties of this hybrid compared to carcasses of Cobb 500 and Ross 308 broilers. Contrary to these researches, *Vračar et al. (1997)* and *Hopić et al. (1995)* did not establish in their research significance of differences for carcass conformation traits between studied hybrids.

Table 6. Broiler carcass conformation of tested genotypes (absolute values)

Genotype	Gender	ML, mm	KL, mm	BD, mm	Breast angle, degrees	TG, mm
Hubbard	♂	81.0±4.7	123.7±3.8	92.5±4.0	125.6±4.2	156.7±8.1
	♀	76.0±4.2	119.7±4.4	88.8±3.5	126.5±2.4	150.7±6.6
	♂ + ♀	78.5±5.0^{b}	121.7±4.5	90.7±4.1	126.1±3.4^{b}	153.7±7.9^{b}
Cobb 500	♂	83.3±3.7	127.5±2.0	96.0±4.5	129.5±1.1	161.4±9.8
	♀	75.8±1.6	119.6±3.4	91.7±3.7	127.8±3.4	153.5±6.7
	♂ + ♀	79.5±4.8^{ab}	123.6±4.9	93.8±4.6	128.7±2.6^{a}	157.5±9.1^{ab}
Ross 308	♂	84.5±3.6	126.0±3.4	96.5±5.0	127.2±4.0	167.2±7.9
	♀	78.3±3.3	115.5±6.6	90.0±5.3	126.4±2.9	153.9±8.3
	♂ + ♀	81.4±4.6^{a}	120.8±7.4	93.2±6.0	126.8±3.4^{ab}	160.6±10.4^{a}
Assessment of significance						
Genotype		p<0.05	p>0.05	p>0.05	p<0.05	p<0.05

Values are presented as $\bar{x}$ ± Sd

* a-b Average values in each column without common designations are significantly different at the level of 5%

Table 7. Index values of carcass conformation measures in tested broiler genotypes

Genotype	Gender	BM/ML, g/mm	BM/KL, g/mm	BM/BD, g/mm	BM/TG, g/mm
Hubbard	♂	31.7±2.5	20.7±1.7	27.7±1.9	16.4±1.0
	♀	31.0±2.1	19.7±1.8	26.5±2.2	15.6±1.1
	♂ + ♀	31.3±2.3^{B}	20.2±1.8^{Bb}	27.1±2.1	16.0±1.1^{b}
Cobb 500	♂	35.0±2.2	22.9±1.7	30.4±2.4	18.1±1.6
	♀	32.2±2.1	20.4±1.4	26.6±1.5	15.9±0.8
	♂ + ♀	33.6±2.6 ^{A}	21.6±2.0^{ABa}	28.5±2.8	17.0±1.7^{a}
Ross 308	♂	34.4±1.6	23.1±1.5	30.2±2.4	17.5±1.6
	♀	30.6±2.2	20.7±1.3	26.6±1.5	15.6±1.2
	♂ + ♀	32.5±2.7 ^{AB}	21.9±1.9 ^{Aa}	28.4±2.7	16.5±1.7^{ab}
Assessment of significance					
Genotype		p<0.01	p<0.01	p>0.05	p<0.05

Values are presented as $\bar{x}$ ± Sd

* A-B Average values in each column without common designations are significantly different at the level of 1%

* a-b Average values in each column without common designations are significantly different at the level of 5%

BM – pre-slaughter body mass ML – metatarsus length KL – keel length
BD – breast depth TG – thigh girth

Conclusion

Based on research results obtained in the study of the effect of broiler genotype on production results, slaughter/carcass yields and share of abdominal fat, the following can be concluded:

Hubbard chickens realized after 42 days of fattening significantly lower average body mass compared to Cobb 500 and Ross 308 chickens.

Lower mortality, better feed conversion and higher value of production index were recorded in chickens of Cobb 500 and Ross 308 provenience compared to Hubbard chickens.

Yields "conventional processing" and "ready to roast" were not under the significant influence of genotype, whereas the yield »ready to grill« was significantly higher in chickens of Cobb 500 provenience compared to Hubbard chickens.

Values of conformation measures were under the influence of genotype. The greatest breast angle was recorded in Cobb 500 chickens, the longest metatarsus and the highest value of thigh girth in Ross 308 chickens, whereas the lowest index value for keel length and breast depth was recorded in chickens of Hubbard genotype.

Genotype also had no statistically significant effect on the share of abdominal fat in carcass.

Based on established results it can be concluded that the results obtained for Hubbard genotype chickens in this investigation were inferior to those obtained for Cobb 500 and Ross 308.

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Uticaj genotipa na proizvodne i klanične karakteristike brojlerskih pilića

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Rezime

Cilj istraživanja bio je da se ispita uticaj genotipa na proizvodne i klanične karakteristike brojlerskih pilića. Primenjena je uobičajena tehnologija gajenja tako da je tov trajao 42 dana. Ogledom je obuhvaćeno ukupno 2070 brojlerskih pilića hibrida: Cobb 500, Ross 308 i Hubbard Classic. Pilići genotipa Cobb 500 i Ross 308 su postigli značajno veće prosečne telesne mase od pilića genotipa Hubbard. Najveći procenat mortaliteta u toku ogleda uočen je kod genotipa Hubbard. Istu konverziju hrane i statistički neznačajno različite proizvodne indekse postigli su pilići Cobb 500 i Ross 308, dok su vrednosti ovih parametara kod pilića Hubbard bile značajno nepovoljnije.

Dubina grudi kao mera konformacije trupa se nije statistički značajno razlikovala među genotipovima. Značajno veće vrednosti grudnog ugla imali su pilići Cobb 500 u odnosu na ostala dva genotipa, dok su najveće obime bataka i najveće dužine piska imali pilići Ross 308.

Bolji klanični rezultati su dobijeni na trupovima oba pola genotipa Cobb 500. Procentualno učešće abdominalne masti je slično kod genotipova, tako da genotip nije imao značajan uticaj na variranje ove osobine.

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NATURAL SEPIOLITE EFFICIENCY IN REDUCING ^{137}Cs TRANSFER AND DEPOSITION INTO MEAT AND EDIBLE ORGANS OF BROILER CHICKENS

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Abstract: The objectives of the present study were to examine the level of radiocesium deposit in meat and edible organs of broiler chickens as well as to investigate efficiency of natural sepiolite in reducing ^{137}Cs deposition in meat, liver and gizzard of alimentary contaminated broiler chickens. Broiler chickens (six weeks of age) were fed with the standard diet and each broiler was given a single oral dose of ^{137}Cs, total activity of 3750 Bq. The broilers were divided into two groups (10 broilers per group). The group 1 was control (received only radiocesium). The broilers of the group 2, in addition to radiocesium received natural sepiolite solution (2 g sepiolite per bird). After 24 hours, all broilers, from each group, were stunned and killed. The samples of meat, (breast and legs), liver and gizzard were taken from each broiler, for gamma spectrometry determination of radiocesium activity. After 24 hours of contamination, 56 % of introduced ^{137}Cs radioactivity was deposited in the meat (breast and legs muscles), 1 % in the liver and 2,4 % in the gizzard of broiler chickens 42 days of age.Natural sepiolite demonstrated insufficient protective action. Compared to the control group, percentage reduction (decreasing percentage) of ^{137}Cs deposition in meat was 16 %, in liver 5 % and in gizzard 12 %.

Key words: ^{137}Cs, deposition, broilers, sepiolite

Introduction

Practical experience gained after the Chernobyl accident has shown that natural clay minerals were effective in preventing high radiocesium levels in animal products. Natural zeolite – clinoptilolite, as one of the most important natural ion exchangers, has been used for adsorption of radioactive caesium in broiler chicks *(Vitorović et. al., 2002; Mitrović et al. 2007)* and pheasant

(Vićentijević e al., 2006). There were determined that ^{137}Cs binding efficiency of natural zeolite ranged from 50.0 % to 70.0 % *(Lonin, 2009)*. Modified clinoptilolite *(Poschl and Øezáè, 2003)* also showed significant efficiency in reducing ^{137}Cs transfer from feed to chicken meat.

Sepiolite is naturally occuring clay mineral of sedimentary origin. It is porous clay with a large specific surface area and with high ability to adsorb inorganic as well as organic compounds and radionuclides *(Lazarević et al., 2007; Lazarević et al. 2009)*. Sepiolite aditives provide viscous fluids when they are dispersed in watre or other liquid systems. These properties make it a valuable material for wide range of applications such as pet litters, absorbents and animal feed additives. In a practice, sepiolite used as a pellet binders for improving pellet quality *(Angulo et al., 1995)*. Addition in broiler chicke diets, sepiolite increase the nutritive value of diets by retaining digesta longer in the gastrointestinal tract. This could increase the ability of the digestive tract ti hydrolize enzymatically dietary polymers, especially in growing broilers which have faster transit times than adults *(Ouhida et al., 2000)*.

There is low information about sepiolite efficiency in radiocaesium sorption in contaminated broiler chickens.

The objectives of the present study were to examine the level of radiocesium deposit in meat and edible organs of broiler chickens as well as to investigate efficiency of natural sepiolite in reducing ^{137}Cs deposition in meat, liver and gizzard of alimentary contaminated broiler chickens.

Materials and Methods

In this experiment 42 days of age male Hubbard broiler chickens were used. The birds were divided into two groups (10 birds per group) and reared in the cages. Body weights of broilers were uniform (2.0 – 2.2 kg). Food and water intake was ad libidum. The birds in all groups were orally contaminated (using gastric tube) receiving a single dose of 3 ml CsCl solution activity of 1250 Bq/ml (total 3750 Bq/bird). The broiler in the group 2 ,simultaneously with the ^{137}Cs was applied, received 5 ml natural sepiolite solution, using gastric tube (2 g sepiolite per bird). After 24 hours, the broilers from each group were stunned and killed by cervical dislocation. The samples of meat (breast and legs muscles), liver and gizzard were taken from each broiler. The samples were homogenized and placed in the counting vessels of defined geometry and kept frozen at the temperature of -18ºC. The ^{137}Cs activity was determined in the thawed samples using gamma spectrometry system (HPGe, ORTEC) with pure germanium vertical gamma detector with 30.3% efficiency. The measurement time was 12000 s. For each broiler, level of radiocesium deposit was determined in samples of meat (breast and legs), liver and gizzard and expresed in Bq/sample and in percents of ingested (given) ^{137}Cs activity (%). For examination the effectiveness of sepiolite, ^{137}Cs

activity concentration (Bq/kg fresh weight) was determined and reduction of ^{137}Cs deposition in the breast meat, liver and gizzard of broiler chicks was calculated in relation to the group 1 (control) expressed in percents (decreasing percentage relative to control).

The experimental data were subjected to the analysis of variance using the STATISTICA for Windows Software (Stat Soft Inc. version 6). The significance of differences between means was tested using Tuckey´s Honestly Significant Test.

Results and Discussion

After oral contamination, radiocesium be deposited in soft tissues. The level of deposition in meat (breast and legs muscle), liver and gizzard of chickens, after 24 hours of contamination is shown in Table 1.

Table 1. ^{137}Cs distribution and deposition into the meat, liver and gizzard of broiler chicken

Tissue/ organs	^{137}Cs activity (Bq/sample)		% of ingested ^{137}Cs activity	
	Group 1 (^{137}Cs)	Group 2 (^{137}Cs+sepiolite)	Group 1 (^{137}Cs)	Group 2 (^{137}Cs+sepiolite)
Meat	2090 ± 112	1660 ± 109*	56.00	44.00
Liver	36 ± 6.1	32 ± 2.1	1.0	0.85
Gizzard	92 ± 8.7	81 ±7.7	2.4	2.16

Means ± Standard deviation;
* Statistically significance of differences ($p < 0.05$)

Obtained results showed that 56 % of introduced ^{137}Cs radioactivity was deposited in the muscle, 1 % in the liver and 2,4 % in the gizzard (group 1, only radiocesium ingested). In the case of oral simultaneous addition of sepiolite, 44 % of introduced ^{137}Cs activity was deposited in the muscle, 0,85 % in the liver and 2,16 % in the gizzard.

The effects of the administration of sepiolite on the decreasing ^{137}Cs deposition in the meat, liver and gizzard of contaminated broiler chicks were presented in Table 2.

Table 2. ^{137}Cs activity concentration (Bq/kg) in broilers meat and edible organs and efficiency of sepiolite in reduction of ^{137}Cs deposition (%)

Tissue/ organs	^{137}Cs activity concentration (Bq/kg)		Decreasing percentage (%)
	Group 1 (^{137}Cs)	Group 2 (^{137}Cs+sepiolite)	
Meat	2614 ± 150	2187 ± 141*	16
Liver	939 ± 67	893 ± 55	5
Gizzard	2298 ± 161	2020 ± 174	12

Means ± Standard deviation;
* Statistically significance of differences ($p < 0.05$)

Broilers in group 2, which received sepiolite, showed significantly ($p< 0,05$) lower ^{137}Cs activity concentration in meat when compared to the broilers in control group (group 1), those who did not received sepiolite. Percentage reduction (decreasing percentage) of ^{137}Cs deposition in meat was 16 % compared to the control group. In cases of liver and gizzard there were no significant differences in ^{137}Cs activity concentration between group1 and group 2. Radiocesium decreasing percentage, relative to the control group, in liver was 5 % and in gizzard 12 %.

Obtained results showed lower degree of broilers protection compared to those achieved with clinoptilolite *Vitorović et. al.* (2002), *Poschl and Øezáè (2003)*, *Vićentijević et al. (2006)* and *Mitrović et al*. *(2007)*. These aouthors stated that ^{137}Cs binding efficiency of natural and modified zeolite ranged from 50.0 % to 70.0 %.

According to the researchers from Ukraine (*Lonin*, 2009) all substances with antiradiation properties can be separated into three categories by the protective action efficiency. The first category (insufficient protective action) consists of substances with protective efficiency of 0-30 %; the second category (efficient) consists of substances with protective efficiency of 30-60 % and the third category (high-efficient) consists a substances with protective efficiency of 60-100 %. On the basis of these divisions, natural sepiolite demonstrated insufficient protective action.

Conclusion

After 24 hours of contamination, 56 % of introduced ^{137}Cs radioactivity was deposited in the meat (breast and legs muscles), 1 % in the liver and 2,4 % in the gizzard of broiler chickens 42 days of age.

Natural sepiolite demonstrated insufficient protective action. Compared to the control group, percentage reduction (decreasing percentage) of ^{137}Cs deposition in meat was 16 %, in liver 5 % and in gizzard 12 %.

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Efikasnost prirodnog sepiolita u smanjenju prelaska i deponovanja ^{137}Cs u meso i jestive organe brojlerskih pilića

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Rezime

Cilj ovog rada je bio da se ispita stepen deponovanja radiocezijuma u mesu i jestivim organima brojlerskih pilića, kao i da se ispita efikasnost prirodnog sepiolita u smanjenju deponovanja ^{137}Cs u mesu, jetri i bubcu, alimentarno kontaminiranih pilića. U radu su korišćeni brojlerski plići (Hubbard) uzrasta 42 dana, koji su dobili po jednu oralnu dozu ^{137}Cs, ukupne aktivnosti 3750 Bq. Pilići su podeljeni u dve grupe (po 10 jedinki u grupi). Grupa 1 je bila kontrolna (dobijala je samo ^{137}Cs). pilići grupe 2, pored radiocezijuma dobijali su, istovremeno, i rastvor sepiolita (2 g sepiolita po piletu). Posle 24 sata, izvršeno je žrtvovanje svih pilića. Uzorci celokupnog mesa (mišići grudi i nogu zajedno), jetre i bubca su uzimani od svakog pileta za gamaspektrometrijsko određivanje nivoa aktivnosti radiocezijuma. Ustanovljeno je da 24 sata posle kontaminacije, u odnosu na unetu aktivnost, u mesu se deponuje 56 % radiocezijuma, u jetri 1 % a u bubcu 2,4 %. Prirodni sepiolit ispoljio je nedovljnu efikasnost zaštite. U odnosu na kontrolnu grupu, procenat smanjenja deponovanja ^{137}Cs u mesu je bio 16 %, u jetri 5 % a u bubcu 12 %.

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SHARED VARIABILITY OF BODY SHAPE CHARACTERS IN ADULT MUSCOVY DUCK

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Original scientific paper

Abstract: In this study body weight and six body measurements namely body length, breast circumference, thigh length, shank length , total leg length and wing length of 150 twenty weeks old male and female Nigeria indigenous muscovy duck, reared under semi intensive system, were subjected to factor analysis. The objectives of the study were to evaluate the main sources of shared variability among body shape characters, to deduce the factors that describe these characteristics and to quantify sex differences in morphometric size and shape in adult muscovy duck. Variation occur in descriptive statistics between male and female traits in favour of the male in almost all traits except shank length. Magnitude of correlation also differ between sexes. Common factor variability in the measured traits in both sexes were accounted for by two factors and are about similar. Body conformation and shape appears to be controlled by common and unique factors. Communalities ranged from 0.671 for shank length to 0.987 for body length.

Key words: muscovy duck, body measurement, communality, shared variability and
factor analysis

Introduction

Biometrical variation into size and shape component in domesticated animal has been an area of growing interest to animal breeders. These concepts are fundamental to the analysis of variation in the animals. Morphological measurement have been found useful in contrasting size and shape of animal *(Mckraken et al., 2000; Latshaw and Bishop 2001)* and to estimate body weight. However, correlation between body dimensions may be different if the dimensions are treated as bivariates rather than multivariates. This is because of the interrelatedness or lack of orthogonality (collinearity) of the explanatory variables. Since body measurements are interrelated both genetically and phenotypically

Blasco et al. (1984), the analysis of these traits should be consider interdependence among these traits *(Shahin and Hassan, 2000)*.

Sexual dimorphism in muscovy duck have been expressed by several authors *(Baeza 2001; Yakubu, 2009; Ogah et al., 2009)*. An attempt have also been carry out in assessing size and shape in muscovy duck using principal component analysis *(Ogah et al., 2009)*, not considering the common and unique factors involved.

The current work was to evaluate main sources of shared variability in male and female adult muscovy duck and to deduce factors that describe body conformation in the duck.

Materials and Methods

One hundred and fifty muscovy ducklings made up of 63 males and 87 females were hatched by 60 dams and 10 sires at the duck unit, Livestock Complex, Teaching and Research Farm of the College of Agriculture Lafia , Nasarawa State, Nigeria. The ducklings used for this study were selected randomly at 3 weeks of age and managed under semi intensive system to 20 weeks of age. The birds were fed on grower marsh formulated at 20% CP and 12058kj/kg and water was supplied *ad libitum*.

The body weight in grams and dimension in centimetre were recorded for each ducklings at 3, 5, 10, 15 and 20 weeks of age. The linear body dimensions considered were body length (BL) length between the base of the neck and that of caudal end, Shank length (SL) distance from the shank joint to the extremity of the digitus pedis, breast circumference (BCC) measured under the wing through the anterior border of the breast bone crest and the central thoracic vertebrae, thigh length (TL) from the end of the drumstick to the body flank , total leg length (TLL), measured as the total length of the leg from the thigh to the extremity of the digitus pedis, wing length (WL) taken from the shoulder joint to the extremity of the terminal phalanx. To ensure accuracy each measurement was taken twice and the mean was use in subsequent analysis. The same person took all measurement and weighing throughout thus eliminating errors due to person differences as suggested by *(Shahin and Hassan, 2000)*.

Statistical analysis;

Mean, standard errors, and coefficient of variation of body weight and linear body measurements were calculated . General linear model (GLM) was used to analyse sex effect. Pearson coefficient of correlation (r) among body weight and various morphometric traits were estimated . For each sex the data was subjected to a factor analysis procedure of SAS (1999)(PROC FACTOR). The main sources of shared variation among the interdependence of body measurements (P) was expressed in terms of fewer mutually uncorrelated common factor F1, F2, Fq

(where q< p) than the original measurement *(Darton, 1980)*.The first factor contained the greatest portion of the original variation while the variables that shows higher power for causing this variation load in factor one. It was designated as a general size factor. The second factor normal have those traits that showed close variability not shown in first factor. Subsequent factors were mutually orthogonal to those preceding and to one another and contained less variation.

The model used is as follow as outline by *Shahin and Hassan (2000)*

$X= \Lambda F+U$

Where X= ap x 1 is a vector observational variables

Λ= ap x q a matrix of factor loading (factor-variate correlations, the degree of correlation of the variables with factor (the pattern matrix); F=aq x 1a

Vector of factors (non observable) and U = ap x 1 a vector of the specific unique factor.

The total variance of a variable was equal to unity and can be written as the form of common variance " Communalities" and unique variance"Uniqueness". The communality represent the portion of the variable variance accounted for by all common factor and the Uniqueness represent the portion of the variable variance not ascribable to its correlation with other variable *(Shahin and Hassan, 2000)*.

Results and Discussion

Original non –independent variables: The mean ±standard error and coefficient of variation of body weight and other morphometric traits of the Nigeria adult muscovy duck based on sex are presented in Table 1. Sex – influence ($P<0.05$) difference were observed in all traits except shank length , with superior values recorded for the drakes. These apparent sex associated differences have been reported earlier in previous studies on muscovy duck *(Baeza et al., 2001; Teguia et al., 2008; Yakubu, 2009)*. The dimorphism might be attributed to the usual between sex associated hormonal effect on growth as reported by *(Deeb and Cahaner 2001)*. *Baeza et al. (1999)* submitted that the degree of divergence between sexes however differ, where there was selection for increase body weight the drakes will mutually twice the size of the female. The average live weight and body measurement obtained in the present study are slightly lower than what *Hu et al.(1999, 2006)* obtained but similar to what *Mopate et al.(1999)* reported on African muscovy duck. The differences obtained here might be due to the non selection and continuous inbreeding in the Nigerian muscovy duck.

Tabe 1. Descriptive statistics of body weight(g) and body measurements(cm) of adult muscovy duck based on sex

Variable	Mean ± SE	Coef.Var	Minimum	Maximum
Male				
BW	2691.6±30.7	3.78	2456.4	2887.0
BL	47.61± 0.17	1.23	46.21	48.14
BCC	39.32±0.18	1.51	38.35	40.20
TL	8.88± 0.03	0.97	8.69	9.01
SL	6.59 ± 0.05	2.49	6.40	6.90
TLL	20.52±0.23	3.68	18.11	22.05
WL	36.99±0.16	1.42	36.67	38.27
Female				
BW	1504.6± 9.60	2.02	1491.2	1590.2
BL	38.61± 0.15	1.18	38.34	39.65
BCC	31.89± 0.29	2.85	31.23	33.98
TL	6.84± 0.12	5.65	6.55	7.76
SL	6.59±0.11	5.03	6.20	7.10
TLL	16.86±0.07	1.31	16.76	17.46
WL	32.94±0.23	2.19	32.40	34.63

BW= body weight , BL= body length ,BCC= breast circumference, TL= thigh length , SL= shank length , TLL= total leg length , and WL= wing length .

Table 2. Correlation matrix between body weight and body measurements of adult muscovy duck male (above diagonal) female(below diagonal)

	BW	BL	BCC	TL	SL	TLL	WL
BW		0.858	0.874	0.899	0.643	0.977	0.868
BL	0.783		0.955	0.918	0.741	0.943	0.868
BCC	0.502	0.278		0.968	0.785	0.927	0.843
TL	0.927	0.687	0.616		0.789	0.922	0.884
SL	0.699	0.338	0.728	0.746		0.671	0.685
TLL	0.901	0.546	0.560	0.833	0.691		0.915
WL	0.765	0.413	0.675	0.704	0.949	0.758	

Pairwise correlation between body weight and biometric traits :Phenotypic correlation of the body weight and body measurements of the muscovy duck of both sexes are presented in Table 2. In the drakes significant($p<0.05$) ($P<0.001$) association existed among body weight and the biometric traits. The coefficient of

correlation ranges between 0.64 to 0.97 while the corresponding in female duck the coefficient ranged from 0.28 to 0.95. the estimate of the correlation coeffiecient in the present study are comparable to previous report *(Teguia et al., 2008; Ogah et al., 2009; Yakubu, 2009)*. The strong relationship observed between body weight and body measurements may be useful as selection criterion, thereby providing a basis for the genetic manipulation and improvement of the muscovy duck. The magnitude of the correlation among variable differ between male and female an indication of variability and relationship between measurements due to sex effect supporting the dimorphism earlier outline *(Baeza, 2001)*.

Table 3. Explained variation associated with rotated factor analysis along with communalities for each variable for male and female adult muscovy duck

Traits	Male				Female			
	common. fac. 1	2	communality	unique fac.	common fac. 1	2	Communal.	unique fac.
Body weight	0.942	0.299	0.976	0.024	0.942	0.248	0.948	0.052
Body length	0.668	0.651	0.870	0.130	-0.042	0992	0.987	0.013
Breast circm.	0.727	-0.464	0.743	0.257	0.965	0.002	0.932	0.932
Thigh length	0.931	0.170	0.895	0.105	0.977	-0.032	0.956	0.068
Shank length	0.873	-0.399	0.922	0.078	0.808	-0.132	0.671	0.044
Total leg length	0.898	0.090	0.815	0.185	0.969	0.140	0.959	0.041
Wing length	0.892	-0.304	0.888	0.112	0.031	-0.203	0.909	0.091
% of total var.	72.7	14.5			74.8	16.1		

Varimax rotated independent factors. Table 3 present the result of the factor analysis in male and female muscovy duck. Two common factors were obtained , contributing between 87.2% to 90.9% of the variability of the seven original variable. The first factor (F1) (general size) was characterized by high positive loading (factor –variate correlation) on all traits considered in the male with total percentage variance of 72.7% while in the female all traits had high positive loading to the (F1) except for body length with total percentage variance of 74.8%. The values obtained for the first factors were higher than what was earlier reported when principal component analysis was applied on eleven morphometric traits in muscovy duck (51.42 and 36.76) for male and female respectively *Ogah et al. (2009)*. *Yakubu et al. (2009)* also uses principal component analysis in examining the covariance of some linear traits in three Nigerian local

chicken genotypes found out that the first principal component general size accounted for 73.9%, 80.95 and 74.6% for normal feather, frizzled and necked neck chicken respectively. The differences in the factor extraction has to do with the way the weight is distributed over the body and genetic adaptation to physiological needs *(Goss ,1981)*.

Table 3 listed the communalities for the various traits, the variances of each trait was partitioned into common portion communality shared with some or all of the other variables and a uniqueness portion, unique to that particular trait and not shared with other variables. 74% to 99% of the variation in the conformation traits was brought about by common factors in both sexes, were as 1 to 16% of their variation were contributed by unique factor specific for each trait. In male the communalities for the conformation traits ranged between 0 .74 for breast circumference to 0.98 for body weight. While in female it ranges between 0.67 to 0.99 for shank length and body length respectively. This is similar to what *Shahin and Hassan (2000)* obtained for Egyptian breeds of rabbit.

In male of the body dimensions , breast circumference had the lowest communality with greatest uniqueness about 74.1% of the variation in breast circumference was brought about by common factor, where as 26% of the variation was contributed by a unique factor specific for this trait. In female shank length had the lowest communality 67% and variation brought about by common factor, while body length had the highest communality about 99%. From the result , communalities for skeletal dimension in male (shank length , thigh length , wing length and body length) were higher than the flesh dimension breast circumference. Similarly in female higher communalities were recorded on skeletal dimension .This finding is similar to what *Shahin et al.(1993)* reported, working with Egyptian buffalo bull, found that the communalities for skeletal dimensions(height at wither and hips were much higher than flesh dimension).The relative high estimates of common variance in both male and female traits is an indication that improving any one of the traits could result in the simultaneous improvement in the remaining traits.

Conclusion

The study have assessed the sources of shared variability of body shape in muscovy duck. It outlined significant morphological differentiation between sexes in favour of the male and similarly showed variability in trait association within sex.

The factor analysis method employed have also sufficiently explored the interdependence in the original seven morphometric traits by analyzing them simultaneously rather than individually and it is useful in consolidating and

describing the correlation and covariance among these interdependence traits in terms of the two interpretable common factor(size and shape) in both sexes.

Varijabilnost osobina telesne razvijenosti odraslih mošusnih pataka

D. M. Ogah

Rezime

U ovom istraživanju su analizirani telesna masa i šest osobina telesne razvijenosti – dužina tela, obim grudi, dužina bataka, dužina piska, ukupna dužina noge i krila, na uzorku od 150 nigerijskih autohtonih mošusnih pataka, ženskog i muškog pola, u uzrastu od dvadeset nedelja, koje su gajene u polu-intenzivnim uslovima/sistemu. Ciljevi ispitivanja su bili da se ocene osnovni izvori podeljene varijabilnosti između osobina telsne razvijenosti, da se odrede faktori koji opisuju ove karakteristike i kvanitifikuju razlike između polova u morfometrijskoj veličini i obliku tela odraslih mošusnih pataka. Varijacije u deskriptivnoj statistici su zabeležene između ženki i mužjaka kod skoro svih osobina osim dužine piska. Jačina korelacije se takođe razlikovala između polova. Konformacija tela je pod uticajem zajedničkog i jedinstvenih faktora, u opsegu od 0.671 za dužinu piska do 0.987 dužinu tela.

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INFLUENCE OF THE PREBIOTIC SALGARD AND A HERB MIXTURE ON PEKIN DUCKLINGS IN ORGANIC POULTRY PRODUCTION: II. HISTOLOGICAL AND MICROBIOLOGICAL INVESTIGATION

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Abstract: The purpose of this investigation was to study the influence of the prebiotic Salgard and an herb mixture (rosemary, thyme, basil, oregano and cinnamon) on the histostructure of some internal organs and on the intestinal microflora of Pekin ducklings in an organic production system. Seventy two Pekin ducklings distributed randomly into 3 groups of 24 birds each and sexed (12 ♂and 12 ♀) were used as followed: group I (control) fed a standard diet; group II – fed the same diet supplemented with the prebiotic Salgard at a concentration of 0.15 %, and group III – fed the same diet supplemented with 0.15 % of a herb mixture in an equal proportion (0.03% of each herb – rosemary, thyme, basil, oregano and cinnamon). At slaughter, material for histological examination was obtained from the ileum, the caeca, the liver and the kidneys of birds. Faecal content from the ileum and the caeca were submitted to microbiological analysis. The addition of Salgard in a concentration of 0.15 % to the diet of Pekin ducklings contributed to significant increasing the length of the villi intestinales ($P<0.001$) and diameter of intestinal glands ($P<0.01$) in the ileum and epithelial height ($P<0.05$) in the caeca, as well as with a reduction of pathogenic intestinal microorganisms in the birds that received the prebiotic and herb mixture.

Key words: duck, prebiotic Salgard, herb mixture, histological characteristics, intestinal microflora

Introduction

The organic production system is in harmony with natural poultry rearing conditions and therefore, a prerequisite for a high level of welfare. Today as growth promoters in poultry nutrition, which have positive effect on poultry growth and feed conversion are in use probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogene additives *(Perić et al., 2009)*.

The prebiotic Salgard is provided by Optivite LTD, UK and composed of propionic acid (20,000 mg/kg), ammonium propionate (85,000 mg/kg), ammonium format (160,000 mg/kg) and formic acid (35,000 mg/kg). Salgard is a feed treatment that helps protect against bacterial and fungal challenges *(Акyurek et al., 2011)*. The prebiotics has a microbicide effect on *Escherichia coli*, *Campylobacter* spp., as well as some Gram-positive microorganisms such as *Staphylococcus* spp, *Streptococcus* spp., *Listeria* spp. and *Clostridium* spp. At the same time it protects the beneficial microflora – *Lactobacillus* spp., *Bifidobacterium* spp. and *Bacteroides* spp. in the animal intestinal tract, resulting in improved health and development, higher utilization of feeds, and a positive effect on their productive qualities in organic animal farming conditions *(Griggs and Jacob, 2005; Biggs et al., 2007; Levic et al., 2008)*. The supplementation with herbs and spices in organic production is an important alternative for improving the health and welfare of animals and poultry. Their active substances stimulate non-specific resistance, increase the appetite and feed conversion, thus increasing productivity *(Loo and Richard, 1992; Jamroz et al., 2006; Mikulski et al., 2008; Frankič et al., 2009)*.

Furthermore, certain herbs (rosemary, thyme, basil, oregano) possess strong anti-inflammatory, antistress and antioxidant properties *(Jamroz et al., 2003, 2006; Lee et al., 2003; Bampidis et al., 2005; Mikulski et al., 2008 ; Windisch et al., 2008)*.

Cinnamon oil and its constituents cinnamaldehyde and eugenol have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa, Enterococus faecalis, Staphylococcus aureus, Staphylococcus epidermis*, *Salmonella sp*. and Parahemolyticus *(Chang et al., 2001)*.

The purpose of our research was to study the influence of the prebiotic Salgard and the herb mixture (rosemary, thyme, basil, oregano and cinnamon) on the histomorphological structure of some internal organs and on the intestinal microflora of Pekin ducklings in an organic production system.

Materials and Methods

The study was carried out in the poultry farm of Department of Animal Science at the Agricultural University – Plovdiv.

Seventy two Pekin ducklings were sexed, individually weighed and distributed randomly into 3 groups of 24 birds (1 control and 2 treatments)(12 ♂and 12 ♀). All birds were fed with diet containing ME – 12.4 MJ/kg and CP – 18.6 % during the starter period (1-28 day of age) and diet containing ME – 12.7 MJ/kg and CP – 16 % during the finisher period (29-63 day of age) and only varied in the feed additives . The ducklings from group I (control) fed a standard diet; group II – fed the same dietsupplemented with the prebiotic Salgard produced by Optivite LTD, Nottinghamshire, UK at a concentration of 0.15 %, and group III – fed the same diet supplemented with 0.15 % of a herb mixture in an equal proportion (0.03% of each herb - rosemary (*Rosmarinus officinalis)*, thyme (*Thymus serpyllum)*, basil (*Ocimum basilicum)*, oregano (*Origanum vulgare L.)*, cinnamon (*Cinnamomum verum)*. All herbs were produced by Bioset LTD, Plovdiv, Bulgaria. The Salgard and herb mixture were added in diet from the 1st day of age to the end of the experiment..

Histological examinations. The material for the histological study – pieces of about 1 cm^3 in size, was obtained from different parts of the intestines (middle part of ileum and both caeca), the liver and the kidneys immediately after the birds were slaughtered – (six birds from each group – 3 males and 3 females). The samples were immediately placed into 10% neutral formalin. After fixation, the samples were washed with running water, dehydrated in an alcohol series, cleared in xylene, and embedded in paraffin. The formed blocks were cut with a paraffin microtome "Reichert." The 6 μm tick sections were stained with hematoxylin and eosin. The observation, micro morphometric examination and photographing were done on a "Hund" microscope. The metric study of the preparations was performed with a standardized eye piece-micrometer. The following parameters were measured – length of villi intestinals, epithelial height of lamina epithelialis, diameter of intestinal glands. It was made 10 measurements per parameter from each slaughtered bird

Microbiological examinations. The faecal content of the ileum and caeca was examined microbiologically by means of routine laboratory methods for isolation, identification, and typizations by genus and species. A part of microbial isolates were identified on the semi-automated identification system CRYSTAL (Becton Dickinson) for enterobacteria and staphylococci, as well as on the API-20 NE system.

Statistical analyses. Micro morphometric histological examinations were expressed as a mean and standard error. Data were subjected to one-way analysis of variance (ANOVA) using GraphPad InStat 3.06 software to determine the level of significance among mean values Tukey's HSD test was performed as a post-hoc test after ANOVA.

Results and Discussion

Light microscope examination revealed that the ileal wall of the ducklings from the three groups was composed of 4 layers: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. The mucous coat is formed of three sublayers: lamina epithelialis – a single-layer columnar epithelium, lamina propria – loose connecting tissue, in which her intestinal glands are located, and lamina muscularis – smooth muscle tissue, which goes into the villi and attains almost up to their tips. The submucosa is a very thin layer whereas the musculature is made of a thicker internal and a thinner external smooth muscle layers. The outermost layer of the intestine is the serous coat – visceral peritoneum.

The micro morphometrical measurments study (Table 1) also shows that the ileum's mucosa forms relatively high villi intestinales, and they are the highest in the ducklings from the group II (Figure 1), while in groups I and III the differences were insignificant (Figure 2). A similar tendency was observed in the height of lamina epithelialis and the diameter of intestinal glands.

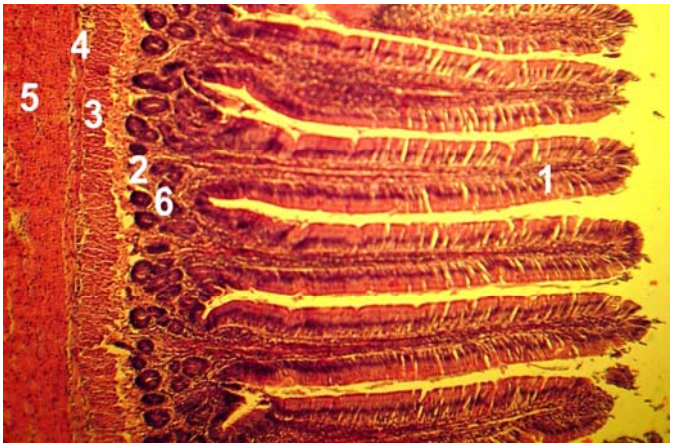

Figure 1. Cross section through the ileal wall (II group): 1 – l. epithelialis. 2 – l. propria. 3 – l. muscularis. 4 – t. submucosa. 5 – t. muscularis. 6 – intestinal glands (bar = 100 μm).

The structure of the caeca wall in the three groups of ducklings is typical for this part of the digestive system. It contains the same 4 layers observed in the ileum. The birds from group II and group III have an increased presence of leucocytes in the propria of the mucosa and in the submucosa (Figure 3). There is no visible difference in the structure of the caeca wall between birds which were fed with Salgard and herbal mixture. The data from the micro morphometrical study indicates that Pekin ducklings, which received the prebiotic Salgard, villi intestinal reach the greatest height (Table 1). The epithelium height and the

diameter of the intestinal glands in this group are also larger, compared to the groups I and III, but the differences are insignificant.

Table 1. Micromorphometri data from the examination of the ileum and caeca (n=10) in ducklings from the different groups

Group	Ileum			Caecum		
	Length of villi, µm	Epithelial height, µm	Glands diameter, µm	Length of villi, µm	Epithelial height, µm	Glands diameter, µm
Group I (control)	892.4±12.5 a_1	57.7±1.3	55.1±0.6 b_2	404.4±10.3	56.8±0.6 b_4. c_1	59.2±1.78
Group II	962.2±16.3 a_1. b_1	59.5±0.7	58.6±0.9 b_2. b_3	418.6±9.4	59.6±0.8 b_4	62.2±1.7
Group III	904.6±15.0 b_1	57.8±1.3	55.8±1.0 b_3	405.2±7.1	58.8±0.8 c_1	60.4±1.6

Note: $P<0.001$ at a_1- a_1; $P<0.01$ at b_1- b_1. b_2- b_2. b_3- b_3. b_4- b_4; $P<0.05$ at c_1- c_1 in the same vertical rank

Light microscope examination of the liver showed no significant differences in the structure of its parenchyma between the different groups of birds. The liver lobules were not clearly differentiated due to the small amount of interstitial connective tissue around them (Figure 4). The glandular tubules were circumferentially located around the central vein and were built from polygonal hepatocytes, with an average diameter of 10-13 µm.

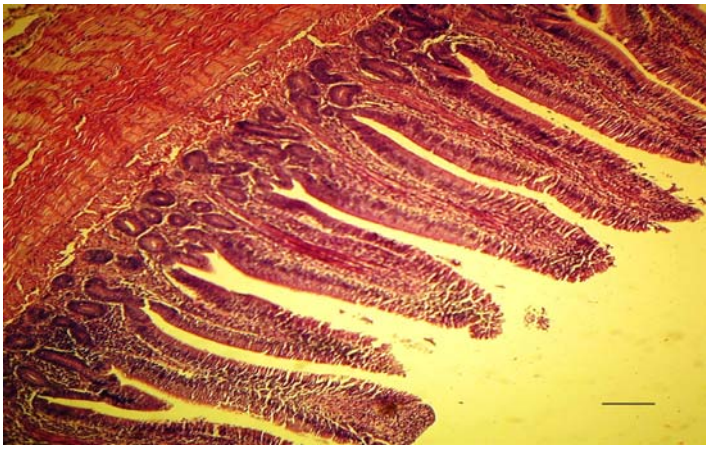

Figure 2. Cross section through the ileal wall (I group): The well-shaped round intestinal villi of the mucous coat could be observed (bar=70 µm).

The histological examination of the kidneys did not reveal any structural differences between the three groups of ducklings could be discovered. The kidney lobules were well differentiated, and each had well formed cortex and medulla (Figure 5).

There is significantly positive effect of prebiotics on performance and height of intestinal villus in small intestines of broilers *(Žikić et al., 2008)*. *Awad et al. (2009)* concluded that dietary treatments influenced the histomorphological parameters of small intestinal villi. The addition of either probiotic or synbiotic increased ($P < 0.05$) the villus height:crypt depth ratio and villus height in both duodenum and ileum. The duodenal crypt depth remained unaffected ($P > 0.05$). However, the ileal crypt depth was decreased by dietary supplementations compared with control.

Garcia et al. (2007) established that the diets with 5,000 and 10,000 ppm of formic acid, and with 200 ppm of plant extract based on a blend of oregano, cinnamon, and pepper essential oils had positive effect on the intestine mucosa and growth performance in broilers.

The results from the microbiological study (Table 2) showed that the addition of Salgard in the birds' diet did not result in impaired content of the intestinal cenosis, which could be expressed by pathological disturbances. The presence of the same microbial varieties from the beneficial microflora, established in healthy untreated birds, was proved. A relative yet transient decrease in the amount of *Bifidobacterium* spp., *Enterobacter* spp., *Enterococcus* spp. and *Clostridium* spp. microorganisms was also evident in the caeca and the ileum of the birds that received the prebiotic.

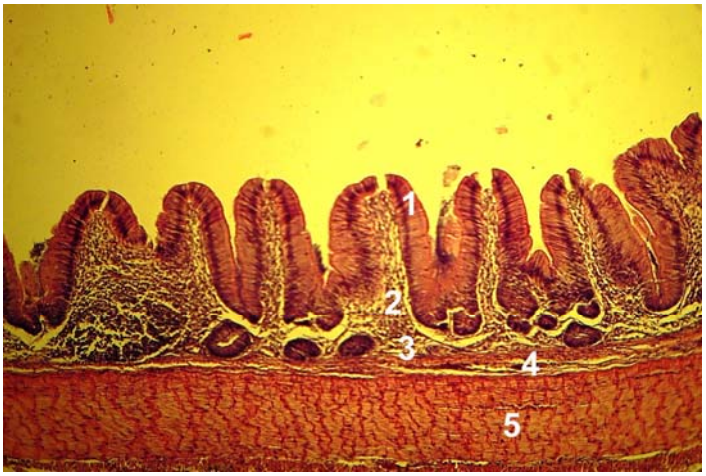

Figure 3. Cross section through the caecal wall (II group): 1 – l. epithelialis. 2 – l. propria. 3 – l. muscularis. 4 – t. submucosa. 5 – t. muscularis; (bar = 100 µm)

The prebiotic has a protective effect on the beneficial microflora and stimulates its development – *Lactobacillus* spp. and *Bacteroides* spp., improving digestion and the nutirent utilization. Salgard has a relatively broad antimicrobial effect on *E. coli*, *Campylobacter* spp, *Listeria* spp. and *Clostridium* spp.

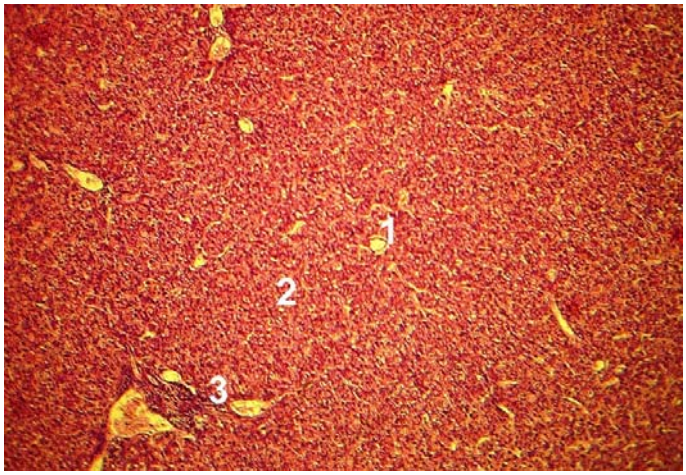

Figure 4. Liver of a duckling from the group II: 1 – v. centralis. 2 – hepatocytes. 3 – interlobular loose connective tissue with artery. vein and biliary duct located within; (bar = 100 µm)

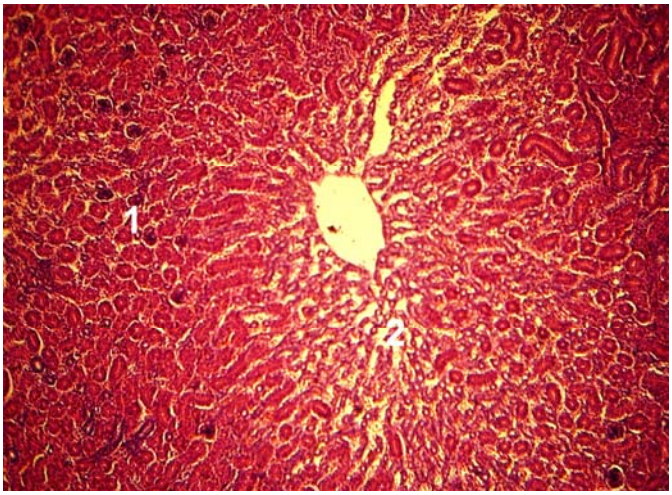

Figure 5. Kidney of a duckling from the group II: 1 –cortex. 2 – core (bar = 100 µm)

Table 2. Microbiological examination of faecal content from Pekin duckings

Group	Sample №	Intestine	*E. coli*	*Bifido bacterium*	*Proteus spp*	*Entero bacter* spp.	*Citro bacter* spp.	*Entero coccus spp.*	*Bacterio ides spp.*	*Clostri dium spp.*
Group I	1	caeca	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)
	2		(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)
	3		(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
	4	ileum	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	5		(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
	6		(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Group II	7	caeca	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
	8		(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
	9		(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)
	10	ileum	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(-)
	11		(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
	12		(+)	(-)	(-)	(-)	(-)	(-)	(+)	(+)
Group III	13	caeca	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
	14		(+)	(+)	(+)	(+)	(-)	(+)	(-)	(+)
	15		(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
	16	ileum	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)
	17		(+)	(-)	(-)	(-)	(+)	(+)	(-)	(-)
	18		(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)

On the other, some aromatic plants (Chinese and Ceylon cinnamon, thyme, St. John's Wort etc.) possess antimicrobial properties against alimentary pathogens and thus suppress the growth and development of enteropathogenic strains of *E. coli*, *Salmonella* spp. and others *(Pasqua et al., 2006; Windisch et al., 2008; Frankič et al. 2009)*. The mentioned aromatic plants and their active substances alter the cell membrane structures of enteropathogenic strains, causing ion leakage out of the cells, and death of pathogens *(Windisch et al., 2008)*. The aldehyde contained in cinnamon (carvacrol) improves the growth of lactobacilli and thus increases the beneficial intestinal microflora *(Castillo et al., 2006)*.

Conclusion

The higher intestinal villi observed during micromorphological analysis and the insignificantly higher lining epithelia of the ileum and the caeca of the ducklings from the 2nd group were probably due to the added prebiotic.

The addition of Salgard and herb mixture to the diet of Pekin ducklings contributed to reduction of pathogenic intestinal microorganisms in the birds.

Supplementation of the feed with prebiotic and herb mixture did not cause significant differences in the histological structure of the liver and kidneys for the investigated period.

Uticaj prebiotika Salgard i biljne smeše u ishrani pekinških pačića u organskoj proizvodnji: II. Histološko i mikrobiološko ispitivanje

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Rezime

Cilj ovog ispitivanja je bilo praćenje uticaja prebiotika Salgarda i biljne smeše (ruzmarin, majčina dušica, bosiljak, origano i cimet) na histološku strukturu nekih unutrašnjih organa i intestinalne mikroflore pekinških pačića u organskom proizvodnom sistemu. U ovom istraživanju, 72 jednodnevna pačeta, podeljena su u 3 grupe od po 24 ptice u svakoj (12 ♂i 12 ♀) na sledeći način: grupa I (kontrola) hranjena standardnom hranom; grupa II – hranjena istom hranom uz dodatak prebiotika Salgard u koncentraciji od 0.15%, i grupa III – hranjena istom hranom uz dodatak 0.15% biljne smeše u jednakim proporcijama (0.03% svake biljke - ruzmarin, majčina dušica, bosiljak, origano i cimet).

Posle klanja, uziman je materijal za histološka ispitivanja iz ileuma, slepog creva, jetre i bubrega ptica. Fekalni sadržaj iz ileuma i slepog creva je podvrgnut mikrobiološkoj analizi.

Dodavanje Salgarda u koncentraciji od 0.15 % u obrok za pekinške pačiće je doprinelo signifikantnom povećanju dužine crevnih resica ($P<0.001$), prečnika crevnih žlezda ($P<0.01$) u ileumu, i visine epitelija ($P<0.05$) u slepom crevu, kao i smanjenja patogenih intestinalnih mikroorganizama kod grla koja su hranjena prebiotikom i biljnom smešom.

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The structure of the caeca wall in the three groups of ducklings is typical for this part of the digestive system. It contains the same 4 layers observed in the ileum. The birds from group II and group III have an increased presence of leucocytes in the propria of the mucosa and in the submucosa (Figure 3). There is no visible difference in the structure of the caeca wall between birds which were fed with Salgard and herbal mixture. The data from the micro morphometrical study indicates that Pekin ducklings, which received the prebiotic Salgard, villi intestinal reach the greatest height (Table 1). The epithelium height and the diameter of the intestinal glands in this group are also larger, compared to the groups I and III, but the differences are insignificant.

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ANALYSIS OF APPLIED BIOSECURITY MEASURES IN BOARS SPERM PRODUCTION

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Abstract: This paper gives a detailed analysis of the applied biosecurity measures in the production of boar sperm at a swine reproduction center. Biosecurity indicators (existence of a written biosecurity plan, isolation, introduction of newly acquired animals into the herd, herd health, assessment of the personnel attitude towards equipment, traffic control, attitude towards visitors, feeding and watering control, manure management, disposal of dead animal carcasses, attitude towards other animals, rodents and birds control, sanitation) were viewed and evaluated by rating scale: (5) - excellent, (4) - very good, (3) - good, (2) - sufficient, (1) - insufficient, there are resources for improvement (0) - insufficient, with no resources for improvement. Obtained data were analyzed in the SWOT process, taking into account all the strengths, weaknesses, threats and opportunities for improving the biosecurity level. The situation in the center is rated as very good, with an average rating of 4.15. However, one disadvantage is serious and related to the boar facilities isolation possibilities, taking into account their location and the presence of two types of male breeding animals (boars and bulls) in the same location. Newly acquired breeding animals are purchased from various sources, but with a rigorous regime of control and not at the same time.

Key words: analysis, biosecurity measures, boars, sperm production

Introduction

Artificial insemination (A.I.) is the routine technological procedure in contemporary swine breeding technology and important biosecurity measure as well. Fresh semen collected from healthy boars necessitates premeditated and correct treatment in order to prevent afterward contamination *(Stanković et al., 2005).*

In epidemiologic terms, sperm is considered to be intermediary source of infection, becoming secondary source after being packed in single doses. Many viruses could be detected in boar semen, primarily during viremic phaze of infection, and some of them, like Foot and Mouth disease virus, PRRS virus, Vesicular Disease virus, Parvovirus, Picornaviruses, Adenoviruses, Japanese Encephalitis virus type B, Aujecsky virus and Reoviruses have great importance. Therefore, quality monitoring of boars before introducing them into herd and during being in reproduction center is essential *(Stanković et al., 2007a)*.

The amount of predicted biosecurity measures have to be defined by protected operation price, but preservation of certain biosecurity level is multifunctional. Primarily, it is essential part of farm programme of food safety. Furthermore, sophisticated and efficient biosecurity measures application leads to better herd health and their higher productivity, which means higher efficacy and profitability, and finally environment protection *(Uhlenhoop, 2007)*. An assessment of all critical spots in technology chain of boar semen collecting, processing and storage, and the most important measures for semen preservation in order to achieve reproductive results were given in this paper.

Materials and Methods

During presented investigations, the effect of applied biosecurity measures in one reproductive center was evaluated, and failures and threats to biological quality of semen were analysed, as well as possibilities to improve present biosecurity level in operation facilities.

There were 75 breeding boars of different breeds (Large White, German, Sweden and Dutch Landrace, Pietrain and Durock), as well as 82 Simmental breeding bulls.

Investigation was performed using combining methods of interview and observation in respect of biosecurity indicators (existence of a written biosecurity plan, isolation, introduction of newly acquired animals into the herd, herd health, assessment of the personnel attitude towards equipment, traffic control, attitude towards visitors, feeding and watering control, manure management, disposal of dead animal carcasses, attitude towards other animals, rodents and birds control, sanitation), which were evaluated according to numerous parameters and other elements. In order to evaluate them, grades were defined: (5) - excellent, (4) - very good, (3) - good, (2) - sufficient, (1) - insufficient, there are resources for improvement (0) - insufficient, with no resources for improvement, and rating scale: 0-1,99 insufficient, 2,00-2,49 sufficient, 2,5-3,49 good, 3,5 – 4,49 very good and 4,5 – 5,00 excellent, were defined. Obtained data were analyzed in the SWOT process, taking into account all the strengths, weaknesses, threats and opportunities for improving the biosecurity level.

Results and Discussion

Results of analysis biosecurity measures application in reproductive center are given in table 1. Biosecurity level was evaluated as very good, with average grade 4.15. Nevertheless, it should be emphasized that this grade is fragile, since there are few severe failures which could threaten the very existence of the center. In the further text will be analysed only those results which were differentiated from optimal values.

There were no written biosecurity plan neither in reproduction center, nor in any previously scrutinized livestock production unit, but only in fragments, concerning certain technological operations and unwritten technological, hygienic or sanitary procedures, as well as semen dilution and conservation technology which were correctly and regularly performed, so this indicator was rated as "good" (3). Furthermore, in spite of international regulations (e.g. EU Directive 90/429/EEC 1990, Directive 92/65/EEC 1992 and Decision 95/176/EC 1995), that in reproduction center may exist only one species male animals, both bulls and boars are present on analysed location, although strictly separated *(Stanković et al., 2005)*.

Table 1. Results of analysis biosecurity measures application in reproduction center

1.	EXISTENCE OF WRITTEN BIOSECURITY PLAN	3
2.	Isolation of entire reproduction center and separate technological segments and operations	2
3.	Introduction of newly acquired animals in herd	3
4.	Herd health status	3
5.	Personnel attitude to equipment	5
6.	Traffic control	3
7.	Visitors policy	5
8.	Control of feeding and watering	5
9.	Manure management	5
10.	Carcases disposal	5
11.	Attitude towards other animals on the farm	5
12.	Rodents and birds control	5
13.	Sanitation	5
	Final rate	4,15

Of course, in this type of the highest technology level production units certain procedures and protocols are in use, concerning visitors policy and especially protocols of sanitation, recommended by manufacturers of sanitation products, as standardized procedures *(Buhman et al., 2005)*.

It must be emphasized that all data concerning acquisitions of animals, exploitation, treatment and diagnostic results and all the other important issues are recorded regularly and systematically, which makes easier to scrutinize and anticipate possible risks and their elimination and prevention *(Stanković et al., 2010)*.

Preservation of desired herd health level is the most important aspect of wanted biosecurity level protection as well as successful production and welfare of boars, consider a line of biosecurity measures as necessary part of technology, including good rearing conditions, good and responsible treatment of boars, and application of prophylactic measures *(Stanković et al., 2007c)*. On the other hand, existence of written biosecurity plan shows how professionals on the farm see and understand potential threats to the production, as well as how can predicted measures answer these changing threats from close and distance environment *(Stanković et al., 2008)*.

Fortunately, according to competences of the professionals in this facility, it could be understood that they clearly see need to provide necessary biosecurity level and the goals to fulfil by undertaking biosecurity measures, which are key for success of production in respect of conserved boar semen quality *(Hristov and Stanković, 2009a)*.

On the other hand, center personnel has no obligation neither not to have animals of their own which may be infected by pathogens common for the boars in the facility, nor not to have contact with swine out of the center, which is not good. Isolation of entire facility or its segments is rated as sufficient (2). Although center has compact organisation, quality perimeters and excellent green protective line, since foundation, its location became questionable because of intensive house building, close heavy used road and international railroad, as well as fodder factory. It can be concluded that this must be corrected, meaning dislocation of center on two separated safer locations for each species *(Seaman and Fangman, 2001, Amass, 2006)*.

It is necessary that new animals acquisition has to be performed under serious veterinary surveillance, from herds with higher or at least same health status level *(Stanković et al., 2005)*. Although new breeding boars are introduced under strict health and sanitary conditions, this indicator is rated as good (3), namely because there is several herds of origin. It should be emphasized that there is permanent risk of introduction of infection into the herd, especially for reproductive transmissible viruses *(Seaman and Fangman, 2001; Stanković et al., 2007c)*.

Surveillance over boars health in center is rigorous, and it could be said that it overcomes norms predicted by law. Still, herd health status is rated as good (3), although noticed failures does not compromise process of collecting, processing and preserving of boar semen, but may affect boars health and shortening their exploitation. Rearing conditions are far from ideal, concerning boars' needs, which

could be considered as technological compromise of boars welfare *(Hristov and Stanković, 2009b)*. It is primarily related to concrete flours on which boar lives, hard and discomfort, and slippery when is wet. Injuries of hooves and joints on this slippery floor happen sometimes, followed by pain during walking, and unwilling to mount the phantom, excluding that boar from production for some time, or even permanently *(Kunavongkrit et al., 2005)*.

Lack of adequate air conditioning or at least cooling during hot season significantly affects both libido and spermatogenesis, decreasing yield and quality of sperm. Having in mind duration of spermatogenesis of 40 to 60 days *(Dobranić and Samardžija, 2010)*, it is obvious that microclimate conditions improvement in boars facilities *(Suriyasomboon et al., 2005)* is more than justified.

It was notified that technicians who collect semen often do not use single use gloves, although they are familiar with this necessity, because it is difficult to fix *glans penis* with them on. This makes invitation to banal infections, which may contaminate semen and make it less viable *(Stanković et al., 2007c)*.

Finally, although separation of present operations *(Stanković et al., 2007b)* was performed in the best possible manner, some crossing of their routes, primarily of those for collecting boar semen and bringing bulls, could not be avoided. This is main reason that makes this indicator rate just good (3).

Conclusion

According to the results and analysis of application biosecurity measures in the production of boar sperm at a reproduction center it can be concluded:

As a result of the application of biosecurity measures, current level of biosecurity could be rated as very good, with a mean score of 4,15;

A number of shortcomings were observed, some of them could be a serious threat to semen, especially the possibility of facilities isolation, taking into account its location and the presence of two species of male breeding animals (boars and bulls);

Newly acquired boars were brought to the center from different herds, but under strict regime of control and not in the same time;

Dislocation of boars facilities to the safer location is complex but priority task, which would remove serious threats to sperm production.

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Analiza primenjenih biosigurnosnih mera u proizvodnji sperme nerastova

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Rezime

U radu je detaljno analizirana primena biosigurnosnih mera u proizvodnji sperme nerastova u jednom centru za veštačko osemenjavanje svinja. Sagledani su i procenjeni svi indikatori biosigurnosti (postojanje pisanog plana biosigurnosti, izolacija, uvođenje novonabavljenih životinja u zapat, zdravstveni status zapata, ocena odnosa osoblja prema opremi, kontrola kretanja i prometa, odnos prema posetiocima, kontrola ishrane i vodosnabdevanja, izđubravanje, uklanjanje leševa uginulih životinja, odnos prema drugim životinjama na farmi, kontrola populacija glodara i ptica, sanitacija), i ocenjeni prema skali ocena: (5) – odličan, (4) – vrlo dobar, (3) – dobar, (2) – dovoljan, (1) – nedovoljan, ima resursa za poboljšanje, (0) nedovoljan, nema resursa za poboljšanje. U razmatranju rezultata primenjena je SWOT analiza i utvrđene prednosti, nedostaci, rizici i mogućnosti za podizanje nivoa biosigurnosti. Stanje u centru je ocenjeno kao vrlo dobro, uz prosečnu ocenu 4,15. Međutim, jedan nedostatak je veoma ozbiljan i odnosi se na mogućnost izolacije objekata, uzimajući u obzir njegovu lokaciju i prisustvo dve vrste muških priplodnih životinja (nerastova i bikova) na istoj lokaciji. Nove priplodne životinje se nabavljaju iz različitih izvora, ali uz rigorozan režim kontrole i ne u isto vreme. Svakako, izmeštanje objekata za držanje priplodnih nerastova na drugu bezbednu lokaciju predstavlja složen ali prioritetan zadatak, kojim bi se otklonile brojne pretnje po proizvodnju sperme.

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CORRELATION BETWEEN BODY MEASUREMENTS AND MILK PRODUCTION OF GOATS IN DIFFERENT LACTATIONS

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Original scientific paper

Abstract: This paper presents the results of the variability and correlation of body measurements and milk production of domestic Balkan goat breed that is reared in the mountain range Sharplanina, depending on the lactation. Studied animals were monitored and lactation, or order of kidding (I, II, III and IV and the next lactation together). Control of milk production, included a total of 290 goats in different lactations (first-81 animals, the second-69 heads, 71 heads third-and fourth and following along latkacije-69 heads). In order to determine the measure of body development in adult goats, one takes values for height at withers, body length, chest depth, chest width, the width of the cross and body weight. The variability of the analyzed characteristics is presented descriptive parameters and the effect of lactation is determined by a factorial analysis of variance. The determined average values for milk production and measures of body development are located within those identified for this population of goats. During these tests showed statistically significant correlation dependence ($P < 0.05$) between all studied variables, except when it comes to length lactation period and individual measures of body development. The correlation coefficient between length of lactation and established measures of body development. are low and reflect the existence uncorrelation dependence, and their values range from 0.08 to 0.11, while they were unjustified and statistically ($P > 0.05$).

Key words: balkan goat, milk production, body development, stage of lactation, correlation

Introduction

The succes of selection in production of goat milk depends not only on direct improvement of the milk traits/performance (milk yield, content of milk fat or

proteins), but also on improvement of other traits, such as body development or functional characteristics which also contribute to improvement of the production efficiency. Therefore, it is necessary to include into the program of improvement of goat milk production through selection measures also those traits which have direct but also indirect impact on this production, such as the traits of body development.

Today, so called linear type and body condition scoring based on visual evaluation of animal according to already determined instructions and standards is generally accepted. In this way certain qualitative traits are transfered into quantitative which enables their statistical processing and analysis. On the other hand, determination of absolute body dimensions is also very important, because they represent morphological and physiological basis not only for linear scoring of body development, but also for optimal expression of production and reproduction traits. Therefore, the knowledge of the correlation between the body development and milk performance in goats is important *(Riva et al., 2002)*.

Phenotype correlations, i.e. correlation between certain traits in goats were studied by numerous authors. Biometrical measuring provides significant information on course of growth of goats or expression of certain traits. In addition, body development of goats and phenotypic expression of milk performance are very important sources of data used in the control of the standards for certain breed *(Riva et al., 2002)*.

Evaluation of goats is based on the expression of their production traits. Therefore, there is the need for assessment of traits, starting with simple traits which can easily be measured (linear body dimensions or milk performance) to the complex ones *(Singh and Mishra, 2004; Khan et al., 2006)*.

Therefore, the objective of this study was to present the impact of the order of lactation on variability and correlation of body development and milk performance in Balkan goat breed. This breed was chosen for this research because of it's importance in goat breeding in Serbia, considering that it still represents the largest goat population (approx. 35% of total population).

Materials and Methods

The research material used in this study were goats of Balkan breed from the territory of northwestern part of the mountain range Šarplanina. Control of production performance included goats of red colour, as authentic representatives of the Domestic Balkan goat. Studied animals were observed in lactations, i.e. order of partus (I, II, III, and IV and subsequent lactations together).

The control of milk performance included total of 290 goats in different lactations (the first - 81 animals, the second - 69 animals, the third - 71 animals, and the fourth and subsequent lactations together - 69 animals). Quantity of

produced milk in all goats was determined 10th day subsequent to partus, at the latest, until the end of lactation (dry off period). All animals were in A control. Measuring of the quantity of milked milk was done using graduated cylinders, with the smallest division of 10 ml.

Measuring of the body development of goats was done at the beginning of September. The following main exterior measures were recorded: height of withers (HW), carcass length (CL), breast depth (BD), breast width (BW), rump width (RW), as well as body mass (BM). Body mass was recorded in adult goats using individual technical weight scale of accuracy of 100g, and body measurements were determined using Lydtin stick/rod.

Variability of analyzed traits was described using parameters of descriptive statistics, correlation between traits was determined by correlation coefficient, whereas the effect of order of lactation was tested using single factor variance analysis.

Results and Discussion

Table 1 presents basic parameters of descriptive statistics for milk yield and individual measures of body development in Balkan goats. Based on obtained data it was established that the average duration of lactation period in Domestic Balkan goat was 230 days, i.e. approx. 8 months, which is slightly shorter lactation compared to value determined by *Bogdanović et al. (2008)*. Based on the value of standard deviation (SD=26,85) it is noticeable that the duration of lactation was very uneven which is result of unequal time of kidding from February to May.

Table 1. Average values and variability of milk performance and measures of body development of goats

Traits	N	M	Minimum	Maximum	SD
Duration of lactation (day)	290	230.1	142.00	287.00	26.85
Height to withers (cm)	290	66.27	57.00	75.00	3.39
Body length (cm)	290	69.91	58.00	80.00	4.32
Breast width (cm)	290	16.84	13.50	21.00	1.39
Breast depth (cm)	290	29.90	24.00	35.50	2.26
Hip width (cm)	290	17.40	14.00	22.00	1.25
Body mass (kg)	290	38.32	23.00	64.00	7.46
Milk yield (kg)	290	169.52	71.90	321.80	46.86

Legend: M = Average, SD = Standard deviation.

Milk performance/yield of the Balkan goat is relatively less expressed compared to pure breeds, even improved transitional breeds. Slightly lower milk yield of Balkan goat was established by *Marković (1997)* in the analysis of

production results from 6 private farms in Montenegro. Average milk yield of Balkan goat in this research was approx. 129 kg, but with somewhat shorter third lactation of around 218 days. However, compared to local, primitive breeds reared in other parts of Europe or world, milk performance of Balkan goat is expressed within expected biological limits for this breed group. In more extensive analysis of the possibilities to use high productive breeds as meliorators in flocks of local, primitive breeds, *Serradilla (2001)* stated that the milk performance of autochthonous Italian breeds and Maltese breeds ranged from 135 to 360 kg, of Greek breeds from 100 to 180 kg and Turkish local goat breeds from 120 to 400 kg. On the other hand, milk performance of Spanish breeds ranged from 425 to 650 kg and lactation period from 200 to 260 days were presented by *Haenlein (2007)* in the critical review of the contemporary development of sheep and goat milk production *(Bogdanović et al., 2008)*.

In Table 2. the average results concerning the milk performance and duration of lactation in Balkan goats depending on the order of lactation are presented. The average quantity of milk in goats in their first lactation was 138,68 kg, aand it increased in subsequent lactations, so in the fourth and later lactations the milk yield was around 192,51 kg. Similar trend in expression of milk traits in different lactations was established by *Bogdanović et al. (2010)*.

Table 2. Milk yield and duration of lactation in Balkan goat depending on the order of lactation

Order of lactation	N	Duration of lactation	Milk yield		
		M	SD	M	SD
L1	81	227.00	26.79	138.69	27.91
L2	69	228.33	28.27	168.10	48.43
L3	71	229.59	28.64	183.74	47.01
L4	69	236.01	22.95	192.51	43.73
Average	290	230.09	26.85	169.52	46.86

Legend: M = Average, SD = Standard deviation

Table 3. presents average results of body development in Balkan goats depending on the order of lactation. According to lactations, average values for certain measures of body development increased from the first lactation to the fourth and later lactations, where the lowest value of all measures of body development, as well as body mass, was recorded in the first lactation, whereas in the fourth and later lactations this value increased.

Table 3. Body development of Balkan goats depending on the order of lactation

Order of lactation	N	Height to withers		Body length		Breast width		Breast depth		Body mass	
		M	SD	M	SD	M	SD	M	SD	M	SD
L1	81	63.38	2.66	65.81	3.29	15.87	0.87	27.75	1.36	30.75	3.97
L2	69	66.18	3.05	70.00	3.75	16.65	1.17	29.53	2.01	36.86	4.77
L3	71	67.71	2.97	72.00	3.26	17.26	1.27	30.71	1.36	41.85	5.68
L4	69	68.26	2.4	72.45	3.23	17.72	1.45	31.94	1.69	45.02	5.72
Average	290	66.27	3.38	69.90	4.32	16.84	1.38	29.90	2.25	38.32	7.45

Legend: M = Average, SD = Standard deviation

In regard to body mass, variation of the average value according to lactation was more distinct (5,72%) in fourth compared to the first lactation (3,97%). In regard to data obtained on the average body mass of goats in the fourth lactation (45,02 kg), it is obvious that it is higher in average than the body mass of goats in the first lactation by approx. 15 kg. Low body mass of goats, primarily in the first lactation, is partially consequence of worsle conditions relating to nutrition and care, especially of progeny which are introduced to breeding with unfinished growth and insufficient body mass, and these consequences are suffered by animals all their life *(Memiši 2000; Khan et al., 2006)*. Obtained values for certain body measures in studied population of Domestic Balkan goat are at the level of results obtained by *Khan et al. (2006)* for body mass, height to withers and body length for Bettal goat breed reared in pakistan, as well as *Ahmed et al. (2004)* for Barki goats in Egypt. Higher values for measures of body development compared to our study are stated by *Helal (2009)* for Damaskus breed goats reared in Egypt.

Table 4 presents results of the variance analysis of the effect of order of lactation on milk performance and body development measures in Balkan goats.

Table 4. The effect of order of lactation on variability of traits

Trait	Sum of the square of model	Degree of fredoom of the model	Mean of the square of model	Sum of the square of the error	Degree of fredoom of the error	Mean of the square of error	F	p
Duration of lactation	3425.8	3	1141.94	204951.5	286	716.614	1.59	ns
HW	1098.3	3	366.09	2222.5	286	7.771	47.11	***
CL	2119.7	3	706.56	3285.6	286	11.488	61.50	***
BW	144.5	3	48.17	411.2	286	1.438	33.50	***
BD	719.8	3	239.93	750.7	286	2.625	91.41	***
RW	83.3	3	27.77	371.8	286	1.300	21.36	***
BM	8763.8	3	2921.28	7307.2	286	25.550	114.33	***
Milk yield	127964.5	3	42654.82	506542.6	286	1771.128	24.08	***

Legend: Significance *** P<0.001.

Differences in milk performance traits and body development measures established under the influence of order of lactation were highly significant at the level of *P<0,001* for all analyzed traits, except duration of lactation, which was not under the statistically significant influence of order of lactation (*P>0,05*).

Table 5 presents data on the magnitude of correlation of studied traits of body development and milk performance in Domestic Balkan goats. Between all studied traits, with the exception of duaration of lactation period and certain measures of body development, statistically significant correlation was determined (*P<0,05*).

Table 5. Results relating to study of the strenght of correlation between certain body development measures and milk performance of goats

Trait	Duration of lactation	HW	CL	BW	BD	RW	BM	Milk yield
Duration of lactation	1.00	0.08 NS	0.11 NS	0.17*	0.09 NS	0.12	0.10 NS	0.51*
HW	0.08 NS	1.00	0.78*	0.51*	0.75*	0.54*	0.78*	0.37*
CL	0.11 NS	0.78*	1.00	0.61*	0.82*	0.63*	0.83*	0.46*
BW	0.17*	0.51*	0.61*	1.00	0.66*	0.76*	0.69*	0.48*
BD	0.09 NS	0.75*	0.82*	0.66*	1.00	0.69*	0.84*	0.49*
RW	0.12*	0.54*	0.63*	0.76*	0.69*	1.00	0.70*	0.48*
BM	0.10 NS	0.78*	0.83*	0.69*	0.84*	0.70*	1.00	0.48*
Milk yield	0.51*	0.37*	0.46*	0.48*	0.49*	0.48*	0.48*	1.00

Legend: HW = height to withers; CL = carcass length; BW = breast width; BD = breast depth; RW = rump width; BM = body mass. Significance: NS = not significant; * P<0,05

Similar values for correlation coefficients are stated in the research by *Marković (1997)* who established the presence of complete correlation between duration of lactation and total milk quantity (P<0,01). *Iloeje and Van Vleck (1978)* stated that the phenotypic correlation between the quantity of milk during the lactation period and body mass in Don goat was 0,39, and in German improved goat 0,132. Similar values of correlation coefficients between body mass and milk performance (0,36) were stated by *Gall (1980)* in the study of the German improved goat, and *Semakula et al. (2010)* in local goat breeds reared in Ugandi.

By investigating the phenotype connection between body mass and some body measures of Domestic white goat and its crosses F_1 generation with Saanen goat from Bulgaria and Switzerland, as well as the influence of different factors on their

expression and variation, *Žujović et al. (1991, 1993)* established positive and statistically very significant correlations (R<0,01) between body mass and height to withers, breast girth and shank circumference, as well as height to withers and breast girth and shank circumference. Mentioned authors stated that the most distinct connection was determined between the body mass and breast girth in all three genotypes (0,708, 0,833 i 0,788). Also, *Pesmen and Yardimci (2008)* stated the statistically significant correlation between certain body development measures and body mass (from 0.5 to 0.94) in goats of Saanen breed which were at the similar level like in this research. *Nemeth et al. (2009*) established medium and very strong (P<0,01) correlation between body mass and body development measures in different breeds reared in Hungary.

Conclusion

Based on results of the investigation of the effect of lactation on variability and correlation between body development traits and milk performance in Balkan breed goats, the following can be concluded:

It was established that average duration of lactation period in Domestic Balkan goat was 230,09 days, i.e. approximately 8 months. Average milk yield in goats in the first lactation was 138,68 kg, and it increased in subsequent lactations, so in the fourth lactation the milk yield was 192,51 kg.

Differences established under the influence of order of lactation were significant at the level of *P<0,*001for all anlyzed traits og body development and milk performance, except for duration of lactation.

The magnitude of correlation between studied traits of body development and milk performance in domestic Balkan goats statistically was significant (P<0,05) between all observed parameters, except duration of lactation and certain body development measures.

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Povezanost telesne razvijenosti i mlečnosti koza u različitim laktacijama

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Rezime

U radu su prikazani rezultati ispitivanja varijabilnosti i povezanosti osobina telesne razvijenosti i mlečnosti domaće balkanske rase koza u zavisnosti od laktacije po redu (I, II, III a IV i naredne laktacije zajedno). Kontrolom proizvodnje mleka bilo je obuhvaćeno ukupno 290 koza u različitim laktacijama (prva- 81 grla, druga- 69 grla, treća- 71 grla, a četvrta i naredne latkacije zajedno- 69 grla). U cilju utvrđivanja mera telesne razvijenosti odraslih koza izmerene su vrednosti za visinu grebena, dužinu trupa, dubinu grudi, širinu grudi, širinu krsta i telesnu masu. Varijabilnost analiziranih osobina opisana je parametrima deskriptivne statistike, a uticaj laktacije po redu je utvrđen jednofaktorijalnom analizom varijanse. Utvrđene prosečne vrednosti za proizvodnju mleka i mere telesne razvijenosti bile se u okviru onih koje su utvrđene za ovu populaciju koza. Ispitivanjem su utvrđene statistički značajne korelacije ($P<0,05$) između svih posmatranih parametara, izuzev kada je u pitanju dužina laktacionog perioda i pojedinih mera telesne razvijenosti koje statistički nisu bile značajne.

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INFLUENCE OF ORDER OF LACTATION ON MILK PRODUCTION AND SOMATIC CELL COUNT IN ALPINE GOATS

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Abstract: In this paper, the annual results of the effect of lactation on milk production, the contents of some chemical parameters in the milk (milk fat, protein and dry matter without fat) and somatic cells in milk goat breeds Alpino in intensive production during one production year. Control is included a total of 82 French Alpine goats in different lactations (first-16 heads, the second-19 heads, 29 heads the third-and fourth and subsequent lactation together-18 heads).). Somatic cell count and chemical quality of milk is controlled on a daily basis in the laboratory for raw milk AD "Dairies" - Subotica on the machine CombiFoss FC 6200. The variability of the analyzed characteristics is presented descriptive parameters and the effect of lactation is determined by a factorial analysis of variance. The average value for somewhat milk goats for the treated population was 362.83 kg, with average milk fat content of 3.31%. Analysis of variance confirmed that the differences that were established under the influence of lactation for all traits analyzed, except for percentage of milk fat, were significant at $P < 0.01$

Key words: alpine goat, lactation, somatic cell, composition of milk

Introduction

Milk performance is polygenic property caused by numerous genes which directly or indirectly have impact on its expression. Production of milk is closely associated with environment factors, such as: nutrition of mothers/dams before and after partus, number of kids, climatic and soil conditions, housing and care, and many other factors. High standard of milk hygiene is required for the purpose of human health protection, maintenance of natrual biological value of raw materials and ensuring of corect technological processes in the processing of milk.

Somatic cell count in milk sample is indicator of udder health and incidence of clicnical and sub-clinical mastitis in the herd of dairy goats. Monitoring and determination of the somatic cell count is very important factor in evaluation of the quality of milk delivered to dairy plant, and it is used, among other parameters, such as chemical composition of milk (milk fat and protein) and total plate, for determination of the price of milk *(Dankov et al., 2003)*. Also, somatic cell count can be excellent indicator in the mastitis monitoring programs.

Increased somatic cell count is associated with decrease of milk quantity and changes in its composition, which can afec its sutiability for processing. Inflammatory process in mammary gland, ocuring as the consequence of the action of pathogen microorganisms, toxins or tissue damage, leads to changes in milk secretion, which results in quantitative and qualitative changes in milk *(Katić et al., 1994; Heeschen, 1995; Bernacka, 2006)*. These changes relate to icnrease of somatic cell count, increase of albumin content in the milk serum, reduction of the secretion of milk components (casein, fat, lactose) and reduction of the quantity of milk. Also, these changes lead to reduction of the milk's thermal stability, longer time for milk coagulation and decrease in milk sustainability/shelf life. Based on above mentioned, it can be concluded with certainty that increase somatic cell count in milk affects its suitability for processing *(Auldist and Hubble, 1998)*. Compared to cow milk, goat milk in average has higher somatic cell count which often amounts to several millions *(Danków et al., 2003)*.

Taking this into consideration, objective of this paper was to analyse the effect of order of lactation on yield, chemical composition and somatic cell count in goat milk.

Materials and Methods

Research was done on goat farm located in the vicinity of municipality of Subotica. Total of 82 heads of Alpine breed goats were included in the control of yield, chemical composition of milk and somatic cell count, in different lactations (the first lactation - 16 animals, the second lactation- 19 animals, the third lactation - 29 animals and fourth and subsequent lactations together - 18 animals). The quantity of produced milk in all goats was determined on the 10^{th} day after partus at the latest, all through to the end of lactation (dry off). All animals were in A control. Milking of goats was mechanized, and carried out in modern milking parlour for goats manufactured by company „Sac“, and control of milk yield was done twice in equal time intervals (7 a.m. and 7 p.m.), and in intervals of 28-32 days. Measuring of milked amount of milk was done using graduated cylinders, the smallest division of 10 ml.

Composition of milk (quantity of milk fat, proteins and fat free dry matter FFDM) was determined by the method of infra-red spectrophotometry using the

apparatus Milkoscan FT 6200, whereas the total somatic cell count (SCC) in milk was determined by fluoro-opto-electronic method on apparatus Fossomatic FC. In order to approach the normal distribution, a logarithm transformation of the somatic cell count was done and new trait was obtained (SCC log).

Goats were housed in closed facilities, and the method of stable feeding was applied. Goats had access to sufficient quantities of alfalfa hay as well as 1 kg of concentrate mixture which was administered twice a day. Chemical composition of used concentrate is presented in Table 1.

Table 1. Chemical composition of concentrate mixture used in goat nutrition

Feed	Share in mixture, %
Corn cracked	64,50
Wheat bran	12,00
Soybean meal (44%)	5,00
Sunflower meal (33%)	16,00
Di-calcium phosphate (16%P)	1,00
Premix	0,5
Salt, iodinated, g	1,0
Dry matter, % NEL MJ/kg CP/g	85.7 6,54 14,5

Variability of analysed properties was determined by method of descriptive statistics, whereas the effect of order of lactation was determined by one factor variance analysis.

Results and Discussion

In Table 2, average values and variability of production of milk, content of certain milk components and somatic cell count are presented.

Table 2. Average values and variability of milk yield, chemical composition and somatic cell count

Variable	N	x	Minimum	Maximum	SD
Duration of lactation	82	252.41	202.00	302.00	18.27
Milk yield	82	362.83	240.30	468.90	52.71
Daily milk yield	82	1.43	1.07	1.72	0.15
MF	82	3.32	2.82	3.62	0.21
% Proteins	82	2.93	2.64	3.19	0.15
FFDM	82	7.94	7.60	8.36	0.16
SCC	82	1,259,682.93	724,000.00	1,865,000.00	272,951.13
SCC, log	82	20.23	19.47	20.83	0.33

MF – Milk fat; FFDM – fat free dry matter; SCC – somatic cell count

Average value for total quantity of milk in investigated population was 362,83 kg, whereas the somatic cell count for entire lactation period in average was $1.26x10^3$. Compared to results obtained by other authors, duration of lactation in our research is similar to the level reported by *Gall (1980*) for duration of lactation in French Alpine breed from 200 to 300 days, as well as *Kompan et al. (1998*) for lactation of 258 days for same goat breed. Lower values were stated by *Pavličeк et al. (2006)* in alpine goats reared in the private sector and whose lactation duration was from 201 to 203 days. Total quantity of milk for goats in the first lactation was the lowest (297.8 kg), and in later lactation it increased, so in the fourth and later lactations it reached 382,6 kg. Average value of total milk quantity of goats in the first lactation was lower by 84,8 kg, compared to established value in older goats (the fourth lactation). Data on average production values, chemical composition and somatic cell count in alpine goats, depending on the order of lactation, is presented in Tables 3 and 4.

Table 3. Analysis of milk traits depending on the order of lactation

Lactation	N	Duration of lactation		Milk yield		Average daily milk yield, kg	
		x	SD	x	SD	x	SD
L1	16	239.37	15.04	297.8	29.09	1.24	0.10
L2	19	252.57	16.13	354.8	31.23	1.40	0.10
L3	29	260.51	18.99	391.5	47.97	1.49	0.10
L4	18	250.77	15.70	382.6	42.30	1.52	0.13
Average	82	252.41	18.27	362.8	52.70	1.43	0.15

Antunac (1994) also established that goats in the first lactation have the lowest milk production/yield (359 l), whereas the highest production was recorded for

goats in the third lactation (588 l). Similar increase in milk production in goats in subsequent lactations was established by *Margetin and Milerski (2000). Finley et al., (1984.)* in their research carried out on three goat breeds (Alpine, Saanen, Toggenburg) in USA, established the highest production of milk in goats at the age between 24 and 50 months. Considering the results of the analysis of the milk fat content in milk from Alpine breed goats, it was determined that the average value for investigated population was 3,31%. Established mean value for milk fat content was the highest in case of goats in the first lactation (3,4%) and it decreased in subsequent lactations, so in the fourth lactation it was 3.24%, however, the differences were not statistically significant (P>0,05).

Order of lactation, like in previous examples, influenced also the content of other milk parameters, e.g. protein percentage and fat free dry matter. Content of proteins in milk decreased from the first lactation (3,04%) to the third (2,85%), but in the fourth and later lactations this value was slightly higher compared to goats in the third lactation (2.92%). Somatic cell count was the lowest in milk obtained from goats in the first lactation ($1.02x10^3$) and it increased in later lactations. Somatic cell count in the second and the third lactation was $1.11x10^3$ and $1.36x10^3$ whereas in the fourth and later lactations it was the highest with average value of $1.42x10^3$.

Table 4. Analysis of the traits of chemical composition and somatic cell count (SCC, log) depending on the order of lactation

Lactation	N	Milk fat, %		Protein,%		Fat free dry matter, %		SCC, log	
		x	SD	x	SD	x	SD	x	SD
L1	16	3.40	0.12	3.04	0.11	8.04	0.16	19.93	0.33
L2	19	3.29	0.17	2.96	0.12	7.98	0.16	20.10	0.26
L3	29	3.33	0.25	2.85	0.14	7.87	0.13	20.36	0.24
L4	18	3.24	0.22	2.92	0.14	7.90	0.11	20.41	0.28
Average	82	3.31	0.21	2.93	0.14	7.94	0.15	20.22	0.33

Similar values in regard to somatic cell count in goat milk, depending on the order of lactation, were reported by *Raynal-Ljutovac et al. (2006). Kozačinski et al. (2002)* established in goat milk average SCC of 1.300.000/ml and concluded that the limit for SCC in goat milk can be over 1.000.000/ml, which is in accordance with results obtained in this study. Increased SCC in milk from dairy goat breeds reared in the USA is often, and above 1.000.000/ml as stated by *Haenlein (2002)*. Similar values, even slightly higher for SCC, depending on the order of lactation are stated by *Pavliček et al. (2006)* in Alpine breed goats. Higher SCC in goat milk (1.589.000/ml) was also established by *Ying et al. (2002.). Antunac et al. (1997)* stated that herds of dairy goats rarely have in the average milk sample SCC below one million.

Tabele 5. The effect of order of lactation on trait variability

Variable	Sum of the model square	Degree of freedom in the model	Model square mean	Sum of the error square	Degree of the freedom of error	Error square mean	F	P
Lactation duration	4.67	3	1.55	2.23	78	2.86	5.42	0.001
Milk yield	9.98	3	3.32	1.25	78	1.60	20.75	0.00
Daily milk yield	8.68	3	2.89	9.65	78	1.23	23.39	0.00
MF, %	2.32	3	7.74	3.37	78	4.32	1.79	0.15
% Proteins	3.92	3	1.30	1.42	78	1.82	7.17	0.00
FFDM	3.78	3	1.26	1.65	78	2.12	5.92	0.001
SCC	1.90	3	6.36	4.12	78	5.28	12.02	0.00
SCC, log	2.80	3	9.35	6.08	78	7.80	11.98	0.00

MF – milk fat; FFDM – fat free dry matter
Significance: *** P<0.01.

Differences established under the influence of order of lactation for all analysed traits, with the exception of milk fat, were significant at the level of P<0.01

Conclusion

Based on results obtained in the study of the effect of order of lactation on production of milk/yield, content of certain chemical parameters in milk (milk fat, proteins and fat free dry matter) and somatic cell count in milk obtained from alpine breed goats in intensive production, the following can be concluded:

Goats in the first lactation produced less milk (297.8 kg) compared to those in the second (354.8kg), the third (391.5kg) and the fourth and later lactations (382.6kg).

The highest content of milk fat, proteins and fat free dry matter was recorded for goats in the first lactation, and the percentage decreased from the first to later lactations.

Somatic cell count was the lowest in milk obtained from goats in the first lactation ($1.02x10^3$) and it increased in later lactations. So, the somatic cell count in the second and the third lactation was $1.11x10^3$ and $1.36x10^3$, whereas in the fourth and later lactations it was the highest, the average value was $1.42x10^3$.

Results of the variance analysis confirm that the differences established under the influence of order of lactation for all analysed traits, with the exception of milk fat content, were significant at the level of P<0.01.

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Uticaj laktacije po redu na proizvodnju mleka i broj somatskih ćelija koza alpina rase

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Rezime

U ovom radu prikazani su rezultati ispitivanja uticaja laktacije po redu na proizvodnju mleka, sadržaj mlečne masti, proteina i suve materije bez masti i broj somatskih ćelija u mleku koza francuske rase Alpina u intenzivnoj proizvodnji u toku jedne proizvodne godine. Kontrolom je obuhvaćeno ukupno 82 grla u različitim laktacijama (prva - 16 grla, druga - 19 grla, treća - 29 grla, a četvrta i naredne latkacije zajedno - 18 grla).

Broj somatskih ćelija, kao i hemijski kvalitet mleka, kontrolisan je svakodnevno u laboratoriji za sirovo mleko AD "Mlekare" – Subotica na aparatu CombiFoss 6200 FC. Varijabilnost analiziranih osobina je prikazana parametrima deskriptivne statistike, a uticaj laktacije po redu je utvrđen jednofaktorijalnom analizom varijanse.

Prosečna vrednost za ukupnu količnu mleka kod ispitivane populacije koza iznosila je 362,83 kg, sa prosečnim sadržajem mlečne masti od 3,31%. Rezultati analize varijanse potvrđuju da su razlike koje su ustanovljene pod uticajem laktacije po redu za sve analizirane osobine, izuzev za procenat mlečne masti, bile značajne na nivou $P<0.01$

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GENETIC VARIATION IN RESISTANCE TO CAPRINE FOOT ROT BY *Dichelobacter nodosus* IN GOATS OF KERALA, INDIA

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Abstract: Foot rot is a highly contagious and economically important disease of sheep and goats, caused predominantly by *Dichelobacter nodosus*. The current investigation was intended to analyse the genetic variation for resistance to caprine foot rot among two purebred native breeds of goats(Malabari and Attappady Black) and crossbred (Malabari crosses with Sannen, Alpine and Boer) goats in Kerala state, India. The cases were identified by observing characteristic symptoms of foot rot in goats, detecting Gram negative large rods from the hoof lesions and by PCR to detect the 783bp amplicon from the 16sRNA gene of *D. nodosus*. Two hundred and four animals were subjected to the study and statistical analysis of the data generated could substantiate that, there is variation in caprine foot rot resistance among genetic groups studied ($p \leq 0.01$) with significantly lower incidence rates in Malabari (14.29%) and Attappady Black (2.29%) compared to the crossbreds (43.75%).

Key words: Attappady Black, caprine foot rot, *Dichelobacter nodosus*, genetic variation, Malabari, resistance

Introduction

Goats are considered as an economically important livestock species in India on account of their short generation interval, higher prolificacy and excellent market potential. In southern states of India like Kerala, goats contribute considerably to the rural economy. Infectious diseases in goats could be regarded as the major cause of production loss, among which those associated with foot are of special importance as the goats are considered to be voracious grazing animals. Foot rot in domestic sheep and goats is a highly contagious disease that results from a mixed bacterial infection of the hoof, in which the obligate parasite *D.*

nodosus is essential for the initiation and establishment of the infection *(Hindmarsh and Fraser, 1985)*. Foot rot is a major concern in northern part of India *(Wani et al., 2004)* with no reports from the southern parts of India, though it is a well known disease of goats world over. The variation in the susceptibility to the disease among breeds may be attributed to the genetic differences in the goat breeds, which are predominantly seen in the native regions. Therefore a work plan has been made to analyze the genetic variation for resistance to caprine foot rot caused by *D. nodosus* among two purebred native breeds (Malabari and Attappady Black) and crossbreds (Malabari crosses with Sannen, Alpine and Boer) from a herd infection of foot rot in University Goat and Sheep farm, Mannuthy, Kerala, India.

Materials and Methods

Animals. Two hundred and four adult goats belonging to three genetic groups, Malabari (63), Attappady Black (45) and Malabari crosses with Sannen, Alpine and Boer (96) maintained at University Goat and Sheep farm, Mannuthy, Kerala were subjected for the study for a period from September to November 2010. The animals were managed through semi intensive system and were usually released for grazing for a minimum of 4 hours daily. They were also provided with a concentrate feed at the rate of 400g/head/day, with mineral supplements at the rate of 600g/100Kg feed/day and fodder grass adlibitum. The three genetic groups under the study were housed in independent pens on raised wooden platforms which were adequately ventilated and optimally illuminated. During the period of illness/ period of study the animals were thoroughly observed for the clinical signs associated with foot rot viz., limping, holding of limbs above the ground, reluctance to walk, presence of dark grey exudate and foul smell from the interdigital spaces *(Stewart et al., 1986)*.

Collection of samples and detection of the pathogen. Exudates/ tissues from foot rot lesions were collected from the goats showing the above clinical signs at random. Gram's staining of the samples revealed large Gram negative rods. The samples were processed to obtain the DNA of the pathogen by crude method i.e., the samples were suspended in 50µl sterile distilled water, boiled for 10 min, snap chilled on ice for 5min and centrifuged at 15000 rpm for 10 min and the supernatant was collected. The DNA obtained was employed in PCR, as it was reported that the PCR based methods using 16 sRNA gene specific primers have been used for the rapid diagnosis of D*. nodosus* from clinical samples *(La Fontaine et al., 1993; Wani et al., 2004; Moore et al., 2005; Wani et al., 2007)*.

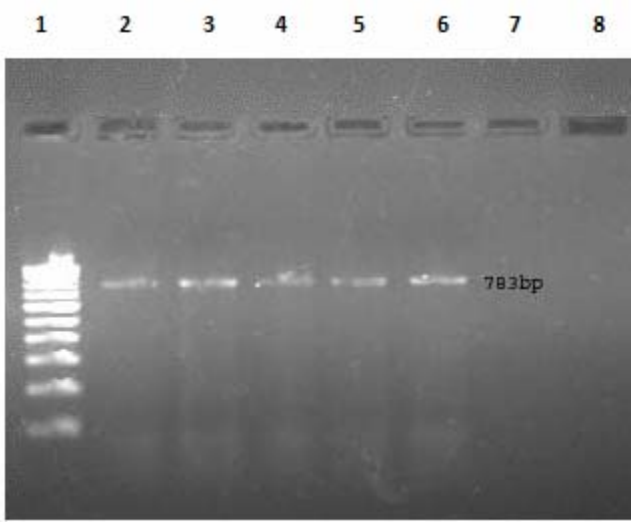
PCR amplifications were carried out in 25µl in 0.2ml thin walled PCR tubes. The PCR mixture contained a final concentration of 5µl of template, 2.5µl of 1X taq buffer (10mM Tris-HCl (pH 9.0), 50mM KCl, 15mM $MgCl_2$), 25pM of forward primer -5' CGGGGTTATGTAG CTTGC 3' and 25pM of reverse primer

-5'TCGGTACCGAGTATTTCTACCCAACACCT 3' (*La Fontaine et al. 1993*), 200µM of each deoxyribonucleotide triphosphate and 1U Taq DNA polymerase. The amplification was carried out in a Thermal Cycler consisted of 94^0C 10 minutes followed by 30 cycles of 94^0C for 1 minute, 58^0C for 30 seconds and 72^0C for 30 seconds and final extension at 72^0C for 5 minutes. PCR products were electrophoresed in 1.5% of Agarose gels, stained with ethidium bromide and visualized under ultraviolet (UV) illumination and photographed with gel documentation system.

Statistical analysis. The significance of genetic group on the incidence of caprine foot rot by *D. nodosus* was tested using Chi-square test. Further the significance difference in proportions of infected animals in each genetic group was tested using Z-test *(Snedecor and Cochran, 1994)*.

Results and Discussion

Among the two hundred and four goats subjected to the study, nine Malabari goats, four Attappady Black goats and 42 crossbred goats were showed the typical symptoms of foot rot. The randomly collected samples yielded PCR amplified products of an expected size of 783 bp, suggestive of 16sRNA gene of *D. nodosus* (Figure 1).



Lane 1 : 100bp DNA ladder
Lane 2-6: 783bp 16sRNA gene of *D. nodosus*
Lane 7,8: Negative controls

Figure 1. PCR amplification of 16sRNA gene of *D. nodosus*

The proportion affected in the three different genetic groups and the comparisons among them are presented in Table 1. The statistical analysis revealed a significant effect for genetic group in the incidence of caprine foot rot by *D. nodosus*, i.e., the incidence in Malabari and Attappady Black goats were significantly lower when compared to crossbreds. Malabari and Attappady Black are the indigenous breeds of Kerala which are well adapted to the local climatic conditions.

Table 1. Proportion of goats affected with foot rot in different genetic groups

Genetic group	Proportion affected	χ^2 value
Malabari	14.29[a]	24.34**
Attappady black	8.89[a]	
Crossbred	43.75[b]	

(** p≤0.01) (Superscripts with different alphabets differ significantly)

In Kerala, goat production is one of the important farm activities for the rural livelihood security. Foot rot is a costly disease in ruminant livestock population particularly during the wet season. Costs of labour, drugs, equipment and treatment, decreased flock productivity, losses from sales of breeding stock etc. make caprine foot rot, a disease of economic importance for producers *(Adama and Kudu, 2008)*. Though goats are very much susceptible to this disease, the susceptibility spectrum varies with different localities/ breeds *(Emery et al., 1984; Shimshony, 1989)*. Therefore, the present study envisages to study whether there is any difference in the susceptibility pattern to this particular disease among the various breeds commonly available in Kerala state.

Research on breeding for disease resistance in goats is very limited in India and disease resistance has not yet been included in breeding programs. There is well-documented evidence for within and between breed genetic variation in resistance to infectious diseases, namely gastrointestinal nematode infections, diseases due to mycotoxins, bacterial diseases including foot root and mastitis, ectoparasites such as flies and lice, and scrapie, the small ruminant transmissible spongiform encephalopathy *(Bishop and Morris, 2007)*.

Conclusion

Though foot rot can be controlled in an effective way through good management practices like the foot bathing, quarantine of the affected animals and vaccinations, adopting them in disease endemic area can make the economics of goat rearing to the non profitable side. Therefore, there is a need for a sustainable and long term solution like building up a flock with genetic resistance to foot rot in

areas where the disease is endemic. The lower incidence rate of caprine foot rot in the purebreds of Kerala as indicated in this study should be extrapolated to its maximum to develop strategies through traditional selection and molecular genetic testing methods to breed for resistance to foot rot in goats of Kerala. The genetic management of disease includes choosing an appropriate breed for the production environment or cross breeding to introduce genes of disease resistance, into a genetic group, which are otherwise well adapted for the required purpose, which is more applicable here. Since the crossbreds excel in their milk production and growth rate when compared to the other two groups, further marker studies are required to introduce foot rot resistant genes of native breeds into the crossbred blood.

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Genetska varijacija u otpornosti na koziju zaraznu šepavost prouzrokovanu sa *Dichelobacter nodosus* kod koza u državi Kerala, Indija

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Rezime

Zarazna šepavost je izuzetno zarazna i ekonomski važna bolest koja pogađa ovde i koze, a izaziva je bakterija *Dichelobacter nodosus*. Istraživanje je imalo za cilj analizu genetske varijacije u pogledu otpornosti na koziju zaraznu šepavost kod dve autohtone rase koze (Malabari i Attappady Black) kao i meleza (melezi Malabari i sanske, alpino i boer rase), u državi Kerala u Indiji. Slučajevi su identifikovani opservacijom karakterističnih simptoma ove bolesti kod koza, otkirvanjem gram negativnih velikih štapićastih bakterija iz lezija na papcima i PCR za otkrivanje 783bp amplikona iz 16sRNA gena *D. nodosus*. U ispitivanje su bile uključene 204 životinje, a statistička analiza dobijenih podataka je potvrdila da postoji varijacija u otpornosti na koziju zaraznu šepavost među ispitivanim genetskim grupama ($p \leq 0.01$), sa signifikantno manjim brojem slučajeva ove bolesti

kod malabri (14.29%) i Attappady Black (2.29%) u poređenju sa melezima (43.75%).

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THE EFFECT OF SPRING SHEARING ON MILK YIELD AND MILK COMPOSITION IN TSIGAI EWES

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Original scientific paper

Abstract: Milk yield and milk composition responses to shearing were assessed in Tsigai ewes kept indoors. Sheep was offered 700g/head daily concentrate and chopped hay administered *ad libitum* and was milked twice daily - at 08:00 and at 18:00 h. Average for the two weeks post-shearing, daily milk yield dropped by 7.2 % due to the 8.2 and 2.2 %, respectively, reduction in the morning and in the afternoon milk yields. Fat and protein concentrations in the morning and in the afternoon milk on day 1, day 7 and day 14 after shearing surpassed vastly pre-shearing values whilst milk lactose concentration showed a trend towards post-shearing reduction. On the three sampling post-shearing days daily output of different milk constituents exceeded the corresponding mean pre-shearing values by 16.2, 11.6 and 9.1 % in milk fat, 1.6, 3.5 and 6.2 % in milk protein, and 4.1, 7.2 and 2.2 % in total solids. The increase in daily output of milk constituents occurred despite the decline in daily milk yield. Lactose daily output, on the contrary, dropped by 6.9, 7.7 and 9.3 % relative to the mean pre- shearing value. Post-shearing adaptive adjustments including changes in nutrient partitioning appear to underlie the alterations in milk yield and milk composition. It may be concluded that post-shearing changes in milk composition makes for the improvement of milk processing characteristics.

Key words: sheep, shearing, milk yield, milk composition

Introduction

Shearing is the common farm routine working disturbances in the thermal homeostasis which brings into action various adaptive responses related to its re-adjustments. Post-shearing metabolic and endocrine adaptations would be expected to influence plasma concentration of metabolites and alter nutrient partitioning that may affect milk yield and milk composition *(McBride and Christopherson, 1984; Symonds, et al., 1990)*. These changes in milk performance may affect energy supply to the offspring and/or processing characteristics of the milk that may,

ultimately, influence the financial performance in dairy farms. In the literature reviewed scarce information was found about shearing effect on milk yield and milk composition in sheep.

The aim of the present study was to elucidate the shearing effect on milk yield and milk composition in Tsigai ewes.

Materials and Methods

Six Tsigai sheep in their 4th month of lactation were involved in the study. Daily ration consisted of 700 g/head concentrate, given in two meals, and chopped hay administered *ad libitum*. Water and salt were freely available. Ewes were shorn at mid-April and were hand milked twice a day at approximately 08:00 h and 18:00 h. Sheep was shorn soon after the morning milking. Milk yield from each ewe was recorded and daily yield was obtained by summing the morning and afternoon yield. Samples for milk composition analysis were taken from the milk of each sheep obtained in the morning and in the afternoon milking on the day before shearing, and on day 1, day 7 and day 14 thereafter. The samples were analyzed for fat, protein, and lactose content using a Milko Scan 133 B, calibrated for sheep milk. Mean daily output of milk constituents in the morning and in the afternoon milk at sampling days was also calculated. Feed and water intake and air temperature in the barn were also recorded twice a day.

The results are presented as mean and standard error of the mean. Significant differences in daily milk yield, concentration and daily output of milk constituents in the milk obtained before and after shearing of ewes were assessed by a Student's *t* test using the mean value for each trait (Snedecor and Cochran, 1989).

Results and Discussion

Mean daily MY in unshorn sheep averaged 604.2 ± 3.5 g/day. The greatest fall in daily yield occurred on day 2 after shearing when it dropped to 85.2 % ($P < 0.01$) of the mean pre-shearing value (Figure 1). Daily MY increased gradually thereafter nearing the pre-shearing level on the sixth day after fleece removal. Afternoon MY showed a sharp, short-term, decline followed by fast recovering. Average for the two weeks post-shearing, daily MY dropped by 7.2 % due to the 8.2 and 2.2 %, respectively, reduction in the morning and in the afternoon milk yields (Figure 1).

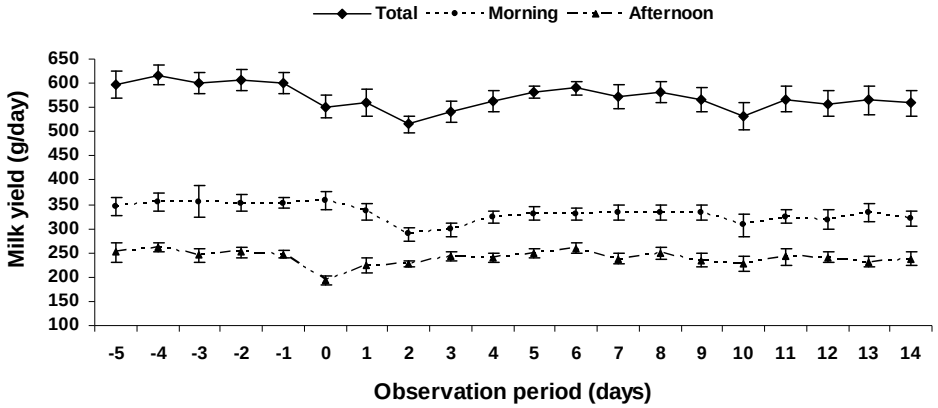

Figure 1. Mean daily milk yield (total), and yield obtained with the morning and afternoon milking. Each point represents the mean for 6 ewes. Vertical bars represent SEM. Day 0 = shearing day

Fat concentration was greater in the milk obtained in the afternoon whilst protein and lactose concentrations were similar in the morning and in the afternoon milk throughout the observation period (Table 1). Of all milk components milk fat exhibited the greatest post-shearing elevation. Before shearing, milk fat concentration averaged 5.74 % in the morning milk, and 6.77 % in the afternoon milk. On day 1, day 7 and day 14 after shearing it increased up to 7.05, 6.81 and 6.91 % ($P < 0.05$) in the morning and up to 8.60 ($P < 0.05$), 7.81 and 7.64 % in the afternoon milk. On the three sampling post-shearing days concentrations of protein and total solids in the morning and in the afternoon milk also surpassed the mean values in unshorn ewes. Daily output of milk constituents followed closely the changes in their concentrations (Table 1). On the three sampling post-shearing days daily output of different constituents exceeded the corresponding pre-shearing values by 16.2, 11.6 and 9.1 % in milk fat, by 1.6, 3.5 and 6.2 % in milk protein, and by 4.1, 7.2 and 2.2 % in total solids, respectively. The increase in daily output of milk constituents occurred despite the decline in daily milk yield. Lactose daily output, on the contrary, dropped by 6.9, 7.7 and 9.3 % compared to the pre-shearing value.

Table 1. Mean daily milk yield, milk composition, and daily output of different constituents in the morning and in the afternoon milk and total for the sampling days before and after shearing (mean ± SEM)

Parameters	Before shearing	After shearing		
		1st day	7th day	14th day
Morning milk				
Milk yield (g/day)	353 ± 12	335 ± 17	333 ± 17	321 ± 16
Fat (%)	5.74 ± 0,38	7.05 ± 0.41	6.81 ± 0.31	6,91 ± 0.25 *
Protein (%)	5.37 ± 0,17	5.92 ± 0.27	5.84 ± 0.26	6.08 ± 0.28
Lactose (%)	5.38 ± 0.05	5.34 ± 0.09	5,20 ± 0.04	5.20 ± 0.06
Total solids (%)	17.08 ± 0.48	18.90 ± 0.59	18.48 ± 0.50	18.78 ± 0.45
Fat output (g/day)	20.26 ± 1.75	23.62 ± 1.87	22.68 ± 1.58	22.18 ± 1.43
Protein output (g/day)	18.96 ± 0.99	19.83 ± 0.51	19.45 ± 1.15	19.52 ± 1.07
Lactose output (g/day)	19.00 ± 0.59	17.89 ± 0.78	17.32 ± 0.79	16.69 ± 0.77
Total solids output (g/day)	60.29 ± 3.01	63.32 ± 3.89	61.54 ± 3.13	60.28 ± 3.05
Afternoon milk				
Milk yield (g/day)	247 ± 7	225 ± 15	238 ± 9	238 ± 14
Fat (%)	6.77 ± 0.35	8.60 ± 0.35 *	7.81 ± 0.40	7.64 ± 0.41
Protein (%)	5.31 ± 0.16	5.67 ± 0.28	5,78 ± 0.25	6.11 ± 0.28
Lactose (%)	5.23 ± 0.05	5.32 ± 0.06	5.10 ± 0.05	5.15 ± 0.06
Total solids (%)	17.89 ± 0.48	20.16 ± 0.52	19.4 ± 0.61	19.51 ± 0.62
Fat output (g/day)	16.72 ± 0.99	19.35 ± 1.65	18,59 ± 1.45	18.18 ± 1.37
Protein output (g/day)	13.12 ± 0.43	12.76 ± 0.92	13,76 ± 0.96	14.54 ± 0.92
Lactose output (g/day)	12.92 ± 0.35	11.97 ±0.79	12,14 ± 0.60	12.26 ± 0.59
Total solids output (g/day)	44.15 ± 1.71	45.38 ± 3.24	50.46 ± 2.86	46.49 ± 2.72
Total for the sampling day				
Milk yield (g/day)	600 ± 22	560 ± 27	572 ± 24	559 ± 27
Fat output (g/day)	36.98 ± 2.59	42.97 ± 3.09	41,27 ± 2.89	40,36 ± 2.61
Protein output (g/day)	32.08 ± 1.34	32.60 ± 2.29	33,21 ± 2.04	34.06 ± 2.01
Lactose output (g/day)	31.92 ± 0.67	29.86 ± 1.37	29.46 ± 1.08	28.95 ± 1.23
Total solids output (g/day)	104.44 ± 4.26	108.70 ± 6.40	112.00 ± 5.72	106,77 ± 5.43

* $P < 0.05$

The initial post-shearing drop in daily MY may be attributed to the cumulative effect of cold stress and emotional disturbance. Fluctuations in daily MY, especially in the afternoon one, provided evidence for the short-term effect of

the psychic stress on milk performance. Over the two weeks post-shearing mean daily temperatures in the barn ranged from 7.8 to 12.7 °C, being below the level of thermo-neutrality *(Yousef, 1985),* that would be expected to drive numerous adaptive adjustments. On the shearing day roughage intake dropped by 7.9 % but the reduction was short-term and on the second day after shearing consumption achieved the pre-shearing level *(Aleksiev, 2010c).* This decrease in energy intake was unlikely to affect substantially plasma substrate concentrations and/or the rate of milk synthesis since the blood metabolite concentrations may be maintained by mobilization of body reserves during a short-term food deprivation. It was found *(McBride and Christopherson, 1984)* cold stress to affect the mammary blood flow that could influence the rate of supply of precursors and the rate of milk synthesis. Conversely, *Lacasse and Prosser (2007)* reported that the rate of blood flow to the udder is primarily associated with metabolic activity of the mammary gland tissues. Therefore, neither the disturbance in mammary blood flow, nor the alterations in the energy balance, due to the post-shearing changes in feed intake and/or the rate of heat dissipation, seem to account for the variations in daily MY and milk composition. The results of our trial suggest that the most possible underlying mechanism appeared to be the registered 19.8 % post-shearing reduction of water consumption *(Aleksiev, 2010c).* The resultant decrease in total body water content and attendant elevation of blood osmolality may limit water movement from blood to milk causing a decrease in the volume of daily MY. Thus, in short-term, the changes in daily MY may be linked primarily to the psychic stress whereas, in long-term, they may be attributed to the adaptive responses and particularly to the voluntary dehydration of sheep promoting homeothermy maintenance.

Different mechanisms, among which diurnal changes in hormonal profile, alteration of plasma nutrient concentrations and/or their partitioning to/or their uptake by the different body tissues may have contributed to the greater reduction in the morning MY. The greater degree of cold stress experienced by the sheep during the cool night hours and/or the inhibition of milk ejection may also cause a depression in milk secretion rate and reduction in the morning yield. Diurnal pattern of water consumption was associated with the feeding pattern *(Aleksiev, 2010c),* which both exhibited a reduction during the nighttime. This may additionally influence plasma osmolality and water content of milk. Similar post-shearing changes in the morning and in the afternoon MY were found in Danube fine wool breed of sheep shorn in February *(Aleksiev, 2009)* and in Pleven blackhead ewes shorn in March *(Aleksiev, 2010a),* pointing towards the involvement of homeostatic adjustments in regulation of milk synthesis.

It would, however, be misleading to consider the post-shearing changes in daily milk yield separately from the changes in milk composition. Lactational performance correlates with the total production of the individual milk constituents which, in this trial, exhibited a substantial post-shearing increase (Table 1). Nutrition is considered as the major factor related to milk performance since it determines the diurnal dynamic of blood metabolites. Average for the two weeks

post-shearing, roughage intake (on dry matter basis) increased by 2.3 % compared to the mean pre-shearing level *(Aleksiev, 2010c),* that was not likely to affect measurably milk composition. Possible post-shearing changes in the rate of digesta passage and digestibility, particularly in roughages, what was found in newly shorn sheep by *Christopherson (1985)*, would not be expected to affect considerably the concentration and/or proportion of volatile fatty acids in the rumen and blood concentration of precursors. Clearly, other mechanisms appear to take part in regulations of the plasma concentration of metabolites, nutrient partitioning to/or their uptake by the mammary gland, and the rate of biochemical reactions related to the synthesis of milk constituents. Changes in milk composition may be attributed to the post-shearing adaptive adjustments associated with alterations in the homeorhetic capacity of the ewe. This contention corresponds with similar post-shearing changes in milk composition observed in Pleven blackhead ewes, shorn in March *(Aleksiev, 2010c)* and in Tsigai sheep, shorn at the beginning of April *(Aleksiev, 2010b).* Our results, in general**,** were in agreement with the findings of *Knight et al. (1993)* in lactating Dorset ewes, kept on natural pastures and shorn at different months of the year. *Rassu et al. (2007)* also found a considerable post-shearing increase in milk fat and milk casein concentrations in Sarda ewes shorn in June at. Similarly, *Peana et al. (2007*) noticed that even in unshorn sheep the drop in ambient temperatures from the thermoneutral values of 9 – 12 °C down to – 3 °C during the winter period influenced negatively milk yield causing the 25 % decrease from the value obtained in optimal temperature conditions.

The greater fat content in the milk obtained in the afternoon before and after shearing of ewes resulted from the higher fat concentration of the milk remaining in the alveoli after milking compared to cisternal milk Afternoon milking was performed only 10 hours after the morning one and it was found *(Labussiere, 1988)* that the shorter the interval between milking the greater milk fat content. *Benchini and Pulina (1997)* noticed that milk composition may be affected more substantially by the milking interval in breeds not selected for dairy production as was the case with Tsigai sheep used in the current study.

Yield of milk fat, milk protein and total solids largely depends on MY. In this respect of particular interest was the post-shearing increase in daily output of different milk constituents. It may be suggested that changes in the plasma concentration of milk precursors and alteration of homeorhetic capacity both coacted in generation of the changes in milk composition**.** Our results were in agreement with the contention of *Symonds et al. (1990)* who noticed that post-shearing endocrine alterations may increase the partition of nutrients towards milk production improving energy content of the milk. Another noteworthy point in our trial was the reduction in lactose concentration and lactose daily output that may be ascribed to the post-shearing changes in the whole body glucose metabolism and/or to the alteration in the blood substrate availability. Lactose output is related to the rate of glucose uptake which, however, may not be proportional to its plasma concentration, and subsequent output in the form of lactose. A possible depression

in glucose uptake by the udder in shorn ewes may influence lactose secretion since the blood glucose is the main precursor of milk lactose. Lactose daily output showed a greater decline in the morning milk exhibiting greater post-shearing reduction (Fig. 1). In the afternoon milk daily output of lactose did not differ substantially from the pre-shearing value. Lactose is the major osmotic compound of the milk and an increase of its concentration, proportionally to the post-shearing elevation of other constituents, could lead to an increased osmotic movement of water from blood to the milk. In a nursing ewe, such a raise of daily MY may increase the lamb's total body water content and thermal conductivity of the peripheral tissues. Therefore, the post-shearing decline in daily MY accompanied by an increase in daily output of milk fat and milk protein may be assumed as an adaptive, anticipatory response improving energy supply to the offspring and its thermoregulatory capacity. The stated changes in milk yield and milk composition may be considered to indicate central pathways in the post-shearing adaptive adjustments including milk synthesis control mechanisms.

Conclusion

Daily milk yield over the two weeks post-shearing exhibited an average drop of 7.2 % due to the 8.2 and 2.2 %, respectively, reduction in the morning and in the afternoon yields. Milk fat and milk protein concentrations increased compared to the corresponding mean pre-shearing values.

Mean daily output of milk fat, milk protein and total solids after shearing increased despite the reduction in daily milk yield, whereas lactose daily output dropped relative to its pre-shearing value

The data suggest that post-shearing changes in milk composition make for the improvement of milk processing characteristics.

Uticaj prolećne striže na prinos i sastav mleka ovaca rase cigaja

Y. Aleksiev

Rezime

Ocenjivana je reakcija prinosa i sastava mleka na strižu kod cigaja ovaca koje se drže u zatvorenom prostoru. Ovce su dobijale 700g/po grlu dnevno koncentrata i iseckanog sena, *ad libitum* i mužene su dva puta dnevno - 08:00 i

18:00 h. U proseku, za period od dve nedelja nakon striže, dnevni prinos mleka je smanjen za 7.2% zbog 8.2 i 2.2%, respektivno, smanjenja prinosa mleka u jutarnjoj i večernjoj muži. Koncentracije mlečne masti i proteina u jutarnjoj i večernjoj muži 1., 7. i 14. dana nakon striže su bile znatno više od vrednosti pre striže, dok je vrednost koncentracije laktoze pokazao trend smanjenja nakon striže. U tri uzorka uzeta nakon striže, dnevni prinosi različitih konstituenata mleka su nadmašili odgovarajuće srednje vrednosti pre striže za 16.2, 11.6 i 9.1 % kod mlečne masti, 1.6, 3.5 i 6.2 % kod proteina u mleku, i 4.1, 7.2 i 2.2 % kod ukupne čvrste materije. Povećanje dnevne proizvodnje konstituenata mleka se desilo uprkos smanjenju prinosa mleka. Dnevna proizvodnja laktoze, suprotno gore navedenim vrednostima, je pala za 6.9, 7.7 i 9.3 % u odnosu na srednje vrednosti pre striže. Adaptivne modifikacije nakon striže uključujući promene u balansiranju hrane su u osnovi promena u prinosu i sastavu mleka. Može se zaključiti da promene u prinosu i sastavu mleka nakon striže utiču na poboljšanje osobina mleka koje su važne za njegovu preradu.

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DETERMINATION OF LEAD, CADMIUM AND ZINC APPLYING THE STRIPPING ANALYSIS ON BIOMASS OF NATURAL GRASSLANDS

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Abstract: This study is focused on mechanisms of voltammperometric determination of lead, cadmium and zinc in the natural grassland biomass in territory of northern Kosovo, applying the Stripping analysis. Two types of determinations have been researched: individual and simultaneous research of all three metals. The preliminary researches had been conducted prior determinations on real samples defining the determination conditions: extraction potential, value of analyzed pH solution, metal extraction time, time for creating of working electrode, as well as solution mixing velocity. It was found that, with accuracy of ±2%, determinations were done for 22-900 μg dm^{-3} of lead, 16-960 μg dm^{-3} of cadmium and 18-750 μg dm^{-3} of zinc. The simultaneous metal determinations had less accurate results for lead and cadmium, whereas they ranged within accuracy limits for zinc. Heavy metal determination in biomass indicated existence of lead, cadmium and zinc in natural grasslands in northern parts of Kosovo and Metohija.

Key words: Biomass, natural grasslands, Stripping analysis, heavy metals.

Introduction

The natural grasslands are comprised of enormous variety of plant species *(Stošić et al., 2005)* and it is a very important factor in development of forage production *(Đorđević-Milošević et al., 1997)*. The growth of plant species and yields results are tightly linked to absorption of mineral elements from soil, mineral transport and distribution within the plant as well as to participation in biochemical reactions *(Jakovljević, Antić-Mladenović, 2000)*. Besides essential elements, the plants also absorb heavy metals which decrease their photosynthetic activities, mineral nutrition, protein metabolism and enzyme activity, membrane function, water regime and some other biochemical processes *(Nikolić, 2009)*. Absorption of these elements is mainly done via root where they are mainly kept if they are not

present in too high concentrations *(Bogdanović et al., 1997)*. Root epidermis represents a barrier for lead absorption, whereas zinc and cadmium are known for their great mobility through the plants and they are hardly ever kept in the root itself, which leads to slow plant growth *(Kastori et al., 2000)*.

The aim of the study is analysis of conditions and mechanisms of voltammpermetric determination of lead, cadmium and zinc in biomass, including the determination of lead, cadmium and zinc contamination levels in natural grasslands in northern part of Kosovo.

Materials and Methods

The metal content was determined applying system for the Stripping analysis, Stripping analyzer M1 (Faculty of Technical Sciences, Novi Sad and Elektrouniverzal, Leskovac, Serbia). This system functions applying three electrodes: glass carbon electrode, reference electrode (Ag/AgCl/KCl/3.5 mol dm^{-3}) and platinum wire acting as auxiliary electrode. Solutions of HCl, HNO_3 (cc), as well as standard Pb, Cd, Zn, Hg (1.000 g dm^{-3}), (suprapur), Merck (Darmstadt, Germany) and working standard solutions of: 90000 µg dm^{-3} for lead, 65000 µg dm^{-3} for cadmium and 75000 µg dm^{-3} for zinc were used.

Results for determination of lead, cadmium and zinc in standard solutions is shown on the Table 1.

Table 1. Determination of lead, cadmium and zinc in standard solutions

	Metal content			Kv (%)		S (µg)		Er (%)	
	Xs	$\overline{X}$ -p	$\overline{X}$ -i	Kv-p	Kv-i	S-p	S-i	Er-p	Er-i
Pb	4.45	4.70	4.15	12.34	14.94	0.58	0.62	5.62	-6.74
	22.48	24.98	21.90	11.34	12.02	2.52	2.58	1.12	-2.54
	44.96	45.42	43.84	11.34	11.78	5.15	5.27	1.02	-2.49
	224.3	226.6	218.54	8.01	8.51	18.15	18.61	1.03	-2.57
	447.5	452.2	434.30	7.28	7.80	32.92	33.89	1.05	-2.95
	890.8	880.6	862,79	7.26	7.64	63.93	65.93	-1.14	-3.14
Cd	3.25	3.45	3.47	13.74	14.49	0.47	0.50	6.15	6.77
	16.23	16.54	16.63	9.45	9.61	1.56	1.60	1.91	2.45
	32.47	33.09	33.25	7.54	7.67	2.49	2.55	1.90	2.41
	162.0	165.1	165.95	7.39	7.53	12.20	12.49	1.90	2.43
	323.2	329.3	330.99	7.43	7.56	24.45	25.03	1.88	2.40
	643.3	634.2	628.55	7.19	7.42	45.59	46.63	-1.41	-2.28
	960.1	941.5	936.41	7.30	7.52	68.73	70.43	-1.93	-2.47
Zn	3.75	3.97	3.99	11.59	12.28	0.46	0.49	5.86	6.40
	18.75	19.09	19.11	10.42	10.62	1.99	2.03	1.81	1.92
	37.50	38.21	38.24	10.36	10.56	3.96	4.04	1.89	1.97
	187.5	191.2	191.19	8.35	8.51	15.96	16.27	1.95	1.97
	375.0	379.8	381.85	7.43	7.53	28.22	28.74	1.27	1.83
	750.0	737.7	736.74	7.79	7.94	57.47	58.48	-1.63	-1.77

Xs-element content in standard solutions, $\overline{X}$ -average determination value–number of determinations=5, p-individual determinations, i-simultaneous determinations

The working electrode was created by extracting mercury from acid mercury(II) ion solution, content of 10 mg dm^{-3} and power of -48.90 μA during 240 seconds on surface of glass carbon. Series of 20 cm^3 water solutions and 0.5-200 μdm^3 working standards were prepared for all determinations. Extraction of heavy metals on working electrode was done individually at potential of -0.999 V for lead; -1.106 V for cadmium and -1.350 V for zinc *(Babincev et al., 2010).* Determination of all three elements simultaneously was done at potential of -1.400 V. The best results were attained for reduction time of 300 seconds, mixing velocity of 4000 min^{-1} and pH: 1.6 for lead; from 1.6 to 2.0 for cadmium and from 2.1 to 3.5 for zinc. Determination for all three elements simultaneously is most effective for pH 2.1 *(Babincev and Rajaković, 2009).*

The herbal material was collected from the natural grasslands of southern exposition of the Kopaonik mountain, at elevation of 466, 512 and 1040-1100 m from the hay stacks in September. The average samples (elevation of 1100 m) were made of average samples of nearby villages. At elevation of 466, the samples were collected from various differences from the landfills (Bostanište, Leposavić). The biomass sampling at elevation of 512 m was done in direct vicinity of the landfill (Žitkovac, Zvecan). The samples were also collected along the main road Lešak-Kosovska Mitrovica, on places 1-2 m way from the road, depending on terrain accessibility.

Following washing, the biomass was dried at 105 °C until the permanent mass was made, then it was burnt at 500 °C. One gram of ash was moisten with water and then treated with 5 cm^3 HNO_3 (cc).After evaporation, the concentrated HCl was added followed by evaporation. The remaining white mass was dissolved with 5 cm^3 HCl 2 % and prepared for analysis in a normal vessel of 100 cm^3 *(Babincev and Rajaković, 2009)*. Content of lead, cadmium and zinc was determined by standard supplements methods.

Results and Discussion

For all determinations, five measurement results were approved, based on which the average value ($\overline{X}$, μg dm^{-3}), standard deviation values (S, μg),variation coefficient (K_v, %), reproduction measure and determination error (Er, %) were calculated.

The Stripping analysis defined the metal contents of: 4-900 μg dm^{-3} for lead, 3-960 μg dm^{-3} for cadmium and 3-750 μg dm^{-3} for zinc. Both individual and simultaneous determinations of all three elements were done. Contents higher than 20 μg dm^{-3} for lead, 16 μg dm^{-3} for cadmium and 18 μg dm^{-3} for zinc were determined with ±2% of accuracy. The most accurate results of simultaneous determinations were obtained for zinc. The results of metal content determination of natural grassland biomass in northern part of Kosovo and Metohija are given in Table 2.

Lead, cadmium and zinc contents in natural grassland biomass taken form different distances from the active Trepča landfill are given in Table 3.

In researches of *Kadovića and Kneževića (2002),* the concentration of lead in actively growing grass ranged between 0.3-1.5 μg g^{-1}, 10 μg g^{-1} at the end of summer and 30 μg g^{-1} of dry mass in the end of winter.

Table 2. Content of heavy metals in biomass of natural grasslands in northern Kosmet

Locations	μg g^{-1} *SM*		
	Pb	Cd	Zn
Elevation higher than 1000 m	2.35	-	55.40
	4.56	-	78.32
	8.95	0.22	93.71
Along main road Lešak-Kosovska Mitrovica	10.32	1.62	130.24
	11.15	3.25	141.01
	12.39	6.49	135.49
In zone of passive Trepca landfill-Žitkovac	17.48	5.09	206.71
	32.94	10.12	158.55
	27.32	8.45	116.17

SM-dry material

Table 3. Content of heavy metals in biomass of natural grasslands on different distances from the active Trepca landfill-Bostanište, Leposavić

Element content μg g^{-1} SM		Distance from flotation landfil, m				
		500	750	1000	1500	3000
Determined individually	Pb	135.65	101.98	76.48	57.23	21.62
	Cd	3.90	2.11	1.96	1.18	0.74
	Zn	717.01	539.43	227.11	144.86	116.26
Determined simultaneously	Pb	130.82	98.35	73.75	55.24	20.83
	Cd	3.93	2.12	1.97	1.18	0.74
	Zn	715.98	542.41	226.79	144.89	116.28

SM-dry material

The vegetation of biomass we tested was over at the end of summer, thus the content of 10 μg g^{-1} of dry mass was anticipated. *Kabata-Pendias and Pendias (1989)*, found 0.19-9 μg g^{-1} of lead in underground parts of grasses collected from unpolluted areas, whereas there was 63-232 μg g^{-1} of lead dry mass in polluted areas. They stated that the natural lead content in plants range between 5-10 μg g^{-1} of dry mass. Our researches indicated that the grassland biomass at elevation of 1000 m contains 2-9 μg g^{-1} of lead dry mass. Absorption of lead by plants growing close to highway depends upon distance, level of biomass covering, wind speed, traffic frequency and vehicle halting time *(Filipović-Trajković et al, 2001)*. Along the main highway Kosovska Mitrovica-Lesak, the lead content in biomass was 10-12.4 μg g^{-1} of dry material. It was even 33 μg g^{-1} of dry material in grassland biomass collected directly close to passive landfill. The greatest lead content was

found in grassland biomass taken 500 m away from landfill, and it was also found in biomass taken 3000 m away from landfill.

In many plant species, the intensity of adoption of cadmium and zinc has been correlated with their concentrations in the medium. Zinc content in plants of natural grassland is different and varies in the range of 0.6 to 83 mg g^{-1} dry matter. Content of 15-30 mg g^{-1} dry matter is the border of zinc deficiency. Excess zinc from 200-500 mg g^{-1} dry matter, is rare and that in acid soils and near the mine and ore dumps *(Vukadinovic et al, 1998)*. The content of cadmium and zinc in natural grassland plants progressively decreased at different distances from the smelting of lead and zinc. Plants sampled at a distance of 750 m contained 523 mg g^{-1} dry matter of zinc. At twice the distance of zinc content was 118 mg g-1 dry matter. For the same distance cadmium content decreased from 2.1 to 0.2 mg g^{-1} dry matter *(Kastori et al., 1997)*. Biomass analyzed in this paper, a distance of 750 m from the landfill Bostanište, showed the content of 539.43 mg g^{-1} dry matter of zinc and 2.11 mg g^{-1} dry matter of cadmium. At a distance of 1500 m zinc content was 144.86 mg g^{-1} dry matter, while cadmium was 1.18 mg g^{-1} dry matter. And at a distance of 3000 m it is evident that the presence of all three metals.

Conclusion

According to the conducted tests, the increased presence of lead, cadmium and zinc in natural grassland biomass in northern Kosovo and Metohija was identified, especially on locations close to the existing polluters. In order to have the comprehensive overview of the existing polluters impact, it is necessary to conduct further researches to monitor the content of heavy metals in plant roots and soils of grasslands which take an important part in forage production in this area.

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Određivanje olova, kadmijuma i cinka striping analizom u biomasi prirodnih travnjaka

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Rezime

Ovaj rad je fokusiran na voltametrijsko određivanje olova, kadmijuma i cinka u biomasi prirodnih travnjaka. Istraživana su dva načina određivanja: svakog

metala posebno i sva tri metala istovremeno. Određivanjima u realnim uzorcima prethodila su preliminarna ispitivanja na osnovu kojih su utvrđeni uslovi određivanja: potencijali izdvajanja, pH vrednosti rastvora za analizu, potrebno vreme za izdvajanje metala, potrebno vreme za formiranje radne elektrode kao i optimalne brzine mešanja rastvora. Utvrđeno je da se, sa tačnošću od ±2%, može odrediti olovo od 22-900 μg dm^{-3}, kadmijum od 16-960 μg dm^{-3} i cink od 18-750 μg dm^{-3}. Istovremenim određivanjem metala dobijaju se manje tačni rezultati za olovo i kadmijum, a u granicama tačnosti za cink.

Određivanjem teških metala u biomasi ustanovljeno je povećano prisustvo olova, kadmijuma i cinka u prirodnim travnjacima severnog dela Kosova i Metohije.

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PHENOTYPIC CONNECTION OF THE MAIN BODY PARTS OF RABBITS AND LAYERS

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Abstract: Nowadays in some west European countries increase the need of rabbit meat .The production of rabbit's meat in these countries is based on some practical knowledge and methods of selection and breeding. The success in production of this kind of meat depends from the rabbit's fat or from the quality of the body during the transport (confectioning meat). That is the reason why in these countries the selection of rabbits is making with a big attention. That is why is important to examine the phenotypic connection of the slaughter's characteristics during the confirmation of the optimal selective criterion. The mass of the thighs and the mass of the muscle layer of the thighs are very important for the rabbit's selection. The thigh's mass has strong phenotypic correlation and full genetic correlation with the mass of the clean body. The purpose of this investigation is to find the range of connection of the thigh's dissection based on correlative and regressive analysis in the prediction of the thigh's meat contribution and also from the clean body. Based on the obtained results we can conclude that the mass of the thighs is a reliable indicator as to the yield of the thigh as for the whole body musculature.

Key words: rabbit's meat, phenotypic correlation, genotypic correlation

Introduction

Production of rabbit meat is based on practical knowledge and methods of breeding and selection. In order to determine the optimal criteria selection studying the relationship between rabbit phenotypic characteristics has great significance *(Kapitan 2006).*Feeding of rabbits have a major impact on the quality of rabbit meat Selection of rabbits coefficients are significant phenotypic in genotypic correlation between age and weight of the carcass clean, the mass of the thighs and the mass of the muscle tissue of the thighs *(Flank et al., 1979*). The mass of the thighs is very strong phenotypic and genotypic correlation with the weight of the

carcass clean. The mass of the thighs is very strong indicator of the yield of meat in the body *(Niadzviadek et al., 1980, 1983)*.

All of authors examined the phenotypic and genotypic correlations *(Flak et.al., 1979; Niedzvwiadek 1980; Niedzvwiadek 1983; Panic et.al., 1989).* These authors discovered that the coefficients of phenotypic correlations between the age and the mass of the cleaned body.

The aim of our research is based on correlation and regression analysis to determine the degree of safety of dissection of the thigh in the assessment of yield of thigh meat and the carcass clean.

Materials and Methods

For testing we used 22 hybrids of California and New Zealand rabbit. Rabbits are fed with at libidum balanced food containing alfalfa, barley, corn, wheat, soy, granules sunflower premixes, salt, vitamins and minerals.

Slaughtering and primary processing of rabbits was performed in the usual way. After 24 hours cooling of carcass at a temperature of +4°C the bodies were cut in basic peaces and measuring them on an electronic balance with accuracy of 0.1 g. After the slaughtering we get the following parts: pelvic thigh part, groin part, shoulder part and part of back and chest.

After slaughtering the carcass of basic parts is performed the dissection of the right thigh and it is determined the participation of fat, bone and muscle connective tissue in the total mass of the thigh.

Average values and variability of indicators of basic parts of the body and tissues is determined with variation statistical method and with the correlation and regress ion analysis is determined their mutual connection .

Results and Discussion

The average mass of rabbit's carcass was 2467 grams. Most of body was formed with the parts with the best quality of meat. So the mass of the pelvic part of the thigh averaged 420.5 g or 30.67 % . The groin part mass was 398.5 g. or 29.04%. The forelegs part of 300.5 g. or 21.94% and neck –breast part with 267.75 g. or 18,35% of the average mass of the body. Participation of the basic parts of a carcass in these rabbit pox in the research are consistent with results obtained from *(Panic et al.,1989)*.

With the dissection of the right thigh is found that the average mass of muscle tissue was 168 g or 76.42%, fat tissue 3.65 g. Or 1, 72% connective tissue 5.60 or 2.5% and 43.5g bone tisueor19.5%.Unlike the basic parts of the body, muscle tissue and bones of the thigh which varied from 5.7 to 10. 6% mass of fat and connective tissue manifested very high phenotypic variability (42.5% and 56.2%) indicating that these properties do not have a normal frequency

distribution. The determined participation of the bones is higher and the muscle and fat tissue is smaller than the results indicated by *(Niedzviadek et .al., 1979, 1980)*. The results obtained from dissection are in accordance with the results given *(Panic et al., 1989)* who examined New Zealand white rabbit. Proceeds of the basic parts of the body and tissues of the thigh is shown in Table 1.

Table 1. Proceeds of the basic parts of the body and tissues of the thigh

Properties	x	sx	Sd	CV(%)
Carcass weight g	2467	0.012	0.052	8.52
Pelvic - thigh part	420.5	4.2	22	4.72
Flank part	398.5	6.2	29.5	11.8
Forelegs	300.5	4.72	22.0	11.6
Neck - breast part	267.0	4.52	19.20	6.78
Meat boot g	168	2.2	12	7.2
Bones of thigh g.	43.5	0.8	4.2	9.8
Connective tissue boot g	5.60	0.4	2.4	42.5
Adipose tissue from thigh g	3.65	0.4	2.0	56.2

The mass of the trunk had a strong positive correlation with the mass of the thighs and shoulder part (r = 0.673+++ and 0.650+++) and very strong statistically significant correlation with the mass of groin part, shoulder section and the mass of the musculature of the thigh (r=0.833+++-0.857+++).

The mass of the thighs and the amount of muscle from the thigh stand in strong correlation (r = 0.840+++) groin part mass and chest neck part in a strong (r = 0.629 and 0.702) shoulder part mass in middle correlation (r = 0.480++) with a mass of muscle tissue from the thighs. Coefficients of phenotypic correlations (above diagonal) and linear regression (below diagonal) yields the basic parts of the body and tissues of the thigh is shown in Table 2.

Table 2. Coefficients of phenotypic correlations (above diagonal) and linear regression (below diagonal) yields the basic parts of the body and tissues of the thigh

Properties	X1	X2	X3	X4	X5	X6	X7	X8	X9
Weight of carcass т (x1)	-	0.673***	0.840***	0.650***	0.833***	0.857***	0.343	0.083	0,404*
Pelvic thigh part (x2)	0.226	-	0.458***	0.271	0.653***	0.840***	0.349	0.171	0,035
Flank part (x3)	0.326	0.533	-	0.370*	0.671***	0.629***	0.132	-0.180	0,269
ForelegsГ'р (x4)	0.179	0.223	0.263	-	0.336	0.480**	0.002	0.000	0,527**
Neck breast part (x5)	0.204	0.480	0.424	0.299	-	0.702***	0.474***	0.080	0,104
Mass of thigh (x6)	0.138	0.407	0.261	0.282	0.462	-	0.335	0.073	0,306
Bones of thigh (x7)	0.019	0.057	0.019	0.000	0.105	0.113	-	0.127	0,005
Connective tissue boot g (x8)	0.02	0.016	-0.014	0.000	0.010	0.014	0.066	-	0,229
Adipose tissue from thigh (x9)	0.012	0.003	0.018	0.050	0.011	0.050	0.002	0.198	-

*=p<0,05 ; **=p<0,01 ; ***=p<0,001 ;

Flak et al. (1979) , found a strong correlation between the mass of clean carcass with the mass of the basic parts of rabbit's carcass and fully genetic correlation of the mass of the cleaned carcass and its basic parts (r = 0,941-0,987). *Panic et.al. (1989),* found that the mass of the clean carcass and the mass of the thighs are reliable indicators of rand man of thigh muscle part. Because this is in complete correlation with the yield of total body musculature we can conclude with high accuracy that the mass of clean hot and cooled carcass and the mass of the thighs are also quite reliable indicators for the total amount of muscle tissue in the body.

The results obtained can be used for evaluating the body of rabbits and in selection of breeding material for constructing of selection index.

Conclusion

Based on the examinations and the obtained results we find the following conclusions

The mass of the cleaned carcass can be considered a reliable indicator of total yield of the thighs and the thigh's musculature. The mass of the thighs is a reliable indicator as to the yield of the thigh as for the whole body musculature. Linear regression coefficients obtained from these tests can be useful for the construction of the selection index for evaluating properties of slaughter characteristics of rabbits in the combined test and progenitors testing.

Fenotipska povezanost osnovnih delova trupa i tkiva kod kunića

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Rezime

U poslednje vreme u nekim zapadno evropskim zemljama sve više raste potražnja mesa kunića. Proizvodnja mesa kunića u ovim zemljama se zasniva na praktičnim saznanjima i metodama selekcije i odgajivanja. Uspeh u proizvodnji ovog vida mesa zavisi od utovljenosti kunića ili kvalitetu trupa pri izvozu (konfekcionirano meso). Zato se u ovim zemljama posvećuje velika pažnja selekciji kunića. U tu svrhu veliki značaj ima proučavanje fenotipske povezanosti klaničnih osobina pri utvrđivanju optimalnih selekcijskih kriterijuma.

Sa ispitivanjem fenotipskih i genotipskih korelacija bavilo se više autora *(Flak i sar.1979; Niedzvwiadek, 1980, 1983; Panic i sar. 1989).* Ovi auori navode da za selekciju kunića od posebnog značaja su koeficijenti fenotipskih i

genotipskih korelacija između uzrasta i mase očišćenog trupa, masa butova i masa mišićnog tkiva butova . Masa butova je u mnogo jačoj fentipsko korelaciji i potpunoj genetskoj korelaciji sa masom očišćenog trupa.

Cilj ovog rada je da na bazi korelaciona regresiona analiza se utvrdi stepen povezanosti disekcije buta kako u oceni prinosa mesa buta tako i u očišćenom trupu.

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A STUDY OF BIOCHEMICAL POLYMORPHISM IN CARP (*Cyprinus carpio*): DETECT NEW ALLELES IN TRANSFERRIN

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Original scientific paper

Abstract: This study was carried out at the college of agriculture and marine science centre, Basrah university. The present study was conducted to investigate the existence of polymorphism at transferrin (**Tf**) locus in the Carp (*Cyprinus carpio*). A polyacrylamide gel electrophoresis (PAGE) under alkaline condition method was used to distinguish Carp **Tf** alleles. Use the gel documentation program in this study. Analysis of **116** animals revealed that all animals were polymorphic, showing many genotypes. There was very clear biodiversity in the **Tf** gene. Seven **Tf** genotypes consisting of 4 homozygote types (**CC, DD, FF** and **GG**) and two heterozygote types (CD, DG and FG) were detected. These fractions are controlled by co-dominant autosomal genes according to the Mendelian laws of inheritance. The highest gene frequencies were calculated 0.50 for **Tf** D, 0.26 for **Tf** F and 0.12 for C and G. thus, carp (*Cyprinus carpio*) assemblages consistently tended to be more predominant to D allele. Differences between expected number and observed number for transferrin genotypes were no significant. This is useful in genetic improvement process through the selection. As far as we know, this is the first large-scale analysis on the genetic polymorphism in carp (*Cyprinus carpio*). Polyacrylamide electrophoresis, the technique employed in this study, allows rapid and efficient screening for the presence of polymorphism in **Tf**.

Key words: Carp, Genetic Polymorphism, Transferrin, biodiversity

Introduction

Transferrin, one of class I genetic markers, is the most heterogeneous polymorphic blood protein in Carp *(Valenta et al., 1976 and Csizmadia et al., 1995)*, in goose *(Valenta and Stratil, 1978)*, in chicken *(Vyshinsky and Muravjev, 1970)* and sheep *(Jaayid et al., 2011)*, a total of 7 co-dominant alleles have been

found in its locus. Transferrin polymorphism was demonstrated in different breeds in fish. Since then, several reports have been published concerning the gene frequencies in this systems and about the possible influence of these polymorphism on disease resistance *(Jurecka et al., 2009)*. This protein, belonging to the group of beta-globulins, is found not only in blood serum, but also milk and semen. The main function of transferrin in the organism is to participate in iron metabolism and in immune responses. Conservation of genetic variety of strains maintained in live gene banks is a high-priority task. Description of the genetic structure should be the first step in this work. By applying different biochemical-genetic markers such as transferrin and isoenzymes, the individuals and the populations could be well characterised genetically. Based on this, breeding programs as well as conservation of races can be carried out without disappearance of genes from the pool. The conservation of genetic resources is based on two different concepts, namely in situ and ex situ conservation methods. For actual implementation of these conservation methods a sound knowledge of the genetic structure. This knowledge will guarantee that the applied conservation measures will cover the genetic variation of that particular species.

The term "genetic polymorphism" defines the fact that each protein presents two or more forms genetically determined by autosomal and co dominant alleles. The study of polymorphism has many uses in medicine, biological research, and law enforcement. Over the last 10-20 years considerable interest has developed in blood protein polymorphism as well as increasing basic knowledge on protein fraction. A related use of polymorphism is widely employed in agriculture. Electrophoretical techniques have been used extensively as a method to analyse the biochemical, systematic and ecological characteristics of marine and freshwater fishes *(Wiegertjes et al., 1995; Ford, 2001; Kohlmann et al., 2003)*. The aim of these survey was to describe the polymorphism of transferrins of carp strains in the live gene bank. Many gaps still exist in the understanding of identification and conservation of breeds as well as the genes controlling these traits in Iraqi fish. Identification and conservation are not sufficiently characterized, they are underutilized in conventional breeding programmes, and there is insufficient research on the ways to select breeds or individuals carrying the most advantageous traits. Transferrin gene frequencies have not been studied in Iraqi Carp populations only one paper *(Jaayid and Aziz, 2009)*, This paper (1) describes transferrin polymorphisms in Carp (*Cyprinus carpio*), (2) presents evidence of multiple a phenotypes in Carp (*Cyprinus carpio*) and (3) investigate and propose management and utilisation strategies for fish resources in Iraq.

Materials and Methods

Electrophoresis. A polyacrylamide gel electrophoresis (PAGE) of transferrin protein fractions was carried out on 13-cm x 22-cm x 4-mm with 24 wells according to the method developed by *Khaertdinov and Gataulin (2000)*. After applying an output voltage of 200 volts for 10 minutes, the inserts were removed and the same voltage continued for a further 15 minutes. The output voltage was then increased to 250 volts and continued until the brown line had migrated 9 cm beyond the insert line. The gel was then removed, sliced and stained for 10 min. with 0.1 % (w/v) Amido Black in methanol-acetic acid-water (50/7/43 by vol.). The gel was distained with a solution containing methanol-acetic acid-water (40/10/50 by vol.).

Statistical analysis. The allele frequencies in the transferrin were estimated by direct counting of the phenotypes. To test differences between observed and expected genotypes frequencies, a chi-square (χ^2) analysis was performed on the basis of the Hardy-Weinberg law.

Results and Discussion

Figure 1 shows the electrophoretical patterns of some individual carp (*Cyprinus carpio*) protein samples. two bands were detected when transferrin was run and stained in Amido Black in methanol-acetic acid-water (50/7/43 by vol.). The Carp (*Cyprinus carpio*) transferrin types were named according to the nomenclature suggested by Irnazarow and *Bialowas (1994)* and *Jurecka et al. (2009)*. The results obtained for the transferrin that show variation in the sample of Carp (*Cyprinus carpio*) are presented in Table 1. Gene frequencies were calculated by the method of gene counting as the mode of inheritance of each of the systems that do show variation is that of codominant alleles at an autosomal locus *(Khaertdinov, 2000)*.

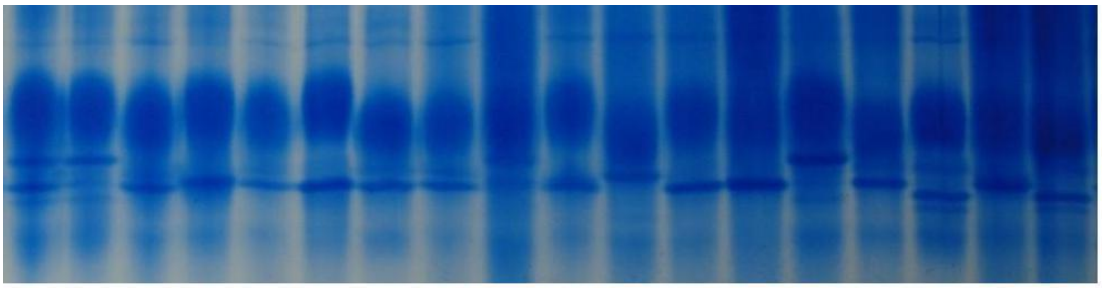
Table 1. Distribution of transferrin Frequency and gene frequency transferrin locus in Carp (*Cyprinus carpio*)

	Transferrin genotypes, n= 116							χ^2	**Gene frequency**			
	CC	**DD**	**GG**	**FF**	**CD**	**DG**	**FD**		**C**	**D**	**G**	**F**
Number	8	45	23	24	8	8	15	21.54	0.12	0.50	0.12	0.26
%	6.72	37.82	6.72	20.71	6.72	6.72	12.61					

The Carp (*Cyprinus carpio*) transferrin phenotypes are due to an autosomal locus with four co-dominant alleles, TfC , TfD, TfG and F. The D and F alleles were most frequent (0.5 and 0.26) respectively, while the C and G alleles were rare

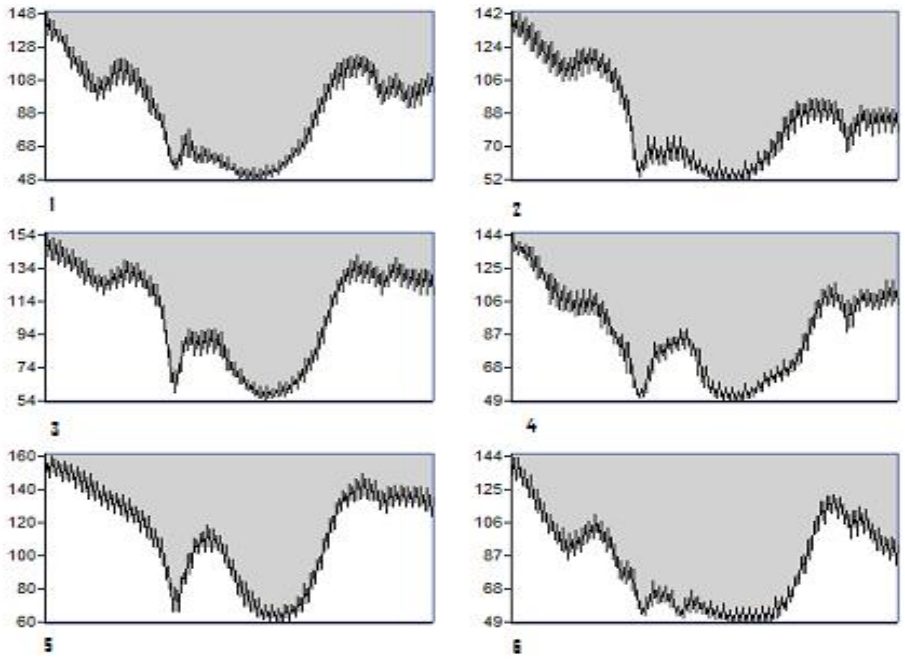
alleles (0.12) (Figure 2). The gene frequency for D allele obtained in the sample is within the range of those observed in *Jurecka et al. (2008), Csizmadia et al. (1995)* and *Wojtczak et al. (2007)* while *Valenta et al. (1976)* have been found Seven transferrin variants (A,B,C,D,E,F, and G) in Carp.

Tf



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure 1. Different transferrin genotypes as detected by polyacrylamide gel disc electrophoresis patterns at 8.6 in Iraqi Carp: 1-DG, 2-GG, 3-8-DD, 9-DG,10-DD, 11-FF, 12-13-DD, 14-GG, 15-FF, 16-CD, 17-FF, 18-CD.



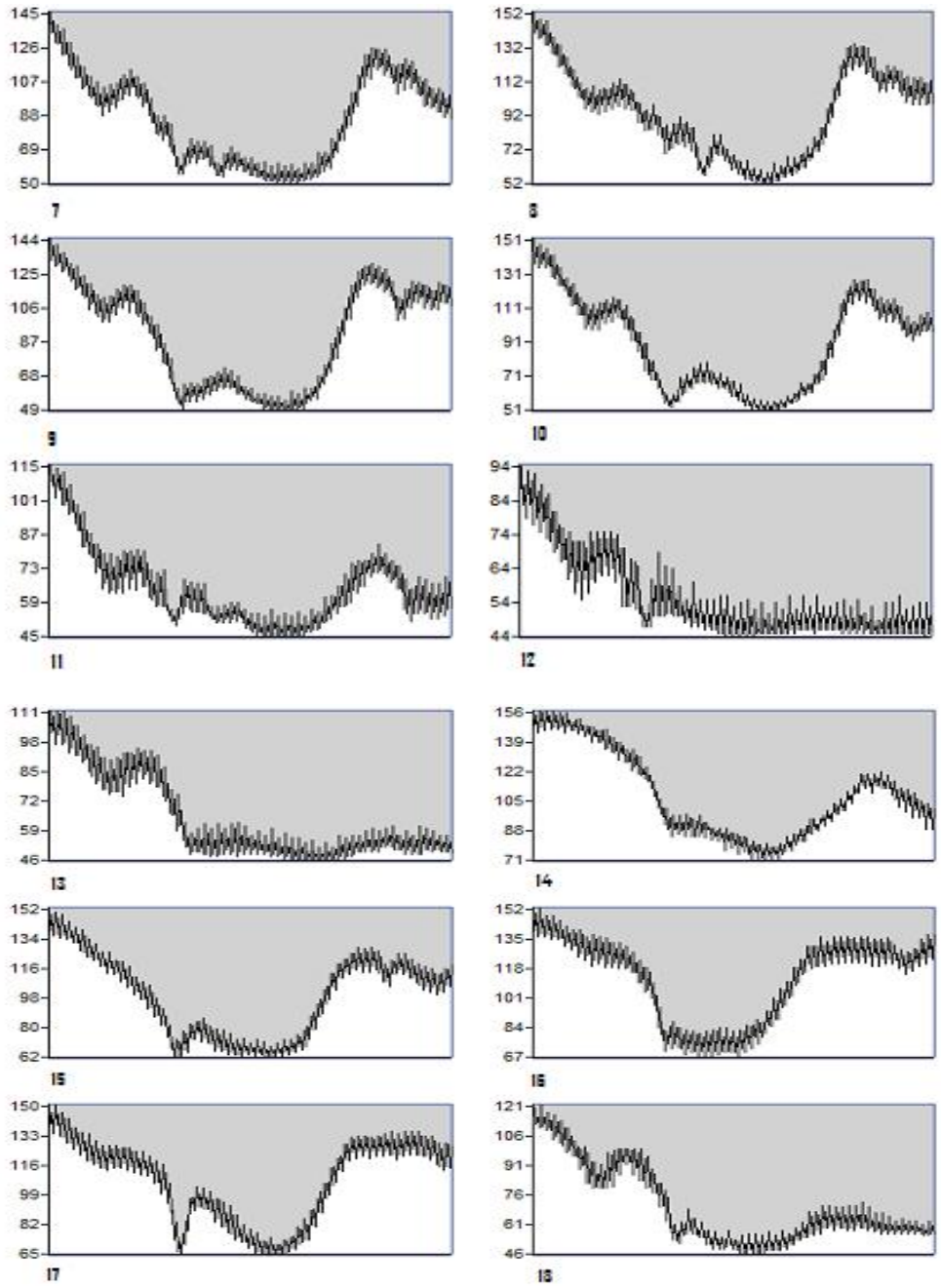


Figure 2. Some of pictures for Fig. no. 1 showed the density of transferrin for lanes no. 1-18 in Iraqi Carp.

Seven genotypes were identified for the transferrin (CC, DD, GG, FF, DC, DG and FD). The genotypes of transferrin alleles obtain in this study are similar to those reporter in *Csizmadia et al. (1995)*. They have found 20 transferrin genotypes (AA, BB, DD, EE, FF, GG, AB, AD, AF, AG, BD, BE, BG, DE, DF, DG, EF, EG, FG, and FH) caused by 7 alleles (A, B, D, E, F, H and G).

Conclusion

As far as we know, this is the first large-scale analysis on the genetic polymorphism in carp (*Cyprinus carpio*). Seven **Tf** genotypes consisting of 4 homozygote types (**CC, DD, FF** and **GG**) and two heterozygote types (CD, DG and FG) were detected

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Ispitivanje biohemijskog polimorfizma šarana (*Cyprinus carpio*): detekcija novih alela u transferinu

T. A. Jaayid, M.Y. Yakoub, J. M. Owaid and N. M. Aziz

Rezime

Ipsitivanje je izvedeno na Univerzitetu u Basri, Poljoprivrednom koledžu i Centru za morska istraživanja. Studija je urađena u cilju ispitivanja postojanja polimorfizma na transferin (**Tf**) lokusu kod šarana (*Cyprinus carpio*). Korišćena je metoda poli-akrilamid-gel elektroforeze (PAGE) u alkalnim uslovima za određivanje **Tf** alela šarana. Analiza **116** životinja je pokazala da su sve životinje bile polimorfne, i pokazivale više genotipova. Postojala je jasna biološka raznolikost (bio-diverzitet) kod **Tf** gena. Sedam **Tf** genotipova koji su se sastojali od 4 tipa homozigota (**CC, DD, FF** i **GG**) i dva tipa heterozigota (CD, DG i FG) je otkriveno. Ove frakcije kontorlišu ko-dominantni autozomni geni prema Mendeljejevom zakonu nasledstva. Najveća učestalost/frekvencija gena je utvrđena za **Tf** D - 0.50, **Tf** F - 0.26, i za C i G - 0.12. Prema tome, asamblaži šarana

(*Cyprinus carpio*) su dosledno pokazivali tendenciju da budu više dominantni na D alelu. Razlike između očekivanih brojeva i brojeva utvrđenih za transferin genotipove nisu bile signifikantne. Ovo je korisno za proces genetskog poboljšanja kroz selekciju. Koliko je poznato, ovo je prva velika analiza genetskog polimorfizma šarana (*Cyprinus carpio*). Poli-akrilamid elektroforeza, metoda koja je primenjena u ovoj studiji, omogućava brz i efikasan skrining prisustva polimorfizma na **Tf**.

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THE CORRELATION BETWEEN HYGIENIC PARAMETERS OF MILK AND WEIGHT LOSS OF SEMIHARD CHEESE

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Abstract: The purpose of the paper was to examine weight loss and correlation between total bacteria count and the somatic cells count and weight loss of semihard naturally dried cheese, product of dairy plant ZZ"Cijevna" in Podgorica. Weigt loss was calculated on the base of difference in mass of cheese at the beginning of ripening and after specified period of ripening, exposed in percents. Examination of weight loss was done on total six product series of cheese during 60 days of ripening on temperature 14,2^{0}C and RH of 89%. Obtained average values for weight loss of cheese were: after 10 days 4.723%, 20 days 8.789% and after 30 days of ripening 11.020%. Weight loss of cheese in period of ripening 10-20 days was 4.266%, in period 20-30 days 2,445% and in period of ripening 30-60 days 5.507%. The total bacteria count in milk was determined on apparatus BactoScan FC 100 and the somatic cells count on apparatus Fossomatic 5000. The middle positive correlation between somatic cells count in milk and weight loss of cheese in period 1-10 days of ripening (0.69156), as well as middle positive correlation (0.767336) between total bacteria count and weight loss of cheese 1-10 days of ripening were determined. The obtained results show that weight loss of cheese was highest in period 1-10 days of ripening and that there is significant influence of hygienic quality of milk on weight loss of cheese and economy of production.

Key words: total bacterial count, somatic cells, cheese, weight loss, yield

Introduction

The weight loss of cheese during the ripening process is called weight loss, and is an important factor affecting the yield of cheese. Weight loss of cheese

arises partly due to mechanical processes during care and ripening of cheese, mostly as a result of continuous evaporative processes that occur between cheese and the air in the ripening room *(Kirin, 2002)*.

Obtaining the optimal amount of cheese, as well as control of the quantity are very important to achieve economically successful production of cheese. Considering the very high share price of raw milk in the total cost production of cheese, even very small variations in the obtained amount of cheese has a significant effect on the realized profit.

Considering that the price of making cheese is 20% of the price of milk and normal profit 10% of the cost of making cheese, reduced quantity of cheese for 1% makes 50% of normal profit *(Emmons, 1991)*. In addition, changes in the quantity of cheese affect its quality, so that without understanding this relationship can not be achieved optimizing the production of cheese *(Walstra, 2000)*. For these reasons knowledge of the factors that influence the amount of cheese is great importance.

Humidity is the most important factor affecting the amount of cheese, which can be controlled during the manufacturing process. During ripening cheeses lose a significant percentage of water by evaporation. Certain percentage of water is converted to a dry matter of cheese due to hydrolysis, usually proteolysis. Each connection interrupted during hydrolysis involves binding of one molecule of water. On the other hand, during ripening creates carbon dioxide and ammonia, which are mostly lost *(Walstra, 2000)*.

The degree of evaporation depends on the relative humidity in the room for ripening, and the temperature of ripening *(Lawrence, 1991)*.

The difference in moisture content between cheeses from different production lines are due to the variation of parameters of production, seasonal changes in the quality of milk, as well as changes in the characteristics of ingredients (extra material). Seasonal variations in the quality of milk affect the degree of acidification, coagulation characteristics and syneresis and thus directly affect the final moisture in cheese. Tests conducted in the Netherlands have shown that the average standard deviation for the percentage of moisture in cheese Chedar was approximately 1.05%, for Gouda cheese 0.8% and 0.95% Edam *(Lacroix et al., 1991)*.

Salting process leads to the absorption of salt, but also to the mass loss of cheese. This is because the water is removed from the cheese during the process of osmosis. The higher the moisture content in cheese, salt diffusion there is faster *(Srbinovska et al., 2001)*. The size of cheese directly affects the quantity of moisture in it *(Bijeljac et al., 2003)*.

The quality of milk to produce cheese, except of the chemical composition, determine its sanitary quality parameters: total number of microorganisms and somatic cells.

The number and types of microorganisms in raw milk and products of their metabolism have an important role in the formation of organoleptic characteristics and quality of finished products *(Kirin, 2001)*.

The somatic cells count in milk is most often due to increased occurrence of mastitis. The primary changes in the quality of milk due to this disease are manifested by reducing the protein content (casein) and / or fat. Active agents who contribute to the reduction of these components are proteases and lipases originating from milk. It also leads to reduction of lactose and calcium content and increasing of sodium, chlorine and serum proteins content.

Somatic cells present in milk during infection are involved in the transformation of plasminogen into plasmin. This enzyme breaks down casein and thus affect the reduction of the amount of cheese. Thus, somatic cells count indirectly indicates the amount of active plasmin in milk. There is a significant reduction in the casein content when the somatic cells count is over 100.000/ml of milk. Besides the impact on the activation of plasmin, many of somatic cells are damaged and release enzymes and antibacterial components in milk *(Barbano, 2000)*.

Considering the importance of weight loss and hygienic quality of milk in cheese production, we investigated the weight loss and impact of hygienic parameters of milk on the weight loss of semihard naturally dried cheese.

Materials and Methods

Weight loss of cheese is calculated by the difference in weight before the beginning of semihard cheese ripening, and after production of cheese and expressed in percentages.

Cheese ripening was carried out in the ripening chamber on wooden planks, without protective layer, in the environment with 89% RH and a temperature of 14.2^0 C for a period of 60 days.

Actual cheese yield is calculated as the weight of cheese obtained from 100L of milk, expressed in percentages. Yield is determined by measuring cheese after removing from the moulds and before salting.

Six of bulk milk samples of cows of six days are tested on total bacteria count and somatic cells count. Samples were transported in laboratory on ice to 1 hour after sampling.

The total bacteria count in milk was determined by the device BactoScan FC 100, and the somatic cells count in the device Fossomatic 5000.

For the obtained results the basic statistical parameters: average (X), maximum (max) and minimum value (min), Sd and the correlation coefficient were determined *(Microsoft Office Excel Programme, 2003)*.

Results and Discussion

Results of measuring the mass of cheese during 30 days of ripening are shown in Table 1. Rresults in Table 1 show that the difference in the mass of cheese in the period 1-10 days of ripening ranged from 0.645 to 3.010kg, 10-20

days from 0.730 to 2.180kg, 20-30 days from 0.395 to 1.205kg and in the period 1-30 days of ripening 1.885 to 6.395kg.

Table 1. The results of measuring cheese mass during first 30 days of ripening

Sign of series	*Number of cheeses*	*Total mass of cheeses during ripening (kg)*				*Difference in mass of cheese at the beginning and on the end of ripening (kg)*			
		1 day	*10th day*	*20th day*	*30th day*	*1-10 days*	*10-20 days*	*20-30 days*	*1-30 days*
6	30	53.055	50.045	47.865	46.660	3.010	2.180	1.205	6.395
7	12	20.180	19. 535	18.640	18.150	0.645	0.895	0.490	2.030
8	12	20.365	19.700	18.795	18.365	0.665	0.905	0.430	2.000
9	12	20.415	19.455	18.670	18.180	0.960	0.785	0.490	2.235
10	12	19.440	18.680	17.950	17.555	0.760	0.730	0.395	1.885
11	12	20.345	18.800	18.025	17.590	1.545	0.775	0.435	2.755
X		25.633	25.336	25.720	25.089	1.264	1.045	0.574	2.883
Max		53.055	50.045	47.865	46.66	3.010	2.180	1.205	6.395
Min		19.440	18.680	17.950	17.555	0.645	0.730	0.395	1.885
Sd		13.4387	13.8194	12.0277	11.7181	0.9182	0.5603	0.3112	1.7477

The results of examination of actual cheese yield are shown in Table 2.

Table 2. The results of examination of actual cheese yield

Sign of cheese	Quantity of milk (L)	Mass of cheese (kg)	Actual yield of cheese after pressing (%)
6	482	55.485	11.51
7	401	43.935	10.95
8	389	44.360	11.40
9	382	44.905	11.75
10	371	41.095	11.07
11	461	50.200	10.88
X	414,3	46.66	11.26
max	482	55.485	11.51
min	371	41.095	10.88
Sd	45.8243	5.2378	0.2786

The results in Table 2 show that for average quantity of milk of 414,3 l and mass of cheese 46,66 kg, actual yield of cheese amounted 11,26%.
Results of examination weight loss of cheese in period 1-30 days are shown in Table 3.

Table 3. The results examination weight loss of cheese first 30 days

Sign of series	Weight loss of cheese (%)				
	1- 10 days of ripening	10-20 days of ripening	20-30 days of ripening	1-20 days of ripening	1-30 days of ripening
6	5.673	4.356	2.517	9.782	12.054
7	3.196	4.581	2.628	7.631	10.060
8	3.265	4.594	2.287	7.709	9.821
9	4.702	4.035	2.624	8.547	10.948
10	3.909	3.907	2.200	7.664	9.697
11	7.594	4.122	2.413	11.403	13.541
X	4.723	4.266	2.445	8.789	11.020
Max	7.594	4.594	2.628	11.403	13.541
Min	3.196	3.907	2.2	7.631	9.697
Sd	1.6891	0.2890	0.1770	1.5264	1.5191

The results in Table 3 show that the average values for weight loss of semihard cheese amounted to: for the period of ripening 1-10 days 4.723%, 1-20 days 8.789% and 1-30 days 11. 020 %. The average value of weight loss of cheese in the period 10-20 days of ripening amounted to 4.266% and in the period 20-30 days of ripening 2.445%.

Results of examination weight loss of cheese in the period 30-60 days of ripening are shown in Table 4.

Table 4: The results of examination weight loss of cheese during 30-60 days of ripening

Sign of series	Number of cheeses	Total mass of cheeses during ripening			Weight loss of cheese during 30-60 days ripening
		After 30 days	After 60 days	Difference in mass of cheese during 30-60 days	
6	11	16.340	15.490	0.850	5.201
7	11	16.730	15.765	0.965	5.768
8	11	16.930	16.010	0.920	5.434
9	11	16.820	15.910	0.910	5.411
10	11	16.200	15.190	1.010	6.234
11	11	16.310	15.495	0.815	4.997
X		16.555	15.643	0.912	5.507
Max		16.930	16.010	1.010	6.234
Min		16.200	15.190	0.815	4.997
Sd		0.3078	0.3070	0.0717	0.4393

The results shown in Table 4 show that weight loss of cheese in ripening period 30-60 days ranged from 4.997% to 6.234%, while the average value for weight loss of investigated cheese amounted to 5.507%.

The results of examination of bulk milk samples of cows on total bacteria count and somatic cells count are shown in Table 5.

Table 5. The results of examination of bulk milk samples on total bacteria count and somatic cells count

Sign of milk sample	Total bacterial count x 1000	Total somatic cells count x 1000
6	1156	503
7	2105	444
8	651	552
9	410	552
10	893	489
11	6727	614
X	1990	525
Max	6727	614
Min	410	444
Sd	2393.3520	59.5270

The results in Table 5 show that the average value of total bacteria count amounted to 1.990.000/ml and the somatic cells count 525.000/ml milk.

Results of the correlation between of hygienic parameters of milk and weight loss of cheese are shown in Table 6.

The results in Table 6 show that middle positive correlation was found between of the somatic cells count in milk and weight loss of cheese in period 1-10 days of ripening (0.69156) and middle positive correlation between the total bacteria count in milk and weight loss of cheese in period 1-10 days of ripening (0.767336).

Table 6. Correlation between hygienic parameters of milk and weight loss of cheese and correlation between somatic cells count and actual yield of cheese

Correlation between:	Intensity of correlation	Values for coefficient of correlation
Somatic cells count in milk and weight loss of cheese in period 1- 10 days of ripening	Medium positive	0.69156
Somatic cells count in milk and weight loss of cheese in period 10-20 days of ripening	Low negative	-0.28784
Somatic cells count in milk and weight loss of cheese in period 20- 30 days of ripening	Low negative	-0.18725
Somatic cells count in milk and weight loss of cheese in period 1- 30 days of ripening	Medium positive	0.64732
Somatic cells count in milk and weight loss of cheese in period 30- 60 days of ripening	Medium negative	-0.69243
Total bacteria count and weight loss of cheese in period 1-10 days of ripening	Medium positive	0.76733
Total bacteria count and weight loss of cheese in period 10-20 days of ripening	Low negative	-0.11601
Total bacteria count and weight loss of cheese in period 20-30 days of ripening	Low positive	0.00326
Total bacteria count and weight loss of cheese in period 1-30 days of ripening	Medium positive	0.777787
Total bacteria count and weight loss of cheese in period 1-30 days of ripening	Medium negative	- 0.50956
Between of somatic cells count and actual yield of cheese	Low positive	0.09359

The amount of the obtained cheese depends on a number of factors, such as the composition of raw milk and its hygienic quality, heat treatment and standardization of milk, method of curd processing and conditions of cheese storage.

Kirin (2002) states that the weight loss of trappist cheese after ripening of 30 days is the highest on the traditional way of ripening, during which the cheese is just washed and turned on the shelves (8.974%). The same author in the period 1-10 days of ripening of cheese found weight loss 4.174%, 10-20 days 2.260%, in period 20-30 days of ripening 2.539% and that weight loss after 10 days of cheese ripening was uniform.

In our research we also found the maximum weight loss of cheese in period 1-10 days of ripening (4.723%). However, weight loss of cheese in the period of 10-20 days of ripening was also high and amounted to 4.266%, while in the period of 20-30 days of ripening amounted to 2.445%, which is in agreement with the results received by *Kirin (2002*). Weight loss of cheese investigated in the period 1-20 days of ripening amounted to 8.789%, and in the period 1-30 days of

ripening 11.020%. For the total period of ripening 30-60 days average value for weight loss of cheese amounted to 5.507% (Tables 3 and 4).

Compared to the traditional ripening, *Kirin (2002)* found slightly lower weight loss during ripening of cheese covered by protective grease (7.694%), and lowest weight loss during the ripening of cheese in a plastic bag (0.176%). The author further found that the weight loss of cheese covered with protective grease was also highest in the first 10 days of ripening.

The highest possibility of influence on the weight of cheese is in the first stage of ripening (*Kirin, 2002*). However, our results show that period of ripening 10-20 days is also important for weight loss of cheese.

In our research we obtained the middle positive correlation between of somatic cells count in milk and weight loss of cheese in period 1-10 days of ripening (0.69156) and middle positive correlation (0.767336) between the total bacteria count and weight loss of cheese in period 1-10 days of ripening (Table 6.) These results can be attributed to the negative influence of somatic cells and the total bacteria count on the quality of milk, and thus the composition and properties of the produced cheese. Changes in the composition of milk, which are correlated with an increase of the somatic cells count in milk, significantly affect the coagulation time, curd firmness, increased activity of bacteria, changed the taste of the finished product and reduced yield of cheese *(Niketić et al., 2003)*.

Effect of somatic cells count and the total bacteria count on weight loss of cheese can best be shown through the example of the results for weight loss of cheese of production line number 11, which was significantly higher (7.594% in the period 1-10 days, or 13.541% in the period 1-30 days of ripening) compared to the value of weight loss of cheese other production lines (Table 3). Milk for production of this cheese had the highest somatic cells count (614.000/mL milk) and the highest total bacteria count (6.727.000/mL).

Changes in chemical composition and physical properties of milk caused by mastitis cause the appearance of prolonged coagulation time and a lower hardness of curd. Low hardness of curd after cutting leads to loss of small curd particules. Decomposition of milk casein also affects the process of syneresis during making of cheese and thus result in increased moisture content (*Barbano, 2000*).

Somatic cells count in milk positively related to moisture in nonfat substances of cheese. Curd moisture increased with increasing SCC in milk (Politis and Ng-Kwai-Hang, 1988a).

No significant trend between of somatic cells count and actual yield of cheese. In the case of actual yield, the high moisture content masked the results *(Politis and Ng-Kwai-Hang, 1988b)*. Our results showed that there is not correlation between somatic cells count and actual yield of cheese, too (Table 6).

This facts affects the increased weight loss of cheese produced from milk with an increased somatic cells count. These cheeses at the beginning of ripening contain a higher percentage of moisture in nonfat substances, and therefore will have a greater weight loss during ripening. In addition, leucocytes contain antibacterial components that inhibit the growth of starter cultures during cheese making, which has a significant influence on the properties of curd or cheese.

Microorganisms, their enzymes and other products affect the technological properties of milk *(Oljačić and Kasalica, 2006)*. The most numerous in cooled milk are the psychrotroph bacteria with the number above 10^5/mL of milk which affect the loss of weight cheese (*Kasalica et al., 2005).* These microorganisms by enzymes break down milk proteins, which also resulted in weaker syneresis of curd, a higher content of moisture in cheese, and therefore more weight loss of cheese. Milk intended for cheese production must not contain more than 10^4-10^6/mL psychrotroph microorganisms.

We should have in mind the fact that the milk for cheese production in the manufacturing line number 11 represented a mixture of cooled milk from the previous day and fresh morning milk. Given that households do not have the conditions for cooling milk on the appropriate temperature (lower than 4^0C), during storage of milk a rapid multiplication primarily of psychrotroph microorganisms occures, leading to degradation of milk components by their enzymes. Growth rate of these bacteria is very weak at temperatures up to 4^0C, but progressively increases at temperatures of 5-10^0C, especially at temperatures above 6^0C. Psychrotroph bacteria generation time, depending on temperature, ranges from several hours to about 15 minutes. If mixed fresh milk with milk that is stored 24 hours or longer, and then continued saving, psychrotroph bacteria count increases more rapidly than when stored only fresh raw milk *(Robinson, 2002)*. You should also have in mind that on the temperature of milk storage active proteolytic enzymes are released from damaged somatic cells. By these facts significantly lower weight loss of cheese produced in the production line number 11 can be explained.

The quality of milk is further aggravated if the time of saving cooled milk is longer, if milk comes from cows that were in the late stage of lactation, or from older cows or cows that have suffered mastitis several times. Rapid cooling of milk allows the preservation of enzymes that damage proteins and fats of milk *(Barbano, 2000)*.

The significant influence of the manner and length of storage-cooling of milk on weight loss can be shown through the example of the results for weight loss of cheese from production line number 6. This cheese is also produced from mixture of the cooled milk from the previous day and fresh morning milk. Weight loss of cheese in the period 1-10 days of ripening amounted to 5.673%, in the

period 1-30 days 12.054%, which were also significantly higher value compared to weight loss of cheeses produced only from fresh milk (Table 3).

Milk from which this cheese is produced had a total bacteria count of 1.156.000/mL and somatic cells count 503.000/mL, which are significantly less than the value of milk used for cheese production line number 11 (Table 5).
The results indicate a significant influence of the hygienic quality of milk on weight loss of cheese and therefore on the cost of production.

Conclusion

Weight loss of semihard cheese in period of ripening 1-10 days was 4.723%, 1-20 days 8.789% and during period of ripening 1-30 days 11.020%. In the ripening period of 10-20 days weight loss of cheese amounted to 4.266% and in the period of ripening 20-30 days 2.445%.

We found a middle positive correlation (0.69156) between the somatic cells count in milk and weight loss of cheese in period 1-10 days of ripening and the middle positive correlation (0.767336) between the total bacteria count in milk and weight loss of cheese between 1-10 days of ripening.

Somatic cells and the total bacteria count in milk have a significant impact on weight loss of cheese and therefore on the cost of production.

Korelacija između higijenskih parametara mleka i kala polutvrdog sira

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Rezime

Cilj rada je bio da se ispita kalo i korelacija između ukupnog broja bakterija i broja somatskih ćelija i kala polutvrdog prirodno sušenog sira, proizvoda sirare ZZ „Cijevna“ u Podgorici.

Kalo sira je izračunat na osnovu razlike u masi sira pre početka zrenja i nakon određenog perioda zrenja i izražen u procentima. Ispitivanje kala je vršeno na ukupno šest proizvodnih serija sira u toku 60 dana zrenja na temperaturi od $14,2^0$C i vlažnosti vazduha od 89%.

Dobijene srednje vrednosti za kalo sira su iznosile: nakon 10 dana zrenja 4,723%, 20 dana 8,789% i nakon 30 dana zrenja 11,020%. Kalo sira u periodu 10-20 dana zrenja je iznosio 4,266%, u periodu od 20-30 dana 2,445% i u periodu 30-60 dana zrenja 5,507%.

Ukupan broj bakterija u mleku je određivan na aparatu BactoScan FC 100, a broj somatskih ćelija na aparatu Fossomatic 5000.

Utvrđena je srednja pozitivna korelacija između broja somatskih ćelija u mleku i kala sira u periodu 1-10 dana zrenja (0,69156) i srednja pozitivna korelacija između ukupnog broja bakterija u mleku i kala sira u periodu 1-10 zrenja (0,767336).

Dobijeni rezultati su pokazali da je najveći kalo sira bio u prvih deset dana zrenja i da postoji značajan uticaj higijenskog kvaliteta mleka na kalo sira, a samim tim i na ekonomičnost proizvodnje.

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PHYSIOLOGICAL ASPECTS OF BEHAVIOUR OF SOWS AND PIGLETS DURING THE LACTATION PERIOD

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Review paper

Abstract: Maternal ability represents complex interactions between different forms of behaviour and physiological characteristics. Behaviour and metabolic processes are partly under the control of endocrine and nervous systems. Sows are different from other mammals, in that sows bear a large number mature baby, capable immediately to suckling. Post partum anabolic processes become catabolic, giving priority to dairy gland in relation to other tissues. Maximum secretated milk takes place betwen 2. and 3. week. Sows lost in body mass and it is appeared the change in value of some bloods parameters. Haemotologic analyses have suggested the increase in total number of leukocytes and neutrophilic granulocytes and the decrease in the number of lymphocytes, monocytes and eosinophils on the first day postpartum. In the third and fourth week of lactation a statistically significant fall in the number of leukocytes and granulocytes in relation to the first day was observed, along with a significant increase in limphocytes, monocytes and eosinophils. Erythrocyte count, haemoglobin concentration and haematocrit value decreased during the lactation period. Level of glucose in plasma is low (it is decreasing for more than 50%), while the level of urea is high, due to huge proteins available. It can also be noticed that there is a high mobilisation of body fat an increasing level of NEFA (non-esterified fatty acid). Secretion of milk is under control prolactin and oxytocin. Oxytocin affects on maternal behaviour and prolactin is linked with timing of suck.

Key words: behaviour, sow, piglet, lactation.

Introduction

Hormonal changes, prior and post parturition do impel the sow to build a nest. It is one of the main characteristics of sow behaviour started by an individual 24h or 15h prior parturition at the latest *(Damm et al., 2003)*, and is the result of increased secretion of prostoglandin F_{2alfa} *(Burne at al., 2001)*. Enclosed housing

systems hinder this need what is frustrating to sows, especially in the phase when pregnant sows are being removed into buildings in which they are kept individually in impacted boxes *(Vučinić and Hristov, 2002; Hötzel et al., 2005)*. In order to avoid these stress situations, a week prior parturition it is necessary to supply sow with suitable material for nest building-up *(Stanković et al., 2007)*. The use of jammed boxes in farrowing houses represents the problem discussed for many years to the purpose to protect the welfare of both sows and piglets *(Hristov et al., 2001)*.

The increased level of cortisol can indicate frustrations when the sows deprive themselves of natural behaviour *(Mason at al., 2001; Thodberg et al., 2002)*. Numerous physiological and biochemical studies have been used for the estimation of welfare and they are mostly concerned with the determination of adrenal gland and hypophysis hormones concentration, especially glucocorticosteroids (GC) in blood *(Hristov and Bešlin, 1991; Hristov et al., 2008)*. Increased levels of cortisol, insulin and thyroid hormones indicate the presence of stress *(Joksimović Todorović et al., 2007)*. Changes occurring in the hypothalamus-hypophysis-adrenal cortex system or vegetative nervous system are not reflected in the form of some psychological reaction but they are reflected on the homeostatic metabolic processes and animal immunity system *(Joksimović Todorović et al., 2008)*.

It is necessary to bring the sow into a certain condition by the farrowing term. Diets must be of good quality, protected against contamination and development of mycotoxins *(Jokić et al., 2003; Joksimović Todorović et al., 2004; Petrović et al., 2007)*. After parturition the organism of individual passes from anabolic to catabolic state when sows lose their body mass and the concentrations of some hormones, concentration of glucose, urea and non-esterificated fatty acids (NEFA) in blood changes *(Algers and Uvnäs-Moberg, 2007)*. The most intensive catabolic processes in sows occur in the 3rd and 4th lactation week when piglets reach the plateau in their daily liveweight gain.

Metabolic changes in sows from prepartal period to weaning

Maternal ability represents complex interactions between different forms of behaviour and physiological characteristics. Factors that influence sow maternal behaviour are: litter size, vitality, health and regime of nutrition. Sows differ from other domestic mammals because they bring forth a large number of mature youngs capable for immediate suckling. Suckling is initiated by sow by calling the piglets making some grunting sounds while piglets try to stimulate sow` s teats for milk ejection by tactile teasing *(Vučinić and Radenković-Damjanović, 2000)*. During gestation mother`s body reserves are increasing in order to provide milk production after parturition. After parturition the organism passes from anabolic into catabolic

state so that mammary gland gains priority in relation to other tissues *(Špinka et al., 2002)*. Level of catabolic processes during lactation depends on nutritive value and quantity of milk secreted and it is most intensive between 2nd and 3rd lactation week. Sows lose their body mass and there also occurs the change in the concentration of some blood plasma ingredients (lower activity of lipoproteinlipases and the rate of fatty acids synthesis) even when sows are fed ad libitum. Diet intake cannot compensate completely the needs for milk production and energy balance is usually negative. High demands for energy lead to abundant catabolic processes especially in the third week *(Kraetzl et al., 1998)*.

During lactation the level of glucose in plasma is low since about 50% glucose is used by mammary gland for milk production. Level of glucose in blood may be increased in the first week of lactation but after that it significantly decreases in the third and fourth week. It is possible that glucose is not directly used from body reserves for milk production since pigs obtain high levels of starch by nutrition. High levels of urea indicate the use of high levels of muscular proteins although this assertion is inconsistent and is not in direct relationship with loss of body mass. Unsaturated fatty acids as a product of fats metabolism are reliable sign of catabolic state and only small quantities come from food. Level of NEFA increases at the end of gestation and it is the highest in the middle and at the end of lactation. Their concentration is used mostly as a measure of energy status post – parturition and catabolism of fats in sows *(Hulten et al., 1993)*.

During the lactation period, there occurs in sows a significant change in the values of haematologic parameters. On the day of parturition (1-6h after parturition) was found leukocytosis, neutrophilia and lymphopenia in sows *(Thorn, 2000; Damgaard et al., 2009; Joksimovic Todorovići et al., 2010a)*. In the third and fourth week of lactation a statistically significant fall in the number of leukocytes and granulocytes in relation to the first day was observed, along with a significant increase in limphocytes, monocytes and eosinophils *(Joksimovic Todorovići et al., 2010a)*. Research conducted by *Dungan et al. (1995), Thorn (2000), Žvorc et al. (2006)* suggest that in sows during lactation period reduces the number of red blood cells, haemoglobin and haematocrit value, which is consistent with studies *Joksimovic Todorovići et al. (2010b)*.

Sow endocrine system

Maternal behaviour and metabolic processes are partly under the control of endocrine system. Building-up of nest for farrowing is induced by the increase of the concentration of prolactin, while the increase of oxitocyne is perceived at the end of nest building. Maintaining of lactation depends on the level of prolactin, while the oxitocin is esential for the reflex of secreting milk. Concentration of oxitocin increases abruptly during suckling, reaches peak levels during milk

ejection when the stimulation of teats by piglets is sufficient. After milk ejection the oxitocin returns to its basic level.

Prolactin is the most important hormone which promotes and maintains lactation. Level of prolactin increases in the last week of gestation and attains maximal concentration immediately prior parturition and in the first two weeks of lactation *(Kraetzl et al., 1998)*. Secretion of prolactin is stimulated by teats massage, its concentration increases gradually, not abruptly as is the case with the level of oxitocin, and greatest confirmed level is 10-20 min after suckling. During lactation the level of prolactin decreases as a consequence of reduced teats massage. This has a positive effect on the number of insuline receptors in mammary gland, and it is thought that it plays role in determination of metabolic functions, especially in arranging sources for milk production.

Insulin is a promoter of glucose and aminoacids utilization from peripheral body tissues by reduced use of nutritive matters for milk production. The insuline reduction during maximal lactation is a physiological response to abundant use of glucose for milk production. Levels of insuline are higher in sows that have lost less body mass during lactation so that catabolic processes are of less intensity *(Kraetzl et al., 1998)*. Insulin directly effects milk production because after parturition the number of insuline receptors in fat tissue decreases while their number in mammary complex increases considerably. This leads to reduced lipogenesis in fat tissue, but increases the sensitivity of mammary tissue against insuline in spite of its reduced concentration in plasma.

Mortality in piglets

Mortality in piglets is ethicaly and economicaly unacceptable in modern pig production. Research must be focused towards reducing this problem. Mortality in piglets is directly or indirectly associated with maternal abilities: piglets starvation, crushing of piglets and other factors to which they are exposed during lactation period. Farrowing in jammed housing system has decreased the mortality of piglets but on the other hand by this system a welfare of sows is influenced in a negative way. Building of nest and natural parturition are impossible in this housing system because of which situation the sows undergo stress situation (level of cortisol and adenocorticotropine hormone is increased) *(Broom and Johnson, 1993)*. Impacted housing system also has negative consequences on the health of pigs (incidence of MMA syndrome) and difficulties during farrowing (prolonged farrowing especially in gilts) *(Hristov et al., 2008)*.

Mortality in piglets differs depending on housing system. Expressed in percentages it ranges from 10 and 20% (perinatal mortality, starvation and crushing). Hungry piglets stay longer on teats trying to stimulate milk secretion. These piglets are more easily crushed by mother or they die of starvation and/or

trauma caused by mother. Starvation is a cause of piglets death in about 50% cases being the greatest on the first day after farrowing. Approximately about 75% total mortality occurs in the first week of lactation. Physical factors such as suffocating in the course of parturition, trauma, starvation and septicaemia are the most often causes in the first four days of the life of piglets. Viral and bacterial infections are the causes of piglets mortality in later period of lactation.

Behaviour of piglets during nursing

Social hierarchy is being established by piglets immediately after their birth. The first pair of teats which produce more milk than other teats are sucked by the dominant piglets. They save their places during suckling and behave agressively towards piglets who try to take over their places. Suckling includes several phases. First the sow makes grunting sounds by which it calls piglets to come and then they arrange themselves in a certain way. In the beginning the piglets squeeze teats by their mouths, hit them by head, and then hold them tightly, drawing them into their mouths and making vacuum. In this way milk reaches the cistern of mammary complex and piglets are starting to suckle very rapidly *(Špinka et al., 2002)*. At the expiration of one hour after previous suckling piglets wake up and initiate suckling again. The sow lies down on its sides allowing in this way hungry piglets to suckle again. In order that suckling should be successfull the piglets indicate to sow by their behaviour that they all are present and ready for suckling *(Vučinić, 2006)*.

Not every breast-feeding is successful. Unsuccessful breast-feeding is recognized by more intensive sow grunting during the entire time of breast-feeding. It is caused by non-releasing of oxitocin in which case the milk may not reach mammary cistern. Permanent and intensive sow grunting may indicate the presence of pain in mammary gland or that the release of oxitocin was omitted. In these cases the sow does not allow the access to piglets or she lies down on its abdominal side. By lying on the stomach the sow is cooling its mammary complex in case the acute inflammation has occurred there.

Conclusion

Behaviour of animals depends on: cognitive, neurophysiological, motor, motivation components and subjective feelings. In order that sows could express their natural behaviour it is necessary to provide them with quality diet in sufficient quantities, suitable space and protection against infective and non-infective diseases. If the environment is less restrictive the sows can express their natural

behaviour but if maternal ability is impaired it cannot be compensated by subsequent improvement of environment.

Fiziološki aspekti ponašanja krmača i prasadi u toku laktacionog perioda

M. Joksimović Todorović, V. Davidović, B. Živković

Rezime

Materinska sposobnost predstavlja kompleksne interakcije između različitih oblika ponašanja i fizioloških karakteristika. Ponašanje i metabolički procesi su delimično pod kontrolom endokrinog i nervnog sistema. Krmače se razlikuju od drugih sisara po tome što rađaju veliki broj zrelih mladih sposobnih da odmah sisaju. Nakon partusa anabolički procesi prelaze u kataboličke, dajući tako prioritet mlečnoj žlezdi u odnosu na druga tkiva. Maksimalna sekrecija mleka odvija se između 2. i 3. nedelje. Krmače gube telesnu masu i dolazi do promena vrednosti pojedinih parametara u krvi. Hematološke analize su ukazale na povećanje ukupnog broja leukocita i neutrofilnih granulocita i smanjenje broja limfocita, monocita i eozinofila prvog dana po partusu. Treće i četvrte nedelje laktacije, došlo je do statistički značajnog pada broja leukocita i granulocita u odnosu na prvi dan, a značajnog porasta limfocita, monocita i eozinofila. Broj eritrocita, koncentracija hemoglobina i vrednost hematokrita smanjuju se tokom laktacionog perida. Nivo glukoze u plazmi je veoma nizak (niži je za preko 50%), dok je nivo ureje visok usled obimnog iskorišćavanja proteina. Takođe je ustanovljeno da značajna mobilizacija telesnih masti dovodi do povećanja nivoa NEFA (nezasićenih masnih kiselina). Sekrecija mleka je pod kontrolom prolaktina i oksitocina. Oksitocin utiče na materinsko ponašanje a prolaktin je u korelaciji sa dužinom sisanja.

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THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

M. D. Petrović[1], Z. Skalicki[2], V. Bogdanović[2], M. M. Petrović[3]

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5.-7. октобар 2011. године, Београд, Република Србија

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ДРУГО ОБАВЕШТЕЊЕ

Трећи међународни конгрес о сточарству **“Нове перспективе и изазови одрживог сточарства ”** одржаће се од 5. до 7. октобра 2011. године у хотелу Парк, у Београду, Србија, у организацији Института за сточарство, Београд-Земун.

Радове, припремљене за штампу према упутству часописа *Biotechnology in Animal Husbandry* (www.istocar.bg.ac.rs), послати до **30. јуна 2011.** године. (Радови који нису припремљени према упутству, биће враћени на измену или неће бити разматрани). Аутори би требало да наведу начин презентације рада, усмено предавање или постер.

Радове послати на енглеском језику на CD-у, на е-маил адресу:
biotechnology.izs@gmail.com

или поштом на адресу**:**

Институт за сточарство,
Аутопут 16, П. фах 23,
Земун-Београд 11080,
Србија,

За секцију постера прилози се припремају у величини 60 x 90 cm (ширина x висина).

Службени језик
Службени језик конгреса је **српски и енглески**. Сва усмена излагања се симултано преводе.

Котизација

Котизацију уплаћују сви учесници конгреса изузев учесника са Пленарним предавањем

- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал конгреса, учествовање у свим секцијама конгреса, коктел, кафу/чај у паузама, износи **100 EUR** (за домаће учеснике у динарима на дан уплате по важећем средњем курсу). Ниједан рад неће бити штампан без уплаћене котизације.
- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал конгреса, учествовање у свим секцијама конгреса, коктел, кафу/чај у паузама, туристички програм и свечану вечеру, износи **150 EUR**.

  Рок за уплату котизација је **31. јул 2011.** године.

Уколико Вам је потребна профактура за уплату котизације можете нам се обратити телефоном, централа 011 2670 121, локали 220, 203 или на е-маил адресу: biotechnology.izs@gmail.com

Међународни научни комитет конгреса ће након пријема радова извршити две рецензије по раду. Рецензирани и прихваћени радови за штампу биће штампани у целости на енглеском језику у часопису *Biotechnology in Animal Husbandry*.

Уплату котизације потребно је у наведеном року извршити на рачун:

Институт за сточарство, Београд-Земун
11080 Земун, Аутопут 16

Текући рачун бр. 205-65958-94

Комерцијална банка

Место одржавања конгреса и смештај

Конгрес ће се одржати у хотелу Парк, Његошева 2, 11000, Београд, Србија

- За учеснике конгреса резервација смештаја до 31. августа 2011. године је по знатно нижим ценама:

Једнокреветна соба (ноћење са доручком) **37 EUR**
Двокреветна соба (ноћење са доручком) **60 EUR**

Цена боравишне таксе није укључена у наведене цене.
Рок за резервацију смештаја по нижим ценама је **31. август 2011**. године

Адреса: Хотел Парк, Његошева 2, 11000 Београд
Тел. 011/3640-385; 011/3640-393
Фах. 011/3640-393

Контакт особа: Данијела Петровић, емаил: hotelpark.recepcija@bvcom.net

Председник
Организационог одбора

Др Милош Лукић, научни сарадник
Србија

Председник
Међународног научног комитета

Проф. др Martin Wahner
Немачка