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ECOLOGICAL TRENDS AT ANIMAL HUSBANDRY NITROGEN UTILIZATION

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Original scientific paper

Abstract: The aim of current work was a part of study for animal husbandry effects on emissions of greenhouse gases and some mitigation strategies between the end of XX and the beginning of XXI century. It's emphasized on nitrogen (N) balance and its fluctuated values, as well as brings forward attendant factors. As a result, we deducted strong correlation models (R^2> 0.89, 0.85, 0.99), as an estimator of the N_2O emissions ($Gg.CO_2^{-eq}$), generated by manure management in relation to animal population (monogastric, ruminant, total) among the investigated middle-term periods throughout 1989 – 2011 y for the Bulgarian realities.

Key words: agro-ecological, strategies, microclimate, ammonia, manure, farming, balance, animal, population

Introduction

The microclimate pooled physical (temperature, humidity, air flow), chemical (toxic gases) and biological (bacteria, viruses, fungi) factors. It's influenced animal health status, e.g. animal performance and could be assumed as an important livestock stressor (*Morgan and Tromborg, 2006*). The productive systems and technologies determined limiting factors as breeding and nutrition strategies, environmental conditions, production need, etc. to be taken into account. In this regards must be promoted the following role „*hygiene = health = efficiency = profitability*".

Thereby, the common air gases pollutants are ammonia, carbon dioxide, hydrosulphide and methane. The atmospheric ammonia concentrations developed animal response in terms of health problems and reduced performance. Thus, we emphasized on a number of worldwide and local mitigation strategies (genetic, nutritional, herd, technological, etc.) and some ecological aspects of ammonia.

The ammonia is a strongly alkaline, colourless, soluble in water and with irritant odour gas. Its molecular weight (17.03), absolute (0.771) and relative to air (0.5967 $g.l^{-1}$) density under pressure liquidified at ammonium hydroxide. The main

concentrations of atmospheric ammonia are generated from animal manure as excreted fecal protein-N and urinary urea-N. These amounts are bio-transformed by bacterial urease enzymes at high temperature (49 ^{o}C) and alkaline optimum (7.7 – 8.0 units).

The amounts generated by manure and the rate and extent parameters depends on the equilibrium in the liquid – gas phase as follow (Eq. 1):

$$NH_4^+ \longrightarrow NH_3 + H^+ \quad (1)$$

The air ammonia emissions could be calculated by different exemplified data models (as software Package *STANK, 1999; HadCM3, 2007* etc.) for ammonia losses from livestock manure (fig. 1), but can depict the situation and Bulgarian

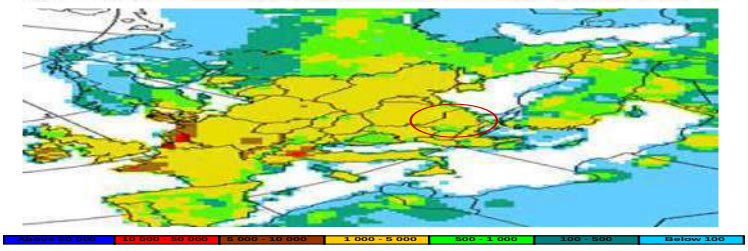


Figure 1. Ammonia emissions (50 km x 50 km EMEP grip, 1997)

place also in regards to ammonia losses among livestock species distributed as follow percentage ranges:

- cattle – 68 %;
- pigs – 15%;
- other – 17 %.

The manure management could be used as a beneficial tool for a sustainable farming system with environmental-friendly practices (*Van Passel et al., 2007*) maintaining the European Common Agricultural Policy. As a support of this, the manure ammonia losses from different livestock species and categories within the barn, we could depicted the situation with, as a percent of total manure N content, summarized on following graph – fig. 2:

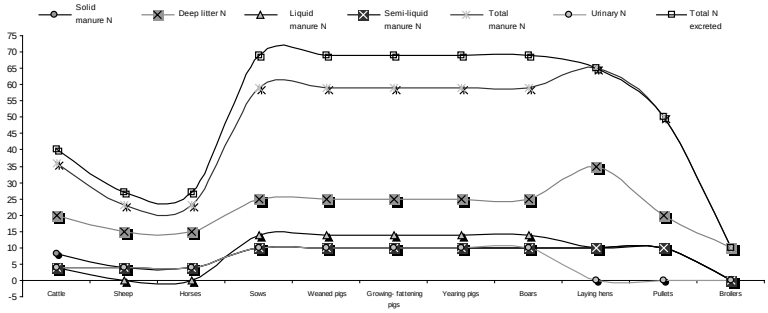


Figure 2. Different forms of manure ammonia losses from different livestock species and categories within the barn (% of total nitrogen content of the manure, STANK 1999)

The diets, provided for productive animals are formulated to maintain higher productivity based on economic limits and ecological restrictions. Likewise, the dietary protein inputs affected total tract protein digestibility and modified the ratio fecal-N/urinary-Noutput (N_f/N_e) (*Accioly et al., 2002; Yossifov and Kozelov, 2011; Yossifov and Kozelov, 2011a; Yossifov, 2014a*). An admitted pollutant values for ammonia are summarized in table below (*Bulgarian Regulation N44, 2014*):

Table 1. Optimal microclimate standards in animal vitality zone - ammonia*

Species / NH_3	(mg/m^3)	(ppm)
Ruminants		
Cattle	up to 20	up to 28.7
Buffalo	up to 20	up to 28.7
Small ruminants		
Lactating	up to 10	up to 14.4
Suckling	up to 10	up to 14.4
Fattening	up to 10	up to 14.4
Yearling	up to 10	up to 14.4
Monogastric		
Pigs	up to 5	up to 7.18
Birds		
Turkey	up to 15	up to 21.5
Goose	up to 15	up to 21.5

* Bulgarian Regulation N44-20/04/2006 (2014)

As a result, the surpass air ammonia values affected animal welfare and animal response (*CIGR, 1984*). In this regards, we aimed to investigate the animal husbandry effects on emissions of N-related greenhouse gases. It's emphasized on nitrogen (N) balance, agro-ecological fluctuates and mitigation strategies, as well

as brings forward attendant factors for the middle-term period throughout 1989 – 2011. Also, we underlined on a number of worldwide and local mitigation strategies (genetic, nutritional, herd, technological, etc.) and some ecological aspects of ammonia.

Materials and Methods

We conducted our study based on following items, contributed to the ammonia losses, associated with livestock farming management:

2. Dietary protein supply – content, subfractions, etc.;
3. Species, categories, individuals, etc.;
4. Farm building management;
5. Manure management – content, storage, conditions, etc.;
6. Manure N content – fractions, spreading, etc.

All obtained data were equalized by *NISTC (2014).* The values were interpreted and correlated by Statistical Package of *MS, 2007*.

Results and Discussion

The flows and cycling of biogenic nutrients – i.e. nitrogen (N), carbon (C), potassium (K), phosphorus (P), and their excessive levels are preconditions to generate ecological problems. Also, the cumulative capacity of N- (NH_3, N_xO_x, NO_3^-), C- (CO_2, CH_4), P- containing (P_xO_x), and K^+ derivatives in atmosphere lead to disproportion and imbalance, resulting in disturbed ecosystem stability. But, the productive systems affecting environment in different order (*Steinfeld et al., 2006*). Thus, the main emissions of gases in ruminant sectors are related to N, as limiting factor (*Bouwman et al., 1997*). Near ½ of greenhouse gases emissions from agriculture (5 %) in EC_{28} are generated by enteric fermentation and manure management (*Freibauer, 2003; European Environment Agency, 2013*). Also, Bulgarian values are near to EC_{28} means (near 10 and 5 % for gases emissions from agriculture and total amounts from enteric fermentation (near 1/3 of emissions from agriculture) and manure management (near 1/6 of emissions from agriculture), respectively).

The leading negative effects of animal husbandry and agriculture could be summarized as a source of different atmospheric pollutants by various nature – chemical, physical, biological, etc. (*Foer, 2009*). The feed lot and dairy industries excreted 27.1 кg CO_2 Eq /кg feed intake and 39.3 кg from total gases emissions (*Hamerschlag and Venkat, 2011*). Likewise, the animal husbandry sector is common environmental pollutant, e.g. source of ecological risks (*Steinfeld et al., 2006*). Therefore, we awaited harmer scenarios with deeper problems, because the

future prognosis indicated food production (meat and milk) to be increased at twice till 2050^{s} (*www.fao.org*). The total amount of greenhouse gases emissions, estimated as CO_2 Eq, are near 18 %, and 4.6 billion t CO_2 Eq are generated in EC_{28} (*European Environment Agency, 2013*). Also, the 4^{th} Assessment Report of the Intergovernmental Panel of Climate Change (*AR4*) generalized the atmospheric concentrations increment: CH_4 – doubled, CO_2 – by 35%, N_2O – by 18%, compared with the pre-industrial era (*IPCC, 2007*). Thus, in terms of Common Agricultural Policy (*CAP, 2014-2020*) and under the limitations and requirements of *Nitrate Directive* (*1991*), and *Bulgarian Regulation N44-20/04/2006* (*2014*) farmers must to control their N flows and cycling (*EC, 1991; COM, 2006*). Otherwise, the environmental pollution with agricultural N becomes from imbalanced cycling at input/output criteria. The N excretion, as a function of input/output ratio, is related with breeding and nutritional systems, physiological status, environmental conditions, etc. So, the manipulation of these factors could modify animal production systems by increasing N utilization and decreasing N pollution.

The N levels, at Earth layers, are established as 4_x10^{21} g. The reactive form (reactive N), as N-fixing organisms, is calculated under 1 % (*Mackenzie, 1998*). Simultaneously, the total amounts of excreted N in animal husbandry, is estimated on 75 $Tg.y^{-1}$ (*Smil, 1999*). So, the ammonia values, as a part of undesirable atmospheric components, are affected by N utilization in farming sector (*Bussink and Oenema, 1998; James et al., 1999*). The low N efficacy is based on higher input levels of crude protein in ruminant diets, e.g. higher output values of excreted N as fecal-N and urinary-N. It's proved by evidence that increased dietary protein per 1 % was followed by $2.8g.d^{-1}$ and $35.7g.d^{-1}$ acceleration in milk N and excreted urinary and fecal N (*Hristov and Huhtanen, 2008*). This confirmed the models from last 50^{s} y at the XX_s century. So, dairy nutrition provided dietary N/milk N ratio near 2 at the ends of the 40_s, and increased up to 7 at the end of the period (*Ketelaars and Van de Ven, 1992; Rotz, 2004*). The leading role in this process was a result of intensification in animal husbandry and farming sector, e.g. constantly increased consumer requirements to achieve unreal levels of animal performance and productivity in short-term periods.

The excessive dietary protein supply in ruminant nutrition with higher N excretion resulted in subsequent ammonization of run-off water, atmospheric ammonia and nitrate contamination, and ecosystem acidulation and eutrophication (*Galloway and Cowling, 2002*). Simultaneously, the N_2O and NO_3^- concentrations correlated positively with the level and rate of N fertilization, and fertilizer' N amounts (*Tamminga, 2003*).

The right approach to the problem might be found with modified productive systems in regards to breeding (*Yossifov, 2014c*), scheme of weaning (*Yossifov, 2013; Yossifov and Kozelov, 2013; Yossifov and Kozelov, 2013a*), diet balancing (*Kozelov and Yossifov, 2013*), zoo-hygiene conditions (*Krastev and*

Petrova, 2000), etc. biotic and abiotic factors. In some articles, aimed at N balance estimation (*Yossifov and Kozelov, 2011; Yossifov and Kozelov, 2011a; Yossifov, 2013b; Yossifov, 2014a; Yossifov, 2014b*) in regards to dietary incorporation of non-traditional and alternative protein forages in feedlot (*Erickson et al., 2000; Yossifov et al., 2012; Yossifov and Kozelov, 2012a*) and dairy (*Kohn et al., 1997; Yossifov, 2012a*) productive systems exhibited adequate terms of reference to so called smart farming and excellent agriculture, based on precision balanced diets (*Rotz, 2004*). The perspective drawings and situations, based on decelerated intensification in agriculture, and aspects of biological farming systems and its subdivisions (*Yossifov, 2014d*), are oriented to achieve sustainable ecosystems related to co–factorial symbiosis in terms of agronomic, ecological, economic, social, etc. (*Van Passel et al., 2007; Yossifov, 2014e*). Nevertheless, animal husbandry sector, as a result of intensification in productive systems, generated near 65 % N_2O, 64 % NH_3, 37 % CH_4, and 9 % CO_2, excreted by human activities (anthropogenic) in the sector. Also, the total amount of generated CH_4 и N_2O emissions in EC_{28} are 194 and 271 million t, respectively (*European Environment Agency, 2013*). Thus, our efforts are exerted to different mitigation strategies and environment protection (*Meadows et al., 1992*). The main tools to decrease EC_{28} emissions of greenhouse gases are based on (*Tamminga, 2003*):

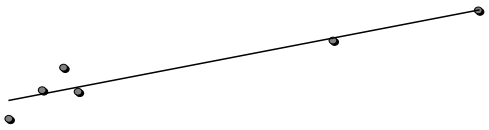
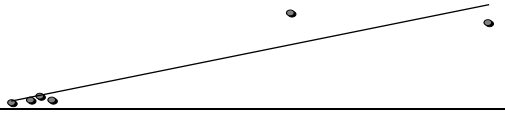
- Population / herd management (*St Pierre and Thraen, 2001*);
- Efficiency of farming systems and productive systems (*Kozelov and Yossifov, 2013*);
- Excellent agriculture, smart farming and precision balanced diets (*Jonker et al., 2002; Avery, 2010*);
- Reduced N-containing fertilizers (*CEAS/EFNCP, 2002*);
- Effective manure management practices (*Ipharraguerre and Clark, 2005*).

The main decisions must be made for more effective and profitable productive systems, based on precision and balanced feeding (*Avery, 2010*). As we mentioned above, farming systems (feedlot and dairy) are ineffective N consumer. This misbalancing employment of N, as a result of disturbed input/output ratio, related to amount of retained N (milk and meat) and N costs for expensive protein forages and excessive N fertilization (*Yossifov, 2013c*). Environmental pollution related to enormous N losses inside the cycling units and between the nutrient flows, based on poor manure management, accelerated N excretion, etc. The goals of the nutritionists' exerted efforts will be to balance livestock diets' by cheapest N sources (*Yossifov, 2013c*) with digestibility surpassed traditional ones (*Kozelov and Yossifov, 2013*). These efforts will gain higher N retention (milk, meat) or lower N excretion either (*Yossifov, 2012a; Yossifov, 2014a*). The potential benefits with better utilization of dietary N will modified both ecological and economic effects (*Oenema and Pietrzak, 2002*). Also, the N biotransformation must be expected at N

fixation (N compounds), ammonification (air NH_3), nitrification (water NO_3^-) and denitrification (N_xO_x) processes.

Our database shown that an overall emission reduction in the agriculture amounted to 70 % in the period 1989 – 2011, and 2011 the sector contributed 9 % to the total of the Bulgaria' GHG_s. The downward trends were driven by livestock population and arable land reduction (table 2). The most important agricultural categories as well as the contribution to the total GHG emissions 1989 – 2011 are agricultural soils (58 %), enteric fermentation (21 %), manure management (19 %).

Table 2. Correlation model – animal population per period (thous)/ N_2O values ($Gg.CO_2^{-eq}$), as generated by Bulgarian manure management

Population (thous) / $Gg.CO_2^{-eq}$ N_2O	Year						R^2
	1989	*1991*	*1997*	*2001*	*2007*	*2011*	
Monogastric							0.89
Ruminant							0.85
Total							0.99

A good parity between investigated parameters was observed among the deducted correlation models. The regressive analysis shows that estimated N_2O values ($Gg.CO_2^{-eq}$), as emissions generated by manure management (y) are manifested by close relationship with animal population (x) among the investigated middle-term periods throughout 1989 – 2011 y. The smooth diversion rate among the investigated parameters allows being comparable with strong correlation (R^2> 0.85 – 0.99).

Conclusion

The N excretion, as a function of input/output ratio, is related with breeding and nutritional systems, physiological status, environmental conditions, etc. So, the manipulation of these factors could modify animal production systems by increasing N utilization and decreasing N pollution. The main decisions must be made for more effective and profitable productive systems, based on precision and

balanced feeding. In regards to deducted correlation models, as an estimator of the N_2O emissions ($Gg.CO_2^{-eq}$), generated by manure management (*y*), are manifested by close relationship with animal population (*x*) among the investigated middle-term periods throughout 1989 – 2011 y for the Bulgarian realities.

Ekološki trendovi korišćenja azota u stočarstvu

K. Krastev

Rezime

Cilj ovog istraživanja je bio deo ispitivanja uticaja stočarstva na emisiju gasova staklene bašte i nekih strategija ublažavanja tog uticaja na kraju XX i početkom XXI veka. Pažnja je usmerena na ravnotežu azota (N) i njegove oscilirajuće vrednosti, kao i prateće faktore. Kao rezultat, utvrdili smo jake modele korelacije (R^2> 0,89; 0,85; 0,99) kao estimatorom emisija N_2O ($Gg.CO_2^{-eq}$), nastalu upravljanjem prirodnim đubrivom/stajnjakom u odnosu na životinjske populacije (ne-preživare, preživare, ukupno) u ispitivanim srednje-ročnim periodima tokom 1989 - 2011 god. u Bugarskoj.

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THE INFLUENCE OF THE FACTOR «GENETIC VALUE OF THE FATHER» ON THE PRODUCTIVE QUALITIES OF THE ROMANOV BREED SHEEP

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Abstract: Sheep farming plays an important role in the production of meat. Romanov breed is known for its high fertility and therefore is used all over the world due to increased production of lambs and lamb meat**.** Meat products are the main food elements of the man. Most of the inhabitants of industrialized countries cannot imagine their menu without meat. Value of meat for human health is known, it supplies protein to the body. The Yaroslavl Region is a leading region of the Romanov sheep breed. Therefore, the aim of our research was to determine the strength and reliability of the influence of the factor «genetic value of the father» on productive characteristics of animals as a factor that helps to increase the productivity of animals. Upon determining the strength of the influence of factors for statistical data have used the procedure of generalized linear models (General Linear Models - GLM), and evaluation components of phenotypic variation attributes were analyzed by multivariate dispersive analysis. Our research has allowed allocating rams with genetic value that has the improving effect. Using the recommended lines the farmers of the Yaroslavl region may increase productive characteristics of animals and the profit of the farms and improve the efficiency of breeding.

Keywords: sheep, genetic factors, productive characteristics, efficiency of breeding

Introduction

Intensification of agricultural production, including sheep and the increase in the demand of products in this sector has accompanied by the creation of new more productive and profitable breeds *(Petrovic, 2006).* With the division of animals into breeds during the last few hundred years, animal breeding has

witnessed a dramatic change. A major role in this process has evaluation of genetic modifications in herds and populations of animals *(Kuznetsov, 1983; Moskalenko et al., 2012).* Most recently, the identification of superior rams and their disproportionate genetic contribution via artificial insemination has lifted the pace of genetic gain for production traits *(El Hanafy and El Saadani, 2009; Kijas et al., 2012).*

The preservation of the gene pool of sheep as well as known, highly productive, rare and unique species for use in crossbreeding is important in the development of sheep breeding *(Moskalenko and Nikolaeva, 2013).*

Currently the rams rated by their own productivity and origin are often used for breeding in small farms. Sometimes well-known parents do not give offspring of the same quality, as they are *(Arseniev, 2011; Caro Petrovic et al., 2013).*

Accelerating the race of genetic improvement of the breed by breeding and productive indicators is possible by using rams improvers having high productive offspring *(Mazepkin and Lebedko, 2000; Moskalenko and Konovalov, 2010; Akhtar et al., 2014).* A large part of the phenotypic variation of the main economically important characteristics of sheep due to the influence of genetic components of variation – «mother's line», "father's line» and «genetic value of the father». The influence of the factor «genetic value of father» on productive qualities of sheep ranged from 8 to 17.3% (*Moskalenko and Nikolaeva, 2013).*

The Yaroslavl region it has 6 gene pool farms of Romanov sheep breed. Therefore, the aim of our research was to determine the strength and reliability of the impact of the factor «genetic value of the father» on breeding characteristics of the Romanov sheep breed.

Material and Methods

Selected farms of Uglich municipal district of Yaroslavl region were involved for this study such as «Agrofirm Avangard», PAC «Rodina», LLC «Friendship», LLC «Zarechye». The object of the study were the first Romanov breed ewes lambing (with a total of 856^{-th} - fishing). As the material of our investigations, we used the data of individual breeding ewes' cards - form № 2, periodical of mating, offspring, individual appraisal and productivity of sheep. According to the genealogical structure of the samples, we studied 13 lines: 3, 13, 18, 20, 25, 29, 34, 115, 267, 450, 508, 541 and 600.

The following evaluation methods of breeding ewes' signs of the study sample have used: a multivariate analysis of variance, selective genetic parameters. During determination of the strength of the influence of factors for statistical data were used the procedure of generalized linear models (General Linear Models - GLM), and evaluation components of phenotypic variation attributes had analyzed by multivariate dispersive analysis. The influence of the factor «genetic value of

rams» on breeding characteristics of Romanov breed ewes was studied by linear model of mixed type**.** The evaluation of components of phenotypic variability was performed using a multi- factorial dispersive analysis *(Kuznetsov, 2006).*

Results and Discussion

We estimated the influence of the factor «genetic value of the father» on the variability of productivity characteristics of Romanov breed ewes in gene pool farms of Yaroslavl region according **t**o the methodology of the research. During the investigations, it was established that the phenotypic variability of the studied characteristics of ewes is determined by strong and significant influence of such factors as the «genetic value of the father». The power of influence of this factor is from 5.6% to 17.3% , including live weight – 9.2 %, shearings –17.3 % , awn length - 10.0% ,down length - 16.8 %, the ratio of awn length to the length of down - 8% and the proportion of spine and down – 5% *(Moskalenko and Nikolaeva, 2012).* Assessing the effects of the gradation of the factor «genetic value of the father» on the studied characteristics of ewes is presented in the table 1.

The ram number 5 (line 34) provided significantly plus effect on body weight of studied offspring in comparison with the average for the sample, also - № 190 (line 3) provided, the gradation effect was 6.76 kg (P> 0.95) kg and 8.7 kg (P> 0.99) kg respectively. The ram number 2 (line 18) provided significantly negative influence, also - № 37 (line 541), № 74 (line 115), № 947 (line 3), the gradation effect was - 8.18 kg (P> 0.95) -7.82 kg (P> 0.95); -8.23 kg (P> 0.95); -6.68 kg (P> 0.95) respectively.

According to *Banerjee et al., (2010*), ewe productivity, defined as number (or total weight) of lambs weaned per ewe exposed, is dependent upon the component traits of fertility, litter size, lamb survival and growth and is also a major concern of the sheep industry. In our study, the ram number 86 (line 541) significantly increased the fertility of ewes, the gradation effect was 0.97 lambs (P> 0.95), lowered – the ram number 31 (line 508), the gradation effect - 0.67 lambs. (P> 0.95). *Shaoqi (1997)* stated that fertility maybe dependent on a maternal and a paternal genetic component because mating behaviors of both parents and the quality of their gem cells are responsible for the success of a mating.

The ram number 84 (line 508) provided significantly positive impact on shearing's of ewes, the gradation effect was 0.33 kg (P> 0.99). The ram number 2 (line 20) provided significantly negative impact on the shearing's, also - № 108 (line 29), the gradation effect - 0.47 kg (P> 0.95) and -0.38 kg (P> 0.95) respectively.

In determining the complex breeding value on the basis of productivity of sheep and sheep-skin coat qualities did not found rams with category of «absolute improver» (category «A») in the sample during the study period (table 2).

Table 1. The Effect of the gradation of the factor «genetic value of the father» on productive characteristics of ewes

The factor «genetic value of the father» / Number of the ram	№ Of the daughter	Daughter's characteristics						Category Of the father
		Live weight, kg		Fertility, lambs		Shearings, kg/year		
		SI′	(μ+SI′)±m	SI′	(μ+SI′)±m	SI′	(μ+SI′)±m	
1	2	3	4	5	6	7	8	9
μ (the average value of the sample)	856	-	48.02±0.20	-	1.81±0.02	-	1.90±0.01	-
1	11	-1.13	46.89±2.83	0.33	2.14±0.32	0.17	2.07±0.13	D
2	62	-8.18	39.84±3.98*	-0.04	1.77±0.45	-0.47	1.43±0.18*	D
5	29	6.76	54.78±2.71*	0.23	2.04±0.31	-0.02	1.88±0.13	D
6	33	2.71	50.73±2.27	0.00	1.81±0.26	-0.03	1.87±0.11	D
7	19	-2.29	45.73±2.43	-0.02	1.79±0.28	0.26	2.16±0.11	D
10	34	0.16	48.18±2.26	0.15	1.96±0.26	0.05	1.95±0.10	D
16	7	-1.05	46.97±3.82	-0.78	1.03±0.44	0.24	2.14±0.18	D
19	5	2.81	50.83±3.85	-0.18	1.63±0.44	0.07	1.97±0.18	B
31	10	2.45	50.47±3.07	-0.67	1.14±0.35*	0.11	2.01±0.14	D
34	13	-1.17	46.85±4.13	0.51	2.32±0.47	-0.21	1.69±0.19	B
37	8	-8.24	39.78±3.90*	-0.03	1.78±0.44	-0.29	1.61±0.18	D
60	21	0.68	48.70±2.67	-0.46	1.35±0.30	0.11	2.01±0.12	D
65	34	-3.15	44.87±2.15	-0.33	1.48±0.25	0.15	2.05±0.10	D
74	12	-7.82	40.20±3.00*	0.19	2.00±0.34	0.02	1.92±0.14	D
84	20	-0.73	47.29±2.43	-0.16	1.65±0.28	0.33	2.23±0.14**	D
86	19	2.12	50.14±3.98	0.97	2.78±0.45*	0.19	2.09±0.18	B
94	15	3.09	51.11±3.99	0.73	2.54±0.46	0.10	2.00±0.19	B
100	8	-2.97	45.05±2.86	-0.20	1.61±0.33	-0.12	1.78±0.13	D
105	19	0.40	48.42±3.25	-0.62	1.19±0.37	0.07	1.97±0.15	D
108	29	-5.33	42.69±3.78	0.05	1.86±0.43	-0.38	1.52±0.18*	D
110	10	-1.04	46.98±4.14	0.01	1.82±0.47	-0.01	1.89±0.19	D
111	25	-1.81	46.21±2.66	-0.08	1.73±0.30	0.25	2.15±0.12	D
113	7	4.66	52.68±3.26	-0.16	1.65±0.37	-0.08	1.82±0.15	D
128	14	-4.99	43.03±2.75	0.35	2.16±0.31	0.06	1.96±0.13	D
140	44	2.51	50.53±2.11	-0.32	1.49±0.24	-0.07	1.83±0.10	D
155	29	2.11	50.13±2.39	-0.32	1.49±0.27	-0.13	1.77±0.11	D
186	30	3.53	51.55±2.11	0.12	1.93±0.24	0.04	1.94±0.10	D
190	12	8.70	56.72±3.08**	0.26	2.07±0.35	-0.04	1.86±0.14	D
196	19	-0.76	47.26±2.46	-0.24	1.57±0.28	0.10	2.01±0.11	D
240	46	2.49	50.51±2.19	-0.25	1.56±0.25	0.05	1.95±0.10	D
247	16	1.90	49.92±2.38	-0.06	1.75±0.27	0.04	1.94±0.11	D
252	21	1.27	49.29±2.29	-0.04	1.77±0.26	-0.03	1.87±0.11	D
331	42	0.98	49.00±2.24	-0.44	1.37±0.26	-0.10	1.80±0.10	D
354	37	0.35	48.37±2.44	-0.40	1.41±0.28	0.11	2.01±0.11	D
369	20	-6.32	41.70±2.97	-0.32	1.49±0.34	-0.11	1.79±0.14	D
416	7	0.59	48.61±4.31	0.56	2.37±0.49	0.05	1.95±0.20	B
615	15	-1.42	46.60±4.00	0.31	2.12±0.46	-0.25	1.65±0.19	D
618	10	-5.41	42.61±3.07	-0.05	1.76±0.35	0.06	1.96±0.14	D
907	14	6.75	54.77±3.03	0.42	2.23±0.35	-0.12	1.78±0.14	D
947	9	-6.68	41.34±3.00*	-0.50	1.31±0.34	0.14	2.04±0.14	D
1067	13	5.40	53.42±2.91	0.27	2.08±0.33	-0.07	1.83±0.14	B
1098	8	2.36	50.38±3.29	0.50	2.31±0.38	-0.16	1.74±0.15	D

Note: The difference between the index and the average value of the sample is reliable when * - P> 0.95; ** - P> 0.99; *** - P> 0.999.

We used information about productive and sheep- skin and wool characteristics of the ewes (daughters) to define the complex breeding value of the rams. It was found that the rams were not with the category «Absolute improver» (category «A») in the studied sample during the study period. Factor «genetic value of the father» did not have a significant positive impact on features such as the length of awn, the length of down and the proportion of awn and down.

Fogarty et al. (2005) commented that ewe flock productivity has a major impact on lamb enterprise profitability and stocking rate. Profit is from the sale of lambs (determined by number produced, carcass weight and fat level), skins and ewe wool (weight and fibre diameter). Potential productivity of the ewes for these traits is determined by their genetic merit. The ranking of the sire breeds (and some sires) varied with the production system and environment in which their daughters were evaluated. The said authors also stressed in their study that there were some significant differences between the maternal sire breeds in performance of their progeny; the variation among individual sires within the breeds was far greater for most production traits.

Table 2. The Effect of the gradation of the factor «genetic value of the father» on fur characteristics of ewes

The factor «genetic Value of the father» Number of the ram	№ Of the daughter	Daughter's characteristics								Category oft he father
		The length of awn, cm		The length of down, cm		The ratio of awn length to the length of down		The proportion of awn and down		
		SI′	(μ+SI′)±m	SI′	(μ+SI′)±m	SI′	(μ+SI′)±m	SI′	(μ+ SI′)±m	
1	2	3	4	5	6	7	8	9	10	11
μ (the average value of the sample)	856	-	2,98±0,03	-	4,75±0,02	-	0,63±0,003	-	7,25±0,04	-
1	11	-0,22	2,76±0,24	0,04	4,79±0,30	-0,02	0,61±0,04	-0,46	6,79±0,60	D
2	62	-0,29	2,69±0,34	0,21	4,96±0,42	-0,08	0,55±0,06	-0,28	6,97±0,84	D
5	29	0,21	3,19±0,23	0,49	5,24±0,28	-0,03	0,60±0,04	0,08	7,33±0,57	D
6	33	0,09	3,07±0,19	-0,14	4,61±0,24	0,04	0,67±0,03	-0,50	6,75±0,48	D
7	19	-0,13	2,85±0,20	0,19	4,94±0,25	-0,02	0,61±0,03	0,01	7,26±0,51	D
10	34	0,05	3,03±0,19	-0,10	4,65±0,24	0,02	0,65±0,03	-0,48	6,77±0,48	D
16	7	0,23	3,21±0,32	-0,14	4,61±0,40	0,06	0,69±0,05	-0,78	6,47±0,81	D
19	5	0,29	3,27±0,32	0,04	4,79±0,40	0,03	0,66±0,05	-0,02	7,23±0,81	B
31	10	-0,25	2,72±0,26	-0,31	4,44±0,32	-0,03	0,60±0,04	0,24	7,49±0,65	D
34	13	-0,09	2,89±0,35	0,99	5,74±0,43	-0,15	0,48±0,06**	0,14	7,39±0,87	B
37	8	-0,31	2,67±0,33	0,03	4,78±0,41	-0,03	0,60±0,05	-0,24	7,01±0,82	D
60	21	-0,14	2,84±0,22	-0,40	4,35±0,28	0,01	0,64±0,04	-0,23	7,02±0,57	D
65	34	0,22	3,20±0,18	0,16	4,91±0,23	0,03	0,66±0,03	-0,78	6,47±0,45	D
74	12	0,03	3,01±0,25	-0,09	4,66±0,32	0,01	0,64±0,04	0,17	7,42±0,64	D
84	20	-0,31	2,67±0,20	-0,87	3,88±0,26***	0,07	0,70±0,03	0,33	7,58±0,51	D
86	19	-0,16	2,82±0,33	0,64	5,39±0,42	-0,13	0,50±0,06*	-0,35	6,90±0,84	B
94	15	0,02	3,00±0,34	0,58	5,33±0,42	-0,10	0,53±0,06	0,18	7,43±0,85	B
100	8	0,20	3,18±0,24	0,24	4,99±0,30	0,01	0,64±0,04	0,13	7,38±0,60	D
105	19	0,34	3,32±0,27	-0,21	4,54±0,34	0,08	0,71±0,05	0,47	7,72±0,69	D
108	29	-0,20	2,78±0,32	0,13	4,88±0,40	-0,05	0,58±0,05	0,09	7,34±0,80	D
110	10	0,06	3,04±0,35	0,84	5,59±0,43	-0,09	0,54±0,06	0,71	7,96±0,88	D
111	25	0,23	3,21±0,22	0,02	4,77±0,28	0,03	0,66±0,04	0,47	7,72±0,56	D
113	7	-0,49	2,49±0,27	-1,28	3,47±0,34***	0,08	0,71±0,05	0,46	7,71±0,69	D
128	14	-0,18	2,80±0,23	0,08	4,83±0,29	-0,02	0,61±0,04	-0,48	6,77±0,58	D
140	44	0,00	2,98±0,18	-0,29	4,46±0,22	0,04	0,67±0,03	0,03	7,28±0,45	D
155	29	0,07	3,05±0,20	-0,03	4,72±0,25	0,02	0,65±0,03	0,71	7,96±0,50	D
186	30	0,12	3,10±0,18	-0,26	4,49±0,22	0,05	0,68±0,03	-0,07	7,18±0,45	D
190	12	0,26	3,24±0,26	0,41	5,17±0,32	-0,03	0,60±0,04	0,09	7,34±0,65	D
196	19	-0,51	2,47±0,21*	-1,03	3,72±0,26***	0,04	0,67±0,03	0,20	7,45±0,52	D
240	46	-0,47	2,51±0,18*	-1,08	3,67±0,23***	0,07	0,70±0,03*	-0,08	7,17±0,46	D
247	16	0,11	3,09±0,20	-0,11	4,64±0,25	0,03	0,66±0,03	-0,20	7,05±0,50	D
252	21	0,01	2,97±0,19	-0,18	4,57±0,24	0,02	0,65±0,03	-0,49	6,76±0,48	D
331	42	0,32	3,30±0,19	0,03	4,78±0,24	0,06	0,69±0,03*	0,23	7,48±0,47	D
354	37	-0,17	2,81±0,21	-0,45	4,30±0,26	0,02	0,65±0,03	0,17	7,42±0,52	D
369	20	0,33	3,31±0,25	-0,13	4,62±0,31	0,05	0,68±0,04	-0,43	6,82±0,63	D
416	7	0,07	3,05±0,36	0,55	5,30±0,45	-0,06	0,57±0,06	-0,07	7,18±0,91	B
615	15	-0,18	2,80±0,34	0,64	5,39±0,42	-0,12	0,51±0,06*	0,09	7,34±0,85	D
618	10	0,21	3,19±0,26	0,15	4,90±0,32	0,02	0,65±0,04	0,04	7,29±0,65	D
907	14	0,12	3,10±0,26	0,31	5,06±0,32	-0,02	0,61±0,04	-0,21	7,04±0,64	D
947	9	-0,13	2,85±0,25	0,07	4,82±0,32	0,00	0,63±0,04	-0,13	7,12±0,64	D
1067	13	0,00	2,98±0,25	0,01	4,76±0,31	-0,01	0,62±0,04	0,04	7,29±0,62	B
1098	8	0,12	3,10±0,28	0,49	5,24±0,35	-0,05	0,58±0,05	1,35	8,60±0,70	D

Conclusion

Our studies have allowed allocating rams having genetic value, which has the effect of improving on the productivity of the flock in which they are used. We recommend using lines and their animal representatives to increase breeding efficiency and preserve the gene pool of Romanov breed sheep.

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Uticaj faktora «genetska vrednost oca» na produktivne kvalitete romanovske rase ovaca

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Rezime

Ovčarstvo igra važnu ulogu u proizvodnji mesa. Romanovska rasa je poznata po visokoj fertilnosti i zato se koristi svuda u svetu radi povećane produkcije jagnjadi i jagnjećeg mesa.Mesni proizvodi su glavni elementi hrane za čoveka. Većina stanovnika u industrijskim zemljama ne mogu da zamisle svoj meni bez mesa. Značaj mesa za ljudsko zdravlje je pre svega u tome što snabdeva organizam proteinima. Jaroslavski Region je vodeći region u gajenju romanovskih ovaca. Dakle, cilj našeg istraživanja bio je da se utvrdi snaga i pouzdanost uticaja « genetske vrednosti oca » na produktivne osobine životinja kao faktora koji pomaže da se poveća produktivnost životinja. Za utvrđivanje jačine uticaja faktora primenjena je statistička obrada podataka. Tom prilikom je korišćen postupak generalnog linearnog modela (Opšti Linearni modeli - GLM). Za evaluaciju komponenti fenotipske varijabilnosti atributa upotrebljene su multivarijacione disperzivne analize. Naše istraživanje je omogućilo identifikaciju genetske vrednosti ovnova sa koji imaju poboljšavajući efekat u potomstvu. Naša saznanja mogu pored naučnog doprinosa biti i od praktične koristi. Upotrebom preporučenih linija odgajivači ovaca u Jaroslavskom regionu mogu unaprediti proizvodne

karakteristike životinja, poboljšati efikasnost odgajivanja i povećati dobit od farmi. Ovaj metod se može primeniti i na drugim populacijama ovaca.

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GENETIC PARAMETERS AND GENETIC GAINS FOR REPRODUCTIVE TRAITS OF ARABI SHEEP

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Abstract: The current study reports, for the first time, the genetic parameters and genetic, phenotypic and environmental correlations and trends of reproductive traits in Arabi sheep. Data were collected at Animal Science Research Station of Khuzestan Ramin Agricultural and Natural Resources University (ASRSKRANRU), south-west of Iran from 2001 to 2008. Litter size at birth (LSB), litter size at weaning (LSW), litter mean weight per lamb born (LMWLB), litter mean weight per lamb weaned (LMWLW), total litter weight at birth (TLWB) and total litter weight at weaning (TLWW) averaged 1.11 lambs, 1.01 lambs, 3.83 kg, 19.43 kg, 4.16 kg and 20.12 kg, respectively. Genetic parameters and correlations were estimated with univariate and bivariate models using restricted maximum likelihood, breeding values of animals were estimated with best linear unbiased prediction (BLUP) and genetic- and phenotypic trends by regression of ewes' average breeding values and phenotypic least square means on year of birth respectively. Random effects were fitted by additive direct genetic effects and permanent environment related to the ewe as well as service sire effects, in addition to fixed effects of ewe age at lambing and lambing year. Heritability estimates of 0.05, 0.02, 0.13, 0.12, 0.04, and 0.06, and repeatability estimates of 0.08, 0.06, 0.17, 0.16, 0.14 and 0.21 for the six traits, respectively. Genetic correlations between traits varied from −0.82 to 0.94. Phenotypic correlations were lower, ranging from −0.33 to 0.52. Estimated annual genetic progress was very low; −0.003 lambs for LSW and 15 g for TLWW. Annual phenotypic trend was only significant for LSW being 0.007 lambs. The study concluded that indirect selection based on total litter weight at weaning could be efficient for the traits studied.

Keywords: genetic parameters; genetic trends; reproductive traits; Arabi sheep

Introduction

Knowledge of genetic parameters is the basis of sound livestock improvement programs. Estimates of heritabilities and genetic correlations are essential population parameters required in animal breeding research and in design and application of practical animal breeding programs *(Imbayarwo-Chikosi, 2010)*. Moreover, repeatability is an important genetic parameter, which is frequently used to measure the animals' ability to repeat their level of production at successive intervals in time, although a high repeatability coefficient does not mean that the animals will strictly demonstrate the same performance in the next productive seasons; it could be predicted in the subsequent performance of the animals under stable environmental conditions *(Mohammadi et al., 2013)*.

Ewe productivity defined as the total weight of lambs weaned by a ewe is one of the most important economic traits and has been proposed as a biologically optimum index to improve overall flock productivity *(Snowder, 2002)*. Also, ewe productivity is a key target in sheep breeding and could be improved by increasing the number of lambs weaned and weight of lambs weaned per ewe within a specific year *(Duguma et al., 2002)*.

Arabi sheep is one of the most important dual-purpose sheep (meat and wool) native breeds of Iran. Most of these sheep are raised in Khuzestan province in southwest of Iran (numbering more than 1.8 million head). They are well adapted to humid-tropical environmental conditions *(Shokrollahi and Baneh, 2012)*. The Arabi breed is characterised as white, cream, black and dark/bright brown colour, horned rams and polled ewes, fat-tailed, medium-sized (mature weight of ewe and ram is 45-50 and 60-65 kg, respectively).

There is no published research on reproductive traits of Arabi sheep, to date. Thus, this paper analyzed data from Animal Science Research Station of Khuzestan Ramin Agricultural and Natural Resources University (ASRSKRANRU), and estimated genetic parameters, and correlations (genetic, phenotypic and environmental) for reproductive traits, providing a scientific evidence for breed selection on this station. In addition genetic-, phenotypic- and environmental trends were estimated.

Materials and methods

Geographical location and management

The data set used in the present study were collected from ASRSKRANRU in Mollasani town, located between Ahvaz and Shoushtar cities, from 2001 to 2008. Climate of Mollasani town is humid-tropical and the maximum temperature recorded is approximately 50 ° C in summer, while the temperature drops to 5 °C

in winter. The mean annual rainfall is around 210 mm, mainly occurring during December – January. The animals were raised on pasture in spring and summer and with access to farm residual feeds during autumn and housed at night, typically. The environmental-, nutritional-, and management conditions were the same for all of the animals. Maiden ewes were exposed to rams at about 18 months of age and kept in the flock until death or apparent infertility. The selected rams were 3-4 years of age and kept separated from ewes, generally. During the breeding season, single-sire pens were used allocating 20-25 ewes per ram. The mating season was from early August to October. Lambing took place from early January to February, consequently. Lambs were weighed, ear-tagged early after birth. The date, sex and type of birth were recorded. Lambs were weaned from their mothers at an average age of 120 days. The ewes and young animals were kept on natural pastures as separate flocks, after weaning. Supplemental feeding was offered during mating and late pregnancy. Selection was based on weight at six months.

Studied traits

The traits analysed were classified as basic and composite. Basic traits were litter size at birth (LSB, the number of lambs born alive, coded by 1 or 2 for lamb alive at birth), litter size at weaning (LSW, the number of lambs weaned per ewe lambing, coded by 0 for lamb dead and 1 or 2 for lamb alive at weaning), litter mean weight per lamb born (LMWLB, the average weight of lambs at birth from the same parity), litter mean weight per lamb weaned (LMWLW, the average weight of lambs at weaning from the same parity), and composite traits were total litter weight at birth (TLWB, the sum of the birth weights of all lambs born per ewe lambed) and total litter weight at weaning (TLWW, the sum of the weights of all lambs weaned per ewe lambed). Summary statistics for reproductive traits is presented in Table1.

Table 1. Summary of descriptive statistics for reproductive traits of Iranian Arabi sheep

	Traits					
	LSB (lamb)	LSW (lamb)	LMWLB (kg)	LMWLW (kg)	TLWB (kg)	TLWW (kg)
No. of records	1690	1690	1690	1388	1690	1388
No. of ewes	473	473	473	408	473	408
No. of sires of the ewes	133	133	133	138	133	138
Mean	1.11	1.01	3.83	19.43	4.16	20.12
S.D.	0.31	0.46	0.77	3.27	1.33	5.16
C.V. (%)	27.93	45.54	20.10	16.83	31.97	25.65

LSB: Litter size at birth, LSW: litter size at weaning, LMWLB: litter mean weight per lamb born, LMWLW: litter mean weight per lamb weaned, TLWB: total litter weight at birth, TLWW: total litter weight at weaning

Statistical analysis

The general linear model (GLM) procedure of SAS *(SAS Institute, 2004)* was used to determine the fixed effects in the final models. These effects were included ewe age at lambing in 6 classes (2–7 years old) and lambing year in 8 classes (2001–2008). The lamb age at weaning (in days) was fitted as a covariate for LMWLW and TLWW traits. The interaction between fixed effects was not significant.

The traits were analyzed by WOMBAT software *(Meyer, 2006)* via AI-REML algorithm. The following models were applied to each trait:

Model 1 $\mathbf{y = Xb + Z_a a + e}$

Model 2 $\mathbf{y = Xb + Z_a a + Wpe + e}$

Model 3 $\mathbf{y = Xb + Z_a a + Z_s s + e}$

Model 4 $\mathbf{y = Xb + Z_a a + Z_s s + Wpe + e}$

where **y** is a vector of records on the respective traits; **b**, **a**, **pe**, **s** and **e** are vectors of fixed effects, direct additive genetic effects, permanent environmental related to repeated records of the ewes, service sire, and residual, respectively. The **X**, $\mathbf{Z_a}$, $\mathbf{W_{pe}}$ and $\mathbf{Z_s}$ stand for design matrices associating with the corresponding effects with elements of **y**, as well. The (co)variance structure for the random effects was:

$$\text{Var}\begin{bmatrix}\mathbf{a}\\ \mathbf{pe}\\ \mathbf{s}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{I_d}\sigma_{pe}^2 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I_s}\sigma_s^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I_n}\sigma_e^2\end{bmatrix}$$

It was assumed that the additive genetic effects, permanent environmental related to repeated records of the ewes, service sire, and residual to be normally distributed with a mean of zero and variances are $\mathbf{A\sigma^2_a}$, $\mathbf{I_d\ \sigma^2_{pe}}$, $\mathbf{I_s\ \sigma^2_s}$ and $\mathbf{In\ \sigma^2 e}$, respectively. Also, $\mathbf{\sigma^2_a}$, $\mathbf{\sigma^2_{pe}}$, $\mathbf{\sigma^2_s}$ and $\mathbf{\sigma^2_e}$ are the direct additive genetic variance, permanent environmental related to repeated records of the ewes, service sire, and residual, respectively. **A** is the additive numerator relationship matrix. $\mathbf{I_d}$, $\mathbf{I_s}$ and $\mathbf{I_n}$ are identity matrices with the order equal to the number of ewes, sires and records, respectively.

Repeatability (r) was calculated using the following formula:

$$\mathbf{r}=\frac{\mathbf{\sigma^2_a}+\ \mathbf{\sigma^2_{pe}}}{\mathbf{\sigma^2_p}}$$

In order to determine the most apposite model, Akaike's information criterion (AIC) was used *(Akaike, 1974)*:

$$AIC_i = -2 \log L_i + 2p_i$$

where log L_i is the maximised log likelihood of model i at convergence and p_i is the number of parameters obtained from each model; the model with the lowest AIC was chosen as the most suitable model.

Genetic-, phenotypic-, and environmental correlations were estimated using bivariate analysis with the same fixed effects as univariate models. Annual genetic and phenotypic trends of the traits were obtained as regression of ewes' means breeding and phenotypic values on their birth year, respectively. The subtraction of ewes' breeding value mean was computed from their phenotypic, and the regression of obtained value on birth year considered as environmental trend.

Results and discussion

Fixed effects

The least squares mean and standard errors of ewe age at lambing are presented in Table 2. The significant effect of ewe age was observed for all traits ($P<0.05$). The lowest reproductive performance was observed in 2- and 3-year-old dams. The significant influence of this effect could be explained by differences in maternal effects, nursing, and maternal behaviour of dams at different ages. All the studied traits were significantly influenced by lambing year ($P<0.05$). This effect may be explained by the variations in climate conditions and nutritional quality over the years.

Table 2. Least-squares mean ± standard errors for ewe age on reproductive traits

Fixed effects	Traits[a]					
	LSB (lamb)	LSW (lamb)	LMWLB (kg)	LMWLW (kg)	TLWB (kg)	TLWW (kg)
Ewe age (year)	*	*	*	*	*	*
2	1.09 [bc] ± 0.01	0.92 [b] ± 0.07	3.50 [e] ± 0.04	18.64 [c] ± 0.19	3.71 [d] ± 0.06	18.93 [c] ± 0.27
3	1.08 [c] ± 0.01	0.97 [ab] ± 0.04	3.77 [d] ± 0.04	18.49 [c] ± 0.18	3.95 [c] ± 0.06	18.59 [c] ± 0.24
4	1.12 [abc] ±0.02	0.99 [ab] ± 0.04	3.92 [bc] ± 0.04	19.47 [b] ± 0.24	4.28 [b] ± 0.08	20.25 [b] ± 0.39
5	1.14 [ab] ± 0.01	1.07 [ab] ± 0.05	4.02 [ab] ± 0.05	19.45 [b] ± 0.22	4.50 [ab] ± 0.09	20.70 [ab] ± 0.38
6	1.14 [ab] ± 0.01	1.13 [a] ± 0.04	4.06 [a] ± 0.05	20.24 [a] ±0.23	4.51 [a] ± 0.10	21.10 [ab] ±0.35
7	1.15[a] ± 0.03	1.12 [a] ± 0.05	3.89 [cd] ± 0.05	20.36 [a] ± 0.20	4.37 [ab] ± 0.11	21.26 [a] ± 0.35
Lambing year	*	*	*	*	*	*

Means with similar letters in each subclass within a column do not differ.
LSB: Litter size at birth, LSW: litter size at weaning, LMWLB: litter mean weight per lamb born, LMWLW: litter mean weight per lamb weaned, TLWB: total litter weight at birth, TLWW: total litter weight at weaning, *: $P < 0.05$

The significant effects ($P<0.01$) of lamb age at weaning demonstrated on traits included weaning weight (i.e. LMWLW and TLWW). Similar to our findings, significant effects of ewe age at lambing and lambing year on

reproductive traits of sheep have been reported in literature *(Hanford et al., 2006; Vatankhah et al., 2008; Mokhtari et al., 2010, Rashidi et al., 2011; Mohammadi et al., 2012; Mohammadi et al., 2013; Amou Posht-e- Masari et al., 2013; Nabavi et al., 2014).*

Univariate analysis

Variance components and genetic parameters for reproductive traits are presented in Table 3. Response to direct selection for litter size is limited by low heritability of the trait, due to its discrete phenotypic expression *(Hill, 1985)*. Heritability estimates for litter traits were low. They were 0.05 and 0.02 for LSB and LSW, respectively. Heritability estimates for litter traits obtained in the current study are close to those of *Ekiz at al. (2005)* for LSB in the Turkish Merino Sheep and *van Wyk et al. (2003); Cyhan et al. (2009)* and *Rashidi et al. (2011)* for LSW in Dormer, Sakiz and Moghani sheep breeds, respectively. However, they are lower than those reported for LSB in other sheep breeds, such as Katahdin *(Vanimisetti et al., 2007)*, Boer *(Zhang et al., 2009)*, Moghani *(Rashidi et al., 2011)*, and Ghezel *(Nabavi et al., 2014).* Moreover, reported heritability for LSW in Turkish Merino, Lori-Bakhtiari, Boer and Makooei sheep reported by *Ekiz et al. (2005), Vatankhah et al. (2008), Zhang et al. (2009)* and *Mohammadi et al. (2012)* was 0.0430, 0.06, 0.10, 0.06, respectively. These findings indicate that, the loss of lambs from birth to weaning is mainly affected by environmental factors and lamb's genotype rather than ewe's genotype.

The value obtained for heritability of LMWLB (0.13) was in accordance with the study of *Mokhtari et al. (2010)*. Nonetheless, higher estimates have been reported by some authors *(Vatankhah et al., 2008; Rashidi et al., 2011; Amou Posht-e- Masari et al., 2013)*. Our finding for heritability of LMWLW (0.12) corresponded to those reported by *Vanimisetti et al. (2007); Vatankhah et al. (2008)* and *Mohammadi et al. (2013)*; also, lower and higher estimates were recorded by *Rashidi et al. (2011)* and *Mokhtari et al. (2010)*, respectively.

Without considering litter size at birth, the ewe capacity to produce lamb weight at birth is measured by total litter weight at birth per ewe lambing. The heritability of TLWB was estimated to 0.04, in consistence with the studies of *Ekiz et al. (2005)* and *Shiotsuki et al. (2014)*. Higher values were reported by several authors *(Zhang et al., 2009; Mokhtari et al., 2010; Rashidi et al., 2011; Mohammadi et al., 2013; Nabavi et al., 2014)*, also. The combined influences of reproduction and pre weaning growth are considered as total litter weight at weaning. In agreement with the study of *Rashidi et al. (2011)*, the estimate of heritability of TLWW was 0.06. Heritability estimates for this trait varied from 0.0255 to 0.195 in different studies *(Rosati et al., 2002; Matika et al., 2003; vanWyk et al., 2003; Ekiz et al., 2005; Mokhtari et al., 2010; Amou Posht-e-Masari et al., 2013; Nabavi et al., 2014; Shiotsuki et al., 2014). Mohammadi et al.*

(2013) estimated heritability of this trait in Zandi sheep at 0.14 (i.e. higher than found in this study). Low heritability of reproductive traits is probably due to the greater proportional influence of environmental effects *(Turner and Young, 1969)*, thus their improvement by selection would be difficult even though they have great economic importance.

Table 3. Estimates of variance components and genetic parameters for reproductive traits.

Traits [a]	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_p	$h^2_d \pm$ S.E.	$pe^2 \pm$ S.E.	r
LSB	1.152	0.711	19.771	21.634	0.05 ± 0.02	0.03 ± 0.01	0.08
LSW	0.587	1.129	25.673	27.389	0.02 ± 0.02	0.04 ± 0.01	0.06
LMWLB	5.146	1.595	32.468	39.209	0.13 ± 0.02	0.04 ± 0.01	0.17
LMWLW	2.247	0.761	15.420	18.428	0.12 ± 0.02	0.04 ± 0.02	0.16
TLWB	1.112	2.768	23.350	27.230	0.04 ± 0.02	0.10 ± 0.02	0.14
TLWW	2.250	5.088	27.879	35.217	0.06 ± 0.02	0.14 ± 0.02	0.21

σ^2_a: direct genetic variance, σ^2_{pe}: permanent environmental variance, σ^2_e: residual variance, σ^2_p: phenotypic variance, h^2_d: direct heritability, pe^2: ratio of permanent environmental variance on phenotypic variance, r: repeatability, S.E: standard error

LSB: Litter size at birth, LSW: litter size at weaning, LMWLB: litter mean weight per lamb born, LMWLW: litter mean weight per lamb weaned, TLWB: total litter weight at birth, TLWW: total litter weight at weaning

Similar to the findings reported by several authors *(Vatankhah et al., 2008; Mokhtari et al., 2010; Rashidi et al., 2011; Mohammadi et al., 2012; Amou Posht-e- Masari et al., 2013; Mohammadi et al., 2013)*, the most appropriate models in the current study included both direct genetic and permanent environmental effects related to the ewes.

The estimated fraction of variance due to permanent environmental effects were lower than the estimates of direct genetic effects, ranging from 0.03 to 0.14, suggesting that additive genetic effects are more important, totally. These fractions for reproductive traits in Zandi sheep reported by *Mohammadi et al. (2013)* ranged from 0.03 to 0.08. Our results were compatible with the reports of *Vatankhah et al. (2008)*, generally. Results showed that composite traits were more affected by permanent environmental effects and environmental factors such as nutrition and management. Consequently, the repeatability values observed in this study ranged from 0.06 to 0.21 that were congruent with the studies of *Vatankhah et al. (2008)*, *Mokhtari et al. (2010)* and *Mohammadi et al. (2013).* Current findings indicate that environmental factors have a highly significant effect on the expression of reproductive traits.

Bivariate analysis

Estimates of correlations are presented in Table 4. The estimate of genetic correlation between litter traits was positive in sign, high in magnitude, despite the traits having low heritability; which is consistent with the studies of *Rashidi et al. (2011)* and *Mohammadi et al. (2012).* Lower values were reported by some

researchers *(Vatankhah et al., 2008; Mokhtari et al., 2010; Amou Posht-e- Masari et al., 2013).* Obtained negative genetic- and phenotypic correlations of both litter traits with litter mean weight traits indicate that lambs born as single tend to be heavier than twins and is an indication that selection for large litter size would be accompanied by a reduction in litter mean weight traits. Positive and high genetic correlations were observed between litter traits with composite traits, similar to those of Zandi sheep reported by *Mohammadi et al. (2013).* These findings could be explained by the fact that the ewes with more number of lambs born in each litter would have a heavier total weaning weight and indicate that indirect selection for each trait will cause an improvement in the other traits. The genetic correlation estimates between litter mean weight traits and composite traits were 0.83, 0.93 and 0.41, respectively, showing that the ewes having lambs with heavier mean birth weight are likely to produce more TLWB and TLWW. As TLWB has high genetic correlation with other reproductive traits (Table 4), selection for reproductive traits could be performed through it. The high genetic correlation (0.82) between composite traits showed that genes controlling the litter size and their weight at birth might control milk production and mothering ability of dams from birth to weaning, also. Similar results were obtained by the studies of *Vatankhah et al. (2008); Mohammadi et al. (2013)* and *Amou Posht-e- Masari et al. (2013).*

Table 4. Correlation estimates among reproductive traits

Trait[a] 1	Trait 2	r_{g12}[b]	r_{p12}	r_{pe12}	r_{e12}
LSB	NLAW	0.71	0.12	0.73	0.25
LSB	LMWLB	-0.45	-0.08	0.77	0.22
LSB	LMWLW	-0.82	-0.24	0.82	0.06
LSB	TLWB	0.94	0.52	0.95	0.14
LSB	TLWW	0.81	0.36	0.84	0.09
LSW	LMWLB	-0.64	-0.33	0.28	0.07
LSW	LMWLW	-0.31	-0.35	0.34	0.22
LSW	TLWB	0.37	0.15	0.85	0.04
LSW	TLWW	0.87	0.36	0.77	0.22
LMWLB	LMWLW	0.83	0.08	0.79	0.05
LMWLB	TLWB	0.93	0.25	0.93	0.12
LMWLB	TLWW	0.41	-0.06	0.84	0.12
LMWLW	TLWB	0.46	0.17	0.66	0.07
LMWLW	TLWW	0.51	0.11	0.86	0.13
TLWB	TLWW	0.82	0.34	0.81	0.31

r_{g12}: genetic correlation between trait 1 and trait 2, r_{p12}: phenotypic correlations between traits 1and 2, r_{pe12}: permanent environmental correlations between traits 1and 2, r_{e12}: environmental correlations between traits 1and 2.
LSB: Litter size at birth, LSW: litter size at weaning, LMWLB: litter mean weight per lamb born, LMWLW: litter mean weight per lamb weaned, TLWB: total litter weight at birth, TLWW: total litter weight at weaning

Low- and negative (in some cases) phenotypic correlations between studied traits were observed. Permanent environmental correlations between traits were positive and medium to high, but higher than genetic correlations, generally. The main objective of selection is to produce heavier lambs at weaning for the sheep meat industry. To reach this aim, the selection index should include litter traits and composite traits. Similar correlations for Moghani and Zandi sheep breeds were reported by *Rashidi et al. (2011)* and *Mohammadi et al. (2013)*, respectively.

Estimation of genetic gain

Predictions of breeding values mean for litter traits of Arabi sheep in each year of birth are illustrated in Figs. 1 and 2. Also, estimations of realised annual genetic progress for the traits are demonstrated in Table 5. Fluctuations were observed during the 8-year period, but annual genetic trend of LSB (0.00025 lambs) was insignificant.

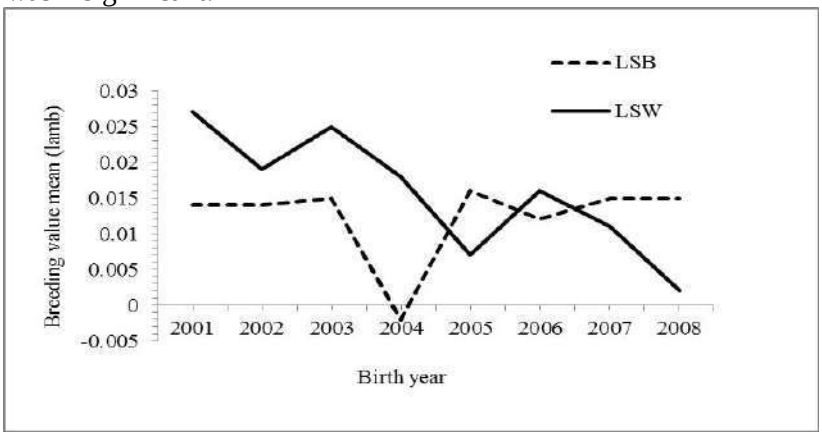

Fig. 1. Predictions of breeding values mean for litter traits of Arabi sheep by year of birth

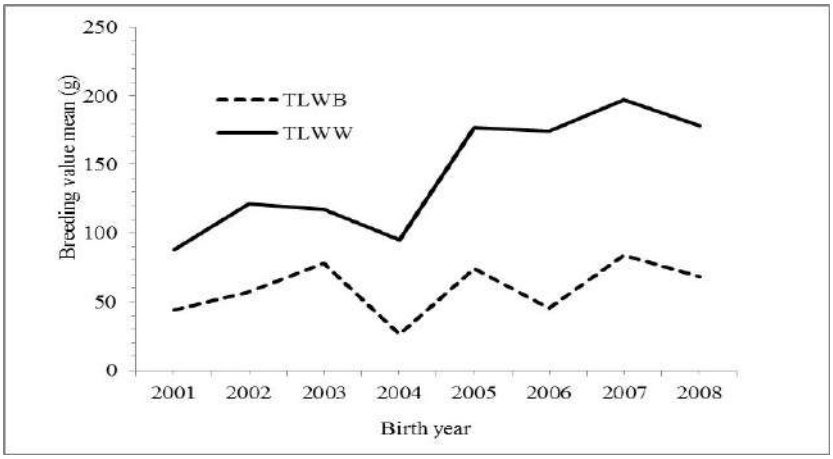

Fig. 2. Predictions of breeding values mean for composite traits of Arabi sheep by year of birth

This finding was compatible with the studies of *Vatankhah et al. (2007)* and *Savar Sofla et al. (2010).* Our estimate for annual genetic trend of LSW was negative and significant (-0.003 lambs. In contrast, an insignificant genetic trend was found by *Vatankhah et al. (2007).* However, *Hanford et al. (2002; 2005)* estimated the annual genetic trends of LSW as 0.3 and 0.4 head per year, for Columbia- and Rambouillet sheep breeds, respectively. In Fig. 2, substantial fluctuations were observed in annual genetic trend of composite traits. Positive and insignificant annual genetic trend was observed for TLWB (3 g), opposite to the study of *Savar Sofla et al. (2010).* Genetic trend varied from 0.5 to 3 per cent of phenotypic mean through selection within-breed in each year *(Smith, 1984).* In accordance with the aforementioned literature, annual genetic trend of TLWW should have become between 100 g to 600 g. Nonetheless, our estimate of genetic trend for TLWW (15.0 g) was higher than reported by *Savar Sofla et al. (2010).* There are few reports to compare the genetic trend of reproductive traits.

Table 5. Annual genetic, phenotypic and environmental trends for reproductive traits

reproductive traits	GT ± S.E.	R^2 (%)	PT ± S.E.	R^2 (%)	ET ± S.E.	R^2 (%)
LSB (lamb)	0.00025 ± 0.001 ^{ns}	1.0	0.007 ± 0.01 ^{ns}	5.8	0.007 ± 0.01 ^{ns}	4.8
LSW (lamb)	-0.003 ± 0.0007 *	73.8	0.007 ± 0.01 *	79.3	0.01 ± 0.01 ^{ns}	11.3
TLWB (g)	3 ± 3.1 ^{ns}	13.5	25.5 ± 72.0 ^{ns}	2.0	22.5 ± 74.0 ^{ns}	1.5
TLWW (g)	15 ± 3.6 *	74.5	-216.0 ± 267.4 ^{ns}	9.8	-231.1 ± 265.3 ^{ns}	11.2

GT: genetic trend, PT: phenotypic trend, ET: environmental trend, R^2: coefficient of determination, *: significant effect at $p < 0.05$, ^{ns}: non-significant ($p > 0.05$)
LSB: Litter size at birth, LSW: litter size at weaning, LMWLB: litter mean weight per lamb born, LMWLW: litter mean weight per lamb weaned, TLWB: total litter weight at birth, TLWW: total litter weight at weaning

Phenotypic least squares mean for reproductive traits are portrayed in Figs. 3 and 4 by year of birth. Phenotypic trend was only significant for LSW (0.007

lamb per year). Nevertheless, negative- and insignificant phenotypic trend was reported by *Savar Sofla et al. (2010)*. In contrast to our finding, phenotypic trend of LSB and composite traits were significant for Moghani sheep *(Savar Sofla et al., 2010).* Annual environmental trends were non-significant for all traits.

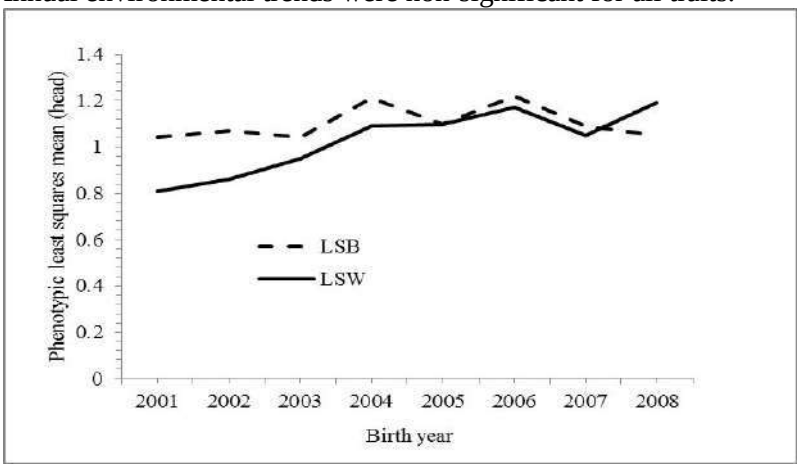

Fig. 3. Phenotypic least squares mean for litter traits of Arabi sheep by year of birth

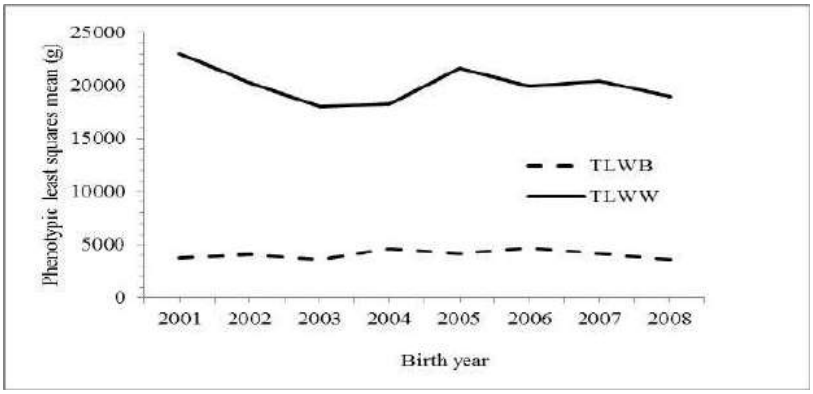

Fig. 4. Phenotypic least squares mean for composite traits of Arabi sheep by year of birth.

Conclusions

Low heritabilities for litter traits were found and might be partly attributed to their discontinuous distribution. A high coefficient of variation for LSW was found, suggesting that high selection differentials could be achieved in effective breeding programs. The genetic correlations between litter traits with composite traits were positive and moderate to high, indicating that selection would be done based on such traits. The insignificant or low genetic trends indicate that selection for the traits studied has been unsuccessful in Arabi sheep in recent years. There is

room to improve the breeding program for Arabi sheep based on the genetic parameters estimated in this study.

Genetski parametri i genetski napredak reproduktivnih osobina arabi ovaca

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Rezime

Aktuelna studija, po prvi put, izveštava o genetskim parametrima i genetskim, fenotipskim i ekološkim korelacijama i trendovima reproduktivnih osobina ovaca rase arabi. Podaci su prikupljeni u Istraživačkoj stanici Khuzestan Ramin Univerziteta poljoprivrednih i prirodnih nauka (Animal Science Research Station of Khuzestan Ramin Agricultural and Natural Resources University - ASRSKRANRU), jugozapadno od Irana, u periodu od 2001. do 2008. godine. Veličina legla na rođenju (LSB), veličina legla na zalučenju (LSV), srednja masa legla po rođenom jagnjetu (LMVLB), srednja masa legla po zalučenom jagnjetu (LMVLV), ukupna težina legla na rođenju (TLVB) i ukupne težine legla na zalučenju (TLVV) u proseku su bile 1,11 jaganjadi, 1,01 jagnjadi, 3,83 kg, 19,43 kg, 4,16 kg i 20.12 kg, respektivno. Genetski parametri i korelacije su ocenjeni korišćenjem univarijatnog i bivarijatnog modela koji koriste ograničenu maksimalnu verovatnoću, priplodne vrednosti su procenjene korišćenjem BLUP-a i genetskih i fenotipskih trendova regresijom prosečnih priplodnih vrednosti ovaca i fenotipskih srednjih vrednosti najmanjih kvadrata u godini rođenja respektivno. Slučajnim uticajima su dodati aditivni direktni uticaj gena i stalnog okruženja ovaca, kao i uticaj oca, pored fiksnih uticaja starosti ovaca na jagnjenju i godine jagnjenja. Procena heritabiliteta od 0,05; 0,02; 0,13; 0,12; 0,04 i 0.06, i ponovljivosti od 0,08; 0,06; 0,17; 0,16; 0,14 i 0,21 za šest osobina, respektivno. Genetske korelacije između osobina su bile u rasponu od -0,82 do 0,94. Fenotipske korelacije su bile niže, u rasponu od -0,33 do 0,52. Procenjen godišnji genetski napredak je bio veoma nizak: -0.003 jagnjadi za LSV i 15 g za TLVV. Godišnji fenotipski trend je bio značajan samo za LSV, 0,007 jagnjadi. Zaključak istraživanja je da bi indirektna selekcija na osnovu ukupne težine legla na odbijanju mogla biti efikasna u slučaju ispitivanih osobina.

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GENOTYPE, SEX AND INTERACTION EFFECT ON LAMB GROWTH TRAITS

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Original scientific paper

Abstract: The pure breeds and crossing have an important role in production. It is essential in sheep meat production to maintain the genetic diversity of the adapted breeds, pure breeds and their crosses. Objective of the study is to determine the impact of genotype and sex on growth traits of lambs. Results of the study showed that male of all genotypes were dominant on body weight in all ages (from BWB to BW90). The highest birth weight (BWB) got male of genotype 2 (W). The lowest body weight at birth was the female lambs of genotype 1 (P). Body weights at ages 30, 60 and 90 days, male and female of genotype 4 (PxWxF) were dominant among other genotypes. Genotype 1 (P) of both sexes had the lowest bodyweights in all ages (BWB, BW30, BW60, BW90). The result showed better growth efficiency from males compared with females. The results of fixed factors and its impact on body weights of lambs showed very significant effect of genotype ($P<0.01$) on body weights of lambs at birth (BWB), ages 30, 60 and 90 days (BW30, BW60, BW90). The effect of sex had highly significant effects ($P<0.001$) on all ages. The interaction between genotype x sex showed a very significant effect ($P\leq0.01$) on body weight at birth (BWB) but were not significant ($P\geq0.05$) on body weights of lambs at ages BW30, BW60 and BW90. Superiority on growth traits of genotype 4 (PxWxF) at ages 30, 60 and 90 days, indicating that three-bred crossing resulted in high growth traits.

Key words: genotype, sex, interaction effect, growth traits, body weights, lamb

Introduction

Breed differences in performance characteristics are important genetic resources for improving efficiency of sheep meat production *(Demeke et al., 2004)*. Breed diversity is a valuable resource for sheep industry. Pure breeds and crossings

have significant role in production *(Pajor et al., 2009)*. Crossbreeding systems utilize breed diversity to increase productivity comparable to purebred flocks *(Petrovic et al., 2011; Fathala et al., 2014)*. In sheep meat production, it is essential to maintain the genetic diversity of the adapted breeds as well as the pure breeds and their crosses with recognized meat breeds *(De Vargas et al., 2014).*

The growth of lambs during the periods from birth to weaning is particularly highly conditioned by the genotype, accessible food-milk, hay, concentrate, in other words the ambience in which the young organism develops *(Bathaei and Leroy, 1998; Burfening and Kress, 1993; Gardner et al., 2007; Caro Petrovic et al., 2013*; *Ilic et al., 2013).* The bodyweight at birth had determined by numerous factors, genetic and paragenetic nature *(Petrovic et al., 2009).*

A great number of different factors influence the growth of lambs while nutrition, health condition and genotype belong to the most important ones. Other factors that can influence the growth ability of lambs to a greater or lesser extent are for example sex, litter size, month or season of lambing, age of dam and year of lamb birth *(Kuchtík and Dobeš 2006).* Sex hormones also influence the growth pattern of lambs **(***Cloete et al., 2012).* The growth advantage of male lambs is attributed to the presence of testicular hormones, particularly testosterone *(Scanbacher et al 1980*; *Cloete et al., 2012*).Good mothering ability, easy lambing, high twinning rate is required from the ewes, nevertheless the mothers should be able to produce milk of sufficient quantity and quality, in order to achieve good lamb growth *(Csizmar et al., 2013).*

Considering the above mentioned the aim of this study is to evaluate the influence of genotype and sex on growth traits of lambs.

Material and Methods

The research was executed in the region of Stara Planina Mountain and at the experimental farm of the Institute for Animal Husbandry. In the evaluation of the growth traits, data from 200 heads per genotype were utilized (100 male, 100 female lambs) of the following:

- Purebred: Pirot Pramenka (P) as genotype 1, Wurtemberg (W) as genotype 2
- Crossbred F1 generation: Pirot x Wurtemberg (PxW) as genotype 3, (Pirot x Wurtemberg) x Il de France (PxWxF) as genotype 4.

All the lambs had weighed at birth and every 30 days intervals thereafter. For the determination of lambs' growth traits, the body weights considered for this study were: the weight at birth (BWB), at the age of 30 days (BW30), 60 days (BW60) and at 90 days (BW90) of the above mentioned genotypes.

The lambs was driven by technology two times short suckling milk with their mothers during the day and also supplemented with alfalfa hay including a

concentrate mixture for lambs with 18% protein. Feeding of lambs had been ad libitum up to 90 days of age.

Statistical analysis was performed by the GLM procedure of Statistical Package for the Social Sciences (SPSS) version 20.

Results and Discussion

Means and standard errors of body weights (BW) according to genotype of lambs are presented in Tables 1 and 2. The results of this study showed that male of all genotypes were dominant on body weight in all ages (from BWB to BW90). In table 1, the highest birth weight (BWB) got male of genotype 2 with the following differences: 1.09 kg - 1.20 kg; 0.49 kg - 0.75 kg; 0.31 kg – 0.57 kg from male and female of genotypes 1; 3; 4 and a difference of 0.62 kg from female of same genotype. Male of genotype 4 got the second place on BWB followed by male of genotype 3 and female lambs of genotypes 4, 2 and 3. The lowest body weight at birth, the female lambs of genotype 1.

Table 1. Mean values of purebred and crossbred lambs' body weight at birth and 30 days, kg

Genotype	Sex	BWB		BW30	
		Mean	±SE	Mean	±SE
P (1)	Male	3.70	0.05	9.71	0.15
	Female	3.59	0.04	9.24	0.17
W (2)	Male	4.79	0.12	11.27	0.23
	Female	4.17	0.09	10.39	0.24
PxW (3)	Male	4.30	0.07	11.13	0.16
	Female	4.04	0.05	10.84	0.15
PxWxF (4)	Male	4.48	0.06	13.32	0.15
	Female	4.22	0.05	12.43	0.12

Table 2. Mean values of purebred and crossbred lambs' body weight at 60 days and 90 days, kg

Genotype	Sex	BW60		BW90	
		Mean	±SE	Mean	±SE
P (1)	Male	15.48	0.16	22.68	0.33
	Female	14.50	0.19	21.25	0.32
W (2)	Male	19.59	0.35	28.16	0.20
	Female	18.62	0.40	27.24	0.21
PxW (3)	Male	18.79	0.29	26.88	0.33
	Female	18.28	0.31	26.22	0.27
PxWxF (4)	Male	22.65	0.39	33.11	0.29
	Female	21.37	0.31	31.28	0.24

Looking at the body weights at 30, 60 and 90 days of age, male and female of genotype 4 were dominant among other genotypes (table 2). Leading on body weights at ages 30, 60 and 90 days, the male lambs' of genotype 4. The differences on body weights with other genotypes (1, 2, 3) of both sexes (male-female) at age 30 days were: 3.61 kg - 4.08 kg, ; 2.05 kg – 2.93 kg, 2.19 kg – 2.48 kg. The difference from female of the same genotype was 0.9 kg. At age 60 days were 7.17 - 8.15kg, 3.06 – 4.03 kg, 3.86 -4.37 kg and a difference of 1.28 kg with female of same genotype.

At 90 days of age, the differences were 10.43- 11.86 kg, 4.95-5.87 kg, 6.23- 6.89 kg with female of same genotype a difference of 1.83 kg.

In tables, 1 and 2 indicating that genotype 1 (P) of both sexes had the lowest body weights in all ages (BWB, BW30, BW60, BW90), which is in agreement with results obtained by *Petrovic et al., (2011).*

It can be noticed as well that the ranking of lambs' genotype were consistent with the levels of heterozygosity. The result of *Mohammadi et al, (2010); De Vargas et al., (2014),* showed better growth efficiency from males compared with females was in accordance with the result we had obtained. Relevant with our result was that an additional remark by *Mohammadi et al, (2010),* the differences in sexual chromosomes probably in the position of genes related growth physiological characteristics, difference in endocrinal system (type and measure of hormone secretion especially sexual hormones) lead to difference of animal growth.

Table 3. Results of testing fixed factors and its impact on the body weight of lambs

Factor	Traits	Sum squares	df	Average squares	F	P
Genotype	BWB	79.866	3	26.622	49.015	0.000**
	BW30	1169.734	3	389.911	122.051	0.000**
	BWB60	4980.492	3	1660.164	172.392	0.000**
	BWB90	10611.933	3	3537.311	457.148	0.000**
Sex	BWB	19.406	1	19.406	35.730	0.000**
	BW30	79.493	1	79.493	24.883	0.000**
	BWB60	173.893	1	173.893	18.057	0.000**
	BWB90	292.457	1	292.457	37.796	0.000**
interaction genotype x sex	BWB	6.674	3	2.225	4.096	0.007**
	BW30	13.414	3	4.471	1.400	0.242n.s.
	BWB60	15.323	3	5.108	.530	0.662n.s.
	BWB90	41.044	3	13.681	1.768	0.152n.s.

**- very significant (P≤0.01) *- significant (P≤0.05) n.s.- not significant (P≥0.05)

The results of fixed factors and its impact on body weights of lambs (table 3), showed very significant effect of genotype ($P<0.01$) on body weights of lambs at birth (BWB), ages 30, 60 and 90 days (BW30, BW60, BW90). *Freking and Leymaster (2004); Kuchtík et al., (2010);* Caro *Petrović et al., (2012),* reported a significant effect of genotype on growth traits of lambs are amenable with our results. An agreeable result also found by *El-Fadilli et al. (2000); Margetín et al. (2004),* who reported that the genotype significantly influenced the majority of growth traits. *Unal et al., (2006),* had an opposite result with ours, they ratified in their study that lamb genotype had no significant effect on live weights of lambs at birth and 90 days of age. Another contrary result found by *Kuchtic and Dobes, (2006)* emphasizing that the evaluation of the effect of genotype on growth showed that this factor did not have a significant effect on the majority of growth traits. In the study of the following authors: *Burke et al. (2003);Karaoglu et al.(2002); Momani et al. (2010); Fathala et al., (2014),* they reported that growth performance (body weight) significantly differ among genotypes of sheep and that the desirable crossbreeding effects might be due to heterosis and breed complementarity. The recently statements rationalized the result of our study. Likewise, supporting our study was that with *Demeke et al., (2004),* informing the effect of genotype was an important source of variation for body weight at all ages and that sex influenced bodyweight at birth.

In our study regarding effect of sex (table 3), was in complement with the result achieved by *Unal et al., (2006),* that sex had highly significant effects ($P<0.001$) on all ages. *Gökdal et al., (2004)* reported in their study that sex of lamb affected lamb weights at the various stages. The same authors commented that the effect of sex on live weight might be attributing to different physiological functions in the two sexes.

Regarding the interaction between genotype x sex (table 3), showed a very significant effect ($P\leq0.01$) on body weight at birth (BWB) but were not significant ($P\geq0.05$) on body weights of lambs at ages BW30, BW60 and BW90.

Conclusion

The study results showed highly significant effect of genotype on lambs' body weights at birth, at ages 30, 60 and 90 days. Male lambs of all genotypes showed better growth efficiency compared with females. There were highly significant effects of sex on all ages. Likewise obtained result showed that the interaction between genotype x sex showed a very significant effect on birth weight.

Based on the results as heavier lambs in both sexes of genotypes 4 showed superiority on growth traits at ages 30, 60 and 90 days, it served as an indicator that three-bred crossing resulted in high growth traits could be a showcase for farmers to realize the important role of system of crossing in sheep industry.

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Efekat genotipa, pola i njihovih interakcija na osobine porasta jagnjadi

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Rezime

Metode odgajivanja imaju važnu ulogu u ovčarskoj proizvodnji. Od suštinskog značaja u proizvodnji mesa ovaca je održavanje genetske raznovrsnosti i varijabilnosti populacija. Cilj ovih istraživanja je da se utvrdi uticaj genotipa i pola, kao i njihovih interakcija na osobine porasta jagnjadi. Rezultati studije pokazali su da muški pol svih genotipova je dominantan u pogledu mase tela u svim uzrastima (od BWB do BW90). Najveća telesna masa (BWB) bila je kod muških grla genotipa 2 (W). Najnižu telesnu masu na rođenju, imala su ženska jagnjad genotipa 1. Masa tela pri uzrastu od 30, 60 i 90 dana, kod oba pola je bila dominantna kod genotipa 4 (PxWxF). Genotip 1 (P)- oba pola, imao je najniže vrednosti masa u svim uzrastima (BWB, BW30, BW60, BW90). Rezultati istraživanja su pokazali bolju efikasnost rasta kod muških u odnosu sa ženska grla. Rezultati fiksnih faktora i njihovog uticaju na telesnu masu jagnjadi pokazali su veoma značajan efekat genotipa ($P < 0,01$) pri rođenju (BWB), kod uzrasta 30, 60 i 90 dana (BW30, BW60, BW90). Pol je imao visoko značajne efekte ($P < 0,001$) kod svih uzrasta. Interakcija između genotipa i pola pokazala je veoma značajan efekat ($P \leq 0.01$) na telesnu masu pri rođenju (BWB), ali dobijene vrednosti razlika nisu bile značajne ($P \geq 0.05$) u dobi BW30, BW60 i BW90. Superiornost genotipa 4 (PxWxF) pokazala se u uzrastu od 30, 60 i 90 dana, što može biti izazov i praktična korist za farmere koji koriste ukrštanje.

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FATTY ACID COMPOSITION OF MILK FAT IN MILK OF TZIGAY SHEEP AND THEIR F2 CROSS-BREEDS WITH CHIOS

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Abstract: The study was conducted on aggregate milk samples, which were taken every month during the milking period from Tzigay sheep and their F2 cross-breeds of Chios, raised in the conditions of the Central Balkan Mountain. The fat extraction of milk samples was done by the Rose-Gottlieb method. Fatty acid composition was determined on a gas chromatograph with flame ionization detector and capillary column. The aim of the study was to follow the changes in the composition of fatty acids in the milk fat of milk of Tzigay sheep and their F2 cross-breeds. The saturated fatty acids in milk of the two groups had high values during both consecutive years, as they varied from 67.05% in milk of Tzigay sheep in the second lactation up to 70.87% at their F2 cross-breeds. The content of myristic acid was correspondingly 8.22-8.88% at Tzigay sheep and 8.45-8.74% at their F2 cross-breeds. The total amount of polyunsaturated fatty acids in the examined milk for the two types of sheep was comparatively low with near concentrations (4.39-5.20%) in the period of the two years. The milk of the two groups had high values of the correlation SFA/PUSFA (15.71 and 13.17) and low values of PUSFA/SFA (0.06-0.08). Monounsaturated fatty acids, represented mainly by the oleic acid (C18:1) varied during both periods from 21.92% to 25.32% and appeared as a substratum in the synthesis of CLA. The short-chain fatty acids (C4:0-C11:0) had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids (C17iso-C25:0) maintained close concentration in the milk of Tzigay breed, while their content in the milk of F2 cross-breeds was increased.

Key words: sheep milk, milk fat, fatty acids

Introduction

For many years fatty acids in milk and milk products were associated with raised serum cholesterol, obesity and illness *(Talpur, 2007)*. Extensive researches

on the effects of various fatty acids on human health indicate that only a few fatty acids are responsible for the negative effects on the health of consumers *(Simopoulos, 2002)*. Some saturated fatty acids as lauric, miristic and palmitic acids may have effect on the total cholesterol but only C16:0 has a proven impact of raising coronary heart diseases *(Williams, 2000)*. Research however is not focused only on the negatives of the fatty acid profile of milk. It contains unsaturated fatty acids including conjugated linoleic acid (CLA), which can reduce total cholesterol, exert anticarcenigenic and antidiabetic activity and has immunomodulating effect *(Mills et al., 2011)*. It is important to compare quantitative contents of saturated and unsaturated fatty acids, by calculating the atherogenic index recommended by *Ulbright and Southgate (1991)*. Another way for estimating fatty acid composition is the ratio omega 6/omega 3, which has antagonistic physiological functions necessary for the human body *(Simopoulos, 2002)*.

Many studies have focused on the various factors affecting the content of the different fatty acids in milk – breed, nutrition, stage of lactation and others. *Signorelli et al. (2008)*, found that there were no differences in the content of conjugated linoleic acid (CLA) and polysaturated fatty acids in local Italians breeds, while differences were reported in monounsaturated ones. In studying the composition of milk fat in sheep of Churra breed, *Sanchez et al. (2010)* found a low heritability in saturated and monounsaturated fatty acids and a potential for genetic variations in polyunsaturated fatty acid. Studying the fatty acid profile of four sheep breeds (Avasi, Lakaune, Friesland and Chios), *Tsiplakou et al. (2008)* proved that the breed had not influenced the fatty acid region of the Central Balkan Mountains profile. The same author found that when using pastures that led to lower correlations of saturated and higher shares of unsaturated fatty acids.

The aim of the study was to follow out the changes in the milk fatty acid profile during the two consecutive lactating periods of Tzigay sheep and their F2 cross-breeds of Chios breed, raised in the conditions of the Central Balkan Mountain, and to determine the value of the atherogenic index and ratio omega 6/omega 3 as indicators for assessment of the functional qualities of the milk.

Materials and Methods

The study was conducted on aggregate milk samples, which were taken from Tzigay sheep and their F2 cross-breeds of Chios breed, raised in the conditions of the Central Balkan Mountain. The milk samples were taken once a month during the milking period at the time of the three milk controls. The milk was analysed during the May-July period, which covered the grazing raising of sheep on mountain pastures, and after June on high-mountain ones. The samples for analysis were taken from the total milk quantity obtained from 12 sheep in groups at second lactation.

The extraction of fat from the milk samples was performed in the laboratory of Dairying Department at the Agrarian Faculty of the Thracian

University, city of Stara Zagora by the method of Röse-Gottlieb. The methyl esters of the fatty acids were separated by a gas chromatograph. Fatty acid composition was determined on a gas chromatograph 'Pay-Unicam 304' with flame ionization detector and capillary column ECTM-WAX, 30 m, ID 0.25 mm, Film: 0.25μm.

Atherogenicity index was calculated as the content ratio of SFA/unsaturated FA using the following formula proposed by *(Ulbricht and Southgate, 1991)*:

$$IA = \frac{4xC14:0 + C16:0 + C18:0}{\sum MUFA + \sum PUFA}$$

The data was processed in a variance statistical way through Statistica for Windows (Release. 4.3. stat. soft. Inc., 1994), and the average values were compared according to the tables of t-test of Student-Fisher.

Results and Discussion

Table 1 shows the results for the content of saturated fatty acids in milk of Tzigay sheep and their F2 cross-breeds. Close values were observed for butyric acid (C4:0), the highest (5.30) was in cross-breeds in the second lactation ($p>0.05$).

Table 1. Saturated fatty acids, g/100 g (n=3)

Fatty acids	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
C4:0	4.66	0.19	4.40	0.31	4.23	0.67	5.30	0.68
C6:0	4.58*	0.30	3.07	0.46	3.31	0.36	3.55	0.54
C7:0	0.29	0.03	0.24	0.12	0.25	0.01	0.44	0.16
C8:0	3.22	0.83	3.40	0.48	3.41	0.74	3.55	0.40
C9:0	0.14	0.04	0.14	0.06	0.33	0.13	0.31	0.11
C10:0	10.63**	0.52	9.62*	1.17	7.34	0.36	6.79	0.17
C12:0	3.47	0.33	3.25	0.06	2.36	1.14	3.59	0.67
C13:0	-	-	0.5	-	0.74	0.30	0.63	0.27
C14:0	8.22	0.20	8.88	0.40	8.74	0.13	8.45	0.23
C15:0	0.65	0.23	0.72	0.34	0.89	0.35	0.83	0.19
C16:0	24.28	1.20	22.87	0.96	23.40	0.61	25.73	3.05
C17:0	0.86	0.10	0.86	0.10	1.74***	0.04	1.66***	0.25
C18:0	8.01	0.27	9.10	0.82	10.62*	1.21	10.04*	1.48

*p<0.05; ** p<0.01; *** p<0.001

The concentration of caproic acid (C6:0) was the highest in the first lactation of Tzigay sheep (at $p<0.05$) and the lowest in the second lactation ($p<0.05$) – 4.58 and 3.07, respectively. This is probably due to the difference of the

feeding during the consecutive years**.** In enanthic acid (C:7) and nonylic acid (C:9) the values were low and without significant differences among the experimental groups and years. The level of caprylic acid (C8:0) in both breeds varied in the range 3.22-3.55 at $p>0.05$. In C10:0 the results were higher in the groups of Tzigay sheep in comparison with the cross-breeds, respectively with reliability $p<0.01$ for the first lactation (10.63 vs 7.34) and $p<0.05$ for the second lactation (9.62 vs 6.79), which is probably due to the breed effects ($p>0.05$).

Close values were measured in lauric acid (C12:0) for Tzigay sheep, with some variation in the F2 cross-breeds – from 2.36 in the first lactation to 3.59 in the second lactation. Traces of C13:0 were registered in Tzigay sheep, while in cross-breeds the content of that acid was minimal. The content of myristic acid (C14:0) in milk, had almost the same concentration in all groups of sheep. It was close to the values in the milk of Karakachan breed found by *Mihaylova et al., (2008)* (6.55+0.29 to 10.11+0.29) and lower than that in Srednostaroplaninska breed *(Gerchev et al., 2011)* (9.09+0.64 to 12.29+0.77).

The saturated fatty acids with an odd number of carbon atoms – C15:0 and C17:0 had higher levels in cross-breeds, with differences registered only in the last fatty acid ($p<0.001$). The palmitic acid (C16:0) was with the highest values in all of the four groups of sheep but no reliable differences were reported between the separate years. In stearic acid (C18:0), which determines to a certain degree the hardness of fat and its melting point, the tendency was similar to margaric acid (C17:0). Higher content of that fatty acid in the milk of cross-breed sheep was reported for first lactation with reliable difference $p<0.05$. The concentrations of saturated fatty acids C16:0, C17:0 and C18:0, in sheep milk of Tzigay breed and their cross-breeds were comparatively higher than those of Karakachanska breed *(Mihaylova, 2008),* respectively 21.02+0.49, 0.90+0.03 и 9.24+1.19 and lower, compared to the results for milk of Srednostaroplaninska breed *(Gerchev et al., 2011),* respectively 26.24+0.74, 2.27+0.19 and 13.03+0.10.

The content of unsaturated fatty acids C10:1, C12:1 and C14:1 in the milk fat in both groups was low with close values between the groups in the milking period of both lactations (Table 2).

Table 2. Unsaturated fatty acids (n=3)

Fatty acids	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
C10:1	0.23	0.03	0.39	0.08	0.23	0.03	0.39	0.08
C12:1	0.13	0.08	0.24	0.06	0.13	0.06	0.24	0.05
C14:1	0.28	0.08	0.41	0.13	0.28	0.08	0.41	0.13
C16:1	0.69***	0.05	0.45	0.16	0.54	0.20	0.33***	0.07
C18:1	25.32	0.90	21.92	1.71	25.32	0.90	21.92	1.71
C18:2	3.04	0.07	3.29	0.26	3.04	0.07	3.29	0.26
C18:3	1.35	0.16	1.80	0.09	1.50	0.02	1.91	0.35

*** $p<0.001$

The concentration of palmitic acid (C16:1) in the examined milk was higher in Tzigay breed in both lactations. The difference was significant at p <0.001 only between the group of Tzigay sheep at the first lactation and the group of cross-breeds at the second lactation. That was due to the feeding up of the animals in the beginning of the grazing period in the first lactation. The content of oleic acid (C18:1) in milk fat had close values and unidirectional tendencies (lower level at second lactation) in the period of lactation of sheep during both years. Typical for oleic acid (C18:1) is the high percent (particularly of some isomers) in the beginning of the grazing period. Many studies on milk, obtained during the grazing period *(Atti et al., 2006; Tsiplakou et al., 2006)* reported a positive relation between the concentration of C18:1 in milk fat and the amount of CLA, where C18:1 was a substrate in the synthesis of the latter.

In polyunsaturated fatty acids – linoleic (C18:2) and linolenic acid (C18:3) an increase was reported during the second lactation, which was unidirectional for both groups of sheep. We should mention that their values in milk fat depend mainly on feeding of animals, because they are not synthesized in their organism, as their lack cause a series of biological disorders in the organism. The found concentration of these two acids in the milk of Tzigay and their F2 cross-breeds was higher than this in Karakachan and Srednostaroplaninska sheep raised in the conditions of the Balkan Mountain *(Mihaylova et al., 2008; Gerchev et al., 2011).*

The distribution of fatty acids in groups is shown in Table 3. The total amount of saturated fatty acids (SFA) during period of lactation was comparatively high, but in different directions and close values during the corresponding years.

Table 3. Groups of fatty acids in milk from sheep (n=3)

Fatty acid groups	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
Σ SFA	69.01	4.24	67.05	5.28	67.36	6.05	70.87	8.20
Σ MUFA	26.65	1.14	23.41	2.14	26.50	1.54	23.29	2.04
Σ PUFA	4.39	0.23	5.09	0.35	4.54	0.09	5.20	0.61
Σ C4:0-C11:0	23.52	1.91	20.87	2.60	18.87	2.27	19.94	2.06
Σ C12:0-C16:1	37.95	2.20	37.71	2.19	36.71	2.90	40.60	4.74
Σ C17iso-C25:0	37.58	1.50	36.97	2.98	42.82*	2.21	38.82*	4.05

* p<0.05

The high level of SFA in milk of Tzigay sheep and their cross-breeds corresponded with low values of MUSFA. The amount of monounsaturated fatty acids (MUSFA) was higher in the first year of lactation and decreased in next year in both groups of sheep. While in the content of polyunsaturated fatty acids (PUSFA) was reported the opposite tendency of lower content of the first lactation and increase in the next

one. The high values of the ratio SFA/PUSFA in both years (15.71 and 13.17 in milk of Tzigay sheep and 14.83 and 13.62 in their F2 cross-breeds) showed good acidic stability in milk, which was better expressed in milk of Tzigay sheep. MUSFA have a preventive action against coronary and cardio-vascular diseases and the action of PUSFA is analogous, but they are more unstable to oxidation due to their greater non-saturation. PUSFA protect the membranes of cells from the oxygen radical almost as much as tocopherol and less than carotene.

The short-chain fatty acids had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids kept close concentrations in the milk of Tzigay sheep, while their content in the milk of F2 cross-breeds at the 1st lactation was higher compared to milk of Tzigay sheep at the 2nd lactation in reliability of the difference ($p<0.05$).

The biologically important ratio of PUSFA/SFA, or the so-called P/S ratio, in the sheep milk was low and varied in narrow limits – from 0.06-0.08 in Tzigay sheep to 0.7 in cross-breeds of Chios. The low values of these ratios showed that the breed had not had any significant influence over the content of particular acids, in case of grazing in one herd.

One of the criteria for evaluating the preventive value of milk is atherogenic index and the ratio omega 6/omega 3. Foods with a high index and ratio are considered to be harmful to human health *(Tsipakou and Zervas, 2008).*

Milk fat is usually considered to be proatherogenic, mainly because of the presence of a large amount of saturated fatty acids (mainly lauric, myristic and palmitic acids). The atherogenic index is a criterion for the level and interrelation of some fatty acids that may have atherogenic properties *(Mierlita, 2011).*

In this study, the atherogenic index was within 2.1-2.4 for Tzigay breed and 2.2-2.7 for cross-breeds of Chios (Figure 1). Our data were lower and close to those obtained by other authors for sheep milk *(Mierlita, 2012; de Renobles et al., 2012).*

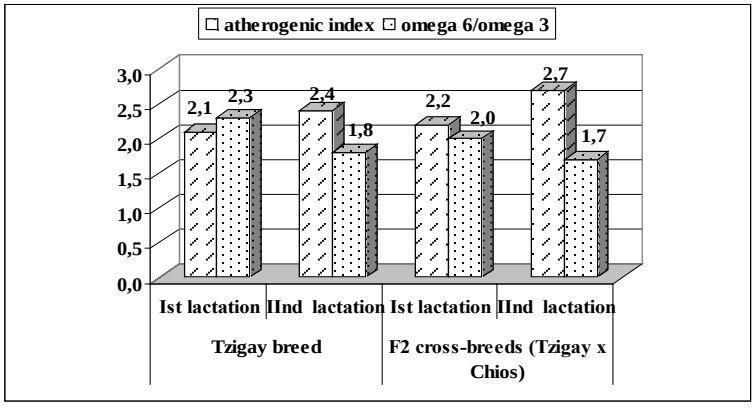

Figure 1. Atherogenic index and ratio omega 6/omega 3 of sheep milk

The ratio of omega 6/amega 3 fatty acids was not significantly different for both breeds over the years – respectively 2.3:1 for Tzigay breed and 2:1 for the cross-breeds of the first lactation and 1.8:1 for Tzigay and 1.7:1 for the cross-breeds of Chios of the second lactation. These data were very close to the ratio omega 6/amega 3 recommended for human health – from 2:1 to 4:1 *(Sretenovic et al., 2009)* and comparable to those reported by other authors for sheep milk *(Mierlita et al., 2011).*

Conclusions

The saturated fatty acids in milk of Tzigay sheep and their F2 cross-breeds had high values during both consecutive years, as they varied from 67.05% to 70.87%, with a lower content of myristic acid correspondingly 8.22-8.88%.

The total amount of polyunsaturated fatty acids in the examined milk for the two types of sheep was comparatively low with near concentrations (4.39-5.20%) in the period of the two years and maintained high values of the correlation SFA/PUSFA (15.71 and 13.17) and low values of the correlation PUSFA/SFA (0.06-0.08). Monounsaturated fatty acids, represented mainly by the oleic acid (C18:1) varied during both periods from 21.92% to 25.32% and appeared as a substratum in the synthesis of CLA.

The short-chain fatty acids (ΣC4:0-C11:0) had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids (ΣC17iso-C25:0) maintained close concentration in the milk of Tzigay breed, while their content in the milk of F2 cross-breeds was increased.

Sastav masnih kiselina mlečne masti ovaca rase cigaja i njihovih F2 meleza sa rasom hios

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Rezime

Istraživanje je sprovedeno na zbirnim uzorcima mleka, koji su uzimani svakog meseca u periodu muže, od ovaca rase cigaja i njihovih F2 meleza sa rasom hios, gajenih u uslovima centralnih Balkanskih planina. Ekstrakcija masti uzoraka mleka je urađena po metodi Rouz-Gottlieb. Sastav masnih kiselina je određen na gasnom hromatografu sa jonizacionim detektorom i kapilarnim kolonama. Cilj istraživanja je bio da prati promene u sastavu masnih kiselina mlečne masti cigaja ovaca i njihovih F2 meleza.

Zasićene masne kiseline u mleku od dve grupe imale su visoke vrednosti tokom obe godina uzastopno, jer su varirale od 67,05% u mleku cigaja ovaca u

drugoj laktaciji do 70,87% kod njihovih F2 meleza. Sadržaj miristinske kiseline je bio 8,22-8,88% u mleku cigaja ovaca i 8,45-8,74% njihovih F2 meleza.

Ukupan sadržaj poli-nezasićenih masnih kiselina u ispitivanom mleku dva tipa ovaca je bio relativno nizak sa približnim koncentracijama (4,39-5,20%) u periodu od dve godine. Mleko od dve grupe je imalo visoke vrednosti korelacije SFA/PUSFA (15,71 i 13,17) i niske vrednosti PUSFA/SFA (0,06-0,08).

Mono nezasićene masne kiseline, zastupljene uglavnom kroz oleinsku kiseline (C18: 1) varirale su tokom oba perioda od 21,92% do 25,32% i pojavile su se kao supstrat u sintezi CLA.

Masne kiseline kratkog lanca (C4:0 - C11:0) su imale veće vrednosti kod cigaja ovaca u poređenju sa F2 melezima sa rasom hios. Masne kiseline dugačkog lanca (C17iso - C25:0) su imale približnu koncentraciju u mleku cigaja rase, dok je njihov sadržaj u mleku F2 meleza bio povećan.

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VARIABILITY OF BLOOD SERUM BIOCHEMICAL PARAMETERS IN KARAKACHAN SHEEP

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Original scientific paper

Abstract: Karakachan sheep represents an endangered, indigenous sheep breed from Balkan Peninsula. There is relatively little information about the characteristics of this sheep breed in the veterinary scientific literature. The aim of this research was an examination of certain metabolic profile parameters of the Karakachan sheep blood serum, and variability of their concentrations in comparison to age and some other indigenous sheep breeds from Balkans. Examination was conducted on 14 clinically healthy sheep divided in two age groups. Blood samples were collected by puncture of *v. jugularis* and blood serum was separated after spontaneous coagulation. The concentrations of total protein, albumin, calcium, inorganic phosphorus, aspartate amino transferase (AST) and γ-glutamyl transferase (GGT) were determined. In relation to age of Karakachan sheep, statistically significant difference between the calculated mean values of examined parameters was not observed. A statistically significant difference was found between the mean concentrations of the studied parameters in Karakachan sheep and other breeds in total protein (Tsigai, Dubrovnik and Dalmatian sheep), albumin (Dalmatian), calcium and inorganic phosphorus (Tsigai, Dubrovnik) and AST's (Dalmatian, Karakachan sheep from Bulgaria).

Key words: Karakachan sheep, blood serum, biochemical parameters

Introduction

Karakachan sheep is one of the oldest sheep breeds in Europe (*Dervisis et al. 2007*). It was created centuries ago, as a result of selection on the primitive living conditions by Karakachans, a nomadic people who lived in the area of present-day countries of South-Eastern Balkans (Greece, Macedonia, Bulgaria and

Serbia). This sheep breed belongs to a primitive type of mountain sheep with a rough constitution, open fleece (Zackel type) and combined production traits. It is also characterized by pronounced vitality, and resistance. During the intensification of sheep breeding, Karakachan sheep lost its importance in modern production. According to the DADIS (Domestic Animal Diversity Information System), there were 130 females and 20 males in reproduction of this breed and total population was estimated at 150–300 individuals in 2012. Most of this sheep population is concentrated in the area of the Stara Planina nature park in South-Eastern part of Serbia.

Determination and monitoring of metabolic profile parameter values may show whether homeostatic mechanisms can maintain blood composition in physiological limits under different conditions of animal husbandry (*Prodanović et al. 2012*). Proper application of metabolic profile, the evaluation of housing conditions, as well as the composition and quality of the meals, can be a reliable method for assessing the condition of the herd, the diagnosis of health disorders and to indicate the etiology of their occurrence. There is a lot of studies that have examined the influence of gender, age, breed, nutrition and housing on blood biochemistry of sheep (*Jovanović et al. 1983; Hrković et al. 2009; Dias et al. 2010; Ouanes et al. 2011; Kiran et al. 2012*). Knowing the values of blood parameters of indigenous breeds is of particular importance because they allow collecting more information about those breeds which are most often threatened. The racial regression is a phenomenon that is present in all species of domestic animals and the disappearance of the primitive breeds is global problem that has resulted in the loss of genetic variability. In Serbia, there is no information about blood biochemistry of Karakachan sheep, so our work represents the beginning of research that aims to contribute in preservation of this threatened sheep breed.

The aim of this study is to determine the basic blood serum biochemical parameters of Karakachan sheep from Stara Planina mountain area, the influence of age on values of certain parameters, and detection of possible differences between Karakachan and other typical zackel sheep breeds from Balkans.

Material and methods

The studied animals belonged to a Karakachan sheep herd from the Stara Planina Nature Park area, and were in the process of conversion to organic production. In the time of blood sampling, nutrition was based solely on pasture (month of July). The study was conducted on 14 sheep (13 females and 1 male). Based on the age, sheep were divided into two groups of 7 individuals: Group I (1 to 3 years old) and group II (3 to 6 years old). Determination of age was based on dentition (*FAO, 2012*).

Blood was collected by puncture of the jugular vein in vacutainer tubes (10 ml) and spontaneous coagulation and centrifugation (3000 rpm for 10 minutes) led to separation of the blood serum. Samples of blood serum were stored at -18 ° C until the analysis were performed. The concentration of the following parameters - total protein g / l, albumin g / l, calcium (Ca) mg / l, inorganic phosphorus (P) mmol / L, aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) IU / l, were determined on a semi-automatic biochemical analyser (Vet evolution, Biosis, Italy).

Basic descriptive data (mean - $\bar{x}$, standard deviation - SD, standard error of the mean – SEM and variation interval - VI), and t-test for comparison of the values of some biochemical parameters in relation to the age, as well as comparison of the studied parameters in Karakachan sheep with the same values in other sheep breeds was obtained by using Graph Pad Prism 5.0 computer program. The reference values of studied biochemical parameters were taken from *Kaneko et al. 1997*.

Results and discussion

The metabolic profile is a diagnostic procedure that determines the concentrations of blood biochemical constituents in order to obtain data on the balance of organic and inorganic substances in the body as well as the function of certain organs. The average concentration values of metabolic profile in blood serum of Karakachan sheep (n = 14) are presented in table 1.

Table 1. Concentrations of studied metabolic profile parameters in blood serum of Karakachan sheep ($\bar{x}$ ±SD)

Parameter	N	$\bar{x}$ ±SD	SEM	VI	RV[1]
Total protein (g/l)	14	61,92±7,41	1,98	49,00-76,00	60,00-79,00
Albumin (g/l)	14	31,03±5,50	1,47	26,50-47,30	24,00-30,00
Ca (mmol/l)	14	2,75±0,29	0,08	2,15-3,22	2,70-3,20
P (mmol/l)	14	1,70±0,35	0,09	0,89-2,18	1,62-2,63
AST (UI/l)	14	97,66±28,87	7,72	65,00-172,00	60,00-280,00
GGT (UI/l)	14	60,50±16,08	4,30	25,60-86,90	20,00-52,00

[1]Kaneko et al. (1997)

From the table 1 it is observed that the average concentration of total protein and albumin in the blood serum of sheep were within the physiological values. However, from the variation interval is observed that in some animals

(35,71%) the concentrations of total protein values were lower compared to the reference value. In addition to albumin and total protein, for the interpretation of nitrogen metabolism, concentrations of urea and creatinine in the blood are also significant. Given the complexity of protein metabolism, nutrition is considered to be of highest influence on the parameters used in the estimation of nitrogen metabolism in domestic ruminants. The results in this study shows that in some animals was present a mild hypoproteinemia in blood serum which can be regarded as a lack of protein in the diet, or poor quality pasture in the summer period on Stara Planina Mountain. This is supported by the results of *Jovanović et al., (1983)* studies which noted that the largest proteinemia in blood serum of sheep was in early spring, because the pasture is richest in proteins at this period of year.

The average concentrations of calcium (Ca) and inorganic phosphorus (P) in the blood serum of sheep were in physiological interval compared to the reference values. From the variation interval of inorganic phosphorus is observed that in some animals (35.71%) was present hypophosphatemia. However, different reference values for concentration of these minerals - Ca 2-3 mmol / l and P 1-2.5 mmol / l – can be found (*Hindson and Agnes, 2002*). Compared to this literature data, concentrations of these minerals were in range of physiological values. The level of minerals in the blood depends on nutrition, which shows that calcemia can be directly correlated to the category and nutrition of sheep (*Hrković et al. 2009*). Levels of calcemia and phosphatemia are primarily regulated with renal excretion. Hypophosphatemia in serum of Karakachan sheep is most likely the result of the reduced alimentary intake of phosphorus which is in agreement with *Jovanović et al. (1983*). According to the recommendations of these authors, hypophosphatemia can be corrected by addition of phosphorus in diet of sheep.

Aspartate aminotransferase is an enzyme that is found in the liver and heart muscle, and plays an important role in the metabolism of amino acids. From the results obtained it can be seen that the activity of AST in serum was within the limits of referent values. The average value of γ-glutamyl transferase (GGT) in the blood serum samples of sheep was slightly elevated compared to reference values. Increased activity of this enzyme in clinically healthy sheep can be considered as a consequence of the intensification of metabolic processes and a response of the body to the negative energy balance (*Hrković et al. 2009*). Also, the increased activity of this enzyme in clinically healthy sheep can be consequence of moderate oxidative stress, related to increased degradation of glutathione (*Hodžić et al. 2011*). Furthermore, the increase of GGT can be a sign of chronic liver dysfunction that may be of different etiology. Due to the high concentration instability of these enzymes in the blood of ruminants, their activity is not a reliable diagnostic indicator for determining the nature of the pathological process in the liver (*Reynolds, 1991*).

Table 2. Comparison of studied metabolic profile parameters in relation to age of Karakachan sheep

Parameter	AG[1]	N	$\bar{x}$ ±SD	VI	t
Total protein (g/l)	1	7	58,55±4,88	49,00-63,00	1.91[ns]
	2	7	65,29±8,28	55,00-76,00	
Albumin (g/l)	1	7	29,05±1,99	26,50-31,02	1.47[ns]
	2	7	33,00±7,24	26,80-47,30	
Ca (mmol/l)	1	7	2,62±0,22	2,15-2,81	1.50 [ns]
	2	7	2,88±0,31	2,30-3,22	
P (mmol/l)	1	7	1,63±0,40	0,89-2,18	0.72 [ns]
	2	7	1,78±0,30	1,30-2,08	
AST (UI/l)	1	7	93,76±36,42	65,00-172,00	0.40 [ns]
	2	7	101,57±21,05	80,00-137,00	
GGT (UI/l)	1	7	54,57±5,96	43,40-60,50	1.51 [ns]
	2	7	66,43±21,03	25,60-86,90	

[1] age group [ns] p>0,05- not significant

Table 3. Comparison of average concentrations of certain metabolic profile parameters in Karakachan sheep with those of the three indigenous breeds in the Balkans.

Parameter	$\bar{x}$ ±SEM			
	Karakachan sheep (N=14)	Dubrovnik sheep (N=10)[1]	Tsigai (N=15)[2]	Dalmatian sheep (N=114)[3]
Total protein (g/l)	61,92±1,98	79,90±2,79**	74,97±1,22**	77,00±0,6**
Albumin (g/l)	31,03±1,47	32,40±0,19	30,31±1,31	38,00±0,30**
Ca (mmol/l)	2,75±0,08	3,01±0,04*	2,55±0,03*	-
P (mmol/l)	1,70±0,09	1,31±0,12*	1,68±0,06	-
AST (UI/l)	97,66±7,72	97,00±6,72	102,20±4,90	127,00±2,20**
GGT (UI/l)	60,50±4,30	52,60±11,08	46,80±2,97*	59,00±1,30

[1]Antunović et al. (2009), [2]Antunović et al. (2011), [3]Vojta et al. (2011)
* p<0,05 – significant, ** p<0,001 – very significant

From the results shown in table 2, it can be observed that there is no statistically significant difference in concentration of examined parameters average values between the two age categories. These results are in agreement with the results obtained by *Kiran et al., (2012)* and suggesting that age has no substantial effect on the concentration of specific blood parameters.

Statistically significant difference between the values of certain parameters in Karakachan sheep blood serum and other indigenous breeds of the Balkans were observed (table 3.).

The average total protein concentration in the blood serum of the Karakachan sheep was significantly lower ($p < 0.001$) compared to other breeds. The average albumin concentration of Karakachan sheep was significantly lower ($p < 0.001$) compared to Dalmatian sheep. The difference in the protein can be explained by the fact that the authors sampled blood of Dalmatian breed in late spring when the pasture is still rich in proteins (*Vojta et al. 2011*).

The average concentration of calcium in the blood serum of the Karakachan sheep was statistically lower ($p < 0.05$) compared to Dubrovnik sheep, and higher than the calcium blood serum concentration of Tsigai ($p < 0.05$). Phosphatemia in Karakachan sheep was statistically higher ($p < 0.05$) compared to Dubrovnik sheep. These differences in the concentrations of minerals in blood between examined sheep breeds can be attributed to the different representation of the mineral matter in the soil where animals are grazed.

The mean concentration of AST in Karakachan sheep blood serum was significantly lower ($p < 0.001$) compared to the activity of this enzyme in the Dalmatian breed. The obtained values of AST in Karakachan sheep from Bulgaria were 121.29 ± 32.35 (*Angelov et al*. 2013). By comparing these values with the results of AST in our sample, we obtained statistically significant differences ($p < 0.05$), indicating that the value of AST may vary even within the same breed of sheep.

Conclusion

The concentrations of examined metabolic profile parameters in Karakachan sheep were within the physiological values.

Age status has no significant effect on the concentrations of blood serum biochemical parameters in Karakachan sheep.Nutrition, soil and pasture quality where the sheep graze has a significant effect on the concentration of proteins and minerals in the blood serum.

There was variation of the AST and GGT concentrations of Karakachan sheep in comparison with other breeds, as well as within the same breed.

Varijabilnost biohemijskih parametara krvnog seruma karakačanske ovce

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Rezime

Karakačanska ovca je ugrožena autohtona rasa ovaca sa Balkanskog poluostrva, o čijim rasnim karakteristikama postoji relativno malo podataka. Cilj ovog rada je bio da se u uzorcima krvnog seruma ispitaju određeni biohemijski parametri karakačanske ovce i ustanove odstupanja njihovih vrednosti u odnosu na starost karakačanske ovce, kao i u odnosu na vrednosti istih parametara kod drugih autohtonih rasa ovaca Balkana. Ispitivanjem je obuhvaćeno 14 klinički zdravih ovaca podeljenih u dve starosne grupe. Uzorci krvi uzimani su punkcijom *v. jugularis* iz kojih je, nakon spontane koagulacije i centrifugovanja, izdvojen krvni serum. Određivane su koncentracije ukupnih proteina, albumina, kalcijuma, neorganskog fosfora, aspartat amino transferaze (AST) i γ-glutamil transferaze (GGT). U odnosu na starost karakačanske ovce, nije ustanovljena statistički značajna razlika između izračunatih srednjih vrednosti ispitivanih parametara. Statistički značajna razlika je ustanovljena između srednjih vrednosti koncentracija ispitivanih parametara karakačanske ovce i drugih rasa za: ukupne proteine (cigaja, dubrovačka i dalmatinska ovca), albumin (dalmatinska), kalcijum i neorganski P (cigaja i dubrovačka) i aktivnosti AST-a (dalmatinska, karakačanska ovca iz Bugarske).

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CALCULATING ECONOMIC WEIGHTS FOR GROWTH, REPRODUCTION AND WOOL TRAITS IN MAKUI SHEEP BREED BY ECOWEIGHT SOFTWARE

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Abstract: Production, reproduction, management and economical parameters obtained from data collected from 1993 to 2012 Makui sheep research station of West Azerbaijan province in Iran were evaluated in the present study. Traits included of fertility, pregnancy rate, lamb weights from birth to the end of period, survival rate of lambs, wool production weight, average daily gain and milk production. The present value of profit computed as the difference between total revenues and total costs per ewe per year. The numeric derivation of each considered trait is calculated by increasing and decreasing the average value of the trait while was kept the other characters in the average. First all costs, revenues, profits and flock structure determined then interned input files and running the software ECOWEIGHT. The results showed that economic values per unit increase in the traits of birth weight, daily gain from birth until weaning, daily gain from weaning until end of period, conception rates ewes, little size, lamb survival, lifetime for ewes, milk yield and wool yield were 0.66, 0.51, 0.03, 0.66, 0.25, 0.85, 0.93, 0.53 and 1, respectively. Breeding objective in Makui sheep breed were productive wool yield, lifetime, lamb survival at weaning, conception rates ewes, birth weight, milk yield, daily gain from birth until weaning, little size, daily gain from weaning until end of period.

Keywords: Ecoweight, breeding, economic weight, lamb, wool

Introduction

The Makuie sheep is a fat-tailed sheep breed which can be found in the Azerbaijan province of Iran. In 1986, a Makuie sheep breeding station was established in the city of Maku in order to breed, protect and purify this breed. Its total population is estimated at approximately 2.7 million (*Abbasi and Ghafouri*,

2011). It has been adapted to cold and highland environments (*Safari, 1986*). They are fat-tailed sheep with a medium-sized body, white in color with black rings around the eyes, nose and feet (*Saadatnoori and Siahmansor, 1986*). They are kept in the Eastern and Western provinces Azerbaijan and their main products are meat, wool and milk (*Saadatnoori and Siahmansoor, 1986*). The rearing system is mostly extensive-migratory from April to September (on natural pastures in spring and summer), and semi-intensive from October to March (on stations and fed in barns during autumn and winter). Alfalfa, barley, corn silage, concentrates and grass are the main feedstuffs used in the semi-intensive rearing period.

The first step in designing a breeding program is definition of the selection object function. In order to do this economic values of traits affecting incomes and costs of production system should be determined. Until now, such a process has not been done for Makui sheep. Need to include the aim of the study. Purpose of the cost adjustment is to provide broad applicability to obtained results in relation to investigated farm (*Okanović et al., 2008*).

One of the useful tools for estimating economic values for traits is a bio-economic model which provides a very powerful tool to estimate the economic value of genetic changes in various traits, and also to investigate the robustness of these values to changes in nutrition, management and market prices (*Jones et al., 2004*).

Materials and Methods

The program package ECOWEIGHT is intended for the calculation of economic values of economically important traits in livestock. At the given stage, in its fifth version, two programs for cattle and three programs for sheep are available. The two programs for cattle (EWBC and EWDC) are described in the first part of the manual. Whereas in the present manual (which forms the second part of the documentation) the stand-alone program EWSH1 for sheep with one lambing per year is presented, the third part of the program package which is documented in two manuals is formed by the program EWSH2 which is a modification of EWSH1 and by the program GFSH which models gene flow in sheep. As the programs EWSH2 and GFSH are run together they are in a joint installation package. The program EWSH1 is the implementation of a bio-economic model on the PC to simulate effects on life-cycle efficiency from genetic change in production and functional traits of sheep under alternative management systems with one lambing per year. The flock structure is described in terms of animal categories and probabilities of transitions among them. The Markov chain approach is used to calculate the stationary state of the ewe flock. Up to 47 categories of progeny may be defined whereby pure-bred and cross-bred animals may occur in most categories if cross-breeding is used in the system.

The algorithm includes both deterministic and stochastic components. Performance for most traits is emulated as the population mean, but variation in several traits is taken into account.

Profit estimated as the difference between the total revenues and total costs per ewe per reproductive cycle is used as criterion of the economic efficiency of the production system in the stationary state. The economic importance (economic values) of up to 35 traits (milk production traits, growth traits, carcass traits, functional traits and wool traits) may be estimated. These economic values are intended for developing a breeding objective for sheep.

Table 1. Input files Ecoweight (EWSH1)

Input file	Introduction
1	parameters for calculating the ewe flock structure
2	parameters for calculating the structure of the ram population
3	parameters for calculating the structure of the progeny of the ewe flock
4	parameters for the progeny of the ewe flock reared for breeding for the interval from weaning to mature weight or to selling
5	parameters for surplus progeny
6	parameters for the calculation of milk production on the basis of the Wood function
7	parameters for the calculation of milk production if the lactation curve is unknown
8	parameters for calculating the nutrition costs
9	parameters for the calculation of non-feed costs in the sheep flock and in lamb fattening
10	parameters for calculating revenues except of revenues from slaughter animals and milk
11	parameters for the calculation of the milk price, revenues from milk and cheese
12	parameters for calculating revenues from adult sheep
13	parameters for calculating revenues from lambs slaughtered after weaning or artificial rearing
14	parameters for calculating revenues from lambs in fattening

The marginal economic value is generally defined as the partial derivative of the profit function with respect to the trait considered. It is expressed per given unit of the trait and per time interval (here per ewe entering a reproductive cycle and per year). When using complex bio-economic models instead of simple profit functions (as in the present program) the exact partial derivative must be replaced by an approximate method, by a numeric derivative (difference quotient). The estimation of economic profit based on weight is calculated for a commercial population of sheep of the given breed or breed combination. The methodology

used for the calculation of economic values is different for traits with continuous variation and for categorical traits.

Economic values for traits with continuous variation

The numeric derivative of profit with respect to the considered trait is calculated by increasing and decreasing the average value of the trait TV_{av} by 0.5%. Let TV_h be the higher value of the trait considered which was derived as $TV_h = 1.005TV_{av}$. Similarly, TV_l is calculated by decreasing the average trait value by the same amount: $TV_l = 0.995TV_{av}$. Furthermore, let TP_h and TP_l be the total profit belonging to the first or the second of these values, respectively. The partial derivative is then approximated by the following difference quotient:

$$ev= (TP_h-TP_l) / (TV_h-TV_l)$$

Some traits are complex quantities which are calculated from a series of parameters. For example, average conception rate of ewes is calculated from the conception rates in the individual lactations. For that reason, the conception rate in all lactations is changed in the way described above for calculating the economic value of the average conception rate. Total milk is calculated from the sum of daily milk yield of ewes on different lactations. In this trait a change in parameter (awo in the program) of the lactation curve proved to be useful for the calculation of economic weights.

Table 2. Number of records, means (± s.d.) for considered traits in Makui sheep

Trait	No. of record	Mean± s.d.
Birth weight	18967	4.11± 0.88
Weaning weight	19297	21.50± 3.50
6 month weight	9957	27.18± 3.53
24 month weight for ewe	395	43.88± 6.45
24 month weight for ram	29	44.59± 7.62
Amount of wool per shearing in ewe (kg/animal)	1826	1.19± 0.47
Amount of wool per shearing in ram (kg/animal)	1389	1.40± 0.55

Non-feed costs include veterinary costs, breeding costs, general labour costs, costs for bedding, costs for shearing, fixed costs, costs for removing and rendering dead animals, costs for buying animals, marketing costs, costs for tanning skins, milking costs and costs for cheese production. The latter two cost components accrue only for dairy ewes. Not each single item of the non-feed costs occurs in all categories of animals. All costs are discounted to the birth date of the animals (Table 3).

In all input files the abbreviation MU is used for monetary unit. All values in the distributed version of the program refer to Euros. It is important to note that the currency used in Iran is the Rial (MU1=25000 Rials).

In order to study the effect of environmental factors on growth traits in Makui sheep, we applied information that was collected from 1993 to 2012 in Makui Breeding Station. Records of birth weight, weaning weight, weight at month 6, weight at month 24 for ewe and ram, Amount of wool per shearing in ewe and ram. Characteristics of the data structure are summarized in Table 2. A univariate procedure of SAS was used to check for normality. The SAS software was used for normality test. The data of all traits was normal.

Table 3. Growth data, nutritional management, production, costs and prices in Makui sheep

Title	Mean	Title	Mean
Birth weight (female/male) kg - Singles - Twins	 4.09- 4.36 3.64- 3.73	Weaning age of lambs (months)	5
Weaning weight (female/male) kg - Singles - Twins	 21.24- 21.31 19.84- 20.54	Age at first mating (months)	18
Survival rate of lambs until weaning (%) - Singles - Twins	 99 96	Number of shearing times in per year	1
Mature weight (kg) - Female - Male	 50 60	Number of years keeping ewes in flock	7
Percentage of singles (%)	85	Number of years keeping rams in flock	5
Percentage of twins (%)	15	Days grazing on rangeland	90
Conception rate (%)	95	Days using pastures of residual crop	120
Average daily gain from birth to weaning (g/d) - Female - Male	 190 197	Days using manual feeding	150
Average daily gain from weaning to six- month (g/d) - Female - Male	 65 61	Number of drenchings against worm (endo-parasites)	2

Survival proportion from weaning to the first breeding season - Female /Male	97	Drug and veterinary service (MU/head/year)	0.4
Number of days gestation	150	Cost for milking per kg milk (MU/kg)	0.8
Costs per shearing (MU)	0.4	Price per kg cheese (MU./kg)	6
Labour (MU./100 head/month)	280	Roughage metabolic energy (Mcal/DM)	1.5
Concentrate metabolic energy (Mcal/DM)	2.8	Roughage price (MU./kg DM)	0.108
Concentrate price (MU/kg DM)	0.192	Price per kg live weight of pure-bred lambs to weaning (MU/kg)	6
Price per kg live weight of pure-bred lambs after weaning (MU/kg)	7.2	Price per kg live weight of ewes (MU/kg)	4.6
Price per kg live weight of rams (MU/kg)	4.4	Price per water(MU/lit)	0

Results and Discussion

In Makui sheep production system, variable costs, i.e. feed and non-feed costs included 99% of the total costs. Fixed costs varied widely between flocks, depending on the type of barn used. Traditional barns were cheap, whereas upgraded or newly build barns were relatively expensive. *Kosgey et al., (2003)* estimated the fixed costs at a proportion of 5%, which was more than the evaluated amount in the present study. The difference may have to be attributed to different assumptions of these models. *Kosgey et al., (2003)* considered only roughage cost as feed costs which resulted to relatively lower variable costs and as a result, the proportion of fixed costs to total costs was higher in comparison with present study. Feeding costs and cost of labour contributed most to the variable costs, which coincided with results of *Khodaee, (2005)* and *Vatankhah, (2005).*

The marginal and relative economic values for all evaluated traits are summarized in Table 4. They express the changes in the present value of profit per ewe present in the flock at lambing and per year that would occur when flock average for the trait was increased by one unit.

Table 4. Marginal economic values and relative economic value of the trait in Makui sheep

Trait acronyms	Trait (units)	Marginal Economic value (MU)	Relative Economic value
BW	Birth weight (kg)	1.032	0.66
DGBW	Average daily gain from birth to weaning (g/d)	0.809	0.51
DGWE	Average daily gain from weaning to six-month (g/d)	0.047	0.03
Cr	Conception rate of ewes (%)	1.035	0.66
Ls	Average litter size per ewe lambing (0.01 lambs)	0.394	0.25
Sr	Survival rate of lambs at lambing (%)	1.341	0.85
LE	Length of productive life of ewes (years)	1.461	0.93
MY	Milk yield in the standardized milking period of 150 d (kg)	0.842	0.53
WY	Wool yield (kg)	1.578	1

Highest economic value in base situation was obtained for wool y yield. The economic value of birth weight trait was positive. Results were in agreement with the results obtained for Harki sheep (*Shiru, 2011*), and Gharagol sheep in Iran (*Zahmatkesh, 2010*) in addition to, the system studied by *Kroupva et al., (2009;2011), Wollfova et al., (2009;2011),* but *Musazadeh, (2012), Haghdoost et al., (2008)* obtained negative economic value for these trait.

Economic value was not worked for trait average daily gain in Iran. Software Ecoweight has found average daily gain. Economic value average daily gain birth to weaning and weaning to six- month traits have intermediate and low level. Average daily gain after weaning is high heritability because appropriately important for these trait.

The economic value of another reproductive trait, that is conception rate have a relatively high economic value. Results were in agreement with the results obtained for Lori Bakhtiari sheep in Iran (*Vatankhah, 2005; Kroupva et al., 2009; 2012; 2013*).

Among the reproductive traits, litter size seems to have an intermediate economic value. On the other hand, survival rates of lambs at lambing and until weaning have high economic values. The economic value for milk yield seems to

have an intermediate economic value. Recently, economic values for milk production as well as for growth and functional traits in dairy sheep have been estimated (*Fuerst-Waltl and Baumung, 2009*).

In many countries with developed agricultural practices, economic outcomes of paratubercusosis were investigated in dairy herds (*Vidić et al., 2013*). In Nigeria, goat production plays an important role in the economic improvement of poor farmers and contributes to poverty alleviation *(Yakubu et al., 2014).*

Results of this research showed that in Makui sheep production system, wool yield, length of productive life of ewes and ewe survival were of the most important traits to increase the profit of the flocks. These traits could be recorded under flock conditions and hence should be considered in genetic improvement programs.

Conclusions

Imposing current breeding programs for various sheep breeds in Iran. These programs were supposed to be performed mainly by the flock owners. Redundant need to reword or change sentences construction because very obvious that only copied the end part of the results and discussion.

Acknowledgments

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Obračun ekonomskih vrednosti za rast, reprodukciju i osobine vune ovaca rase makui korišćenjem „Ecoweight“ softvera

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Rezime

Parametri proizvodnje, reprodukcije, upravljanja i ekonomski parametri dobijeni iz podataka prikupljenih od 1993. do 2012. godine, u istraživačkoj stanici za Makui ovace pokrajine Zapadni Azerbejdžani u Iranu, su ocenjenivani u ovoj studiji. Osobine uključene u istraživanje su osobine plodnosti, stopa jagnjenja, težine jagnjadi od rođenja do kraja perioda, stopa preživljavanje jagnjadi,

proizvodnja vune, prosečni dnevni prirast i proizvodnja mleka. Sadašnja vrednost profita izračunava se kao razlika između ukupnih prihoda i ukupnih troškova po ovci godišnje. Numeričko izvođenje svake posmatrane osobine je izračunato povećavanjem i smanjivanjem prosečne vrednosti osobine, dok su ostale osobine zadržane u prosečnoj vrednosti. Prvo su svi troškovi, prihodi, profit i struktura stado utvrđeni, onda internirani ulazni inputi i pokrenut softver ECOWEIGHT. Rezultati su pokazali da se ekonomske vrednosti po jedinici povećavaju u osobinama težina na rođenju, dnevni prirasti od rođenja do odbijanju, dnevni prirasta od odbijanja do kraja perioda, stope koncepcije ovaca, veličina legla, preživljavanje jagnjadi, životni vek ovaca, prinos mleka i vune prinos za 0,66; 0.51; 0,03; 0,66; 0,25; 0,85; 0,93; 0,53 i 1, respektivno. Odgajivački cilj za ovce rase Makui je prinos vune, životni vek, preživaljavanje jagnjadi kod odbijanja, stope koncepcije ovaca, telesna masa, prinos mleka, dnevni prirast od rođenja do odbijanja, veličina legla, dnevni prirasta od odbijanja do kraja perioda.

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EFFECTS OF WEIGHT AND AGE ON CARCASS YIELD AND CONFORMATION OF CATTLE

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Abstract: The aim of this research was to analyse effects of weight and age of dairy cattle breeds and their crossbreeds (423 bulls, 492 heifers and 567 cows) on carcass yield and conformation scores according SEUROP standard data. All animals were divided into groups according to pre-slaughter weight (50 kg interval) and according to age (2 months interval). For research, average ages of the animals were: bulls 19 months, heifers 20 months and cows 38 months. The highest average weight was determined in cows 521.2 kg, followed by heifers 461.7 kg and the least got bulls 456.2 kg however, on the average carcass yield bulls got the highest value of 51.3 percent, 50.1 percent of heifers and the least 47.8 percent of cows. When animals pre-slaughter weight an average increase of 50 kg, the carcass yield of bulls on average increased by 11.1 percent, of heifers - 0.72 and cows – 0.94 percent. In all studied groups of animals were obtained weak correlation links between age of animal and carcass yield. The strongest correlation links were obtained between the animal's weight and carcass weight of bulls ($r = 0.93$, $p < 0.001$), heifers ($r = 0.94$, $p < 0.001$) and cows groups ($r = 0.91$, $p < 0.01$). It was revealed that, if age of cattle moderately increases for 2 months, then there was a moderate increase on carcass yield such as 0.21 percent in heifers ($p < 0.05$) and 0.12 percent in cows groups ($p < 0.05$). This relationship was not statistically significant in the bulls group. Mostly, the O muscularity class of carcasses were evaluated in all analysed animals groups, both depending from pre-slaughter weight and age. In the group of bulls evaluated O muscularity class carcasses were found moderate (76.8 percent), heifers (74.3 percent) and in group of cows (49.7 percent). However, a trend was observed that with animal's weight and age improved carcass yield and arise carcass muscularity class.

Keywords: cattle, carcass yield, muscularity class

Introduction

In many countries of intensive animal husbandry, the cattle breeding carried out considering not only according quantitative but also qualitative indicators of meat production *(Jukna et al., 2006, Jukna et al., 2010)*. The individual scientists maintain that on edible parts of carcass yield (without bones, tendons, and cartilages) with animal age increases *(Crews et al., 2003, Šmiecinska et al., 2006)*. The animal concerning of uneven individual tissue and organ growth intensity of carcass yield increases. Carcass yield and morphological composition of cattle depend on the breed, sex, feeding different growth periods, of the individual animal characteristics, of animal's condition and age *(Aleksic et al., 2001, Berg et al., 2003, Rotta et al., 2009, Huertas et al., 2010)*. Also, carcass morphological composition depends on the individual tissues ratio. The main are muscle, fat and bone tissues. Muscular tissue consists of about 50-65 percent of carcass. The most influence on carcass composition have animal's obesity degree. Between the two sides of the weight and quality of the categories there are a strong relationship, the major carcass weights associated with better body structure and higher animal obesity *(Aleksic et al., 1999, Culioli et al., 2003, Gečienė et al., 2007)*. Cattle carcasses according to age and sex are divided into categories: A - young (not older than two years) uncastrated bulls, B - other (over two years) uncastrated bulls C - oxen (castrated bulls), D - cows (cows that have calved) and E - heifers carcasses *(Jukna et al., 2009)*.

The muscle growth intensive on young cattle are the first 15-18 months of life. When full-fledged feeding in animal offspring muscle growth rate is significantly higher than the skeleton, this consist favourable conditions for the formation of muscular and heavy carcasses with very high valuable soft parts amount. Slaughter age and feeding system are the two main factors, the most influencing the animals' growth and carcass traits *(Matusevičius et. al., 2006, Bendikas et. al., 2009, Hilton et al., 2010, Veselá et al., 2011, Agastin et. al., 2013)*.

The fundamental purpose of beef cattle growing is to produce high-quality beef. Carcass yield and good muscularity and fat class are important factors for beef production profitability. Muscularity classification indicates of carcasses development, structure and quality. Cattle carcass quality are assessed according six SEUROP muscularity standard classes. In Lithuania is grown many dairy cattle, whose carcasses are of inferior quality, therefore mainly carcasses adequate the O and P muscularity classes *(Pečiulaitis et. al., 2007)*. Individual countries are verified subclasses system and muscularity and fat classes. Higher quality

muscularity class means, that there is more meat in the carcass and the slaughterhouse is usually assessed at a higher price *(Jamieson, 2013).* In order that to get a good carcass yield and higher muscularity class most importantly to slaughter cattle to respective age and weight.

The aim of the study was to determine the pre-slaughter weight and age of cattle, its influence on carcass yield and carcass muscularity class.

Materials and Methods

In order to investigate the cattle weight and age influence on the carcass yield and quality, for the research data of slaughtered cattle in the years 2011-2012 was used from one slaughterhouse in Lithuania. For the study was used a total of 4890 slaughtered different dairy breeds and their crossbreeds with beef breeds cattle data, from the following: 423 bulls, 492 heifers and 567 cows. Cattle pre-slaughter weight was divided for every 50 kg intervals. Animals according to age were divided for every 2 months interval. Bulls, heifers and cows were separately grouped. For study have used different ages of the animals such as bulls 19 months, heifers 20 months and cows 38 months. Carcasses according of muscle development has been classified in accordance with SEUROP standard. Cattle carcasses classified by SEUROP classification with reference of Commission Regulation (EB) Nr. 1249/2008, 2008 on 10 December whereby determinable comprehensive Community of cattle, pig and sheep of carcases classification scale use and the report about the carcass prices instruction. During slaughter was assessed animal pre-slaughter weight, the warm carcass weight, carcass yield and muscularity class.

Carcass yield calculated by the following formula: H=(S*100) / G, where H – carcass yield, %; S – warm carcass weight, kg; G – animal pre-slaughter weight, kg.

Statistical analysis were determined using the R statistical package 2:01 (Gentlemen, Ihaka, 1997) and an Excel spreadsheet. The linear correlation coefficients were calculated to evaluate the investigated signs of mutual relations. The differences between the average parameters of groups evaluated Student's criteria. Carcass yield dependence from pre-slaughter weight and age. To evaluate the aforesaid the analysis of variance (ANOVA) was applied and the average trends were calculated. The difference statistically reliable when $p < 0.05$.

Results and Discussion

Studies have shown that pre-slaughter weight of animals has influence on carcass yield till a certain age of the animal. The study used bulls average age was

19 months, heifers 20 months and cows 38 months. The highest average weight of animal was determined on cows 521,2 kg, slightly lesser heifers 461.7 kg and the least of bulls 456.2 kg however, the highest average carcass yield 51.3 percent was of bulls, slightly lower 50.1 percent of heifers and least 47.8 percent of the cows (Table 1).

Table 1. The bulls, heifers and cows of age, weight and carcass yield analysis

	The number of animals, units	Age of the animals, months	Weight of animals, kg	Carcass weight, kg	Carcass yield, percent
Bulls	423	19.18±0.24 [a]	456.19±3.62 [a]	235.21±2.34 [a]	51.34±0.19 [a]
Heifers	492	20.22±0.15 [a]	461.66±3.56 [a]	232.24±2.13 [a]	50.13±0.16 [b]
Cows	567	38.9±0.32 [c]	521.2±3.73 [b]	256.7±2.44 [b]	47.8±0.20 [c]

In column a, b, c letters to mark averages differed statistically significant p <0.001

In all three studied cattle groups were observed a statistically significant (p <0.001) linear relationship between the animal pre-slaughter weight and carcass yield. The linear averages trends obtained suggest that, an animal's pre-slaughter weight for an average increase of 50 kg, the carcass yield of bulls on average increased 11.1 percent, heifers - 0.72 and cows – 0.94 percent (Fig. 1). Our study data of cattle pre-slaughter weight showed considerable influence to carcass yield coincides with the data of other researchers *(Jukna Č. & Jukna V., 2002; Crews et al., 2003; Serra et al., 2004; Jukna et al., 2009).*

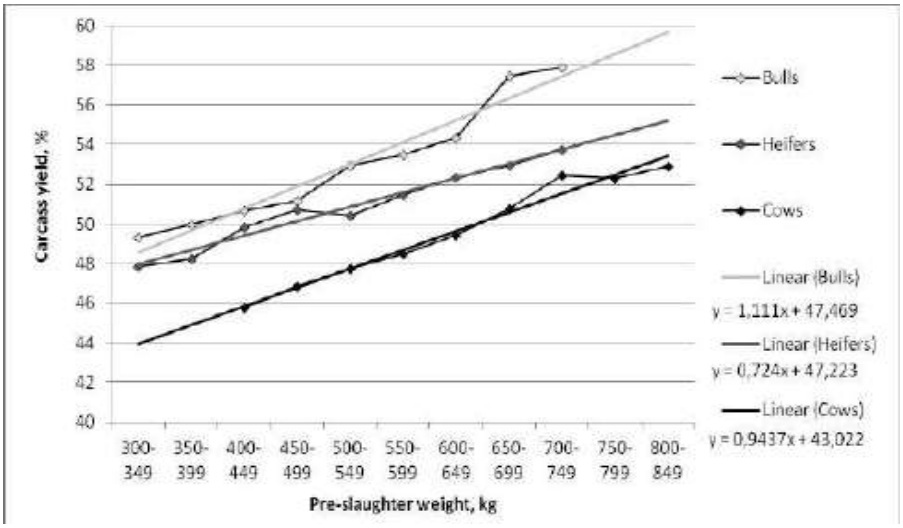

Figure 1. Carcass yield of bulls, heifers and cows depending from pre-slaughter weight

The data in table 2 showed, that correlation link were found stronger between carcass weight and carcass yield in group of bulls ($r = 0.65$, $p < 0.001$) than between pre-slaughter weight and carcass yield ($r = 0.35$, $p < 0.001$). The strongest correlation link was found between the animal's weight and carcass weight ($r = 0.93$, $p < 0.001$). The results obtained show that when weight of animals increased carcass weight and yield also increased. The weakest correlation link was found of bulls group between age of bull and carcass yield ($r = 0.11$, $p < 0.05$). Similar correlation links has been obtained and in group of heifers, between carcass weight and carcass yield was determined stronger correlation link ($r = 0.57$, $p < 0.001$) than between of heifers pre-slaughter weight and carcass yield ($r = 0.27$, $p < 0.001$). The strongest correlation link was obtained between the animal's weight and carcass weight ($r = 0.94$, $p < 0.001$) and weakest link between heifers age and carcass yield ($r = 0.09$, $p < 0.05$). After correlation analysis also strong correlation links were determined between carcass weight and carcass yield ($r = 0.70$, $p < 0.01$) and the animal's weight and carcass weight ($r = 0.91$, $p < 0.01$) in group of cows. The weakest statistically significant correlation link was established between the age and carcass yield of cows. ($r = 0.09$, $p < 0.05$). Although, in all studied groups of animals were obtain weak correlation links between age of animal and carcass yield, but the trend shows that with increasing age, carcass yield also increases. Some researchers argue, when animal grows due to uneven growth of individual tissues and organs intensity, carcass yield also increases *(Alberti et al., 2005; Jukna Č., Jukna V., 2005; Jukna et al., 2009)*.

Table 2. The correlation coefficients between the different signs of cattle in groups

Signs	Weight of animals, kg	Carcass weight, kg	Carcass yield, kg
Bulls group			
Age of the animals, months	0.331***	0.307***	0.113*
Weight of animals, kg		0.934***	0.352***
Carcass weight, kg			0.656***
Heifers group			
Age of the animals, months	0.413***	0.381***	0.094*
Weight of animals, kg		0.944***	0.279***
Carcass weight, kg			0.574***
Cows group			
Age of the animals, months	0.365***	0.317***	0.098*
Weight of animals, kg		0.913***	0.365**
Carcass weight, kg			0.705***

* - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

Analysis have shown that between age and carcass yield linear dependence was expressed slightly. The results presented in figure 2 showed, that this dependence was not statistically significant in the bulls group, while of heifers and cows groups of carcass yield dependence from age describing of linear functions the reliability was evaluated $p < 0.05$. The linear trends averages obtained suggests that, if analysed cattle age moderately increases for 2 months, then moderately carcass yield increased 0.21 percent in heifers group ($p < 0.05$) and 0.12 percent in cows group ($p < 0.05$).

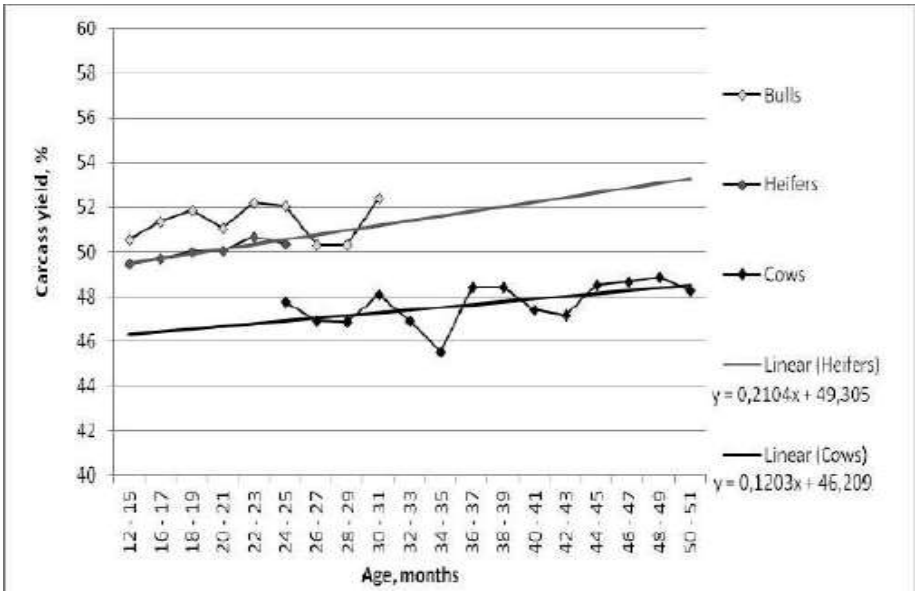

Figure 2. Carcass yield of bulls, heifers and cows depending on the age before slaughter

In figure 3 data show, that increasing pre-slaughter weight of bulls carcass muscularity class also increased. The R muscularity class of bull carcasses assessed moderately accounted 14.4 percent, increasing animal's pre-slaughter weight every 50 kg. The P muscularity class carcasses assessed until 500 kg of bulls pre-slaughter weight was obtained 7.6 percent. Mostly of O muscularity class of bulls carcasses were determined moderately accounted 76.3 percent. The U muscularity class assessed carcasses of bulls appeared only from 500 kg, and they moderately accounted of 1.2 percent. The carcasses muscularity of heifers also increased, when pre-slaughter weight of animals increased. Increasing pre-slaughter weight of heifers every 50 kg from 500 kg weight of heifers carcasses evaluated U muscularity class were obtained moderately of 0.9 percent. It also increased and the R muscularity class evaluated carcasses the increasing pre-slaughter weight of heifers, their were determined moderately of 16.5 percent. From 500 kg of pre-

slaughter heifers' weight P and O muscularity classes evaluated carcass were not observed. Increasing pre-slaughter weight of heifers for every 50 kg until 500 kg on average P muscularity class carcass accounted - 8.3 percent and O class - 74.8 percent. Cows lowest muscularity class of carcass was evaluated which had lowest pre-slaughter weight. When cow's pre-slaughter weight increasing every 50 kg, carcass P muscularity class evaluated declined gradually. Carcass P muscularity class moderately were evaluated 47.4 percent. Similarly as other studied groups, O muscularity class on carcass mainly were evaluated, it accounted of 49.7 percent. From 500 kg pre-slaughter weight of cows emerged 0.4 percent a highest U muscularity class assessed cow carcasses. The R muscularity class obtained 2.5 percent of cows' carcasses. Our research data coincided with the data of other researchers that pre-slaughter weight of animals influenced to carcass quality. Their study found that cattle the larger pre-slaughter weight carcasses were superior quality *(Rios-Utrera et al., 2005; Bendikas et al., 2006; Alberti et al., 2007)*.

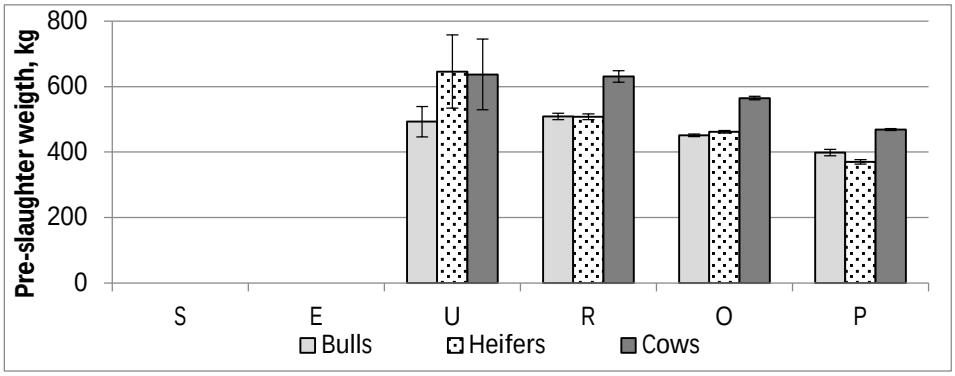

Figure 3. Pre-slaughter weight of cattle distribution according SEUROP of muscularity classes classification

The data presented in Figure 4 showed that with age of the animal carcass muscularity class slightly but rising. The observed, that O muscularity class of carcasses evaluated number increased meanwhile evaluated P class declined. Assessing, every 2 months age bull O muscularity class assessed of bulls carcasses were found moderately 76.8 percent, and carcass P muscularity class moderately accounted of 7.6 percent. The U and R muscularity classes assessed bulls carcasses with age increased. The bulls' carcasses evaluated for R muscularity class moderately accounted of 14.4 percent. The least U muscularity class has been evaluated bulls carcasses (1.2 percent). U muscularity class evaluated heifers were only from 22 months old in heifers group. U class carcass composed of 0.9 percent. The R muscularity class of carcases with age gradually increased and were obtained 16.5 percent. Meanwhile, P muscularity class of heifers' carcases

evaluated with age decreased and accounted moderately of 8.3 percent. The O muscularity class evaluated carcasses were mostly (74.3 percent) in group of heifers. The highest U muscularity class evaluated of cows carcasses, moderately were found 0.4 percent, cows evaluated every 2 age months, R muscularity class obtained 2.5 percent, and P carcass muscularity class accounted of 47.4 percent. At most O muscularity class has been estimated of carcass, and it accounted of 49.7 percent. Other authors also argue, that in order to get a good muscularity class, and thus higher profits, it is best to grow up and realize the animals of a certain age, because muscle tissue later turns into fat tissue *(Berg et al., 2003; Serra et al., 2004; Jukna et al., 2009).*

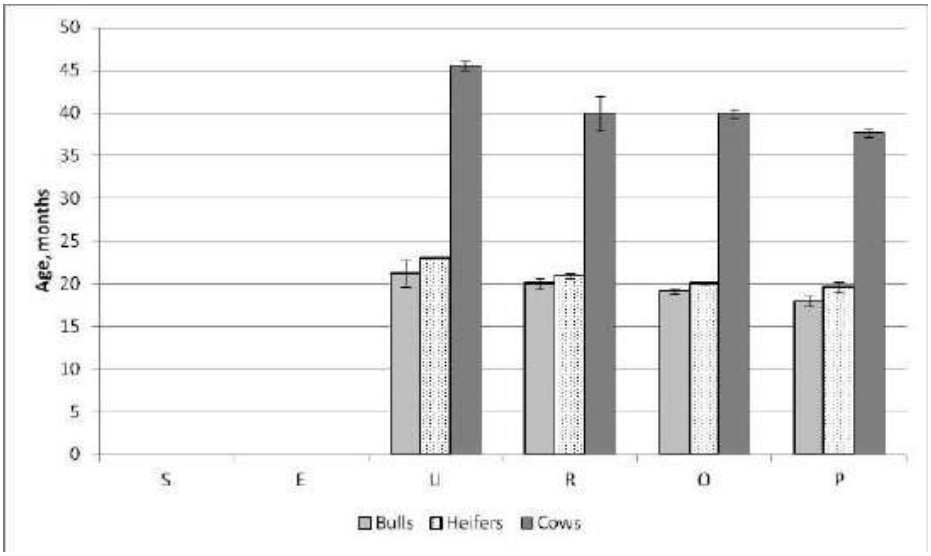

Figure 4. Cattle age distribution according SEUROP of muscularity class classification

Conclusion

Carcass yield of cattle depends on the animal's weight until to a certain age, if increasing mass of animal also increasing carcass yield. The linear averages trends obtained suggest that, an animal's pre-slaughter weight an average increase of 50 kg, carcass yield of bulls on average increased 11.1 percent, of heifers - 0.72 and cows – 0.94 percent.

Although, in all studied groups of animals were obtain weak correlation links between age of animal and carcass yield, of bulls ($r=0.11$; $p<0.05$), heifers ($r=0.09$; $p<0.05$) and cows ($r=0.09$; $p<0.05$), but the trend shows that with increasing age, carcass yield also increases. The strongest correlation links was

identified between the animal's weight and carcass weight of bulls ($r = 0.93$, $p < 0.001$), heifers ($r = 0.94$, $p < 0.001$) and cows groups ($r = 0.91$, $p < 0.01$).

Between age and carcass yield linear dependence was expressed slightly. This dependence was not statistically significant in the bulls group. The linear trends averages obtained suggests that, if analysed cattle age moderately increases for 2 months, then moderately carcass yield increased for 0.21 percent in heifers group ($p < 0.05$) and 0.12 percent in cows group ($p < 0.05$).

Mostly, O muscularity class of carcass were evaluated in all analysed animals groups, both depending from pre-slaughter weight and age, it was apparently, therefore that in a slaughterhouse mostly were slaughtered different dairy breeds cattle and their crossbreeds with beef breeds. In the group of bulls evaluated O muscularity class carcasses were found moderately (76.8 percent), heifers (74.3 percent) and in group of cows (49.7 percent). However, a trend was observed that with animal's weight and age arise muscularity class.

Uticaj telesne mase i godine starosti na randman i konformaciju trupa goveda

N. Pečiulaitienė, V. Jukna, E. Meškinytė-Kaušilienė, S. Kerzienė, S. Moleikaitienė

Rezime

Cilj ovog istraživanja bio je da se analiziraju efekti telesne mase i starosti goveda mlečnih rasa i njihovih meleza (423 bika, 492 junice i 567 krava) na prinos trupa i ocenu konformacije prema SEUROP standardnim podacima. Sve životinje su podeljene u grupe prema težini na klanju (interval od 50 kg) i prema uzrastu (interval od 2 meseca). Za istraživanje, prosečne starosti životinja su: bikovi - 19 meseci, junice - 20 meseci i krave - 38 meseci. Najveća prosečna masa je utvrđeno kod krava 521,2 kg, zatim junica 461,7 kg a najmanja prosečna masa je utvrđena kod bikova 456,2 kg međutim, u prosečmi prinos/randman trupova je bio najviši kod bikova - 51,3 %, 50,1% kod junica i najmanje 47,8 % kod krava. Kada prosečna telesna masa životinja na klanju se poveća za 50 kg, prinos trupova bikova u proseku se poveća za 11,1%, kod junica - 0.72 % i krava - 0.94 %. U svih ispitivanim grupama životinja su dobijene slabe korelacije između starosti životinja i prinosa/randmana trupova. Najjače korelacije su dobijene između telesne mase i težine trupova bikova ($r = 0,93$, $p < 0,001$), junica ($r = 0,94$, $p < 0.001$) i krava ($r = 0,91$, $p < 0,01$). Pokazalo se da, ako se starost goveda umereno povećava za 2 meseca, onda dolazi do umerenog povećanja prinosa trupa od 0,21 %, kao što je u junica ($p < 0,05$) i 0,12 % u grupi sa kravama ($p < 0,05$). Ovaj odnos nije bio statistički značajan u grupi bikova. Uglavnom, O klasa mišićavosti trupova su ocenjeni u svim analiziranim grupama životinja, u zavisnosti od težine na klanju,

kao i starosti. Međutim, registrovan je trend da sa telesnom masom i godinama starosti životinje poboljšava se prinos trupa i povećava klasa mišićavosti trupova.

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MANAGEMENT OF THE STORAGE OF CRYOPRESERVED SPERM ON DAIRY CATTLE FARMS

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Original scientific paper

Abstract: 26 liquid nitrogen tanks were selected from different dairy cattle farms. Three sperm doses were introduced in a frequently used canister, while another three straws were deposited in another canister that did not contain any sperm doses, to determine whether the refilling with liquid nitrogen had been done appropriately. Then, 10 sperm doses belonging to the same freezing lot were stored in our laboratory under ideal conditions to be used as control doses. After certain time period, the doses were collected from the farms and were analysed to obtain data about their total sperm motility and the individual kinetic parameters of each sperm. Four sperm subpopulations (SP) with different patterns of motility were identified using a cluster multivariate analysis. The results show that the mean total sperm motility has hardly decreased for the doses stored in the frequently used canister (45.2 ± 6.9%) in comparison with the doses stored in the rarely used canister (46.9 ± 59.0). However, the decrease in total motility was greater when compared with the control doses (59.0%). As for the sperm SP, (SP4 rapid and progressive sperm) which contained 31% of the total of sperm (control doses), differed the most when control doses were compared to straw stored in farm tanks. The percentage of the latter was reduced to 10 % after being stored in the tanks of the farms for 7 mo.

Such damage in SP 4 is progressive and cumulative and would probably reduce drastically compromising the fertility of the aforementioned sperm doses.

Keywords: motility, sperm subpopulations, management

Introduction

In the last 25 years, we have succeeded in improving the genetics and the management of the dairy cattle, which has resulted in an increase in milk

production. However, this improvement has triggered a decrease in the reproductive efficiency in the livestock farms *(Lucy, 2001; López-Gatius, 2003)*. During the last decade the pregnancy rate has been declining year after year and nowadays it stands at about 35% *(Quintela et al., 1997)* in the northwest of Spain (Galicia). In the beginning, the reasons for the decrease in fertility were attributed only to the genetic selection of the animals in favour of milk production *(Quintela et al., 1997)*. However, the cause may be multifactioral and not only due to genetic selection *(Lucy, 2003; García-Ispierto, 2007*). Several factors could be influencing the reproductive efficiency of the dairy herds. For instance, stress due to heat (*Lucy, 2003; García-Ispierto, 2007)*, nutritional causes *(Roche, 2000)*, the comfort of the cow *(Hansen et al., 1999)* and incorrect practices in the management of the sperm doses in the farm, which could lead to a reproductive decline. The last factor has been widely revised as it could be one of the main reasons for the decrease in fertility *(Larson and Graham, 1958; Pickett and Berndtson, 1974; Senger et al., 1980; Saacke, 1983; Barth, 1993).*Correct management of the storage of cryopreserved sperm is fundamental in achieving suitable pregnancy rates when artificially inseminating dairy cattle. On several occasions, artificial insemination (AI) organisations have published recommendations with the intention of helping farmers and AI technicians in the handling of the doses of frozen-thawed semen. Despite these attempts, incorrect management has been confirmed in several studies in which not only professional inseminators *(Pickett, 1971)* are implicated, but also farmer-inseminators *(López Gatius, 2003)* under the supervision of vets and AI organisations. It seems that the problem is more significant with the farm owner/inseminator who have minimum training and whose technique has not been supervised. As time goes by, even professional inseminators may develop bad habits and a lax attitude towards the management of semen and insemination technique, which means a decrease in fertility.

Table 1. Mean values (±SD) of the kinematic parameters for the four subpopulations identified in frozen-thawed semen of dairy farms

Kinematic Parameters	SP 1	SP 2	SP 3	SP 4
Nº spz (%)	5996 (35.8)	2446 (14.6)	2977 (17.8)	5291 (31.6)
VCL (µm/s)	126.3±20.a	167.9±26.8b	64.3±24.5c	185.6±23.3d
VSL (µm/s)	100.1±17.8a	64.8±24.5b	37.8±20.4c	148.5±23.9d
VAP (µm/s)	111.5±17.2a	129.7±25.1b	48.1±21.7c	166.1±21.3d
LIN (%)	79.9±12.0a	38.8± 14.0b	57.4±23.1c	80.6±12.1d
STR (%)	89.8±8.6a	50.9±19.3b	75.2±21.7c	89.6±9.5d
WOB (%)	88.9±9.1a	77.7±11.6b	73.6±16.9c	89.7±7.4d
ALH (µm)	3.1±1.3a	5.2±1.5b	2.4±1.0c	4.3±1.5d
BCF (Hz)	8.7±3.1a	9.2±3.5b	5.6±3.4c	9.0±2.8d

a, b, c, d: different letters indicate significant differences between subpopulations ($P<0.05$)

So far, the experiments carried out to evaluate the decline in membrane integrity and motility of frozen-thawed semen were based on subjective microscopic assessment. Nowadays, much more precise and objective techniques are available, Computer-assisted semen analyses (CASA), for evaluating the functional characteristics of the sperm. Moreover, they allow us to quantify the different sperm subpopulations *(Muiño et al., 2008)*. The identification and quantification of sperm subpopulations with different patterns of movement can be vital to help estimate the fertilizing capability of the sperm doses of bovine frozen-thawed semen. It is likely that the inappropriate management of the sperm doses has a specific influence on the percentage of sperm subpopulations capable of reaching the female oviducts. Therefore, if the number of sperm is insufficient, the sperm will not be able to cross the barriers of the reproductive tract of the female and fertilize the oocyte *(DeJarnette et al., 1992; Nadir et al., 1993)*.

The main aims of the present study were:

1. To evaluate whether the farmer-inseminators have acquired bad habits and neglected their attitude towards the management of the semen, and consequently, whether they have contributed, together with other factors, to the decline in the fertility of the dairy cattle.
2. To investigate the influence of the management of the sperm doses during the storage on the frequency of distribution of the spermatozoa inside the different sperm subpopulations.

Material and Methods

Experimental design

In this study, 26 herds of Holstein-Friesian high-yielding dairy cows (26 liquid nitrogen tanks) were analysed in Northwest Spain. All the dairy farms were classified into two groups in order to evaluate the sperm stored in their tanks. One of the groups was made up of 17 small farms with an average of 36 cows and 23 heifers. The other group was made up of 9 collaborating farms with an average of 75 cows and 46 heifers. The herds were selected because they represented the largest dairy area in Galicia. Several parameters such as, tank condition and location, were written down in each farm.

An ejaculate from a *toro Asturiano de los Valles*[1] was selected and processed to be cryopreserved. Of the whole lot of frozen-thawed straws, 156 were selected to be deposited in the 26 cryogenic storage tanks (with a capacity of 18-20 L of liquid nitrogen). Three straws from the lot were placed in a canister with

[1] *Asturian Bull from the Valleys*. A breed of bull typical of Asturias that can also be found surrounding areas such as the east of Galicia.

semen which was routinely used by the cattle farmer. Thus, it was subjected to different types of manipulation such as raising and lowering the canister. Another three doses from the lot were placed in a canister without semen to know whether the frequency of refill with nitrogen was correct. The rest of the straws from the lot were stored in our laboratory and were used as control doses. They were also assessed every so often to get a standard of comparison as they were stored under ideal conditions. The liquid nitrogen in the control tank was kept at less than 5 cm below the neck of the tank.

When the doses were deposited, some characteristics related to the management of the tank, such as the state of the tank and its insulation, were investigated.

17 tanks from collaborating farms were classified as tanks in good conditions, with a lack of damage on the surface of the tank and its neck. However, nine tanks were in poor condition due to denting (Table 2).

Table 2. Characteristics related to the management of the tank

Parameters studied			**% Sperm total motility**	**% sperm inside SP**
Condition of the tank	Good Condition N=17	Poor Condition N=9	N.S	N.S
Frequency of refill with liquid nitrogen	30 dias N=18	45 dias N=7	N.S	N.S
Size of the catlle farms	Small farms(36 cows) N=17	Higher farms(75 cows) N=9	N.S	N.S

N.S indicate non-significant differences between the parameters studied and % sperm total motility and % sperm inside SP.

As for tank insulation in the farms, they were classified into 3 categories: on the floor and not protected (9 farms), on the floor but protected inside a box (11 farms) and kept upright, ideal conditions (7 farms) (Figure 2.a and 2.b).

After 7 mo, the doses stored in the tanks of the collaborating farms were collected (6 sperm doses). These were labelled with the farm´s origin. And the doses were transferred to our laboratory in order to evaluate the effects of the management on their sperm quality.

Collection and freezing of semen.

An ejaculate from an *Asturian bull from the Valleys* was collected by using an artificial vagina (internal temperature at 45º C). Then ejaculate volume and sperm concentration were evaluated (Accucell; IMV, L'Aigle, France). The morphology and sperm motility were subjectively assessed. After the initial evaluation, each ejaculate was diluted to a concentration $92x10^6$ sperm/ml using a

commercial extender Bioxcell (IMV, L'Aigle, France). This diluted semen was cooled from 22º C to 5º C for 1 ½ h (the cooling rate was of 0.22ºC/min approximately) and stored at 5ºC for a period of 2½ h. Thus total equilibration time was of 4 h. After dilution, it was packaged in 0.25 ml straws (23x106 sperm/ml) and frozen in vapours of liquid nitrogen inside a programmable freezer. We followed the standard freezing curve IMV Digit-cool, L'Aigle, France) for bovine semen (-5ºC/min from +4ºC to -10ºC; 40ºC/min from -10ºC to -100ºC; and -20ºC/min from -100ºC to -140ºC). Subsequently, the doses were deposited in liquid nitrogen (-196ºC) for a month and an initial evaluation of the motility (59%) was carried out in the Artificial Insemination Centre of Somio (SERIDA), Gijón, Asturias. After this period, the straws were delivered to the collaborating farms.

The experiment

Six straws from each farm were thawed simultaneously in a water bath at 37ºC for 40 s and their content was deposited in 5 ml Falcon tubes. The frozen-thawed semen was incubated at 37ºC for 2 h. One aliquot was evaluated immediately after warming (0 h) and another 2 hours later in order to assess the number of sperm per 5 µL. Sperm kinetic parameters were also assessed using the CASA system (ISAS, Valencia, Spain) in order to determine the existence of sperm subpopulations in the samples of bovine frozen-thawed semen. The analyses were also done to determine if cryogenic storage of the sperm on the farms damaged the structural integrity of the aforementioned sperm subpopulations. The sperm motility parameters of each cryopreserved sample were examined immediately after thawing (0 h) and after 2 h incubation at 37ºC using CASA.

The CASA system used captures 16 consecutive digital photographic images in a fraction of 0.64 s. This implies an image capture rate of one photographic image every 40 milliseconds. The images are taken from a sperm aliquot of 5 µL which is placed on slides and covered with 20 x 20 mm coverslips. Three microscopic fields were analysed in each sample using a phase contrast microscope supplied with a pre-warmed stage at 37ºC and a 100x magnification (Olympus BH2, Olympus Optical Co, U.K.). The total number of the spermatozoa analysed in each sample varied from 100 to 200. Non sperm particles were manually eliminated from each analysis. The total motility was defined as the percentage of spermatozoa with a mean velocity (VAP) of 10 µm/s approximately.
The kinetic parameters analysed for each sperm were the ones described by *Mortimer (1997, 2000)*: curvilinear velocity (VCL, µm/s), which is the distance covered by the spermatozoid along its trajectory according to time; rectilinear velocity (VSL, µm/s), which is the distance covered by the spermatozoid between the first and the last point of its trajectory per unit time; mean velocity (VAP, µm/s) which is, the distance covered by the spermatozoid along its mean trajectory per unit time; linear coefficient (LIN, %), defined as the percentage relation between VSL and VCL; straightness coefficient (STR, %) which is the percentage

relation between VSL and VAP; Wobble coefficient (WOB, %) defined as the percentage relation between VAP and VCL; mean lateral head displacement (ALH, μm), defined as the movement that the head of the sperm makes in its curvilinear trajectory from one point to another of the mean or linear trajectory; frequency of head displacement (BCF, Hz), which is the frequency with curvilinear trajectory that crosses the mean or linear trajectory according to time.

Statistical analysis.

The data of all motile sperm obtained from 104 thawing processes (26 dairy farms x 2 canisters x 2 assessments) were clustered in a single data group that represented 16,710 spermatozoa. Each spermatozoid was defined according to the eight motility patterns described above. A multivariate K-means clustering analysis was carried out in order to classify the 16,710 spermatozoa into a reduced number of subpopulations taking into account their pattern of movement. According to this classification, each sperm belonged to a single cluster. The spermatozoa which were very close to each other were assigned to the same group while those which were further away from each other were classified into different groups. The K-means clustering procedure uses Euclidean distances of 8 quantitative variables so that the core of the clusters was the means of the observations assigned to each cluster. In order to define the exact number of clusters, a prior analysis of hierarchic dendograms was carried out (Holt 1996). This analysis was based on individual ejaculates using the Ward method. For each of the canisters belonging to the 26 sperm tanks, contingency tables were used in order to determine the percentage of spermatozoa assigned to the different clusters at different times (0h and 2h).

A General Linear model (GLM) was used to analyse the frequency of distribution of the spermatozoa inside the subpopulations after subjecting the straws to the post-defrosting incubation and to a manipulation consisting of raising and lowering the canisters inside the sperm tank. The GLM procedure was also used to evaluate the influence exerted on the distribution of spermatozoa inside the subpopulations by dependent variables as the following: the level of liquid nitrogen inside the tanks, insulation of the tank, preservation conditions of the tanks and size of the cattle farms. All the statistical analysis were carried out using the SPSS software (SPSS Inc., Chicago, IL, USA), and the differences were considered significant when $P<0.05$.

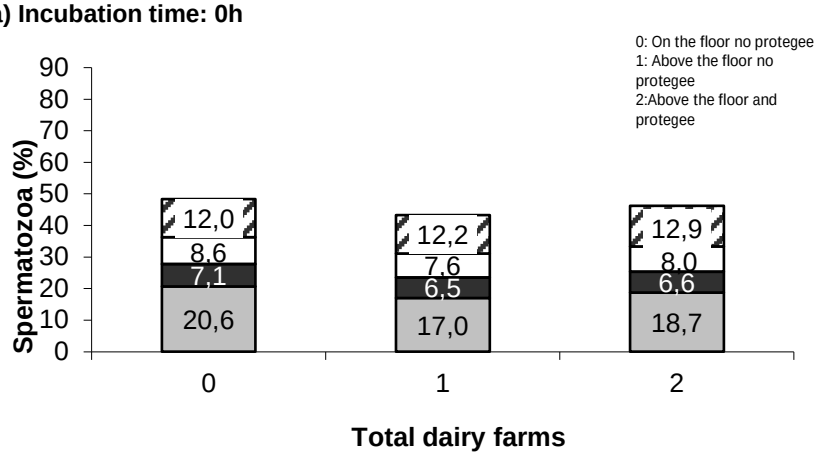
Results

Sperm total motility

The sperm managed in the 26 dairy farms had a lower mean sperm motility, objectively determined with the CASA system, compared to the doses

stored in the AI centre. Immediately after the thawing process (Figure 1.a), the percentage of sperm total motility was 45.2 ± 6.9%, 46.9 ± 8.9% and 59.0%, for thawed samples in frequently used canisters, in rarely used canisters and the control doses respectively. As demonstrated, no significant differences were observed among the treatment groups. The variables, such as the conditions of the storage tank, and the size of the cattle farms, did not have any interaction over the objective sperm motility immediately after thawing (Table 2). However, the differences in the insulation of the tanks of nitrogen had a significant effect on the total sperm motility (Figure 2.a). Thus, the doses stored in the tanks placed on the floor and without insulation showed a mean sperm motility of (48.5 ± 7.2). Similar results were found for the doses stored in tanks insulated inside a box and kept upright (46.3 ± 7.2). A significant decline in sperm motility was obtained for the doses stored in tanks placed on the floor, although insulated inside a box (43.5 ± 8.5). After 2 h of incubation (Figure 2.b), the percentage of total sperm motility diminished in all the tanks located in different places. However, the doses that registered the highest decline were those stored in tanks placed on the floor and not insulated (35.6 ± 9.4) whereas the values of motility for tanks placed on the floor and insulated were 40.3 ± 7.3 and the values of sperm motility for tanks kept upright and insulated were 41.0 ± 8.7. The frequency with which all the tanks of the collaborating farms were refilled was correct. Therefore, it did not influence the total sperm motility, nor did the size of the dairy farms (Table 2).

*indicate significant differences between subpopulations (P<0.05).



*indicate significant differences between subpopulations (P<0.05).

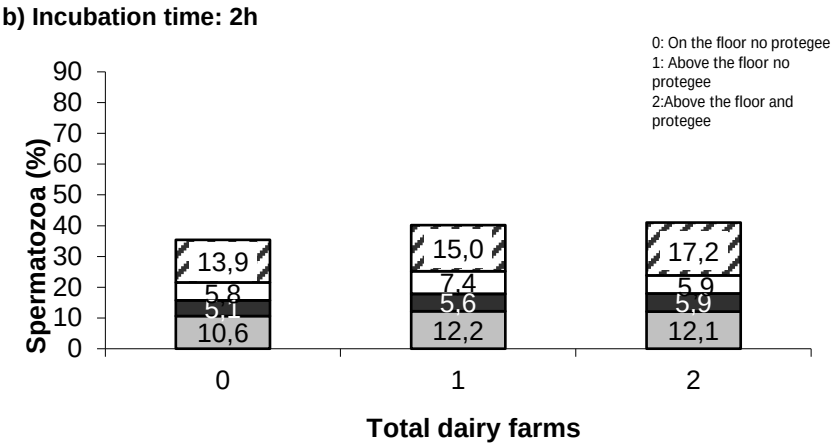


Figure 2. Relative frequency distribution of motile spermatozoa within subpopulations of straws storage in different insulates tanks, thawed at 37ºC during 40s, after 0 (a) and 2 h (b) of incubation at 37ºC.

Motile sperm subpopulations.

Four sperm subpopulations were defined after carrying out a multivariate cluster analysis of a total of 16,710 individual sperm. The characteristics of motility of those subpopulations are shown in table 1 and its patterns of motion can be described as follows:

Subpopulation 1 is represented by spermatozoa with low velocity (mean VCL and VAP) but with high progressiveness (high LIN, STR, WOB and low ALH). This subpopulation represents 35% of the total motile spermatozoa.

Subpopulation 2 is made up of spermatozoa with high activity but with non progressive motion as indicated by high values of VCL and ALH together with low values of LIN and STR and moderate BCF. These spermatozoa could be considered to have a movement similar to hyperkinesis. About 14% of the total motile spermatozoa were assigned to this subpopulation.

Subpopulation 3 is represented by 17% of the total spermatozoa that move slowly and not progressively. These spermatozoa show low values of VCL, ALH and BCF together with low LIN and STR.

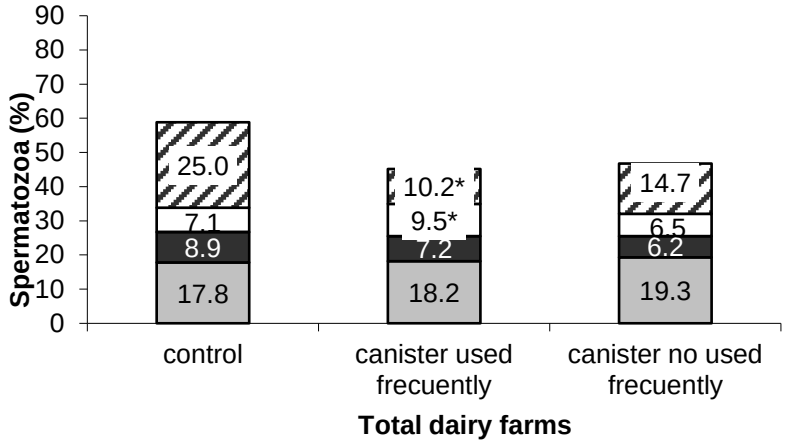
Subpopulation 4, which contains about 31% of the total population, is represented by spermatozoa with faster and progressive movement as its high values of VCL, VAP, LIN, STR and also BCF indicate.

Frequency of distribution of the spermatozoa inside the subpopulations according to management of doses in different canisters.

Immediately after thawing, the use of doses placed in a commonly used canister had a significant effect ($P< 0.05$) on the proportion of spermatozoa assigned to subpopulations 4 and 3. (Figure 1.a). Just after thawing, the samples of

semen placed in a frequently used canister by the farmer showed a significant reduction ($p<0.05$) in the subpopulation of spermatozoa with rapid and progressive movement (SP 4). It also showed an important increase in the proportion of spermatozoa with slow and non progressive movement (SP 3). Meanwhile, the sperm doses stored in our laboratory showed a high proportion of spermatozoa assigned to subpopulation 4. The management of semen did not alter the percentage of spermatozoa assigned to subpopulations 1 and 2.

a) Incubation time: 0h



*indicate significant differences between subpopulations ($P<0.05$).

b) Incubation time: 2h

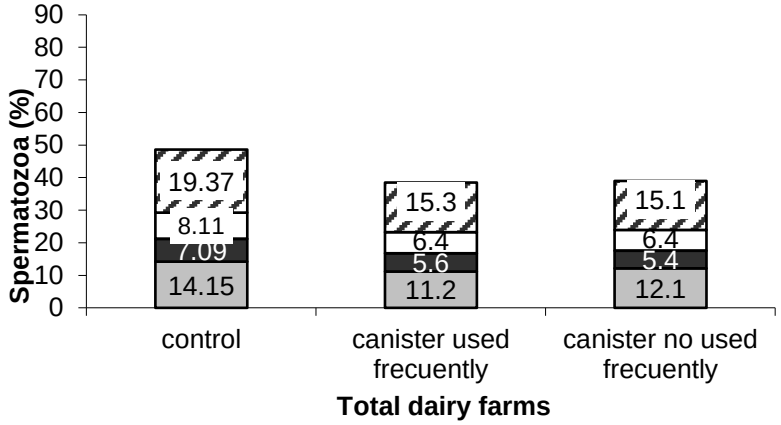


Figure 1. Relative frequency distribution of motile spermatozoa within subpopulations (1: grey columns, 2: black columns, 3: white columns, 4: striped columns) of straws storage in canister used frequently and other canister no used frequently, thawed at 37ºC during 40s, after 0 (a) and 2 h (b) of incubation at 37ºC.

After 2 h of incubation (Figure 1.b), the percentage of sperm assigned to subpopulations 3 and 4 diminished in all the samples of semen placed in the farmer and our laboratory. However, the doses that registered the highest decline were those stored in semen placed in a frequently used canister and rarely used canisters. The best results remained for controlling dose , more sperm number assigned to the SP4, and less proportion of spermatozoa with SP3. No significant differences were observed among the different groups

Other variables, such as the differences in conditions of tank insulation and the frequency of refill with liquid nitrogen, did not have any effect over the frequency of distribution of the spermatozoa inside the subpopulations (Table 2).

Discussion

The results of the present study indicated that, in general terms, there were no significant differences in the post-thaw sperm motility between the control doses and the doses stored in the dairy farms. These results disagree with other previously published studies that show significant differences between the semen stored in the farms and the one stored in the AI centre *(Pace and Sullivan, 1978; Linewear et al., 1979; Senger, 1980)*. In those prior studies, different periods of storage were used as well as different methods of sperm package. Furthermore, the sperm motility was assessed subjectively *(Barth, 1993; Dalton, 2002)*.

In this experiment, four sperm subpopulations were established in samples of frozen-thawed bull semen. They were determined according to the eight kinetic parameters studied. The present study confirms previous studies *(Muiño et al., 2008)* in which a very similar structure was described. That structure consisted of four sperm subpopulations in fresh and frozen-thawed Holstein bull sperm. Despite the fact that both experiments were developed in bulls of different breeds, the cryopreservation protocols used were similar. The presence of three or four well defined sperm subpopulations was shown in other species *(Holt et al., 1996; Abaigar et al., 1999; Quintero et al., 2003; Nuñez Martinez et al., 2006)*.

The doses that were managed and stored for 7 mo induced considerable changes in the distribution of the spermatozoa in the subpopulations. These changes were evident immediately after thawing the samples.

A significant increase in the proportion of the spermatozoa assigned to the subpopulation of spermatozoa with low and non progressive motion (SP 3) was observed in the samples of frozen-thawed semen. In these samples we could also observe a significant decline in the proportion of rapid and progressive spermatozoa (SP 4). This fact has recurred in all the sperm doses that were stored in the tanks of the dairy farms studied.

The damage caused to the frozen sperm when it is exposed to room temperature, around 20º C *(Larson and Graham, 1958; Rapaz, 1966; Holt, 2000),* has been pointed out in several studies. This is common during manipulations such as extracting the straw and then reintroducing it in liquid nitrogen with the aim of transferring the straws from one tank to another, or simply to see their identification. Such manipulations can result in a partial thawing of the content of the straw. As a consequence, the spermatozoa can undergo a phenomenon called re-crystallisation by which the sperm becomes unviable.

The present study suggests that the decline in the percentage of spermatozoa observed in subpopulation 4 at the moment of thawing (the proportion of the most rapid and progressive spermatozoa might be the most appropriate pattern of movement for fertilization to take place) may be caused by the phenomenon of re-crystallisation. The increase in the size of some crystals might damage the sperm membrane and make some of the spermatozoa in SP 4 move to SP 3 (formed by non-progressive spermatozoa with little movement). The transfer from SP 4 to SP 3 could represent a late stage in cell deterioration. As the differences between the doses in the farms of study and the control doses were mainly found in the proportion of the spermatozoa assigned to subpopulation 4, this subpopulation might also determine the differences in fertility on site. For this reason, the doses stored in the AI centre might have a better fertility on site than the ones stored in the collaborating farms. The correlation between subpopulation 4 and the number of spermatozoa bound to the zona pellucida, the penetration rate and the rate of pronucleos formation were determined by *Ferraz et al., (2013).* They found a significant ($P<0.05$) and positive correlation between the zone pellucid, the penetration rate and the pronucleus formation with sperm subpopulation 4 ($r=0.79$, $r=0.66$ and $r=0.63$, respectively).

Berndtson et al., (1976) showed that when the 0.25 ml straws in the globet were exposed to 20 ± 0,6ºC, that is room conditions, the temperature of re-crystallisation was reached in 40-60 s of exposure. However, re-crystallisation was reached in less time, in 10-15 s, when the straws were exposed to the air at 20 ± 0,6 ºC and a forceps was used. *Barth (1993)* suggests that the sperm in the neck of the tank must not be handled for more than 5 s in order to avoid re-crystallisation.

Other studies suggested that the sperm stored and used on site can be subjected to gradual deterioration *(Pace and Sullivan, 1978; Janett et al., 2008).* However, these changes were not observed by other authors *(Lineweaver et al., 1979; Senger et al., 1980)* when they compared sperm stored in farms with sperm stored in AI centres as they obtained similar values of viability and sperm motility. Furthermore, they did not find any differences in viability between the sperm located in the upper part of the canister and the one in the lower part.

Other possible sources of sperm damage have been described by different researchers and taken into account in the present study. For example, the level of liquid nitrogen in the farm´s cryogenic storage tanks, the package of sperm in 0.25

ml straws and the insulation of the tanks. When the level of liquid nitrogen is high enough to cover the globets, the sperm is much less sensitive to changes in temperature. Such changes are produced especially when the sperm doses are removed in order to carry out the AI or to transfer them from one tank to another *(Berndtson et al., 1976)*. In our experiment, the level of liquid nitrogen was always kept above the minimum level. The tanks were frequently refilled and the level of liquid nitrogen was periodically checked with a dipstick. In order to study whether the frequency of refill with liquid nitrogen was correct, we analysed the motility and the subpopulations in the doses stored in canisters. Then, we compared them with the doses stored in the AI centre and no differences were found between them. This result confirmed our hypothesis that the frequency of refill was correct.

With regards to the type of straws for packaging semen, the 0.25 ml straws are the most commonly used in Galicia (in the Northwest of Spain) freeze and store bovine sperm due to their high storage efficiency in liquid nitrogen.

It is well known that 0.25 ml straws respond faster to the changes in temperature than 0.50 ml straws *(Stevenson et al., 2009)*. However, the fertility rate of semen frozen in 0.25 ml straws is the same or higher than that of semen frozen in 0.50 ml straws *(Pickett and Berndtson, 1974; Johnson et al., 1995; Stevenson et al., 2009)*. In this experiment, the 0.25 ml and 0.50 ml doses, which were stored at the same time, did not show any differences between them in sperm motility nor in the distribution of the subpopulations after being stored in the farms for 7 mo. Similar results were obtained by *Berndtson and Foote (1972) and Coulter and Foote (1973)* who did not observe any differences in the sperm motility of the sperm doses stored for 0, 6 and 18 mo.

Finally, when the insulation of the tanks (usually of 18 L) was revised, we could see that most of them were located in the offices of the farms. They were kept upright and protected from direct sunlight in a place where they were easily accessible. The place was fresh, dry and clean, as well as being ventilated and free of dust. Nine tanks were placed directly on the floor and subjected to acid corrosion that might deteriorate them. The total motility after thawing diminished considerably in the tanks kept directly on the floor as compared to those kept upright.

In conclusion, once the sperm doses in the tanks studied in this experiment were assessed, the results obtained in terms of total sperm motility after thawing were better in the case of the doses stored in the AI centre as well as in those kept in the rarely used canister. However, the study of the different sperm subpopulations present in the thawed sperm samples, and especially the dynamic subpopulations, revealed slight functional differences between both ways of handling the doses (frequent management of the canister / no management of the canister). A high percentage of spermatozoa with rapid and progressive movement was shown, after the thawing process, in the doses kept in the AI centre. However, it is not known whether this slight difference (SP 4) observed in these doses with

respect to the ones stored in the farms can affect fertility *in vivo*. Nevertheless, a more detailed study of the sperm subpopulations, which coexist in a sperm sample, might open up new possibilities to improve semen mishandling. Moreover, it could help avoid its harmful effects on the sperm doses kept in the farm tanks and in particular the sperm stored in the commonly used canister. It seems likely that this problem could be accentuated in the farms in which the inseminators are the owners of the herds because they very often have minimum training and no supervision. Consequently, as time goes by they may develop bad habits and a lax attitude towards the sperm management.

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Skladištenje, čuvanje zamrznute sperme na farmama muznih krava

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Rezime

Ukupno 26 kontejnera sa tečnim azotom je izabrano sa različitih farmi muznih krava. Tri doze semena su stavljene u kontejner/kanister koji se često koristi, dok je još tri slamke položeno u drugi kanister koji nije sadržavao nijednu dozu sperme, da se utvrdi da li se dopunjavanje sa tečnim azotom radi na odgovarajući način. Zatim, 10 dozi sperme koje pripadaju istoj zamrznutoj partiji su čuvane u našoj laboratoriji pod idealnim uslovima, i koristile su se kao kontrolne doze. Doze su posle određenog vremensko perioda prikupljene sa farmi i analizirane kako bi se dobili podaci o ukupnom motilitetom sperme i pojedinačnim kinetičkim parametrima svake sperme. Četiri subpopulacije sperme (SP) sa različitim obrascima pokretljivosti su identifikovani pomoću multivariacione analize klastera.

Rezultati pokazuju da je srednja ukupna pokretljivost sperme neznatno opala za doze uskladištene u kontejneru/kanisteru koji se često koristi (45,2 ± 6,9%) u odnosu na doze pohranjene u kontejner/kanister koji se retko koristi (46,9 ± 59,0). Međutim, smanjenje ukupnog motiliteta bilo je veće u poređenju sa kontrolnim dozama (59,0%). Što se tiče sperme SP, (SP4 brza i progresivna sperma) koja je sadržala 31% od ukupnog broja spermatozoida (kontrolne doze), razlikovala se najviše kada su kontrolne doze poređene sa dozama koje se čuvaju u

kontejnerima na farmama. Procenat potonjih je smanjen na 10% nakon što su skladištene u kontejnerima na farmama u periou od 7 meseci.

Takva oštećenje kod SP 4 je progresivno i kumulativno i verovatno će doći do drastičnog smanjenja, što ugrožava plodnost navedenih doza sperme.

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IDENTIFICATION AND DIFFERENTIATION AMONG CHICKEN'S, DUCK'S, QUAIL'S, RABBIT'S AND TURKEY'S MEAT USING PCR-RFLP TECHNIQUE

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Original scientific paper

Abstract: PCR–RFLP technique was developed for identification and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat. DNA from small amount of muscles (0.05 g) was extracted and a region of mitochondrial DNA (cytochrome-*b* gene) in chicken, duck, quail, rabbit and turkey was amplified by PCR. Fragment length of the PCR product was 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in both quail and turkey. Six nucleotides different makes it difficult to differentiate among these five species-specific meat. For differentiation, three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I) were used to digest the PCR products. Restriction analysis showed difference among chicken's, duck's, quail's, rabbit's and turkey's meat. Where, *Dde*I yielded two fragments (291 and 83 bp) only in rabbit's meat. *Msp*I yielded three fragments (221, 85 and 65 bp) in chicken's meat and two fragments (290 and 87 bp) in both quail's and turkey's meat. *Taq*I yielded three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat. The use of Cyt*b*-PCR-RFLP assay allowed a direct and fast authentication and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat.

Key words: Poultry, meat, discrimination, cytochrome-*b*, PCR-RFLP

Introduction

Consumers are concerned by a variety of issues, such as food authenticity and adulteration. The identity of the ingredients in processed or composite mixtures is not always readily apparent and verification that the components are authentic and from sources acceptable to the consumers maybe required (*Lockley and Bardsley, 2000*). This opens the possibility of fraudulent adulteration and substitution of the expected species with others of less value. For protection consumer's rights, the legislation of each country should therefore impose a labelling of food products declaring the species used in the processed foods. Many

different methods such as morphological characteristics, immunological, electrophoretic and chromatographic were previously used for species identification (*Taylor et al., 1993; Andrasko and Rosen, 1994; Espinoza et al., 1999; Czesny et al., 2000*). Application of such protocols has, however, failed to successfully differentiate closely related species, highlighting the need for a method possessing higher specificity and sensitivity (*Bellis et al., 2003*). However, the analysis of molecular genetic variations could potentially provide definitive information regarding animal species origin. Recently, food products such as meat products can be fast and accurate identified using molecular genetic methods such as PCR and PCR-RFLP techniques. Buffalo's, cattle's, sheep's, cat's, dog's, donkey's, horse's and pig's meat were identified using PCR technique (*Ahmed et al., 2007; Abdel-Rahman et al., 2009*), while Cyt*b*-PCR-RFLP technique was used to differentiate between chicken's and turkey's meat (*Lenstra et al., 2001*). In the current study, PCR–RFLP technique was developed for identification and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat using cytochrome-*b* gene oligonucleotide primers.

Materials and methods

DNA extraction. Genomic DNA was extracted from chicken's, duck's, quail's, rabbit's and turkey's muscle samples according to Abdel-Rahman et al., (2009). Where, 50 mg of the tissue was homogenized and suspended in 500 µL STE (0.1 M NaCl, 0.05 M Tris-HCL and 0.01 M EDTA, pH 8). After adding 30 µL 10% SDS and 30 µL proteinase K (10 mg/mL), the mixture was vortexed and incubated at 50°C for 30 min. 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 (50 µL) of autoclaved double distillated water (addH_2O).

PCR amplification. A fragment of cytochrome-*b* gene (377 bp, approximately) in chicken, duck, quail, rabbit and turkey was amplified by PCR with the use of specific primers sequences (Forward/Reverse) (5'-CCCCTCAGAATGATATTTGTCCTCA-3'/5'-CCATCCAACATCTCAGCATGATGAAA-3') (*Bellis et al., 2003*). PCR was performed in a reaction volume of 25 µL using 25 ng of genomic DNA of each specie, 10 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/µL *Taq* DNA polymerase (Promega). Thermal cycling (MyGene Series Peltier Thermal Cycler) was carried out by initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 1 min,

annealing temperature at 57°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% 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).

PCR-RFLP. For digestion, 10 μL of PCR product (371-377 bp of mitochondrial *cytochrome-b* gene) in chicken (371 bp), duck (374 bp), quail (377 bp), rabbit (374 bp) and turkey (377 bp) was accomplished with 10 units of *Dde*I, *Msp*I and *Taq*I restriction enzymes for four hours at 37°C (*Dde*I, *Msp*I) and for one hour at 65°C (*Taq*I). Digested fragments were separated on 3% agarose gels in IX TBE buffer, stained with ethidium bromide, visualized under UV light and photographed.

Results and discussion

In this study, the amplification of mitochondrial DNA segment (*cytochrome-b* gene) generated PCR products with sizes 371 bp in chicken, 374 bp in duck and rabbit, 377 bp in quail and turkey. As a result of the little difference of the nucleotides number (6 bp) among the five species, the positions of the PCR products are approximately the same (Figure 1).

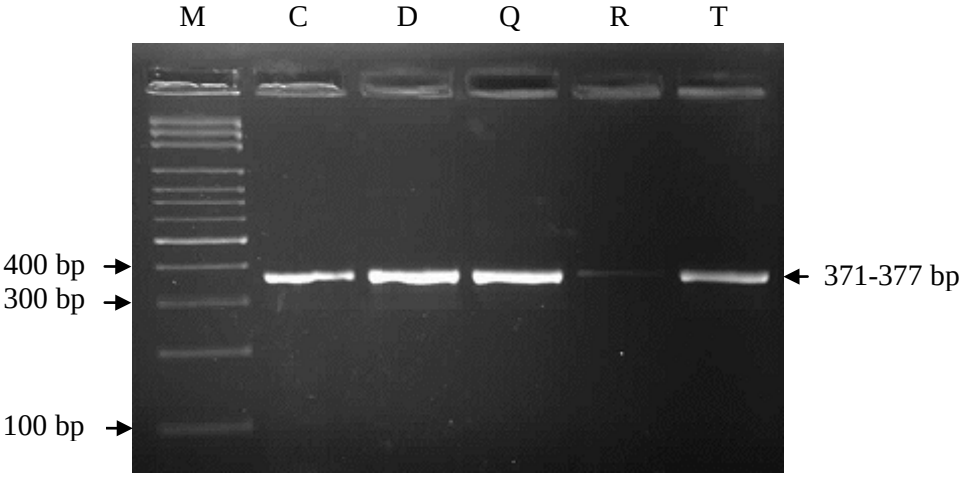

Figure 1. PCR products (371, 374 and 377 bp) of the amplified cytochrome-*b* gene. Lane C is chicken, lane D is duck, lane Q is quail, lane R is rabbit, lane T is turkey and lane M is a molecular weight marker (100 bp).

For differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat, PCR–RFLP technique was used. PCR products (371-377 bp) of the amplified region of the gene encoding cytochrome-*b* were treated with three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I), separately (Table 1). *Dde*I restriction enzyme yielded two fragments (291 and 83 bp) only in rabbit's meat, while in the other species no digestion was obtained (Figure 2).

Table 1. Species PCR products and fragment length of the amplified cytochrome-*b* gene generated by restriction enzymes (*Dde*I, *Msp*I and *Taq*I).

No.	Specie	PCR product (bp)	*Dde*I	*Msp*I	*Taq*I
1	Chicken	371	-	221/85/65	-
2	Duck	374	-	-	146/134/94
3	Quail	377	-	290/87	226/151
4	Rabbit	374	291/83	-	-
5	Turkey	377	-	290/87	-

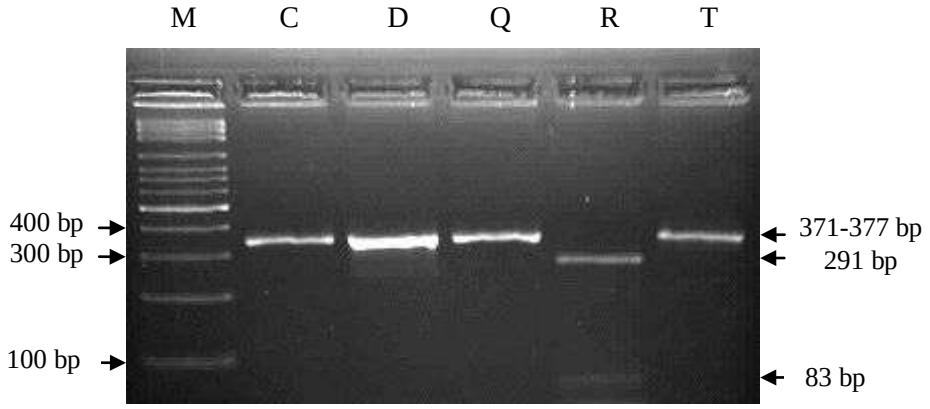

Figure 2. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Dde*I generated two fragments with sizes of 291 and 83 bp in rabbit (lane R). Lane C is chicken, lane D is duck, lane Q is quail, lane T is turkey and lane M is a molecular weight marker (100 bp).

*Msp*I restriction enzyme yielded three fragments (221, 85 and 65 bp) in chicken's meat and two fragments (290 and 87 bp) in both quail's and turkey's meat, while in the other two species (duck's and rabbit's meat) no digestion was obtained (see Figure 3).

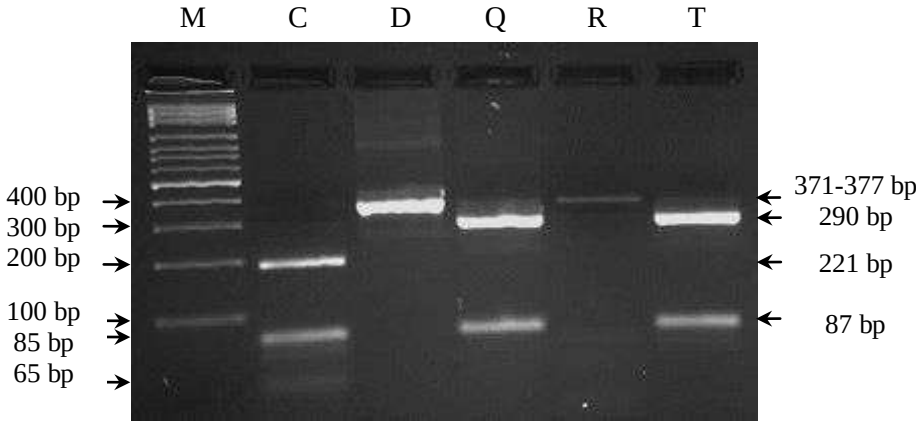

Figure 3. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Msp*I generated three fragments with sizes of 221, 85 and 65 bp in chicken (lane C) and two fragments with sizes of 290 and 87 bp in both quail and turkey (lanes Q and T). Lane D is duck, lane R is rabbit and lane M is a molecular weight marker (100 bp).

As can be seen in Figure 4, *Taq*I restriction enzyme generated three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat, while in the other three species (chicken's, rabbit's and turkey's meat) no digestion was obtained.

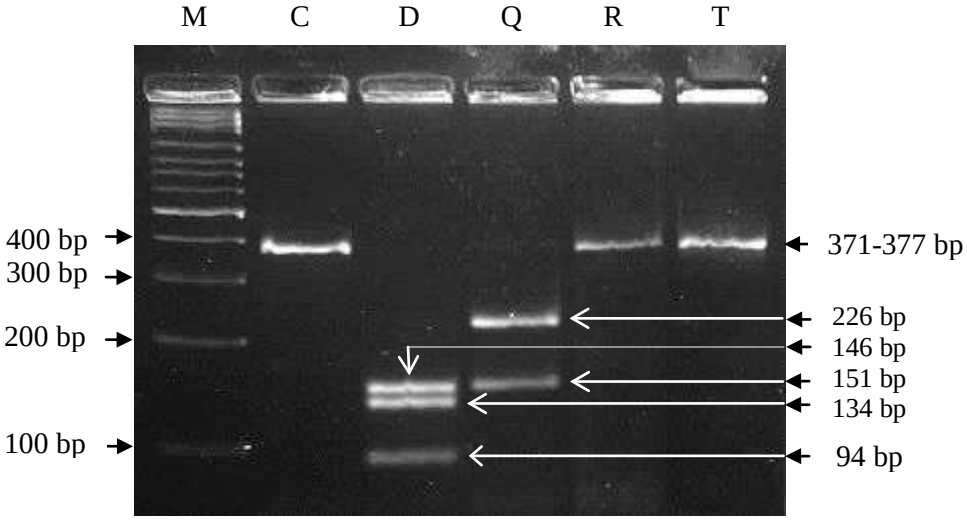

Figure 4. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Taq*I generated three fragments with sizes of 146, 134 and 94 bp in duck (lane D) and two fragments with sizes of 226 and 151 bp in quail (lane Q). Lane C is chicken, lane R is rabbit, lane T is turkey and lane M is a molecular weight marker (100 bp).

From these results, it could be easily identify and differentiate among chicken's, duck's, quail's, rabbit's and turkey's meat using the amplified cytochrome-*b* gene. Where, restriction analysis showed difference among these species using three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I). However, *Dde*I yielded two fragments (291 and 83 bp) only in rabbit's meat. *Msp*I yielded three fragments (221, 85 and 65 bp) in chicken's meat and two fragments (290 and 87 bp) in both quail's and turkey's meat. *Taq*I yielded three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat. It should be noted that *Msp*I yielded two fragments (290 and 87 bp) in both quail's and turkey's meat, discriminated by *Taq*I (see Table 1).

Numerous studies have been previously carried for detection and identification of species-specific meat using molecular genetic methods such as PCR and PCR-RFLP techniques (*Baradakci & Skibinski, 1994; Meyer et al., 1995; Meyer et al., 1996; Hopwood et al. 1999; Partis et al., 2000; Sharma et al., 2000; Lenstra et al., 2001; Abdulmawjood et al., 2003; Ahmed et al., 2007; Ilhak and Arslan, 2007; Abdel-Rahman et al., 2009*). For example, species-specific PCR and Cyt *b*-PCR-RFLP techniques were used to identify buffalo's, cattle's, sheep's, cat's, dog's, donkey's, horse's and pig's meat. The results of PCR amplification were 603 bp in buffalo and cattle, 374 bp in sheep, 672 bp in cat, 808 bp in dog, 221 bp in donkey and horse, and ≤100 bp in pig. To differentiate between buffalo's and cattle's meat, as well donkey's and horse's meat, cytochrome-*b* gene was amplified (359 bp) and digested with restriction enzymes. *Taq*I generated two different fragments (191 bp and 168 bp) in buffalo, whereas no fragments were obtained in cattle. *Alu*I yielded three different patterns in horse (189 bp, 96 bp and 74 bp), while in donkey no digestion was obtained (*Ahmed et al., 2007; Abdel-Rahman et al., 2009*).

Conclusion

PCR–RFLP technique was used to identify and differentiate among chicken's, duck's, quail's, rabbit's and turkey's meat. DNA from small amount of muscles (0.05 g) was extracted and a region of mitochondrial DNA (cytochrome-*b* gene) was amplified by PCR. PCR product was 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in both quail and turkey. For differentiation, three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I) were used to digest the PCR products. *Dde*I yielded two fragments (291 and 83 bp) only in rabbit's meat. *Msp*I yielded three fragments (221, 85 and 65 bp) in chicken's meat and two fragments (290 and 87 bp) in both quail's and turkey's meat. *Taq*I yielded three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat. The

proposed Cyt*b*-PCR-RFLP assay represents a rapid and sensitive method applicable to the detection and authentication of poultry meat species.

Identifikacija i razlikovanje pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa, korišćenjem PCR-RFLP tehnike

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Rezime

PCR-RFLP tehnika je razvijen za identifikaciju i diferencijaciju između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa. DNK iz male količina mišića (0,05 g) je ekstrahovana i region mitohondrijalne DNK (citohrom-b gena) pileta, patka, prepelice, zeca i ćurke je amplifikovana pomoću PCR. Dužina fragmenta PCR proizvoda je bila 371 bp kod pileta, 374 bp patke i zeca i 377 bp kod prepelice i ćurke. Šest nukleotida razlike otežava razlikovanje između ovih pet vrsta mesa. Za diferenciranje, tri različite restriktivna enzima (*Dde*I, *Msp*I i *Taq*I) su korišćeni za digestiju PCR proizvoda. Restriktivna analiza je pokazala razliku između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa, gde je, *Dde*I dala dva fragmenta (291 i 83 bp) samo u mesu zeca. *Msp*I je dala tri fragmenta (221, 85 i 65 bp) u pilećem mesu i dva fragmenta (290 i 87 bp) u mesu prepelice i ćurke. *Taq*I daje tri fragmenta (146, 134 i 94 bp) u pačetini i dva fragmenta (226 i 151 bp) u mesu prepelice. Upotreba Cyt*b*-PCR-RFLP testa omogućavaa direktnu i brzu potvrdu mesa određene vrste i diferencijaciju između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa.

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DEVELOPMENT OF IGY ANTIBODIES FOR CONTROL OF TETANUS

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Abstract: Tetanus is a cut and often highly fatal infectious disease that affects both human and animals; the disease caused by exotoxin which produced by *C. tetani*. In the current study, we try to get hyperimmune IgY in chicken egg against tetanus toxin and use it as prophylaxis and therapeutic treatment for tetanus. The obtained IgY titer after inoculation of tetanus toxin in chicken eggs was 1320 limit of flocculation (Lf-eq) after 72 hr. IgY in adose of 4500 Lf-eq can be protect donkey after artificial infection by 1 minimum lethal dose (MLD) of *C. tetani*. While a dose of 30000 Lf-eq IgY intramuscularly two times daily for 2 injections, with 9500 Limit of flocculation Lf-eq IgY intrathecally in subarachnoid space was 100% curative for a donkey which was challenged with 1 MLD of *C. tetani*. Furthermore, IgY was evaluated experimentally in comparison with IgG in mice. IgY has equally efficacy to IgG in prophylaxis and treatment of tetanus.

Key words: Tetanus, IgY, IgG, Treatment.

Introduction

Tetanus, commonly called lock jaw, is a wound infection disease that is usually accompanied by fatal toxaemia. The toxaemia causes contraction of voluntary muscle, mainly those of the face, neck, body, legs and tail. Spasms are the results of steady and prolonged contractions of the affected muscles. Tetanus is caused by a rod-shaped germ, *C. tetani*, which produces an extremely potent toxin. The germs form highly resistant, large terminal spores, which give the organism a particular drum stick appearance. The tetanus germs and spores remain localized at the place of the wound where they enter the body. They multiply and produce the powerful toxin (*Johnston, 1994; Kahn, 2010*).

The disease is more prevalent in Lower Egypt than upper one with an infectivity rate of 28 per million in the former and 4.7 per million in the latter.

Infection is more common among equines showing an infectivity rate of 1.2 % in mules, 0.4% in horses and 0.025 % in asses. The infectivity rate in males was always greater than in females. The financial losses were LE 19,057.880 which considered to be far less than the actual losses (calculated for 10 years) (*El-Nahas, 1962).*The recorded cases of equine tetanus in Egypt were 6660 case between 1980 and 1990 (*Ahmed, 1991*).

Chicken eggs consider an ideal alternative antibody source to mammals, as the IgY in the chicken's blood is transported to the egg and accumulates in the egg yolk in large quantities. The existence of an IgG like molecule in avian eggs (IgY), has been well documented in recent studies and extensive research has been carried out on its characterization, production and purification (*Hodek and Stiborová, 2003).*

The chicken egg yolk antibodies (IgY) have been applied successfully for scientific (*Schade et al., 1997*), diagnostic (*Di Lonardo et al., 2001*), prophylactic (*Almeida et al., 1998*) and (*Sarker et al., 2001*) and therapeutic purposes (*Lemamy et al., 1999*), and veterinarian therapy against bacteria such as enteropathogenic E. coli (*Amaral et al. 2002*). There are several distinct advantages for using chickens to produce polyclonal antibodies over than other animals, the chicken IgY have higher titers, animal-friendly, cheaper, nearly unlimited quantities, contain a larger glycosylation index, and stability (*Gassmann et al., 1990*).

This study aimed to prepare antitetanic antibody (IgY) in chicken egg and evaluate it as prophylactic and therapeutic treatment against tetanus in experimental infected animals

Material and methods

Development, purification and titration of anti-tetanic IgY:

a. Purification and concentration of tetanus toxin

Tetanus toxin was purified in VACSERA laboratory (Egypt) using ammonium sulphate, and concentrated to the required concentration. Crude tetanus toxin of 1350 Lf/ml determined by (Ramon, 1922) was used.

b. Immunization of hens

Hens were immunized with tetanus toxin according to the following hyperimmunization schedule (Table 1) after modification the method of *(Rűdiger et al., 1996*).

Table1.Immunization schedule of hens using tetanus toxin

Inoculation Day	Inoculation dose	Adjuvant
0	10 lf tetanus toxin in 0.25 ml normal saline	0.25 ml CFA
7	20 lf tetanus toxin in 0.25 ml normal saline	0.25 ml IFA
14	30 lf tetanus toxin in 0.25 ml normal saline	0.25 ml IFA
21	50 lf tetanus toxin in 0.5 ml normal saline	0.5 ml Aluminum hydroxide (1mM)
28	100 lf tetanus toxin in 0.5 ml normal saline	0.5 ml Aluminum hydroxide (1mM)
35	200 lf tetanus toxin in 0.5 ml normal saline	0.5 ml Aluminum hydroxide (1mM)
42	400 lf tetanus toxin in 0.75 ml normal saline	0.75 ml Aluminum hydroxide (1mM)
49	500 lf tetanus toxin in 0.75 ml normal saline	0.75 ml Aluminum hydroxide (1mM)
56	500 lf tetanus toxin in 0.75 ml normal saline	0.75 ml Aluminum hydroxide (1mM)
63	500 lf tetanus toxin in 0.75 ml normal saline	0.75 ml Aluminum hydroxide (1mM)
	Blood for serum sample and collecting eggs for purification	

Sampling

At 9^{th} day after the last inoculation two samples were taken, 1 ml blood sample per hen from wing vein and laid eggs were collected daily.

Serum collection

Whole blood was collected in a covered test tube. Then allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 min. The clot was removed through centrifuging at 1.000-2.000xg for 10 min in a refrigerated centrifuge. The resulting supernatant is the serum. The samples should be maintained at 2-8°C while handling. If the serum is not analysed immediately, the serum should be apportioned into 0.5 ml aliquots, stored, and transported at -20°C or lower. It is important to avoid freeze-thaw cycles because this is detrimental to many serum components. Samples which are haemolysed, icteric or lipemic can invalidate certain tests.

c. Purification of egg yolk IgY

Egg yolk free of egg white obtained from pre-warmed refrigerated eggs was rinsed in triple distilled water and rolled in tissue paper for complete removal of the albumen. After several such washes, the yolk membrane was punctured in a pierced funnel shaped filter paper in glass funnel, allowing the yolk to flow into a

graduated measuring cylinder holding the membrane. The yolk was diluted by adding 9 volumes of pre-cooled distilled water and the pH was adjusted to 5-5.2 with 1M HCl and incubated at 4ºC for 6 to 8 hrs. Following incubation the supernatant was harvested and centrifuged at 3000 x g for 25 min in a refrigerated centrifuge. The resulting immunoglobulin (supernatant) containing filtrates (water-soluble fraction) were collected and estimated for protein concentration by the Modified Biuret and Dumas method (*Dumas, 1971*). The IgY containing water-soluble fractions was purified by the salt precipitation method by titrating against 33% ammonium sulphate solution (Algomhoria co., Egypt) as described by *Hansen et al,. (1998)* in three cycles. The precipitate from the last cycle containing IgY was dissolved in normal saline, dialyzed against the same saline until ammonium sulphate was completely removed (*Gazim and Irena, 2003*).

The Protein concentration of the final suspension containing purified immunoglobulin was estimated using the Modified Biuret and Dumas method (*Dumas, 1971*).

d. Titeration of IgY

Using single radial immunodiffusion test according to the method of **(***Ljungqvist and Lyng, 1987*) and Ramon flocculation test according to the method of (*Ramon, 1922*).

Detection of Clostridium tetani minimum lethal dose

20 ml of *C. tetani* (Harvard strain obtained from Abbasia research institute for veterinary vaccine and antisera production, Egypt) bacteria in its media were centrifuged at 3000 rpm for 20 min to remove media and toxins. The precipitated bacteria suspended in 20 ml PBS saline (PH 7.2). These previous steps repeated 2 times. Tenfold series dilutions of the previous suspension in calcium chloride were prepared to reach 2.5% of each tube (1, 1/10, 1/100, 1/1000, $1/10^4$, $1/10^5$, $1/10^6$, $1/10^7$, $1/10^8$, $1/10^9$). In 10 Swiss mice groups each of five inject 0.2 ml per mouse in the thigh muscles one dilution per one mice group. After 4 days explore the highest dilution group where all mice were dead this is minimum lethal dose.

Evaluation of prophylactic and therapeutic capability of anti-tetanic IgY and anti-tetanic IgG in experimental mice

The mice were divided into four groups (15 mice per group) as in table 2.

Table 2. Proposal therapy of mice groups

Days	Group 1 (IgY prophylaxis)	Group 2 (IgY Therapy)	Group 3 (IgG prophylaxis)	Group 4 (IgY Therapy)
0	1000 IU IgY S/C		1000 IU IgG S/C	
1	2MLD of *C. tetani* in 2.5% $CaCl_2$ I.M. in 0.2 ml, 5 mg metronidazole BID rectally for group 1 and 3			
2	5 mg metronidazole BID rectally		5 mg metronidazole BID rectally	
3	5 mg metronidazole BID rectally	1000 IU IgY subcutaneously plus 10 mg metronidazole BID rectally	5 mg metronidazole BID rectally	1000 IU IgG subcutaneously plus 10 mg metronidazole BID rectally
4-11	5 mg metronidazole BID rectally			
12-16		5 mg metronidazole BID rectally		5 mg metronidazole BID rectally

Evaluation of prophylactic capability of anti-tetanic IgY versus metronidazole in experimental infected mice

The mice were divided into three groups each group 10 mice as in table 3.

Table 3. Proposal of IgY and metronidazole prophylaxis of mice groups

Day	Group1 (IgY prophylaxis)	Group 2 (Metronidazole prophylaxis)	Group 3 (Positive control)
0	1000 IU IgY S/C	5 mg metronidazole BID rectally	-
1	2MLD of *C. tetani* in 2.5% $CaCl_2$ I.M. in 0.2 ml 5 mg metronidazole BID rectally for group 2		
2	-	5 mg metronidazole BID rectally	-
3	-	5 mg metronidazole BID rectally	Death of all mice
4-16	-	5 mg metronidazole BID rectally	-

Prophylactic and therapeutic capability of antitetanic IgY in experimental donkeys

Six donkeys divided into 3 groups; 2 donkeys designed for prophylactic group, 2 donkeys designed for therapeutic group and 2 donkey designed for positive control group. The prophylactic group was injected with 3 ml per donkey of IgY (1500 Lf/ml) I/M and metronidazole in a dose of 25 mg /Kg BW three times daily orally for 7 days. After that, all groups injected with 1 ml of crude *C. tetani* in 2.5% calcium chloride.

Symptoms start to appear in therapeutic and positive control groups at 8^{th}

day. These symptoms start as stiffness causing the donkeys to move reluctantly, head and ears are extended, there is evidence of muscular spasms affecting the muscles of mastication and making eating and drinking difficult. It becomes hypersensitive with the external stimuli (sounds, light, touch), and we can note a hyper salivation.

The treatment starts at 8^{th} days with injection of 20 ml of IgY tetanus antitoxin 1500 Lf/ml I/M for two times daily for 2 injections. After hypnotize donkeys with 10% chloral hydrate in a dose of 100 mg/Kg BW I/V, 7 ml IgY tetanus antitoxin 1500 Lf/ml was directly injected into the subarachnoid space through the atlanto-occipital space after removal of 7 ml of CSF.

In addition to, dissolving metronidazole tablets in 20 ml water in a dose of 25 mg/kg BW and administer it per rectum TID for 10 days. In combination with administration of Diazepam 0.1 mg/kg BW to release muscle stiffness, Vitamin C in a dose of 2 gm I/V per day for 10 days.

Results

The results of preparation, purification and titration of anti-tetanic IgY.

The immunization of the hens with tetanus toxins combined with different types of adjuvant and increasing doses according to the schedule, achieve in the development of protective IgY. The purification of IgY through ammonium sulphate precipitation method gives high immunoglobulin yield with low protein impurity where the total protein of egg yolk before purification was 6.5 mg/ml and after purification was 3.9 mg/ml.

a. The result of single radial immunodiffusion test

Known samples with increasing titres were put in the marginal wells while the unknown sample (anti-tetanic IgY) was put in the central well of Petri dish. The dish was incubated 48 hrs. Precipitation rings appear around all wells. The diameters of the marginal precipitation rings were measured (Table 4).

Table 4. Interpretation of petri dish radial precipitation rings

Well Nr.	Lf	Diameter of precipitation ring
1	240	1.3 cm
2	480	1.6 cm
3	720	1.9 cm
4	960	2 cm
5	1200	2.2 cm
U	Unknown IgY sample (anti-tetanic IgY)	2.3 cm

In figure 1, the data obtained from the experiment was plotted where X axis is the diameter of the precipitation ring and Y is the LF equivalent, then by dropping the unknown sample line we can explore that the unknown sample was 1320 Lf-eq.

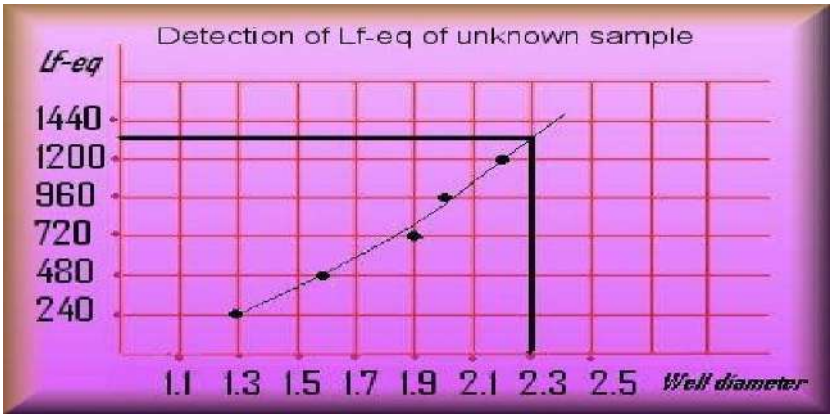

Figure 1. The Ag-Ab precipitation ring diameter was plotted against Known titre to determine unknown sample

b. Results of flocculation test

Results of flocculation test indicated that the first tube that flocculate was test tube No. 6 which contain 1300 Lf is 1 ml purified IgY potency was 1300 Lf-eq.

Results of determination of C. tetani minimum lethal dose

The minimum lethal dose was at the fourth tube with dilution 1/1000 where it's the high dilution that kills all the mice in one group.

Results of evaluation of Prophylactic and therapeutic capability of antitetanic IgY and antitetanic IgG in experimentally infected mice

The mice of therapeutic groups showed signs of at the third day of the experiment which include erected tail, stiffness and rigidity in the injected limb. The therapeutic regime was applied as showed in table 2. The results showed no deaths in the mice of prophylaxis and therapeutic group either with treatment with IgY or IgG. The result indicated that anti-tetanic IgY is as effective as anti-tetanic serum (IgG) originating from equine immunoglobulin.

Results of evaluation of prophylactic capability of antitetanic IgY versus metronidazole in experimental mice

Positive control group results showed that mice were died after 48 hr of experimental infection, while both anti-tetanic IgY prophylaxis mice group and metronidazole prophylaxis mice group still alive till day 10 of the experiment without any tetanus symptoms. The result indicated that anti-tetanic IgY is effective in prophylaxis of experimentally infected mice.

Results of evaluation of prophylactic and therapeutic capability of antitetanic IgY in experimental donkeys

At eighth day of the experiment, symptoms start to appear on both, therapeutic and positive control groups while there was no signs observed on prophylactically IgY treated group.

The prophylactic group showed a dose of 4500 Lf-eq IgY one day before experimental tetanus infection was 100% protective as a prophylactic dose for a donkey of around 100 Kg body weight challenged with 1 MLD of *C. tetani* where no tetanus symptoms appears on this group of animal all over the experiment .

The therapeutic group showed a dose of 30000 Lf-eq IgY I/M BID for one day and 9500 Lf-eq IgY intrathecally was 100% curative for a donkey of around 100 Kg body weight challenged with 1 MLD of *C. tetani* .

The results showed at day 20 of the experiment donkeys of this group starts to open their mouth, and muscle spasms fades but the animals still unable to stand. While at day 27 of the experiment donkeys start to walk and return normal without any spasm but still weak.

The positive control group animal died by respiratory failure due to severe muscle contraction at day 13 of the experiment. The result indicated that anti-tetanic IgY is effective in prophylaxis and treatment of experimentally infected donkeys.

Discussion

Tetanus is a bacterial disease that can affect most animals. Horses are particularly susceptible because of their environment and tendency to incur injuries. Tetanus antitoxin is produced by hyper immunization of donor horses and then harvesting the antibodies. It is used to administer to unvaccinated horses to induce short-lived, immediate, passive protection. The passive immunity usually lasts only 2 to 3 weeks (*Barnett et al., 2001*).

This study was aimed to find a new method of treatment of tetanus using IgY originating from chickens to avoid problems of IgG originating from mammalian origin. Furthermore, the chicken IgY is cheaper, much more in quantity than mammalian antibody and it can be used together with mouse and rabbit antibodies without the danger of cross- reactivity. Secondary antibodies

against chicken IgY's don`t cross react with mammalian IgG's (*Michael et al. 2010*).

There is an increasing interest in the use of chicken egg yolk for polyclonal antibody production for practical and economic reasons (*Bollen et al. 1996; Svendsen et al., 1994; Tini et al., 2002*). The chicken egg yolk antibodies (IgY) have been applied successfully for scientific (*Schade et al., 1997*), diagnostic (*Di Lonardo et al., 2001*), prophylactic (*Sarker et al., 2001; Almeida et al., 1998*) and therapeutic purposes (*Lemamy et al., 1999*).

In this study, hens were immunized with tetanus toxin to hyper-immunization, followed with collection of blood samples and egg at day 9 after last inoculation of tetanus toxin and finally purification of IgY by salt precipitation method using ammonium sulphate solution. Hens could be immunized by tetanus toxin and produce their immunoglobulin in blood which transported to egg yolk in detectable, protective amounts that could be purified and protect other animals by passive immunization (*Marco et al., 2009*).The protein (IgY) concentration in egg yolk after purification with ammonium sulphate determined at week 10 of immunization by spectrophotometer at 280 nm wave length within the absorbance range of 0.2 - 1.5.was 3.9 mg/ml and this agree with the IgY concentration produced with a previous study used ammonium sulphate (*Gazim and Irena, 2003*) where IgY concentration was 3.8 mg/ml. Purification with ammonium sulphate provides high immunoglobulin production, inexpensive and easy to perform.

From a productivity point of view, the yield of IgY obtained by various purification methods is of interest. Literatures reported that the amount of IgY obtained by ammonium sulphate (60% v/v) precipitation was 0.6 mg/ml of egg yolk (Hansen et al., 1998). According to earlier publications (*Hippel and Schleich 1978*) and (*Kabat and Mayer, 1968*) the neutral inorganic salts, such as zinc sulphate and cadmium sulphate induce the precipitation of the proteins.

Although these methods were found effective for IgY purification but had lower IgY production than ammonium sulphate purification.

C. tetani minimum lethal dose in mice was determined for the bacterial culture suspension; the dilution of 1MLD was 1/1000 of the original suspension, this experiment is of great importance to detect the lethality dose of the bacterial strain thus facilitate starting point to the other experiments.

The prophylactic and therapeutic capability of anti-tetanic IgY and anti-tetanic IgG in experimental mice were determined; tetanus symptoms appear on therapeutic groups at the third day of the experiment, the prophylactic and therapeutic effect of both IgG and IgY was identical and effective, this indicate that IgY obtained from tetanus toxin immunized hens has the same effect as IgG that obtained from equine tetanus toxoid hyper immunized serum.

The result of evaluation of Prophylactic and therapeutic capability of anti-tetanic IgY and anti-tetanic IgG in experimental mice are similar to the results

obtained (*Smith and MacIver, 1969*). They found that as the dose of antitoxin was increased, the time at which signs of tetanus first appeared was progressively delayed until, the dose of 500 units. Mice given the largest dose of antitoxin failing to give a high level of protection, (100 units) developed tetanus on average at approximately 9-10 days after injection of the spores. While infected mice with 1 MLD of *C. tetani* in 2.5% calcium chloride and treat them with different units of tetanus antitoxin they found that 1000 units is the best therapeutic dose where all mice in this group were alive.

The prophylactic capability of anti-tetanic IgY versus metronidazole in experimental mice was evaluated as shown in table (3), both anti-tetanic IgY prophylaxis mice group and metronidazole prophylaxis mice group still alive till day 10 of the experiment without any tetanus symptoms, the mode of action of metronidazole is through reduction of their nitro group, however, leads to the production of short-lived cytotoxic intermediates, which finally decompose into nontoxic end products (*Müller, 1983*). That leads to kill *C. tetani* before or shortly after the production of tetanospasmin. While the mode of action of IgY depends on in vivo neutralization of the produced tetanospasmin and modification of effector functions that help the immune system to kill the microorganism.

This experiment agrees with the followings: Metronidazole, a compound widely used in man with minimal side effects, has been shown to be highly active against experimental infections of *C. tetani* and C. welchii in mice (Freeman et al., 1968). Penicillin was used in combination with tetanus antitoxin to reduce the dose of antitoxin required for protection. By preventing multiplication of *C. tetani.*

Other researchers recommend the use of metronidazole (*Cook et al., 2001*) and (*Hsu and Groleau, 2001*). Metronidazole is associated with a better recovery time and a lower mortality rate than penicillin. Penicillin requires adequate blood flow to the site of infection in order to reach effective concentrations. The anaerobic wounds where *C. tetani* thrive often have become devitalized and do not receive enough blood flow. Metronidazole can penetrate devitalized tissue in wounds that penicillin cannot normally reach (*Ahmadsyah and Salim, 1985*).

Prophylactic and therapeutic efficacy of anti-tetanic IgY in experimental infected donkeys was evaluated. At eighth day of the experiment, symptoms start to appear on both, therapeutic and positive control groups, while there was no signs observed on prophylactically IgY treated group.

The results showed that, dose of 4500 Lf-eq of IgY was 100% protective as a prophylactic dose for a donkey of around100 Kg body weight challenged with 1 MLD of *C. tetani* bacteria. While a dose of 30000 Lf-eq IgY intramuscularly BID for 2 shoots, with 9500 Lf-eq IgY intrathecally was 100% curative for a donkey of around100 Kg body weight challenged with 1 MLD of *C. tetani*. Using intrathecal therapy with IgY resulting in the healing of signs, hence this therapy is obviously most beneficial early in the course of illness. In addition, this treatment

was without potential complications as seizures were reported in one of the five horses following intrathecal TAT (*Green et al., 1994*).

Our results indicated that the anti-tetanic IgY had protected both mice and donkeys against experimentally tetanus infection. Therefore, anti-tetanic IgY can be used as an alternative prophylaxis and therapeutic against tetanus infection together with traditional therapy. This study may ensure that the use of anti-tetanic IgY is a new trend in prophylaxis and treatment of animal tetanus in Egypt.

Razvoj IGY antitela za kontrolu tetanusa

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Rezime

Tetanus je česta i veoma fatalna zarazna bolest koja utiče i na ljude i životinje; bolest je uzrokovana egzotoksinom koji proizvodi *C. tetani*. U ovoj studiji, pokušali smo da dobijemo hiperimunih IgY iz kokošijeg jajeta protiv tetanus toksina i koristimo ga kao profilaksu i terapijski tretman za tetanusa. Dobijen IgY titar nakon inokulacije kokošijih jaja tetanusa toksinom bila je 1320 graničnom flokulacije (Lf-eq) posle 72 časa. IgY u dozi od 4500 Lf-eq može zaštititi magarca posle veštačke infekcije sa 1 minimalnom smrtonosnom dozom (MLD) *C. tetani*. Dok doza od 30000 Lf-eq IgY intramuskularno dva puta dnevno za 2 injekcije, sa 9500 graničnom flokulacijom Lf-eq IgY intratekalno u subarahnoidnom prostoru je bila 100% kurativna kod magarca koji je izazvan sa 1 MLD *C. tetani*. Štaviše, IgY je eksperimentalno procenjena u poređenju sa IgG kod miševa. IgY ima jednaku efikasnost/delotvornost kao i IgG, u prevenciji i tretmanu tetanusa.

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FUSARIUM INFECTION AND DEOXYNIVALENOL CONTAMINATION IN WINTER WHEAT

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Original scientific paper

Abstract: In this paper, the incidence of *Fusarium*-infected grain of winter wheat and the content of mycotoxin deoxynivalenol (DON) was studied in two Serbian cultivars Simonida and NS40S, both harvested in 2014. The level of *Fusarium* contamination of wheat grain was determined using phytopathological techniques based on the standard methodology while DON was detected by enzyme-linked immuno-sorbent assay (ELISA).

The incidence of *Fusarium*-infected grain ranged from 12 to 19% for Simonida and NS40S, respectively. *Fusarium graminearum*, as well-known producer of DON mycotoxin, was identified among *Fusarium* species. In addition, *Alternaria spp.* was isolated in high percentage, with an average incidence of 53% (Simonida) to 63% (NS40S).The average content of DON ranged from 424 μg kg^{-1} to 1101 μg kg^{-1} for Simonida and NS40S cultivars, respectively. Statistically insignificant negative correlation ($r = -0.18$) was determined between *Fusarium*-infected grain and DON in the cultivar Simonida and statistically insignificant positive correlation ($r = 0.11$) in the cultivar NS40S.

The mean levels of DON in studied wheat samples of both tested cultivars were not higher than the maximum permitted limit (1250 μg kg^{-1}) although the level of *Fusarium*-infected grain of both cultivars was relatively high. These results indicate that both wheat cultivars are susceptible to *Fusarium* infection and DON mycotoxin production in agro-ecological conditions of Serbia, but the cultivar NS40S being more susceptible compared to cultivar Simonida. In view of all stated above, regular health check of grains and developing strategies for integrated monitoring of incidence of Fusarium head blight are necessary preventive measures in protection of winter wheat.

Key words: *Fusarium* spp., deoxynivalenol, winter wheat

Introduction

Wheat is one of the most important cereal crop grown in Serbia, on approximately 500,000 ha, with average yield of 3,700 kg/ha *(Statistical Yearbook of Serbia, 2012)*. Besides maize, it is the main crop for human consumption, commonly used in the production of bread. It can also be applied in livestock nutrition, as an integral part of the feed mixture, while the straw is used as bedding in barns and stables.

The major fungal wheat disease is Fusarium head blight (FHB) *(Santos et al., 2013)*. In agro-climatic condition in Serbia, *Fusarium graminearum* has been mostly isolated from *Fusarium*-infected grains *(Lević et al., 2008; Krnjaja et al., 2011)*. There are different mycotoxins produced by this species, of which the major contaminant of cereal grains is deoxynivalenol (DON) *(Nakajima, 2007a; Stanković et al., 2012)*.

DON belongs to a larger group of trichothechenes, the type B-trichothecene, with toxic effects on animals and human health. In livestock production, DON is the main cause of reproductive disorders in pigs *(Biagi, 2009)*. In cattle, DON causes reduced food intake and lower milk production *(Trenholm et al., 1985)*. DON concentrations significantly increase in *Fusarium*-damaged grain *(Wegulo, 2012)*.

During the growing seasons in the field, the main factor responsible for FHB development and DON contamination is highly associated with weather conditions during the period of anthesis as the most susceptible growth stage for *Fusarium* infection. Intense rainfall during the anthesis disperses *Fusarium* inoculums from crop residues and promotes FHB infection *(Landschoot et al., 2012)*. Likewise, crop residues and infested debris are reported such as the major sources of the primary inoculum for *Fusarium* spp. involved in FHB epidemics *(Miedaner et al., 2008)*. According to *Blandino et al. (2012)* FHB infection and DON contamination of wheat grains caused by different factors, at first place climatic conditions, but also agronomic factors including previous crop residue management, cultivar susceptibility and fungicide applications. Similar to that, *Eiblmeier and von Gleissenthall (2007)* have stated that weather conditions at flowering, preceding crop, no or minimal tillage, susceptible cultivar, strobilurin as foliar fungicide (EC 31 – EC 59) and late harvest represent risk factors for increasing levels of DON in wheat grains. Therefore an integrated approach to the disease is appropriate to reduce the risk of high DON values in wheat grains.

Taking into consideration the importance of the harmful effect of *Fusarium* species and deoxinivalenol in wheat grains, the presence *Fusarium* spp. and deoxynivalenol (DON) in grains of two Serbian winter wheat cultivars, was analysed.

Materials and Methods

A total of 40 grain samples of two Serbian wheat cultivars, 20 samples of cultivar Simonida and 20 samples of cultivar NS40S were analysed in mycological and mycotoxicological tests. Grain samples were collected during harvest in 2014, from the crops production of the Institute for Animal Husbandry, Belgrade, Serbia. The tested wheat crops were sown in October 2013 in the field where maize was grown previously. During the growing season, the same basic cultural practices and crop protection measures were carried out for wheat crops of both cultivars, fertilization in late February was performed with ammonium nitrate fertilizer, crop protection measures against weeds, insects and pathogenic fungi were carried out at the wheat tillering stage, and treatment with fungicides against FHB was performed at the beginning of flowering. Samples of wheat grains were collected according to the method of the *European Commission (2006a),* and the moisture content of the grain was determined by moisture analyzer (OHAUS MB35, USA).

For the mycological analysis, grains were disinfected in 1% sodium hypochlorite (NaOCl) for 2-3 minutes, and rinsed twice in distilled water. After drying on filter paper, 100 of grains, of each sample, were distributed in Petri dishes with the nutrient medium (10 grains per Petri dish). Plates were incubated for 14 days at 20°C with alternating light and darkness. Two percent (2%) water-agar (WA) was used as nutrient medium, with NaCl (18g NaCl per 1 litre of medium) added to prevent the grain germination. Identification of colonies of fungi that have developed around the grains of wheat was done based on microscopic examination of mycelia and spores, as reported by *Burgess et al. (1994)* and *Watanabe (1994).* The incidence of fungal species were calculated according to *Lević et al. (2012)*: *I (%) = [Number of kernels in one sample in which a species occurred] / [Total number of kernels in the same sample] x 100.*

For the mycotoxicological analysis, the wheat grain samples were ground to a fine powder with an analytical mill (IKA A11, Germany). All the samples were kept at 4ºC in the refrigerator before further analysis. Five grams of powder was mixed with 1 g of NaCl and homogenized in 25 ml of 70% (v/v) methanol in a 250 ml Erlenmeyer flask on the orbital shaker (GFL 3015, Germany). Homogenate was filtered through a Whatman filter paper 1. The level of DON was detected using the competitive ELISA method according to the manufacturer's instructions Celer Tecna ® ELISA kits. Absorbance was determined at a wavelength of 450 nm on an ELISA plate reader spectrophotometer (Biotek EL x 800TM, USA). The limit of detection (LOD) was 40 μg kg^{-1} and the limit of quantification (LOQ) was 125 μg kg^{-1} for wheat for DON. The mean recovery turned to be 104±12%.

The correlation between individual values for moisture content, the incidence of *Fusarium* spp. and DON concentration was determined using the Pearson correlation coefficient.

Results

According to the data of Republic Hydro-meteorological Service of Serbia, for 2014 for Belgrade area, from 1 to 31 May abundant rainfall (278.5 mm) was recorded, when wheat was in the pheno stage of flowering. These were very suitable climatic conditions for the development of *Fusarium* infection on spike wheat.

The average moisture content of the tested samples of wheat was 9.96% (ranged from 9.30 to 10.29%) for the cultivar Simonida and 10.38% (ranged from 9.56 to 11.16%) for the cultivar NS40S. According to mycological analysis the species from the genera *Alternaria* and *Fusarium* were determined with highest incidence. Based on morphological characteristics, *F. graminearum* was identified as only producer of DON mycotoxin. In case of Simonida cultivar, in the examined samples, incidence of *F. graminearum* was 12% (ranged from 7-19%) wheras in the cultivar NS40S it was 19% (ranged from 5-35%). The incidence of *Alternaria* spp. was 53% (ranged from 38-65%) in the cultivar Simonida, and 63% (range 46-72%) in the cultivar NS40S (Table 1).

Table 1. Incidence of *Fusarium graminearum* and *Alternaria* spp. in tested grain samples of two Serbian wheat cultivars

Fungal species	Wheat cultivar			
	Simonida		NS40S	
	Incidence (%)			
	Range	Average	Range	Average
Alternaria spp.	38-65	53	46-72	63
Fusarium graminearum	7-19	12	5-35	19

The presence of DON was detected in 100% of tested samples of both cultivars. The average concentration of DON was 424 μg kg^{-1} (ranged from 175 – 610 μg kg^{-1}) in the cultivar Simonida, and 1101 μg kg^{-1} (ranged from 214-1440 μg kg^{-1}) in the cultivar NS40S (Table 2). The average concentrations of DON in unprocessed wheat grain of both tested cultivars were not above the maximum permitted limit (1250 μg kg^{-1}) adopted by the *European Commission (2006b).*

Table 2. Level of DON in tested grain samples of two Serbian wheat cultivars

Mycotoxin	DON	
Wheat cultivar	Simonida	NS40S
Sample size[a]	20/20	20/20
Incidence %	100	100
Range (μg kg^{-1})	175-610	214-1440
Mean[b] (μg kg^{-1})	424	1101

[a] Number of positive samples/Number of total samples; [b] Mean concentration in positive samples

Statistically insignificant negative correlation (r = - 0.18) was determined between the incidence of *F. graminearum* and concentrations of DON in the cultivar Simonida, and statistically insignificant positive correlation (r = 0.11) in the cultivar NS40S. The correlation between moisture content and incidence of *F. graminearum* evident in both cultivars was statistically insignificant negative, while between moisture content and concentration of DON statistically insignificant positive correlation was found in both tested cultivars.

Discussion

In this study, a high incidence of *F. graminearum* was established in the investigated samples of wheat grains of both studied cultivars, although, cultivar NS40S had higher average incidence of *F. graminearum* (19%) compared to the cultivar Simonida (12%). Similarly, the average concentration of DON in Simonida was lower (424 μg kg^{-1}) in relation to the cultivar NS40S (1101 μg kg^{-1}). These results are similar to those reported by *Krnjaja et al. (2014)* where the average incidence of *F. graminearum* was 14% and the average concentration of DON was 478 μg kg^{-1} in the 19 tested wheat samples of the Serbian cultivar Takovčanka, collected during the harvest 2013. It should be noted that in all these trials, grains sampled originated from the wheat crop treated with fungicide against FHB at the flowering stage of development. Based on earlier research *of Krnjaja et al. (2011a, b)*, the average incidence of *F. graminearum* was higher than 80% in wheat grain samples originating from crops that were not treated with fungicide at the flowering stage of development. The average concentration of mycotoxin DON ranged from 214 to 490 μg kg^{-1}*(Krnjaja et al., 2011a, b)*. These results pointed out that the agro-ecological and agro-climatic conditions in Serbia are very suitable for the occurrence of FHB and production of DON mycotoxin in wheat grain. Furthermore, maize as the previous crop and important host species for *Fusarium* spp. provided more inoculum especially in favourable weather conditions such as heavy precipitation during anthesis of wheat in 2014. In our study, differences were found between the cultivars in terms of the incidence of *F. graminearum* and the DON content. This indicated that the use of less susceptible cultivars to FHB can be an important factor in reducing the occurrence of harmful contaminants in wheat grain.

According to *Mesterházy et al. (1999)* resistance of cultivars has also an impact on the contents of DON. In most resistant cultivars, a low average level of DON was detected, and in some growing seasons, DON was not detected at all. In addition, great differences in the content of DON in the same cultivar of wheat have been also established, that involved a large diversity of pathogenic populations in the field *(Mesterházy et al., 1999)*. According to recent research of *Mesterházy et al. (2002)*, it was concluded that the level of resistance of given

cultivar was more important in accumulation of DON than the aggressiveness of *Fusarium* isolates. This is confirmed by our research in which the differences among the cultivars in terms of accumulation of DON were determined, although the incidence of *F. graminearum* was high in both wheat cultivars. *Blandino et al. (2012)* reported that the main factors affecting the formation of DON in wheat grain were; susceptibility of a wheat cultivar, the preceding crop (especially maize and sorghum), soil tillage, fungicide application at anthesis of wheat. Contrary to the above, *Müller et al. (2010)* pointed out that the monitoring of FHB and DON content requires good knowledge of agricultural factors, primarily crop rotations and tillage practice, then climatic conditions especially annual precipitation and topographic factors (relief position, topographic wetness index TWI). In the research of these authors, the susceptibility ranking of wheat cultivars to *Fusarium* infection had no significant influence on DON accumulation.

According to data from other countries with similar geographical and climatic conditions, *Mankevičiene et al. (2007)* have established the incidence of DON in 62.5% to 100% of cereal samples from harvests 2004 and 2005 in Lithuania, with concentrations ranged up to 1121μg kg^{-1}. The high concentrations of DON were highly associated with abundant rainfall in both investigated years. Likewise, *Pleadin et al. (2012)* revealed the high mean concentrations of DON (up to 1454μg kg^{-1}) in 103 feed samples from three regions of Croatia.

Conclusion

It can be concluded that both tested cultivars were susceptible to FHB, although, NS40S cultivar, showed higher incidence of *F. graminearum* and higher DON content compared to the cultivar Simonida. Although the average DON levels did not exceed the maximum permitted limit, it is necessary to develop and implement the methods for monitoring the risk of harmful contaminants in wheat grain. Therefore, it is essential to conclude that the individual methods such as the application of less sensitive or tolerant cultivars in the strategy of control of FHB and mycotoxins in wheat grain, have lower effect in preventing the risk of harmful contaminants in the food chain, compared to a combination of several measures of crop protection such as crop rotation, tillage practice and fungicide application at anthesis of wheat, especially under favourable climatic conditions, existing in Serbia.

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Fusarium infekcija i deoksinivalenol kontaminacija ozime pšenice

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Rezime

U radu je proučavana incidenca *Fusarium*-zaraženih zrna ozime pšenice iz žetve 2014. godine kod dve domaće komercijalne sorte Simonida i NS40S, kao i sadržaj mikotoksina deoksinivalenola (DON). Nivo fuzariozne kontaminacije zrna pšenice određen je primenom fitopatoloških testova standardne metodologije a DON je detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Incidenca *Fusarium*-zaraženih zrna bila je u proseku za sve ispitivane uzorke od 12% (Simonida) do 19% (NS40S). Od *Fusarium* vrsta identifikovana je jedino *Fusarium graminearum*, kao dobro poznati producent DON mikotoksina. Pored ove gljivične vrste, u visokom procentu izolovana je *Alternaria* spp. sa prosečnom incidencom od 53% (Simonida) do 63% (NS40S). U ispitivanim uzorcima pšenice prosečan sadržaj DON bio je od 424 μg kg^{-1} (Simonida) do 1101 μg kg^{-1} (NS40S). Između *Fusarium*-zaraženih zrna i DON utvrđena je statistički neznačajna negativna korelacija ($r = – 0.18$) kod sorte Simonida i statistički neznačajna pozitivna korelacija ($r = 0.11$) kod sorte NS40S.

Prosečne koncentracije DON u ispitivanim uzorcima pšenice kod obe ispitivane sorte nisu bile iznad maksimalno dozvoljenog limita iako je nivo fuzariozne kontaminacije zrna obe ispitivane sorte bio visok. Ovi rezultati ukazuju da su obe ispitivane sorte pšenice osetljive prema fuzarioznoj infekciji i produkciji DON mikotoksina u agroekološkim uslovima Srbije, s tim što je sorta NS40S osetljivija u odnosu na sortu Simonida. Zbog svega navedenog, redovna zdravstvena kontrola zrna i razvijanje strategije integralnog monitoringa fuzarioze klasa neophodne su preventivne mere borbe u zaštiti pšenice.

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EFFECT OF FOLIAR FERTILIZATION ON SOYBEAN GRAIN YIELD

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Abstract: The aim of this investigation was to estimate the effects of foliar fertilization on quantitative traits (plant height, first pod height, number of nodes per plant, number of pods per plant, number of grain per plant, grain yield per plant, 1000-grain weight and grain yield) in two soybean cultivars (Balkan and Bečejka). Studied cultivars belong to different maturity groups (Balkan - I and Bečejka - 0). Four treatments of fertilization were tested: control (no fertilization), Urea (46 kg N ha^{-1}), Urea (46 kg N ha^{-1}) + Wuxal super (5 l ha^{-1}) and Urea (46 kg N ha^{-1}) + Ferticare I (5 kg ha^{-1}). Wuxal super and Ferticare I were foliar applied two times at the R2-R3 growth stage. The field experiments were carried out in dry land farming in the region of Vojvodina province at location Putinci (45° 00′ N Lat., 19° 58′ E Long.), during the years 2007 and 2008. In both research years, Balkan had higher values for all investigated traits than Bečejka. Results showed that foliar fertilizers significantly increased the values for all quantitative traits. Ferticare I is more effective than Wuxal super in soybean because this fertilizer has higher concentration of macronutrients. Foliar fertilization of soybean reduced the negative impact of small amounts of rainfall during the summer months on grain yield.

Key words: cultivar, foliar fertilization, soybean, qvantitative traits

Introduction

Soybean is the most important legume in Serbia of animal feed and food production. Soybean seeds to make high protein meal which is used largely as a supplement to cereal seeds in feed domesticated livestock such as dairy cows, cattle, pigs, goats, sheep, horses and poultry *(Iqbal et al., 2003; Ranđelović, 2009)*. *Marinković et al. (2010)* reported that nitrogen deficiency in the soil results in significant yield losses and yield quality reduction of soybean. Results by *Starling et al. (2000)* showed that plant growth and grain yield of soybean were higher

when fertilizer nitrogen was applied as starter. Many researchers indicate the importance of foliar feeding of soybean plants. Foliar feeding is the practice of applying liquid fertilizers to plant leaves *(Kovačević, 2003). Silberbush (2002)* reported that foliar fertilization is widely used practice to correct nutritional deficiencies in plants caused by improper supply of nutrients to roots. *Ranđelović (2009)* reported that the uptake of mineral nutrients from the soil and the extent of their utilization by the soybean plant depend on weather conditions during the growing season. In this case, preference should be given to the application of foliar fertilizers. *Camberato et al. (2010)* reported that if the micronutrient deficiencies do occur during the growing season, the most effective method for overcoming these deficiencies is through foliar fertilizer applications. *Rehm et al. (1997)* concluded that foliar fertilization is not a substitute for a program based on soil-applied fertilizers. They suggest that applications of phosphate and potash before planting are the most reliable method for meeting soybean material needs. *Barge (2001)* found that the foliar fertilizers (ElamMax (27% Mn), Folizyme (12% N, 3% K, 3% Ca and 3% Mn), Keylate (5% Mn), White Label (6% Mn) and Harvest More Urea Mate (N, P, K, Ca, Mg, B, Co, Cu, Mn, Mo and Zn) increased grain yield of soybean than control. *Mallarino et al. (2005)* reported that the foliar fertilization at early vegetative stages of soybean increased grain yield in 15 to 30% of the fields depending on the year. *Ashour and Thalooth (1983)* studied the effect of soil-fertilized with 35, 70 or 105 kg N ha^{-1} or foliar-sprayed with 0.5 or 1.0% urea at the R6 stage of plants development of soybean cv. Clark. Their results showed that foliar application of 1.0% urea the most increased fruit-set, weight of pods, and the yield of oil and protein in seed. *Garcia and Hanway (1976)* evaluated various nutrient combinations for foliar application at the R2 to R7 growth stages and found that a 10-1-3-0.5 N-P-K-S ratio increased yields by 441 to 504 kg ha^{-1}. They concluded that the optimum time of foliar application was between growth stages R5 and R6. *Haq and Mallarino (2000)* showed that N–P–K foliar fertilization of relatively small amounts sprayed at the V5 stage affected yields significantly at 6 or 27 sites. However, researches by *Schmitt et al. (2001)* and *Binford et al. (2004)* reported that foliar applications of N-P-K show decreases or no significant soybean grain yield differences. *Kaiser et al. (2007)* found that foliar fertilization with two fluid 3-8-15 and 28-0-0 (N-P-K) fertilizers at V5 or R2 growth stages, did not affect the grain yield of soybean cultivars.

The aim of this investigation was to the effects of soil and foliar fertilization on agronomic traits and grain yield in two soybean cultivars and to determine better foliar fertilizer and genotype in agro-climatic condition of Srem.

Materials and Methods

The experiments were carried out in dry land farming in the region of Srem, localitety Putinci (45° 00′ N Lat., 19° 58′ E Long.), on the calcareous chernozem soil of the loess terrace, during years 2007 and 2008. The main

characteristics of the soil (depth: 0-50 cm) were: pH in KCl – 6.1; pH u H_2O – 7.2; $CaCO_3$ – 7.2% (carbonate); humus – 2.01, total N – 0.11%. The soil contained 11.9 and 20.1 mg/100g soil phosphorus and potassium, respectively. Two soybean cultivars Balkan (maturity group I) and Bečejka (maturity group 0) were used as material. Plots were organized as a randomized block system design in four replications.

Meteorological conditions have a major impact on plant growth *(Popović et al., 2013a, Ikanović et al., 2014)*. Weather conditions had varying between 2007 and 2008 year, especially true of the amount and distribution of rainfall (Table 1). In 2007, average annual rainfall (358.8 mm) was higher 45.5 mm (14.5%) than in 2008 (313.3 mm). In both year researches monthly air temperatures was higher than long-term monthly air temperatures.

Table 1. Mean monthly air temperatures and sum of rainfall

Months	Temperature (^oC)			Rainfall (mm)		
Year	2007	2008	1981-2010	2007	2008	1981-2010
X - III	-	-	-	254.6	260.4	260.5
IV	13.0	12.9	11,8	0	52.4	48.4
V	18.5	18.3	17,2	79.0	42.4	56.2
VI	22.0	21.7	19.9	85.2	58.1	84.4
VII	22.6	21.7	21.5	38.7	61.0	61.6
VIII	22.3	21.5	21.2	62.5	22.7	52.8
IX	14.3	15.4	16.6	93.4	76.7	50.3
Mean	18.8	18.6	18.0	-	-	-
Growing season	-	-	-	358.8	313.3	353.7
Annual	-	-	-	613.4	573.7	614.2

Four treatments of fertilization were tested: control, Urea, Urea + Wuxal super and Urea + Ferticare I. The Urea (46 kg N ha^{-1}) was incorporated into the soil before sowing. Foliar fertilizers Wuxal super (5 l ha^{-1}) and Ferticare I (5 kg ha^{-1}) were applied two times at 10 days interval at the R2-R3 growth stages. Foliar fertilizers contain microelements in the form of chelate complexes, which ensures high utilization and good mobility of adopted elements. Wuxal super is a liquid fertilizer that contains macronutrients (8% N, 8% P_2O_5, and 6% K_2O) and micronutrients (0.01% B, 0.015% Fe, 0.007% Cu, 0.013% Mn, 0.001% Mo, and 0.005% Zn). Ferticare I is a crystal fertilizer that contains macronutrients (14% N, 11% P_2O_5, 25% K_2O, and 2.3% MgO) and micronutrients (0.02% B, 0.01% Cu, 0.1% Fe, 0.1% Mn, 0.002% Mo, and 0.01% Zn). Preceding crop was winter wheat. Soybean planting was done on April 12 in 2007 and April 16 in 2008. The plant densities were used 450000 plants ha^{-1} (Balkan) and 500000 plants ha^{-1} (Bečejka). Plot size was $10m^2$ (5 m x 2 m), and the row-to-row spacing was 50 cm. Plots were rolled after sowing. A standard cultivation practice was applied.

Soybean harvest was performed manually. Ten plants randomly selected from each plot were used to record data seven quantitative traits (plant height, first pod height, number of nodes per plant, number of pods per plant, number of grain

per plant, grain yield per plant and 1000-seed weight). After harvesting grain yield was converted into t ha^{-1}. Grain yield is calculated on a 13% moisture basis.

Data were processed using analysis of variance (ANOVA) according to a linear model which included effects of cultivar and fertilizer treatments, and the interaction between them. The statistical tests were carried out using STATISTICA (version 10; StatSoft, Tulsa, Oklahoma, USA). The significance level was set at $P \leq 0.05$ and $P \leq 0.01$. Differences between traits means were assessed using Duncan's Multiple Range Test at $P \leq 0.05$ level.

Results and Discussion

Results showed that soybean cultivar Balkan, in average for years and fertilizer treatments, produced higher plant height (108.8 cm), first pod height (12.3 cm), number of nodes per plant (13,0), number of pods per plant (56.4), number of grain per plant (121.0), grain yield per plant (20.76 g), 1000-grain weight (181.89 g) and grain yield (3950 kg ha^{-1}) than cultivar Bečejka (92.6 cm, 10.6 cm, 11,3, 48.8, 104.0, 18,04 g, 169.48 g and 3347 kg ha^{-1} respectively), Table 2, 3 and 4.

Table 2. Effect of different fertilizer treatments on plant height (cm), first pod height (cm) and number of nodes per plant of soybean cultivars

Year	Fertilizer (B)	Traits								
		Plant height			First pod height			No of nodes per plant		
		Cultivar (A)								
		Balkan	Bečejka	M	Balkan	Bečejka	M	Balkan	Bečejka	M
2007	Control	112.4	95.1	103.8^{d}	11.8	9.3	10.6^{c}	13.5	10.5	12.0^{c}
	Urea	127.1	104.1	115.6^{c}	12.9	10.1	11.5^{b}	14.9	12.5	13.7^{b}
	Urea+Wuxal	136.7	104.5	120.6^{b}	14.0	10.1	12.1^{ab}	15.2	12.7	14.0^{b}
	Urea+Ferticare I	139.7	107.4	123.6^{a}	14.2	11.0	12.6^{a}	15.6	13.3	14.4^{a}
	M	129.0^{a}	102.8^{b}	115.9	13.2^{a}	10.1^{b}	11.7	14.8^{a}	12.2^{b}	13.5
F test		A	B	AxB	A	B	AxB	A	B	AxB
		**	**	**	**	**	ns	**	**	ns
2008	Control	83.9	67.0	75.4^{d}	10.5	10.6	10.6^{d}	9.3	8.3	8.8^{c}
	Urea	88.2	83.2	85.7^{c}	11.0	10.8	10.9^{c}	11.1	10.1	10.6^{b}
	Urea+Wuxal	90.5	85.7	88.1^{b}	11.2	11.1	11.2^{b}	12.2	11.5	11.9^{a}
	Urea+Ferticare I	91.3	93.9	92.6^{a}	12.6	12.0	12.3^{a}	12.5	11.6	12.1^{a}
	M	88.5^{a}	82.4^{b}	85.4	11.3^{a}	11.1^{a}	11.2	11.3^{a}	10.4^{b}	10.9
F test		A	B	AxB	A	B	AxB	A	B	AxB
		**	**	**	ns	**	ns	**	**	ns
M	Control	98.2	81.0	89.6	11.2	10.0	10.6	11.4	9.4	10.4
	Urea	107.7	93.7	100.7	12.0	10.4	11.2	13.0	11.3	12.2
	Urea+Wuxal	113.6	95.1	104.4	12.6	10.6	11.7	13.7	12.1	13.0
	Urea+Ferticare I	115.5	100.7	108.1	13.4	11.5	12.4	14.1	12.4	13.2
	M	108.8	92.6	100.7	12.3	10.6	11.5	13.0	11.3	12.2

Mean followed by different letters are significantly different by Duncan's Multiple Range Test at $p \leq 0.05$; ns - not significant; * - significant at $P \leq 0.05$; ** - significant at $P \leq 0.01$

In both research years, cultivar Balkan had higher values for all investigated traits compared to cultivar Bečejka. These differences were statistically significant, except for first pod height in 2008. The higher values for all quantitative traits in 2007 can explain the favorable distribution of rainfall than 2008. In our production conditions is a critical stage of grain filling (August) when lack of rainfall leads to a decrease in soybean grain yield. In 2008 drought stress was in August.

Results showed that treatment fertilizer Urea + Ferticare I, in average for years and cultivars, produced highest plant height (108.1 cm), first pod height (12.4 cm), number of nodes per plant (13.2), number of pods per plant (58.1), number of grain per plant (124.8), grain yield per plant (21.46 g ha^{-1}), 1000-grain weight (185.84 g) and grain yield (3961 kg ha^{-1}). Values of these traits were higher in 2007 (favorable weather conditions) than in 2008.

Table 3. Effect of different fertilizer treatments on number of pods per plant, number of grain per plant and grain yield per plant (g) of soybean cultivars

Year	Fertilizer (B)	Traits								
		No of pods per plant			No of grain per plant			Grain yield per plant		
		Cultivar (A)								
		Balkan	Bečejka	M	Balkan	Bečejka	M	Balkan	Bečejka	M
2007	Control	56.7	46.8	51.8^{d}	122.7	98.8	110.8^{d}	20.98	17.30	19.14^{d}
	Urea	63.1	53.5	58.3^{c}	135.7	115.0	125.4^{c}	23.20	20.24	21.72^{c}
	Urea+Wuxal	66.4	55.9	61.2^{b}	142.8	120.2	131.5^{b}	24.75	21.64	23.20^{b}
	Urea+Ferticare I	68.7	58.5	63.6^{a}	148.4	126.3	137.4^{a}	26.12	21.35	23.74^{a}
	M	63.7^{a}	53.7^{b}	58.7	137.4^{a}	115.1^{b}	126.3	23.76^{a}	20.13^{b}	21.95
F test		A	B	AxB	A	B	AxB	A	B	AxB
		**	**	ns	**	**	**	**	**	**
2008	Control	41.0	36.5	38.8^{c}	86.5	75.8	81.2^{d}	14.62	13.74	13.68^{d}
	Urea	45.8	41.1	43.4^{b}	97.1	87.3	92.2^{c}	16.31	15.21	15.76^{c}
	Urea+Wuxal	53.7	48.0	50.9^{a}	116.0	103.0	109.5^{b}	19.80	17.74	18.77^{b}
	Urea+Ferticare I	55.6	49.5	52.6^{a}	118.5	105.9	112.2^{a}	20.30	18.06	19.18^{a}
	M	49.0^{a}	43.8^{b}	46.4	104.5^{a}	93.0^{b}	98.8	17.76^{a}	15.94^{b}	16.85
F test		A	B	AxB	A	B	AxB	A	B	AxB
		**	**	ns	**	**	ns	**	**	**
M	Control	48.9	41.7	45.3	104.6	87.3	96.0	17.80	15.02	16.41
	Urea	54.4	47.3	50.9	116.4	101.2	108.8	19.76	17.72	18.74
	Urea+Wuxal	60.0	52.0	56.0	129.4	111.6	120.5	22.28	19.69	20.99
	Urea+Ferticare I	62.2	54.0	58.1	133.4	116.1	124.8	23.21	19.71	21.46
	M	56.4	48.8	52.6	121.0	104.0	112.5	20.76	18.04	19.40

Mean followed by different letters are significantly different by Duncan's Multiple Range Test at $p \leq 0.05$; ns - not significant; * - significant at $P \leq 0.05$; ** - significant at $P \leq 0.01$

In both years fertilizer treatments were significantly increased all studied quantitative traits, especially with the foliar treatment. In 2007 the minimum traits values observed in control (plant height 103.8 cm, first pod height 10.6 cm, number of nodes per plant 12.0, number of pods per plant 51.8, number of grain per plant

110.8, grain yield per plant 19.14 g, 1000-grain weight 164.22 g and grain yield 3624 kg ha^{-1}) and maximum in treatment Urea + Ferticare I (123.6 cm, 12.6 cm, 14.4, 63.6, 137.4, 23.74 g, 191.90 g and 4366 kg ha^{-1}, respectively). Also, in 2008 the minimum traits values observed in control (plant height 75.4 cm, first pod height 10.6 cm, number of nodes per plant 8.8, number of pods per plant 38.8, number of grain per plant 81.2, grain yield per plant 13.68 g, 1000-grain weight 156.67 g and grain yield 2919 kg ha^{-1}). The maximum traits values observed in treatment Urea + Ferticare I (plant height 92.6 cm, first pod height 12.3 cm, number of nodes per plant 12.1 number of pods per plant 52.6, number of grain per plant 112.2, grain yield per plant 19.18 g and grain yield 3555 kg ha^{-1}) except 1000-grain weight which highest in treatment Urea + Wuxal super (181.38 g). Our research has showed that foliar feeding should be given priority under conditions of limited uptake of nutrients from the soil. Values of all traits were higher in treatments with foliar fertilization than in control and treatment Urea.

Many researchers have reported that foliar fertilization treatments significantly increase plant height *(Prijić et al., 2003; Ranđelović, 2009; El-Abady et al., 2008; Yildirim et al., 2008; Popović et al., 2013b)*, first pod height *(Ranđelović, 2009)*, number of nodes per plant *(Odeleye et al., 2007; Ranđelović, 2009)*, number of pods per plant *(Schon and Blevins, 1990; Reinbott and Blevins, 1995; El-Abady et al., 2008; Yildirim et al., 2008; Ranđelović, 2009)*, number of grain per plant *(Odeleye et al., 2007; El-Abady et al., 2008; Ranđelović, 2009)*, grain yield per plant *(Schon and Blevins, 1990; El-Abady et al., 2008; Ranđelović, 2009)* and 1000-grain weight *(Ranđelović, 2009; Popović et al., 2013b)*. Contrary, *Abdel-Gawad et al. (1989)* and *Yildirim et al. (2008)* reported that foliar fertilization did not have any statistical effect on 1000-grain weight. Foliar fertilization of soybean at early vegetative stages increased soybean grain yield in approximately 15% *(Haq and Mallarino, 2000; Mallarino et al., 2001)*. *Garcia and Hanway (1976)* found that yield of soybean increases of 27 to 31% when a foliar fertilizer (N, P, K, S) was applied at late reproductive stages. *Woon and Porter (1986)* reported that foliar fertilizers (FF) applied at the reproductive growth stage increased soybean yield but FF formulations 16N + 4P + 4K + 1 S gives higher yield than formulation 12 N + 4 P + 4 K + 0.5 S. *Peele (1997)* reported that the foliar dressing of macronutrients increased soybean grain yield by 30 to 400 kg ha^{-1}. *Oko et al. (2003)* reported that the foliar fertilization of urea at the R2-R3 growth stage increased soybean grain yield between 6 and 68% compared to control. *Ranđelović et al. (2009)* reported that method of foliar feeding has been proved as an effective tool for increasing of grain yield in two soybean cultivars with reduced content of Kunitz trypsin inhibitor (Laura and Lana). *Sultan et al. (2003)* reported that spraying with foliar fertilizers at 45 days after sowing increased grain yield of soybean. *Haq and Mallarino (2005)* found that foliar N fertilization increased protein and oil production because of soybean yield increases. *Popović et al. (2013c)* reported that NS soybean varieties Galina

(maturity group 0), Victoria and Tea (maturity group I) had higher yield and 1000-grain weight in the variant with foliar fertilization with fitofert (composition: 12% N, 4% P_2O_5, 6% K_2O, 0.013% Mn, 0.010% Fe, 0.008% B, 0.006% Cu, and 0.005% Zn) than in the control. Contrary, earlier research *Parker and Boswell (1980)* reported a 10.9% and 17.6% soybean grain yield decrease with application of foliar fertilizers. *Chowdhury et al. (1985)* obtained that the high level of foliar fertilization did not significantly effect on the grain yield in soybean cultivars Williams and Micthel.

Table 4. Effect of different fertilizer treatments on 1000-grain weight (g) and grain yield (kg ha^{-1}) of soybean cultivars

Year	Fertilizer treatments (B)	Traits					
		1000-grain weight			Grain yield		
		Cultivar (A)					
		Balkan	Bečejka	M	Balkan	Bečejka	M
2007	Control	171.39	157.04	164.22^{d}	3889	3358	3624^{b}
	Urea	184.96	165.85	175.40^{c}	4679	3544	4112^{a}
	Urea+Wuxal	198.22	173.82	186.02^{b}	4698	3722	4210^{a}
	Urea+Ferticare I	203.02	180.77	191.90^{a}	4793	3939	4366^{a}
	M	189.40^{a}	169.37^{b}	179.39	4515^{a}	3641^{b}	4078
F test		A	B	AxB	A	B	AxB
		**	**	**	**	**	ns
2008	Control	157.42	155.91	156.67^{c}	3096	2742	2919^{d}
	Urea	171.08	167.10	169.09^{b}	3198	2991	3095^{c}
	Urea+Wuxal	182.89	179.86	181.38^{a}	3458	3159	3309^{b}
	Urea+Ferticare I	184.14	175.44	179.79^{a}	3792	3318	3555^{a}
	M	173.88^{a}	169.58^{b}	171.73	3386^{a}	3053^{b}	3220
F test		A	B	AxB	A	B	AxB
		**	**	*	**	**	ns
M	Control	164.40	156.48	160.44	3493	3050	3272
	Urea	178.02	166.48	172.25	3939	3268	3604
	Urea+Wuxal	190.56	176.84	183.70	4078	3441	3760
	Urea+Ferticare I	193.58	178.10	185.84	4292	3629	3961
	M	181.89	169.48	175.56	3950	3347	3649

Mean followed by different letters are significantly different by Duncan's Multiple Range Test at $p \leq 0.05$; ns - not significant; * - significant at $P \leq 0.05$; ** - significant at $P \leq 0.01$

The interaction of soybean cultivars and fertilizer treatments did not significantly affect the first pod height, number of nodes per plant, number of pods per plant and grain yield in both years, and number of grain per plant in 2008. Contrary, interaction between studied factors was significant effect on plant height, grain yield per plant and 1000-grain weight in both years and number of grain per plant in 2007.

Conclusions

Cultivar Bečejka, with shorter vegetation period, produced lower plant height, first pod height, number of nodes per plant, number of pods per plant, number of grain per plant, grain yield per plant, 1000-grain weight and grain yield then cv. Balkan. From this study it may be concluded that different fertilizer treatments effected the increasing of studied quantitative traits in both soybean cultivars. Method of foliar feeding has been proved as an effective tool for increasing of grain yield in both cultivars. However, Urea + Ferticare I treatment is more effective than Urea + Wuxal super in soybean. This follows from the fact that Ferticare I has higher concentration of macronutrients than Wuxal super. Generally, cultivar Balkan and treatment Urea + Ferticare I may be recommended in soybean production in localities with similar agro-ecological conditions.

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Efekat folijarne ishrane na prinos zrna soje

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Rezime

Cilj istraživanja je bio da se oceni efekat folijarne ishrane na kvantitativne osobine (visina biljke, visine prve mahune, broj nodusa po biljci, broj mahuna po biljci, broj zrna po biljci, prinos zrna po biljci, masa 1000 zrna i prinos zrna) dve sorte soje (Balkan i Bečejka). Ispitivane sorte pripadaju različitim grupama zrenja (Balkan - I, Bečejka - 0). Upoređivane su četiri tretmana ishrane biljaka: kontrola, Urea (46 kg N ha^{-1}), Urea (46 kg N ha^{-1}) + Wuxal super (5 l ha^{-1}) i Urea (100 kg ha^{-1}) + Ferticare I (5 kg ha^{-1}). Wuxal super i Ferticare I primenjeni su folijarno u R2-R3 fazi rastenja i razvića soje. Ogledi su izvedeni u suvom ratarenju u Vojvodini na lokaciji Putinci (45° 00′ SGŠ, 19° 58′ IGD) tokom 2007. i 2008. godine. U obe godine istraživanja sorta Balkan je imala veće vrednosti za sve ispitivane osobine nego Bečejka. Rezultati su pokazali da je folijarna ishrana značajno povećala vrednosti svih ispitivanih kvantitativnih osobina. Viši prinosi postignuti su primenom Ferticare I nego primenom Wuxal super jer sadrži veću koncentraciju

makroelemenata. Folijarno prihranjivanje soje umanjilo je negativan uticaj malih količina padavina tokom letnjih meseci na prinos zrna.

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THE INFLUENCE OF THE FACTOR «GENETIC VALUE OF THE SIRE» ON THE IMPLEMENTATION OF THE GENETIC POTENTIAL OF THE INDICATOR «MILK PRODUCTION OF MAXIMUM LACTATION » OF THE YAROSLAVL BREED COWS

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Abstract: Dairy products are the main food elements of the man. Milk is the sole food for infants and it plays an important role in feeding of the sick, convalescent, and healthy adults. The Yaroslavl Region is a leading region of the Yaroslavl cattle breed. Therefore, the aim of our research was to determine the strength and reliability of the influence of the factor «genetic value of the sire» on productive characteristics of animals as a factor that helps to increase the productivity of animals. When we determine the strength of the influence of factors for statistical data we used the procedure of generalized linear models (General Linear Models - GLM), and evaluation components of phenotypic variation attributes were analyzed by multivariate dispersive analysis. Our research has allowed to allocate bulls with genetic value which has the improving effect. Using the recommended lines the farmers of the Yaroslavl region may increase productive characteristics of animals and the profit of the farms and improve the efficiency of breeding.

Keywords: cattle, productive characteristics, genetic potential

Introduction

Accelerating the race of genetic improvement of the breed by breeding and productive indicators is possible by using bulls - improvers having high productive offspring *(Mazepkin, 2000; Moskalenko and Konovalov, 2010).* The impact of the

productivity of daughters is decisive in the selection process. Improvement of cattle in sense of improvement of the genetic basis of the population of cattle for milk production, production of milk fat and content of milk fat in present conditions is done through bull sires and bull dams. By application of high quality breeding animals with proven genetic capacity (sons of bull dams and bull sires) production and reproduction traits of cattle population can be improved.*(Petrovic et.al., 2006; Pantelic et.al., 2009, Petrovic et.al., 2012)*. Common genetic contribution of bulls in the genetic improvement of dairy cattle population is up to 95% *(Mityukov, 2007; Moskalenko et. al., 2012*). According to native and foreign scholars who study the impact of the factor «genetic value of the sire», 43-46% of the maximum possible selection effect is determined by selection of the bulls sires, 30-35% - by the selection of bulls mothers, 16-20% of cow sires and only 6% is due to the selection of cow mothers (76-78% of breeding progress is determined selection of bulls and only 22-24% - by selection of cows).

Producers used in the herd influence not only on the growth and development of young animals, but also in the future - on productive characteristics of animals. This influence obeys the law: the offspring will be more productive if the difference between the productivity of the mother of the sire and mother of the daughter is small *(Ovchinnikov, 2008).*

We have a large number of literary sources devoted to the study of the environmental and genetic factors influencing on genetic potential and its realization. It is also given a great importance in the existing breeding programs. The Yaroslavl region is the leading district in breeding of the Yaroslavl cattle. Therefore, the aim of our research was to determine the strength and reliability of the impact of the factor «genetic value of the sire» on the implementation of the genetic potential of the indicator «Yield of maximum lactation» of the Yaroslavl cattle breed.

Material and methods

The object of the study were pure-bred cows of the Yaroslavl breed and crossbreed cows of the Holstein x Yaroslavl breed having different thorough-bredness of the Holstein breed, in the amount of 6230 cows, 4776 of them are leavers.

Material is prepared on the basis of the «Information database of the Yaroslavl cattle breed» (№ of the state registration is 2013620064), data of the program ARMZS (up to 2009 year) and ARMS -W (№ of the state registration is 2009613920 from 22.07.2009 year), information software module «PAVKA».

Farms of the Yaroslavl region with different keeping technology were selected for study: LLC breeding plant «Rodina» (Loose - boxed keeping of cows and

equipment of «Westfalia» company), Joint Stock Company, breeding farm named after Dzerzhinsky (captive keeping with the use of installations of «DeLaval» company).

We determined the realization of the genetic potential estimated by Kuznetsovsks algorithm, 1983 **(***Malyukova, 2012).* The evaluation of components of phenotypic variability was performed using a multi- factorial dispersive analysis *(Kuznetsov, 2000).*

Results and discussion

In cattle herds Yaroslavl breed we determined the effect of the factor «genetic value of the sire» on the implementation of the genetic potential of the indicator «milk production of maximum lactation» (picture 1). Assessing the effects of the gradation of the factor «genetic value of the sire» on the studied characteristics of cattle is presented in the table 1.

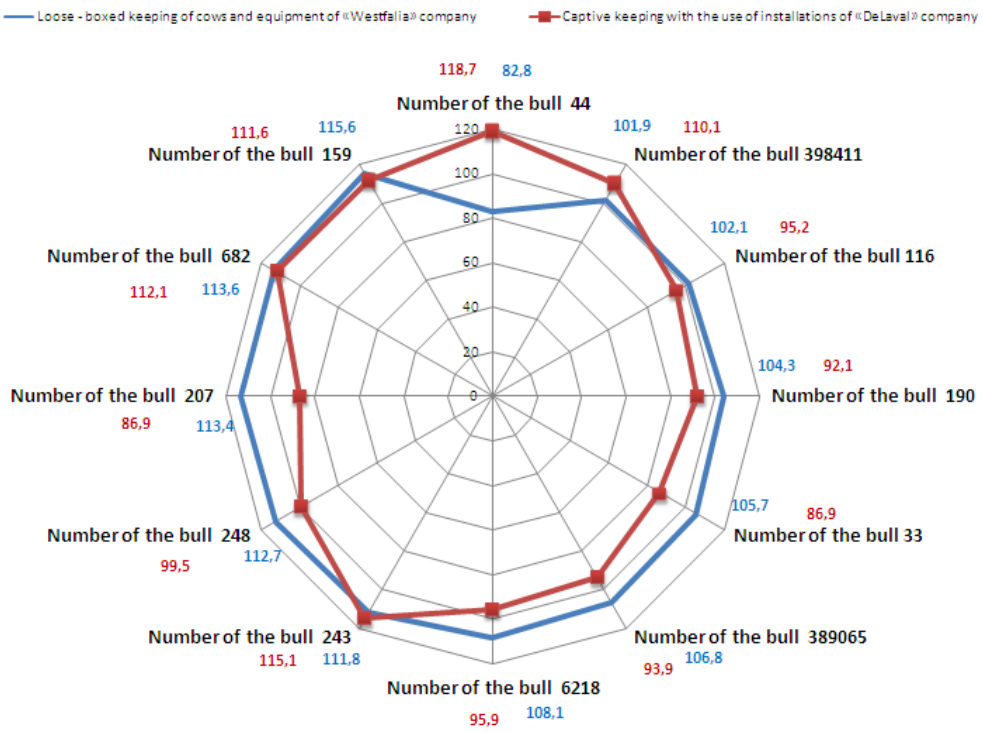

Figure 1. The effect of the factor «genetic value of the sire» on the indicator «milk production of maximum lactation»

Table 1. The Results of the application of various technologies in the context of thoroughbredness of the Holstein breed

Number of the bull	Technology of The keeping cows / Index	Loose - boxed keeping of cows and equipment of «Westfalia»	Captive keeping with the use of installations of «DeLaval» company
116	the number of cows	32	27
	milk production of maximum lactation	6898,6	5288,0
	genetic potential	6756,8	5554,6
	genetic superiority, %	102,1	95,2
159	the number of cows	79	54
	milk production of maximum lactation	6397,2	7519,7
	genetic potential	5533,9	6737,5
	genetic superiority, %	115,6	111,6
190	the number of cows	22	131
	milk production of maximum lactation	7045,4	5129,9
	genetic potential	6754,6	5569,9
	genetic superiority, %	104,3	92,1
207	the number of cows	89	22
	milk production of maximum lactation	6276,0	5885,9
	genetic potential	5534,4	6773,2
	genetic superiority, %	113,4	86,9
243	the number of cows	127	31
	milk production of maximum lactation	7503,9	6386,1
	genetic potential	6711,9	5548,3
	genetic superiority, %	111,8	115,1
248	the number of cows	36	18
	milk production of maximum lactation	7601,3	5526,1
	genetic potential	6744,7	5553,9
	genetic superiority, %	112,7	99,5
33	the number of cows	25	14
	milk production of maximum lactation	7140,5	4829,0
	genetic potential	6755,4	5556,9
	genetic superiority, %	105,7	86,9
389065	the number of cows	80	82
	milk production of maximum lactation	5919,1	6363,8
	genetic potential	5543,2	6777,2
	genetic superiority, %	106,8	93,9
398411	the number of cows	9	71
	milk production of maximum lactation	6133,8	7740,6
	genetic potential	5641,5	7596,3
	genetic superiority, %	110,5	101,9
44	the number of cows	110	53
	milk production of maximum lactation	4621,5	7988,7
	genetic potential	5581,5	6730,2
	genetic superiority, %	82,8	118,7
6218	the number of cows	11	17
	milk production of maximum lactation	7299,3	5326,5
	genetic potential	6752,4	6200,8
	genetic superiority, %	108,1	95,9
682	the number of cows	28	40
	milk production of maximum lactation	6304,2	7561,7
	genetic potential	5549,5	6745,5
	genetic superiority, %	113,6	112,1

Information shown in the picture 1and table 1reflect the results of the evaluation of the quality of bull's offspring. So the bull having nickname Zavetniy 159 has breeding category A3 with a bias toward neutral side, its rating falls in all farms. The bull Jasmine 6218 (category A1B1) using in studied technologies (loose and captive keeping of cows) shows a high implementation of the genetic potential and genetic superiority (95.9-108.1%) of the indicator «milk production of maximum lactation». The bull Stinger 243 has a rating category A1 which has sufficiently manifested in the characteristics of the daughters using in studied technologies (his genetic superiority from 111.8 to 115.1 %). The daughters of the bulls 159 and 207 shows low implementation of genetic potential in the terms of the, breeding farm named after Dzerzhinsky on the variability of the indicator «milk production of maximum lactation». The effect of «genetic value of the sire» had a strong and significant influence (9.2%) on the variability of the indicator «milk production of maximum lactation». The analysis of factors effecting on productive traits were evaluated by *Mazepkin (2000); Moskalenko and Konovalov (2010); Adediran et al., (2010).* *Hric and Pavlik (2012)* in their research find that sire have significant effect on milk production of lactating cows.

Conclusion

Based on research and the results obtained, we can conclude the following:

There is a significant genetic variability of the studied traits. The genetic merit of the sire had a strong influence on the variability of the indicator «milk production of maximum lactation».

Our studies has allowed to allocate bulls having genetic value which has the effect of improving on the productivity of the herds in which they are used. We recommend to use lines and their animal representatives to increase breeding efficiency in the cattle herds.

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Uticaj faktora «genetska vrednost oca» na implementaciju genetskog potencijala indikatora « proizvodnja mleka u maksimalnoj laktaciji » kod krava jaroslavske rase

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Rezime

Mlečni proizvodi su glavni elementi ishrane čoveka. Mleko je jedina hrana za odojčad i igra važnu ulogu u ishranu bolesnika, rekovalescenata i zdravih odraslih osoba. Jaroslavski region je vodeći region u gajenju istoimene – jaroslavske rase goveda. Dakle, cilj našeg istraživanja bio je da se utvrdi snaga i pouzdanost uticaja faktora «genetske vrednosti oca» na produktivne osobine životinja kao faktor koji pomaže da se poveća produktivnost krava. Da bi smo odredili snagu uticaja faktora podatke koje smo koristili, podvrgli smo u proceduri obrade putem generalnog linearnog modela (Opšti Linearni modeli - GLM). Komponente evaluacije fenotipske varijacije atributa su analizirane putem multivarijacione disperzivne analize. Naše istraživanje je omogućilo da se izdvoje bikovi sa genetskim vrednostima koje imaju efekat poboljšanja željenih svojstava populacije. Upotrebom preporučenih linija odgajivači goveda u jaroslavskoj oblasti mogu povećati proizvodne performanse životinja. Sve ovo void ka povećanju profita, a time otvara mogućnost za dalji napredak u proizvodnji.

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Example 2

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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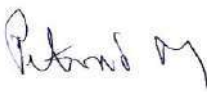

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