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THE EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZABILITY OF SEMEN FROM ANGORA, KILIS AND SAANEN GOATS

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ABSTRACT

The aim of this study was to investigate effect of different glycerol concentrations on freezability of semen from Angora, Kilis and Saanen goats. Three male goats from each breed were selected and ejaculates were collected with artificial vagina. Three ejaculates from each breed were pooled and extended with skim milk-based extender containing 10 % (v/v) egg yolk and 0 %, 3 %, 5 %, 7 % and 9 % (v/v) glycerol (G0, G3, G5, G7 and G9, resp.) as a cryoprotectant. Extended semen from different goat breeds was equilibrated, cryopreserved and stored in liquid nitrogen. The best post-thaw motility for Angora (51.6 %) and Kilis (75.0 %) goats was obtained with G5 concentration, while the best post-thaw motility for Saanen goat (61.6 %) was obtained with G7 concentration (P<0.001). Similar results were recorded for percentage of live spermatozoa for Angora (58.1 % in G5), Kilis (78.5 % in G5) and Saanen (64.0 % in G7) goats (P<0.001). The lowest abnormal spermatozoa percentages were obtained with G5 concentration for Angora (34.3 %), Kilis (30.8 %) and Saanen (40.8 %) goats (P<0.001). While different glycerol concentrations for goat breeds were considered, it was determined that suitable glycerol percentages for Angora, Kilis and Saanen goats were 5 %, 5-9 % and 7 % respectively. It was concluded that glycerol concentration is an important factor affecting freezability of goat semen from different breeds.

Key words: goat breeds; goat semen; glycerol; cryopreservation

INTRODUCTION

In Turkey there are over 6 million heads of goats. Goat livestock is an important source of milk and meat production in Turkey. Anatolian black goats are the most widespread breed but are low in production. Because native breeds are poor producers, one of the approaches to improve is to adopt a genetic strategy of crossbreeding. Hence, the goats of Saanen may be the breed of choice because of their high milk yield and fecundity. Therefore, cryopreservation of Saanen reared in Turkey is important issue to carry out successful genetic strategy of crossbreeding (Kulaksız and Daşkın, 2010).

Angora and Kilis are two main important goat breeds raised in Turkey. There are several advantages of raising these species, such as their ability of adaptation to harsh conditions of hilly and mountainous districts of Turkey. Angora goats (breed is named after the town Ankara, in Central Anatolia) are reared mostly for mohair production and Kilis goats (originate in Kilis province, which lies on the Syrian border) are the major dairy breeds which have an important role in milk and meat production (Atay *et al*., 2011). These breeds are mainly endangered genetic resources of Turkey. In this context,

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the cryopreservation of gametes is important because it would allow us to support a genome resource bank for this breed for an indefinite period of time.

Cryoprotectants used for freezing of sperm cells provide protection from cold shock and the other damages during freezing. Optimum adding rates of cryoprotectants become peculiar to species and require determination of some parameters of cell membrane. Glycerol has been extensively used as a cryoprotectant (Purdy, 2006). Recent studies have demonstrated that glycerol remains to be the most effective cryoprotective compound for freezing goat semen and no enhancement was showed by the addition of other compounds (Farshad *et al*., 2009; Bezerra *et al*., 2011). Therefore, glycerol is the most commonly used cryoprotectant for goat semen.

There are no more studies about different concentrations of glycerol for cryopreservation of goat semen (Deka and Rao, 1986; Biswas *et al*., 2002; Farshad *et al*., 2009). However, a lot of studies about glycerol concentrations have been carried out about freezability of semen from different species (Pena *et al*., 1998; Rota *et al.*, 1998; Baran *et al*., 2000; Buhr *et al*., 2001; Abbas and Andrabi, 2002; Rasul *et al*., 2007; Awad, 2011; Hoffman *et al*., 2011). Moreover, we did not find any study or other information about interaction of goat breed and also other species with glycerol concentrations on freezability of semen until now.

There is no information on the freezability of Kilis goat semen and no study on the effect of different glycerol concentration on the freezebality of Angora, Kilis and Saanen goat semen has been reported. Therefore, the present study was designed to determine the suitable glycerol concentration for cryopreservation of Saanen, Angora, and Kilis goat semen.

MATERIAL AND METHODS

Location

Animals were housed at the Education Research and Practice Farm, Faculty of Veterinary Medicine, University of Ankara, Turkey at 39º57 N, 32º53 E, at an altitude of 850 m.

Experimental animals

Nine healthy male goats of Angora (n=3), Saanen (n=3) and Kilis (n=3) breed, aged between 2 and 3 year, were selected for the study. They were housed in a covered shelter with an open-air run and were allowed to walk freely. Throughout this study the nutrition of the goats remained uniform and constant. Feeding consisted of 750 g commercial concentrate, and 1 kg alfaalfa hay/animal/day; water was provided ‘‘ad libitum’’.

Semen collection, dilution, freezing and thawing

During the breeding season, semen was collected from each goat once a week by means of artificial vagina. Immediately after collection, the ejaculates were placed in a water bath (37ºC) and aliquots were taken for the assessment of semen quality. After individual examination, three ejaculates from the same breed (i.e. Angora, Saanen or Kilis) were pooled and only ejaculates with at least 85 % estimated progressive motility, were used for freezing.

Five extenders were prepared as follows: Skimmed milk-based egg yolk (10 %) (SMEY) added by glycerol 3 % (SMEY3), glycerol 5 % (SMEY5) , glycerol 7 % (SMEY7), glycerol 9 % (SMEY9) and SMEY without glycerol (0 %; control). The pooled ejaculates from different breeds were divided into 5 aliquots, and diluted with SMEG0, SMEG3, SMEG5, SMEG7, or SMEG9 to reach the average semen concentration of $400x10^6$/ml.

The extended semen from different breeds (n=3) and groups (n=5) was separately packaged in 0.25 ml straws, and equilibrated at 4ºC for 2 hours. The straw was frozen in a styrofoam box at 4 cm above the liquid nitrogen (LN) surface for 15 minutes. The frozen semen was stored for 24 hours in LN for further evaluations. The frozen semen straws from different breeds and groups were thawed in a 37ºC water bath for 30 seconds and semen evaluation was carried out as follows.

Semen evaluation

Sperm motility was assessed using a phase-contrast microscope (x 400 magnification, Olympus BH-2, Olympus Optical CO. LTD., Japan), with a warm stage maintained at 37ºC. A wet semen mount was made using 2µL semen placed directly onto a microscope slide and covered by a cover slip. For each sample, at least 5 microscopic viewfields were examined by two trained observers. The mean of the three successive evaluations was calculated as the final motility score (Ax *et al*., 2000).

The viability of sperm in the sample was assessed by means of an eosin-nigrosin staining. The sperm smears were prepared by mixing a drop of semen with two drops of stain on a warm slide and spreading the stain immediately with the aid of a second slide. The viability was assessed by counting 200 sperm cells with a bright-field microscopy (X400, Olympus CX21FS1, Olympus Optical CO. LTD., Japan). The sperm cells showing partial or complete colorization were considered to be non-viable or dead. Only the sperm showing strict exclusion of the stain were considered to be alive (Evans and Maxwell, 1987).

For the assessment of sperm abnormalities, at least three drops of each sample were added to an Eppendorf container with 1 mL Hancock solution (62.5 mL formalin (37 %), 150 mL saline solution, 150 mL buffer solution

and 500 mL double-distilled water). One drop of this semen mixture was put onto a slide and covered with a cover slip. The percentage of sperm abnormalities was determined by counting a total of 200 sperm under phase-contrast using an immersion objective (Schafer and Holzman, 2000).

Statistical analysis

In the present study, totally 6 replications for each glycerol concentration were carried out. All data from 6 replications were examined for normal distribution with Shapiro-Wilk testand homogeneity of variance - with Levene's test. In case of abnormality of the distribution, logarithmic transformation of data was performed (in order to normalize the distribution). Two-way analysis of variance (ANOVA) was conducted to assess the effect of breed and concentration of glycerol on motility, proportion of live sperm and abnormal spermatozoa. *Post hoc* multiple comparisons were performed using Duncan test. *P* values <0.05 were considered to be significant. The results were presented as the least square of means.

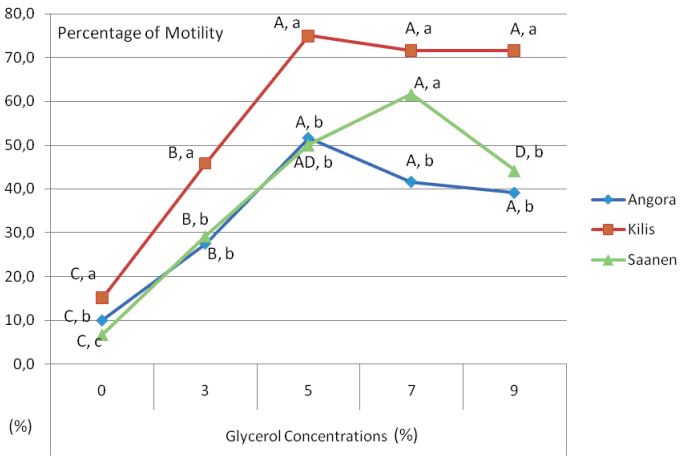
RESULTS AND DISCUSSION

Kilis goat semen had higher motility and viability at different concentrations of glycerol after thawing, compared with Saanen and Angora (P<0.001; Fig. 1 and 2). Motility and viability at 5 % concentration of glycerol was higher than those at other glycerol concentrations (except 7 % glycerol concentration), considered glycerol concentrations in all breeds (P<0.001; Fig. 1 and 2).

When breeds were considered, Saanen goat semen showed higher percentage of abnormal spermatozoa after thawing compared to Angora and Kilis (P<0.001; Fig. 3). Percentage of abnormal spermatozoa at 5 % glycerol concentration was lower than other glycerol concentrations in all breeds, considered glycerol concentrations (P<0.001; Fig.3).

There were statistically significant interactions between concentrations of glycerol and breeds for all spermatologic parameters after thawing (P<0.001; Fig.1, 2 and 3).

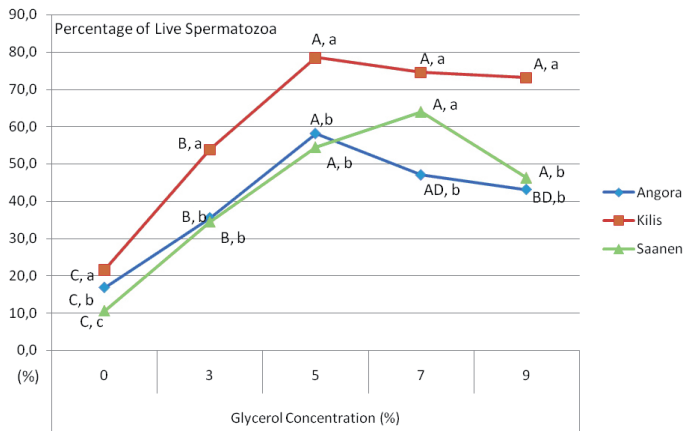
Our results indicated on the interaction between goat breed and glycerol concentration. While 7 % glycerol concentration in Saanen semen cryopreservation provided optimum freezability, Angora goat semen needed 5 % glycerol to provide optimum freezability. Moreover, Kilis goat semen could be successfully cryopreserved using 5, 7 and 9 % glycerol concentrations. In this study, it was determined that 0 and 3 % glycerol concentrations detrimentally affected freezability of semen from three goat breeds. On the other hand, higher concentrations of glycerol (i.e. 7 and 9 %) increased post-thaw semen abnormality in all breeds.



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

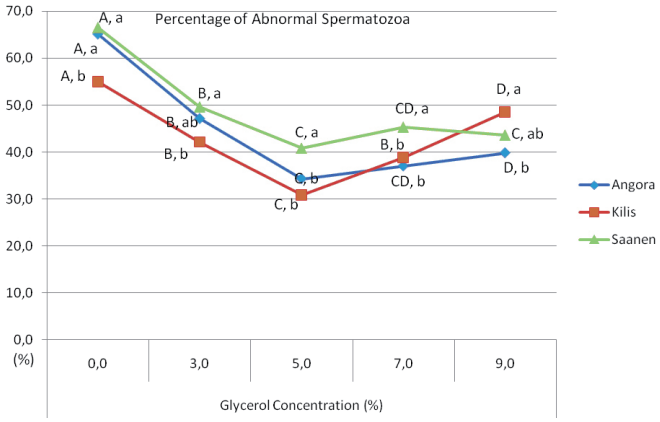
Fig. 1: Post-thawing progressive motility of sperm from Angora, Kilis and Saanen goats at different glycerol concentrations



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

Fig. 2: Post-thawing percentage of live spermatozoa of Angora, Kilis and Saanen goats at different glycerol concentrations



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

Fig. 3: Post-thawing percentage of abnormal spermatozoa for Angora, Kilis and Saanen goats at different glycerol concentrations

There are very limited studies about different concentrations of glycerol for cryopreservation of goat semen. However, a lot of studies have been carried out about freezability of semen from different species (Pena *et al.*, 1998; Rota *et al.*, 1998; Baran *et al.*, 2000; Buhr *et al.*, 2001; Abbas and Andrabi, 2002; Sönmez and Demirci, 2004; Rasul *et al.*, 2007; Awad, 2011; Hoffman *et al.*, 2011, Swelum *et al.*, 2011). Therefore, we had to compare our results with not only those from goat species but also those from other species.

Deka and Rao (1986) showed that the different concentrations (i.e. 4, 6.4, 9 %) of glycerol did not differ in post-thaw sperm motility and abnormalities in goat semen. Results of Deka and Rao (1986) are similar to our results for Kilis goat semen. However, our results for Saanen and Angora goat semen are different. Differences between our results and those by Deka and Rao (1986) may be derived from the differences between goat breeds, extenders used for cryopreservation and also methods of post-thaw semen analyses. Biswas *et al.* (2002) used 5 %, 7 % and 10 % of glycerol for freezing of goat semen. They found that motility and viability of thawed sperm frozen in 7 % glycerol concentration were superior to those of sperm frozen and thawed in

Table 1: Post-thawing progressive motility, viability and abnormality for spermatozoa of Angora, Kilis and Saanen goats at different glycerol concentrations

Breed	N	Concentration of glycerol (%)				
		0	3	5	7	9
				Motility (%)		
Angora	6	$^{C}10.0\pm1.83^{b}$	$^{B}27.5\pm2.14^{b}$	$^{A}51.6\pm1.05^{b}$	$^{A}41.6\pm1.05^{b}$	$^{A}39.1\pm0.83^{b}$
Kilis	6	$^{C}15.0\pm1.29^{a}$	$^{B}45.8\pm1.54^{a}$	$^{A}75.0\pm1.83^{a}$	$^{A}71.6\pm1.05^{a}$	$^{A}71.6\pm1.05^{a}$
Saanen	6	$^{C}6.6\pm1.05^{c}$	$^{B}29.1\pm0.83^{b}$	$^{AD}50.0\pm1.29^{b}$	$^{A}61.6\pm1.05^{a}$	$^{D}44.1\pm1.54^{b}$
				Viability (%)		
Angora	6	$^{C}16.8\pm2.43^{b}$	$^{B}35.5\pm2.86b$	$^{A}58.1\pm2.04^{b}$	$^{AD}47.0\pm1.93^{b}$	$^{DB}43.1\pm0.95^{b}$
Kilis	6	$^{C}21.5\pm1.09^{a}$	$^{B}53.8\pm0.79a$	$^{A}78.5\pm1.80^{a}$	$^{A}74.5\pm0.56^{a}$	$^{A}73.1\pm0.95^{a}$
Saanen	6	$^{C}10.6\pm0.88^{c}$	$^{B}34.5\pm0.76b$	$^{A}54.5\pm0.62^{b}$	$^{A}64.0\pm1.39^{a}$	$^{A}46.3\pm1.63^{b}$
				Abnormality (%)		
Angora	6	$^{A}65.1\pm1.58^{a}$	$^{B}47.1\pm2.12^{ab}$	$^{C}34.3\pm1.43^{b}$	$^{CD}37.0\pm1.61^{b}$	$^{D}39.8\pm1.52^{b}$
Kilis	6	$^{A}55.0\pm1.44^{b}$	$^{B}42.1\pm1.49^{b}$	$^{C}30.8\pm1.40^{b}$	$^{B}38.8\pm1.14^{b}$	$^{D}48.5\pm0.89^{a}$
Saanen	6	$^{A}66.5\pm0.76^{a}$	$^{B}49.6\pm1.28^{a}$	$^{C}40.8\pm1.08^{a}$	$^{CB}45.3\pm0.84^{a}$	$^{C}43.6\pm1.15^{ab}$

$^{A,B,C,D:}$ Means with different superscripts within the same row are significantly different among different glycerol concentrations for same goat breed (P<0.001).

$^{a,b,c:}$ Means with different superscripts within the same column are significantly different among goat breeds for same concentration of glycerol (P<0.001).

5 % and 10 % glycerol concentrations. Moreover, it was determined that glycerol concentrations did not affect individual freezability of goat semen (Biswas *et al.*, 2002). However, Biswas *et al.* (2002) determined sharp increase and then decrease in post-thaw motility for different concentrations of glycerol. We determined that 5 % and higher concentration of glycerol did not sharply affect post-thaw motility of goat semen for all breeds. Farshad *et al*. (2009) found that post-thaw semen quality was higher at 5 and 7 % glycerol compared with other glycerol concentrations (i.e. 1 %, 3%) in Markoz goat semen. These results are similar with our results for Angora (Markhoz) goat semen cryopreservation.

Sönmez and Demirci (2004) tested different concentrations of glycerol and they determined that 5 % glycerol provided successful cryopreservation of ram semen. Furthermore, higher concentration of glycerol (i.e. 7 %) negatively affected post-thaw semen quality. Although we tried different concentrations of glycerol on different species from small ruminant, the findings of Sönmez and Demirci (2004) are similar to those in our study. However, Awad (2011) compared 3 % and 6 % glycerol concentrations in cryopreservation of ram semen and, interestingly, he did not find any differences between concentrations of glycerol.

In other species, except small ruminants, the studies about glycerol concentrations have been carried out. Rota *et al.* (1998) compared 3 % and 5 % concentrations of glycerol in canine semen cryopreservation. They found that 5 % glycerol is suitable to cryopreserve canine semen, while Cardoso *et al.* (2003) did not find any differences among different concentrations of glycerol (i.e. 4, 6 and 8 %) in cryopreservation of canine semen. On the other hand, Baran *et al.* (2000) investigated interactions between different extenders (TRİS vs. skimmed milk-based) and different glycerol concentrations (4 and 7 %) in canine semen cryopreservation. They determined that 7 % concentration of glycerol had toxic effect on cryopreservation of canine semen extended with TRIS and skim milk based extenders. Pena *et al*. (1998) used different concentrations of glycerol and found that 8 % glycerol provided success for cryopreservation of canine semen.

Siwelum *et al.* (2011) investigated interactions between 7 % concentration of glycerol and different extenders (TRIS and skim milk based) in bull semen cryopreservation. They found that 7 % glycerol in TRIS improved post-thaw semen parameters compared to skim milk-based extenders in bull semen cryopreservation. Abbas and Andrabi (2002) used range concentrations (2-12 %) of glycerol for cryopreservation of bufallo semen. They determined that 6 or 7 % concentration of glycerol provided successful cryopreservation of bufallo semen. Rasul *et al.* (2007) compared different concentrations of glycerol for freezability of buffalo semen, and they obtained similar results with Abbas and Andrabi (2002). On the other hand, Rasul *et al.* (2007) determined that lower doses of glycerol (0 and 3 %) adversely affected freezability of buffalo semen. These results are similar to our findings, although the species are different.

Buhr *et al.* (2001) tested different doses of glycerol (0, 2, 4, 8 %) for cryopreservation of boar semen. They found that 2 and 4 % concentrations of glycerol provided higher post-thaw motility and acrosomal integrity. Hoffmann *et al.* (2011) showed that different concentrations of glycerol (1-4 %) for cryopreservation of stallion semen extended with skim milk-based extender did not affect post-thaw semen quality.

Briefly, results from other similar studies pointed out that different concentrations of glycerol may have dissimilar effect on freezability of semen from different breeds and species.

CONCLUSION

Whilst the results obtained from the current study were considered, it was concluded that the different concentrations of glycerol may influence the success of cryopreservation of semen from different goat breeds. It was observed that suitable glycerol percentages for Angora, Kilis and Saanen goats were 5 %, 5-9 % and 7 % respectively. However, these results warrant future fertility studies where semen cryopreserved with different concentrations of glycerol will be used.

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ESTIMATION OF (CO) VARIANCE COMPONENTS OF EWE PRODUCTIVITY TRAITS IN KERMANI SHEEP

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ABSTRACT

The present study was carried out to estimate (co)variance components and genetic parameters for some productivity traits of Kermani ewes using data collected during a 16-year period (1995-2011) at Breeding Station of Kermani sheep, located in Shahrebabak city, Kerman province, Iran. The traits studied were: conception rate (CR), litter size at birth per ewe exposed (LSB/EE), litter size at weaning per ewe exposed (LSW/EE), total litter weight at birth per ewe exposed (TLWB/EE) and total litter weight at weaning per ewe exposed (TLWW/EE). Genetic analysis of the studied traits was performed applying restricted maximum likelihood (REML) procedure under uni- and multivariate repeatability models. Ewe age at lambing and lambing year had significant effects on all the studied traits ($P<0.01$). Weaning age of lambs had significant effect on TLWW/EE as a linear covariate ($P<0.01$). Estimates of direct heritability for CR, LSB/EE, LSW/EE, TLWB/EE and TLWW/EE were 0.08, 0.06, 0.07, 0.11 and 0.15, respectively, while corresponding repeatability estimates were 0.25, 0.19, 0.18, 0.25 and 0.31, respectively. There were found no antagonist relationship among the studied traits in terms of phenotypic, genetic and environmental effects. Direct genetic correlation estimates among the studied traits varied from low estimate of 0.16 for CR-TLWB/EE to high estimate 0.95 for CR-LSB/EE. Low to medium phenotypic correlation estimates of 0.07 (LSB/EE-TLWW/EE) and 0.46 (TLWB/EE-TLWW/EE) were found. It seems that selection based on TLWW/EE, as an efficient selection criterion bring about genetic progress for ewe productivity traits in Kermani sheep.

Key words: reproductive performance; heritability; genetic correlation; sheep

INTRODUCTION

Small ruminants, especially native breed types, play an important role to the livelihoods of a considerable part of human population in the tropics from socio-economic aspects. Therefore, integrated attempt in terms of management and genetic improvement to enhance production is of crucial importance (Kosgey and Okeyo, 2007). Economical and biological efficiency of sheep production enterprises generally improves by increasing productivity and reproductive performance of ewes. The profitability per ewe is mainly determined by reproductive rate and ewe productivity, where mutton and lamb production have sizable influences on profitability (Wang and Dickerson, 1991). Mutton is the main source of red meat in Iran and its production does not meet the increasing demand of the consumers. Generally, the more intensive mutton production system requires the more production of large numbers of lamb per breeding ewe. Reproductive characteristics are of outstanding importance in sheep production enterprises due to their effect on profitability (Matos *et al.*, 1997), especially when meat production is the chief target. The Iranian indigenous sheep breeds are mainly kept by local pastoralists under extensive production systems based on rangelands of low quality and quantity. In such production system low efficiency is common and is caused by several factors, e.g. low reproductive efficiency (Esmailizadeh *et al.*, 2009).

Litter weight weaned per ewe exposed considered as an appropriate indicator of overall ewe productivity and one of the most important economic contributions

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that genetics can made to any sheep breeding system (Vanimisetti *et al.*, 2007). It is a complex trait, influenced by several factors such as age at puberty, ovulation rate, mothering ability, lamb growth and survival (Snowder and Fogarty, 2009). Duguma *et al.* (2002) stated that improvement in ewe productivity could to some extent be achieved by increasing the number and weight of lambs produced per ewe within a specific year. Knowledge of genetic parameters for these decisive traits is of great importance from genetic improvement standpoint. Genetic parameters for reproductive traits of several sheep breeds have been reported (Rosati *et al*., 2002; Ekiz *et al.*, 2005 Vatankhah *et al.*, 2008; Rashidi *et al*., 2011). The genetic characterization of the native breeds is of crucial importance for the conservatory considerations and building up efficient selection and breeding programs (Matika *et al*., 2003). Efficient genetic improvement programs can boost profitability and efficiency for smallholders, within breed selection is an alternative for genetic improvement of small ruminants in the traditional, low-input production systems of the tropics (Kosgey and Okeyo, 2007). Accurate estimates of (co)variance components for economically important traits, such as reproductive ones, are pre-requisites for efficient designing of such strategy.

Kermani sheep is one of the most important Iranian native sheep breeds and is well-adapted to harsh environmental conditions of south-eastern part of country, where dry and hot weather is prevalent and pastures are of low quality and quantity. Kermani sheep is fat-tailed, dual purpose (meat and wool) with mutton production is of primary importance, medium-sized and white-wool breed. In a previous study genetic parameters for some reproductive traits of Kermani sheep, in a ewe lambed basis, were estimated by Mokhtari *et al.* (2010). However, estimation of genetic parameters and (co)variance components for reproductive traits in a ewe exposed basis has not been reported. Such estimates are of biological importance and provide more realistic measures for genetic improvement of ewe productivity with considering conception rate. Therefore, the present study was performed at aiming estimation of genetic parameters including heritability, repeatability and genetic correlation for reproductive traits of Kermani ewe in a ewe-exposed basis using animal model.

MATERIAL AND METHODS

The data set and pedigree information used in the present study were collected for 16 year period from 1995 to 2011 from experimental flock at the Breeding Station of Kermani sheep, located in Shahrebabak city, Kerman province, south-east of Iran.

A controlled mating strategy was designed. During the breeding season - period lasting from mid-August to mid-September, single sire pens were used allocating 25-35 ewes per a fertile ram. The ewes were kept in a flock for a maximum of 5 parities (approximately until the age of 7 years old). In order to avoid inbreeding, rams were allocated rotationally to each group of the ewes in different years. The flock was mainly kept on pastures of low quality and quantity, supplementary feeding was offered especially around mating and during winter (animals kept indoors). The supplemental feeds consist of 1.5 kg alfalfa, 0.5 kg wheat straw and 0.2 kg barley per head per day. The maiden ewes were exposed to the rams at the age of 18 months. Lambing occurs from mid-January to mid- February and new-born lambs were weighed and ear-tagged at the birth time. The lambs were kept indoors during the winter and fed manually. Flocks were grazed during the day and housed at night. Weaning

Table 1: Descriptive statistics for the studied reproductive traits

Item	Traits [a]				
	CR	LSB/EJ	LSW/EJ	TLWB/EJ	TLWW/EJ
No. of records	2683	2683	2683	2658	2597
No. of ewes	993	993	991	991	989
No. of sire of the ewes	71	71	71	71	71
No. of dams	535	535	535	534	534
No. of dams with progeny	507	507	507	504	499
Mean	0.87	0.91	0.84	2.96	17.02
S.D.	0.32	0.40	0.47	1.35	8.96
C. V. (%)	36.78	43.96	47.62	45.61	52.64

[a] CR: conception rate LSB/EJ: litter size at birth per ewe exposed; LSW/EJ: litter size at weaning per ewe exposed; TLWB/EJ: total litter weight at birth per ewe exposed; TLWW/EJ: total litter weight at weaning per ewe exposed

was at approximately 3 months of age. All lambs were weaned at the same day, without necessity at the same age.

Traits investigated can be categorized into two classes; basic and composite. Conception rate (CR) was a basic and binary trait that is measured with values of 0 (a ewe exposed to a ram did not lamb) and 1 (a ewe exposed to a ram did lamb). Other considered traits were composite including litter size at birth per ewe exposed (LSB/EE=CR x LSB), litter size at weaning per ewe exposed (LSW/EE=CR x LSW), total litter weight at birth per ewe exposed (TLWB/EE=CR x TLWB) and total litter weight at weaning per ewe exposed (TLWW/EE=CR x TLWW). LSB/EE was the number of lambs born per ewe exposed within a specific year (0, 1 or 2) and LSW/EE was the number of lambs weaned per ewe exposed within a specific year (0, 1 or 2). TLWB/EE refers to the sum of the birth weights of all lambs born per ewe exposed and TLWW/EE refers to the sum of the weights of all lambs weaned per ewe exposed. The data structure of the studied traits is presented in table 1.

General linear model (GLM) procedure of SAS package (SAS, 2002) was employed for least square analyses and determining of significant fixed effects to be included in final model. The model accounting for fixed effects included lambing year in 17 levels (1995–2011) and ewe age at lambing in 6 classes (2–7 years old). Age of lamb at weaning (in days) was fitted as a linear covariate for corresponding traits. The interaction between lambing year and ewe age was not significant and was removed from the final model. All traits contained lamb weights at birth and/or at weaning were pre-adjusted for sex of lambs using appropriate multiplicative adjustment factors.

The (co)variance components and genetic parameters were estimated by restricted maximum likelihood (REML) method, applying AI-REML method with a convergence criterion of 10^{-8} using WOMBAT program of Meyer (2007) fitting the following repeatability model:

$$y= Xb+ Za+Wpe+ e$$

where **y** is a vector of records for each traits; **b**, **a**, **pe** and **e** are vectors of fixed effects, direct additive genetic effects, permanent environmental effects related to repeated records of ewes and residual effects, respectively. Design matrices of **X**, **Z** and **W** relate the corresponding effects to the vector of **y**.

It was assumed that additive genetic effects, permanent environmental effects related to repeated records of ewes and residual effects to be normally distributed with mean of zero and variances of $\boldsymbol{A}\sigma_a^2$, $\boldsymbol{I}_d\sigma_{pe}^2$ and $\boldsymbol{I}_n\sigma_e^2$ respectively. Also $\boldsymbol{\sigma}_a^2$, $\boldsymbol{\sigma}_{pe}^2$ and $\boldsymbol{\sigma}_e^2$ are direct additive genetic variance, service sire variance, permanent environmental variance related to repeated records of the ewes and residual variance, respectively. $\boldsymbol{A}$ is the additive numerator relationship matrix, $\boldsymbol{I}_d$ and $\boldsymbol{I}_n$ are identity matrices with order equal to the number of ewes and records, respectively. In order to estimate the genetic, environmental and phenotypic correlations a multivariate analysis was performed. The fixed effects included in the multivariate animal model were those in univariate analyses.

Table 2: Least square means with standard error for the studied reproductive traits

Fixed effects	Traits [a]				
	CR	LSB/EJ	LSW/EJ	TLWB/EJ	TLWW/EJ
Overall mean	0.86±0.02	0.94±0.03	0.87±0.01	2.95±0.05	18.26±0.34
Ewe age (year)	**	**	**	**	**
2	0.63±0.02[c]	0.69±0.01[c]	0.62±0.03[c]	1.94±0.05[c]	11.21±0.34[d]
3	0.88±0.01[b]	0.92±0.03[ab]	0.86±0.01[b]	2.90±0.04[b]	17.43±0.36[c]
4	0.91±0.01[ab]	0.95±0.01[a]	0.88±0.01[b]	3.23±0.06[a]	18.61±0.35[b]
5	0.94±0.02[a]	0.99±0.01[a]	0.94±0.02[a]	3.24±0.07[a]	18.90±0.33[b]
6	0.95±0.03[a]	1.00±0.04[a]	0.96±0.03a	3.36±0.09[a]	20.11±0.42[a]
7	0.86±0.05[b]	0.87±0.06[b]	0.84±0.07[b]	2.91±0.19[b]	17.54±1.06[c]
Lambing year	**	**	**	**	**
Birth date [b]	-	ns	ns	-	0.04**±0.01

[a] Abbreviations of the traits are described in footnote of Table 1.
[b] Regression coefficient on day of lamb birth.
[c] resulted from multivariate analysis
Means with similar letters in each subclass within a column do not differ.
ns Non significant (P > 0.05).
** Significant effect at P < 0.01.

RESULTS AND DISCUSSION

The least square means for studied traits are shown in table 2. All traits significantly influenced by lambing year and ewe age at lambing (P<0.01). Age of the lamb at weaning (in days) significantly influenced TLWW/EE (P<0.01). Lambing date turned out not to have any significant effect on LSB/EE and LSW/EE (P>0.05).

Variance components and genetic parameters for considered traits under univariate analysis are presented in table 3. Low direct heritability (h^2_d) estimates were obtained for all traits ranging from 0.06 for LSB/EE to 0.15 for TLWW/EE. Estimates of ratio of permanent environmental variance due to repeated records of ewe on phenotypic variance (pe^2) were also low and varied from 0.11 for LSW/EE to 0.17 for CR and repeatability estimates from 0.18 for LSW/EE to 0.31 for TLWW/EE.

Estimates of direct heritability, obtained from multivariate analysis, are presented in table 4 (on-diagonal values). Corresponding estimated values were lower than those of obtained under univariate analysis and were 0.06, 0.04, .0.04, 0.07 and 0.08 for CR, LSB/EE, LSW/EE, TLWB/EE and TLWW/EE, respectively. Correlation estimates (phenotypic, genetic and environmental) among the traits are presented in table 4. Low to high genetic correlation estimates found among the traits that were ranged from 0.16 for CR-TLWB/EE to 0.95 for CR-LSB/EE. While, phenotypic correlation estimates were low (0.07 between LSB/EE and TLWW/EE) to medium (0.46 between TLWB/EE and TLWW/EE). Also, environmental correlation estimates were low to medium in magnitude and varied from 0.06 for LSW/EE to 0.31 for CR-LSB/EE.

Table 3: Estimates of genetic parameters and variance components for the studied reproductive traits

Traits [a]	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_p	h^2_d ± S.E.	pe^2 ± S.E.	r
CR	0.0085	0.0180	0.0795	0.1060	0.08 ± 0.03	0.17 ± 0.03	0.25
LSB/EJ	0.0085	0.0184	0.1148	0.1417	0.06 ± 0.02	0.13 ± 0.03	0.19
LSW/EJ	0.0119	0.0188	0.1400	0.1707	0.07 ± 0.03	0.11 ± 0.02	0.18
TLWB/EJ	0.1632	0.2078	1.1131	1.4841	0.11 ± 0.02	0.14 ± 0.05	0.25
TLWW/EJ	8.2101	8.7474	37.7766	54.7341	0.15 ± 0.04	0.16 ± 0.02	0.31

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.
[a] Abbreviations of the traits are described in footnote of Table 1.

Table 4: Phenotypic, genetic and environmental correlation [b] estimates and direct heritability [c] estimates for the studied reproductive traits

Traits [a]	CR	LSB/EJ	LSW/EJ	TLWB/EJ	TLWW/EJ
CR	0.06±0.05	0.95±0.26	0.74±0.34	0.16±0.21	0.34±0.23
LSB/EJ	0.44±0.08	(0.31±0.06)	0.04±0.03	0.76±0.29	0.35±0.19
	0.28±0.17				
LSW/EJ	0.27±0.09	(0.16±0.04)	0.36±0.09	(0.16±0.02)	0.04±0.01
	0.39±0.27	0.49±0.26			
TLWB/EJ	0.11±0.04	(0.08±0.02)	0.21±0.06	(0.12±0.02)	0.23±0.04
	(0.06±0.02)	0.07±0.05	0.87±0.15		
TLWW/EJ	0.15±0.04	(0.19±0.05)	0.07±0.03	(0.14±0.04)	0.32±0.03
	(0.14±0.04)	0.46±0.04	(0.22±0.05)	0.08±0.04	

[a] Abbreviations for the traits are presented in footnote of Table 1.
[b] Genetic correlations (above diagonal), phenotypic (below diagonal) and environmental in the parenthesis
[c] resulted from multivariate analysis

A general tendency for improvement of the studied traits with the increase of ewe age was observed until the age of 7 years old (Table 2) and it can be explained partly by differences in maternal effects, nursing and maternal behavior of ewe at different ages. Fourie and Heydenrych (1983) reported that twinning rate and conception rate generally increase with age, followed by a decrease in reproductive performance after approximately 5 parities. Significant effects of ewe age on reproductive traits of sheep have been reported in the literature (Rosati *et al.*, 2002; Ekiz *et al*., 2005, Rashidi *et al.*, 2011). The significant effect of lambing year may be ascribed to variation in climatic conditions and managerial practices through different years. Significant effect of lambing year on ewe productivity traits has been well documented by others (Boujenane *et al*., 1991; Bromley *et al*., 2001; Ekiz *et al*., 2005; Vatankhah *et al*., 2008; Mokhtari *et al*., 2010; Rashidi *et al*., 2011).

A low estimate of 0.08 was obtained for direct heritability of CR, which was in accordance with estimates of Rosati *et al.* (2002) and Safari *et al.* (2005). Lower estimate of 0.01 was found by Vatankhah *et al*. (2008) for direct heritability of CR in Lori-Bakhtiari sheep that was lower than estimated value in the present study. The low estimate may be due to the effect of random environmental factors on variability of the observations and because of the categorical expression of the trait. Therefore, improvement of CR through selection would be difficult even though CR is of great economic importance. Low estimates of direct heritability were found for LSB/EE (0.06) and LSW/EE (0.07) that generally agreed with several authors (Fogarty, 1995; Rosati *et al.*, 2002; Safari *et al*. 2005; Vatankhah *et al.*, 2008). Therefore, the possibility to achieve rapid genetic gain through selection for these traits would be limited. Direct additive genetic variance constitutes 11 % and 15 % of phenotypic variance for TLWB/EE and TLWW/EE, respectively. TLWB/EE indicates the capacity of the ewes to produce weight of lambs at birth after exposure to the ram without taking the number of lambs born into account (Rosati *et al*., 2002). Direct heritability estimate of TLWB/EE (0.11) was in general congruence with estimate of Rosati *et al.* (2002) and Vatankhah *et al*. (2008).

TLWW/EE measures the ability of the ewe to produce weaning weight of lamb after exposure to the ram and is a trait of great economic importance in any sheep breeding production system. Obtained direct heritability (0.15) was concordant with estimate of Rosati *et al.* (2002) and Safari *et al.* (2005). Lower estimate of 0.07 was obtained by Vatankhah *et al*. (2008) in Lori-Bakhtiari sheep. The TLWW/EE could be considered as an efficient selection criterion because it is in a sense, a measure of total productivity of the ewe for lamb-meat production during a specific breeding year (Rosati *et al*., 2002). Furthermore, it is a high economic importance composite trait (Ercanbrack and Knight, 1998) and had components such as fertility, number of lambs at weaning per ewe exposed and number of lambs born. For all practical purposes, it is more desirable to select a component trait than a composite one when a component trait bears a high genetic correlation with composite trait, higher heritability and coefficient variation compared to the composite one (Snowder and Fogarty, 2009).

Comparison between a component trait and a composite one in terms of selection response can be done by comparing the product of the heritability and coefficient of variation (Smith, 1969). Such product can be useful in determining the credence of selection based on a composite trait relative to selection based on its components (Snowder and Fogarty, 2009). Using the obtained values in the present study, the product of the heritability and coefficient variation for TLWW/EE is 7.89, compared with 2.94 for CR, 2.64 for LSB/EE and 3.33 for LSW/EE. Therefore, response to selection for TLWW/EE would be greater than the responses expected for its component traits.

Estimates of repeatability were higher than the heritability ones suggesting that traits are affected more by non-additive genetic effects (dominance and epitasis) and permanent environmental effects. Therefore, the accuracy of selection for these traits especially for TLWW/EE on the first lambing should be medium as repeatability measures correlation between performance records in different lambing of the ewe. Repeatability estimate of CR (0.25) was medium. Contrary to us, Vatankhah *et al.* (2008) estimated low estimate of 0.10 for repeatability of CR in Lori-Bakhtiari sheep. LSB/EE and LSW/EE have relatively similar in magnitude repeatability values of 0.19 and 0.28, respectively. Higher estimates were obtained for repeatability of TLWB/EE (0.25) and TLWW/EE (0.31). Corresponding lower estimates were found by Vatankhah *et al.* (2008).

Genetic correlation estimates among the studied traits were positive and higher than those of phenotypic and environmental ones. CR had high genetic correlation with LSB/EE (0.95) and LSW/EE (0.74), probably because it is a major component of these traits. Lower genetic correlation estimates of CR with TLWB/EE (0.16) and TLWW/EE (0.34) could be explained to some extent by the fact that these traits have records different from zero, only if conception rate is successful (Rosati *et al.*, 2002; Vatankhah *et al*., 2008). Obtained genetic correlations among CR and other studied traits were in general agreement with estimates of Rosati *et al.* (2002). LSB/EE and LSW/EE have positive and relatively high genetic correlation (0.76). Similar to our estimate, Vatankhah *et al.* (2008) obtained a corresponding value of 0.77 in Lori-Bakhtiari sheep. A lower estimate of 0.29 was found by Rosati *et al.* (2002). Medium estimates

of genetic correlations for LSB/EE-TLWB/EE (0.35) and LSB/EE-TLWW/EE (0.28) were generally lower than those of obtained by Vatankhah *et al*. (2008) but generally were in agreement with Rosati *et al.* (2002). Estimates of genetic correlations for LSW/EE-TLWB/EE (0.39) and LSW/EE-TLWW/EE (0.49) were generally agreed with those of obtained by Vatankhah *et al*. (2008) and higher than estimates of Rosati *et al.* (2002). A high genetic correlation (0.87) was estimated between TLWB/EE and TLWW/EE. This high genetic correlation suggests that genes resulting in heavy birth weight of litters, through number and weight of lambs are responsible for genes affecting milk production performance and maternal behavior of ewes throughout pre-weaning period. Selection for TLWB/EE may be desirable, even if the direct heritability is not high because of the high genetic correlation between TLWB/EE and TLWW/EE. Recording of TLWB/EE have taken some weeks earlier than records of TLWW/EE. This time period can be of breeding decision making important, due to the typical seasonal breeding activity of sheep, saving a few weeks may advance selection by one breeding season (Rosati *et al.*, 2002).

CONCLUSION

Large influences of non-genetic effects on the studied traits were observed. Therefore, concentrating on managerial practices such as improvement in ewe nutrition around mating and late pregnancy can result in the improvement of reproductive performance of ewes. In spite of low genetic variations observed for the studied traits building an appropriate breeding program needs to include these traits as an integral part of the program, due to their considerable influence on the profitability of production system. In this account, TLWW/EE is a composite trait incorporating growth ability of lambs as well as their survival from birth to weaning, maternal ability of the ewes and conception rate. The existence of positive genetic correlation estimates between TLWW/EE and the other traits suggests that using TLWW/EE as a selection criterion in plotting out breeding program would be beneficial and could promote the overall productivity of the ewes.

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ESTIMATION OF MATERNAL EFFECTS ON THE NORTH-IRANIAN NATIVE CHICKEN TRAITS USING BAYESIAN AND REML METHODS

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ABSTRACT

In the present research, direct and maternal genetic parameters were estimated for 4 productive and reproductive traits of Iranian native chickens. Six different animal models with restricted maximum likelihood (REML) and Bayesian procedure were applied to estimate genetic parameters. The chickens were investigated considering their phenotypic and genotypic trend after 19 generation selection. Estimated direct and maternal heritability is based on best models using REML methods: 0.55, 0.01 for average egg weight; 0.22, 0.009 for body weight at 8 weeks old; 0.15, 0.02 for egg number and 0.39, 0.045 for age at sexual maturity, respectively. The estimated values of these parameters using Bayesian approach for studied traits are based on best model: 0.54, 0.034 for average egg weight, 0.23 and 0.044 for body weight at 8 weeks old, 0.15, 0.02 for egg number and 0.41, 0.048 for age at sexual maturity, respectively. In this study, the results obtained from the statistical REML method are similar to that of Bayesian approach, but there are differences between the traits regarding the selection of best model due to different ways of models´ evaluation. The result of genetic trend regarding average egg weight, body weight at 8 weeks old, egg number and age at sexual maturity were -0.100 (g), 5.650 (g), 0.721 (number) and -1.558 (day), respectively. Correlation of the direct and maternal genetic effects (r_{am}) for studied traits was estimated. Consequently, a negative correlation between maternal and direct additive genetic effects was observed.

Key words: genetic parameters; native chicken; maternal effects; REML; Bayesian

INTRODUCTION

In recent decades, breeding programs and genetic gain have an important effect on the genetic composition of commercial chickens (Muir *et al.*, 2008). But the question striking to mind is what would be the future of genetic diversity of pure commercial line. Muir *et al.* (2008) showed that 50 % or more of the genetic diversity in ancestral breeds is absent in commercial pure line. This absence resulted from high number of non-corporate breeds. These studies indicate an important role of native chicken in satisfying future genetic diversity needs. The first goal of the Mazandaran breeding center was to conserve the native chicken's gene pool and the second one was to increase the production ability of this breed for that environmental condition in developing the rural industry. It is important to have an accurate (co) variance component and consequent genetic parameters for every animal breeding program. To achieve this aim, several statistical methods have been used during the four past decades. Two powerful statistical methods are still being widely used for different animal breeding researches. The first one is Restricted Maximum Likelihood (REML) using popular algorithm average information REML (AI-REML; Misztal, 2008) and the second one is a Bayesian method using Gibbs sampling (BAGS) technique. The BAGS method has been widely used in different animal breeding programs (Wing, 1993a, 1994b; Jensen, 1994; Sorenson, 1994; Van Tassel and Van Vleck, 1996). Direct and maternal genetic effects are genetically correlated. The maternal effect influenced the progeny phenotype due to genetic and environmental

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differences between dams (Grosso *et al.*, 2010). There is a very high genetic correlation between the maternal effect on chick weight and the direct effect on egg weight (Hartman *et al.*, 2003). In another study by Saatci (2006) strong correlation was detected between direct genetic effect of egg traits and maternal genetic effect of BW trait. Several studies have reported estimates of (co) variance components, additive and maternal genetic parameters considering productive and reproductive traits of native chicken (Kamali *et al*., 2007; Seraj *et al.*, 2006; Xu *et al.*, 2011). The aim of this research was to estimate the genetic parameters and correlation between direct and maternal genetic effect with different animal models using both REML and Bayesian methods. An additional aim was to calculate predicted genetic trend following selection over 19 generations.

MATERIAL AND METHODS

Birds and data

Mazandaran province in the north of Iran has mostly a rainy and humid climate. In this research, data on 74500 Iranian native hens which belong to the breeding center of Mazandaran native chickens from founder generation (G0) to generation 19 (G19) were used to estimate (co) variance component and genetic parameters of productive and reproductive traits. All laying chickens were selected from a small population, with an individual phenotypic value of body weight in 8 weeks (BW8) of age and egg number (EN) during the first 12 weeks of the laying period. For first generation, the eggs were collected from rural areas without any background of birds and were hatched to compose the basic population. Parents were selected on the basis of BW8 and the production of their sisters. All birds were reared on the floor for 8 weeks. Following that, a number of laying native chickens were selected according to their body weights, transferred into the individual cages and their production traits were recorded. The selection intensities were 40 % for hens and 5 % for cocks during the breeding process. The collected data from the individuals included Body Weight at 8 weeks of age (BW8), Egg Number during the first 12 weeks of the laying period due to the average period of Mazandaran native fowl is 12, so that during the selection only 12 weeks of laying period were monitored Average Egg Weight (AEW) at 28, 30 and 32, weeks old of chickens and Age at Sexual Maturity (ASM). The data file of animal consists of sire, dam number, generation, hatch number and sex (for BW8 trait), number of productivity days as a covariate (for EN trait) and information about other traits.

Statistical analysis

The statistical description of the traits is summarized in Table1. Because of missing observation, the number of observations was different between traits. The Proc Univariate was used for editing data to remove outlier values. The GLM procedure of SAS software (SAS, 2003) was used to test the significance of the fixed effects of generation (in 19 levels), hatch number (maximum 6 levels in each generation) and sex (in 2 levels) in the model. As shown in Table 1, fitting these fixed effects in a model as contemporary group effects of generation - hatch (GH) for AEW, ASM and EN and generation - hatch - sex (GHS) for BW8 have significant ($P < 0.0001$) effects in studying these traits. The effect of days in production (DP) was fitted in the model considering EN trait. It should be noted that egg laying period was different among hens; therefore this effect was included in the model as a covariate. For estimation of (co) variance components and genetic parameters six different animal models were used as follows (Meyer, 1998):

Table 1: Basic data statistics

Trait	N	Mean	SD	Min	Max	C.V %
AEW	44183	47.67	4.98	26.50	83.2	10.32
BW8	73726	574.72	166.89	400	1400	17.56
EN	31967	36.65	15.49	15	95	38.61
ASM	42919	163.56	16.72	120	235	1.35

Number of observations (N), calculated mean (Mean), Standard deviation (SD), Coefficient of variation (C.V), Minimum (Min), Maximum (Max) Value of traits and of traits AEW= average egg weight (g), BW8 = body weight at 8 weeks of age (g), EN = egg number and (ASM) age at sexual maturity (day) in 19 generation of selection

$\mathbf{y = Xb + Z_1a + e}$ Model (1)
$\mathbf{y = Xb + Z_1a + Wc + e}$ Model (2)
$\mathbf{y = Xb + Z_1a + Z_2m + e}$ cov (a,m) = 0 Model (3)
$\mathbf{y = Xb + Z_1a + Z_2m + e}$ cov (a,m) ≠ 0 Model (4)
$\mathbf{y = Xb + Z_1a + Z_2m + Wc + e}$ cov (a,m) = 0 Model (5)
$\mathbf{y = Xb + Z_1a + Z_2m + Wc + e}$ cov (a,m) ≠ 0 Model (6)

The first model is a simple animal model including only additive animal genetic effects as random effects. Model 2 included the maternal permanent environmental effect as additional random effects which had no relationship with the other effects. Model 3 included maternal genetic effects as a second random genetic effect having no covariance between the direct and maternal effects, i.e. $\boldsymbol{\sigma_{am} = 0}$. In the model 4, the covariance between them is not set to zero. In the model 5 and 6, both the permanent environmental and maternal genetic effects were considered. The differences between model 5 and 6 are in ignoring and considering covariance between direct and maternal effects, respectively. In these models, **y** = the vector of observations, **b** = vector of fixed effects including (generation, hatch number of all traits and days in product (DP) as a covariate of EN only), **a** = vector of direct genetic effects, **m** = vector of maternal genetic effects, and **e** = vector of residual effects and **X**= incidence matrix relating to the observation obtained from fixed effects. $\mathbf{Z_1}$, $\mathbf{Z_2}$ and **W** are incidence matrices relating observation to the above mentioned random vectors (**a**, **m**, and **c**, respectively). The covariance structure of the full model (M_6) will be as follows:

$$V = \begin{bmatrix} a \\ m \\ c \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & A\sigma_{am} & 0 & 0 \\ A\sigma_{am} & A\sigma_m^2 & 0 & 0 \\ 0 & 0 & I\sigma_c^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix},$$

Where, σ_a^2 = direct additive genetic variance, σ_m^2 = the maternal additive genetic variance, σ_{am}^2 = covariance between, direct and maternal genetic effects, σ_c^2 = maternal permanent environmental variance, σ_e^2 = residual variance. The basic assumptions in these models were: E [**y**] = Xb; E [**a**] = 0; E [**c**] = 0 and E [**e**] = 0. Total phenotypic variance (σ_p^2) was estimated as the while cumulating all (co) variance components. Direct heritability (h^2), maternal genetic heritability (h_m^2) and proportion of maternal permanent environmental variance to phenotypic variance (c^2) were calculated. In Table 2 more information is included to make the statistical importance of effects more clearly.

Total heritability resulting from incorporating direct-maternal genetic covariance and variance from heritable maternal effects was calculated using the formula

$h_{tot}^2 = (\sigma_a^2 + 0.5 * \sigma_m^2 + 1.5 * \sigma_{am}) / \sigma_p^2$ Willham (1972).

Estimation of (co) variance components in the first step was done by REML using an average information algorithm by AI-REMLF90 Misztal (1999b) software. The convergence criterion was set at 10^{-10} for most of the analyses. To test the significance of the random maternal (genetic and permanent environmental) effects in REML method, the likelihood ratio test with k degree of freedom was used, where k is set to the number of additional factors in the complete model of Dobson (1990).

$x_k^2 = 2 \log (L) - 2 \log L (R)$,

where L (F) = the likelihood of full model, and L (R) the likelihood of reduced model. In the second step, BAGS method was applied by GIBBS3F90 Misztal (1999a) software. In each analysis, 500000 rounds of Gibbs sampling were conducted. The first 50000 steps were discarded as a burn-in period, and the thinning interval was constant at 100 cycles. The deviance information

Table 2: Univariate animal model for Mazandaran chicken

Factor	Type [a]	Trait [b]			
		AEW	BW8	EN	ASM
Generation	F	×	×	×	×
Hatch	F	×	×	×	×
Sex	F	-	×	-	-
Days in production	C	-	-	×	-
Additive animal genetic effect	A	×	×	×	×
Additive maternal genetic effect	A	×	×	×	×
Maternal permanent effects	R	×	×	×	×

[a] Type of factor: F, fixed factor, R, random factor, A, random factor with covariance matrix, C, covariable
[b] Traits: see footnotes for Table 1.

criterion (DIC) was used for model compared with BAGS methods. The idea is that models with smaller DIC should be preferred to models with larger DIC. The phenotypic and genetic trend of studied traits was estimated by regression of Least Square Means (LSM) and an average of breeding values of birds on generation in nineteen generations of selection, respectively.

RESULTS AND DISCUSSION

There were different estimation of (co) variance components and genetic parameters considering different models. Results of Restricted maximum likelihood and Bayesian approach using Gibbs sampling regarding AEW, BW8, EN and ASM are shown in Tables 3 and 4, respectively. Estimated direct heritability of AEW using REML method was 0.55 based on the best model (model 6), compared to the BAGS method in which this value was 0.54. From all of the models, model 4 was regarded as a good model. Based on model 6 with REML method, estimates of c^2 and h_m^2 were 0.03, 0.01 respectively. Using Bayesian method estimated h_m^2 proved to be 0.34. According to these methods total heritability was 0.48 and 0.47 for REML and Bayesian method, respectively. As shown in Table 3, estimated h^2, c^2, h_m^2 and r_{am} in BW8 by REML method were 0.22, 0.041, 0.009 and -0.52 respectively. According to the model 4 as the best model used in Bayesian method h^2, h_m^2 and r_{am} of BW8 were 0.23, 0.044, -0.17 respectively. Estimated total heritabilities regarding REML and Bayesian methods of BW8 were 0.19 and 0.23 in that order. According to the same obtained results of EN based on model 6 as a good model in both REML and Bayesian methods estimation of direct heritability, proportion of maternal environmental variance to phenotypic variance (c^2), maternal heritability (h_m^2) and total heritability (h_t^2) of EN were 0.15, 0.02, 0.02 and 0.10, respectively. The estimated correlation between direct additive genetic effect and maternal additive genetic effects (r_{am}) of EN proved to be -0.74 using REML method and -0.77 using BAGS method. As shown in Table 4, estimation of direct and maternal heritability of ASM based on the best model (model 6) in REML method was 0.39 and

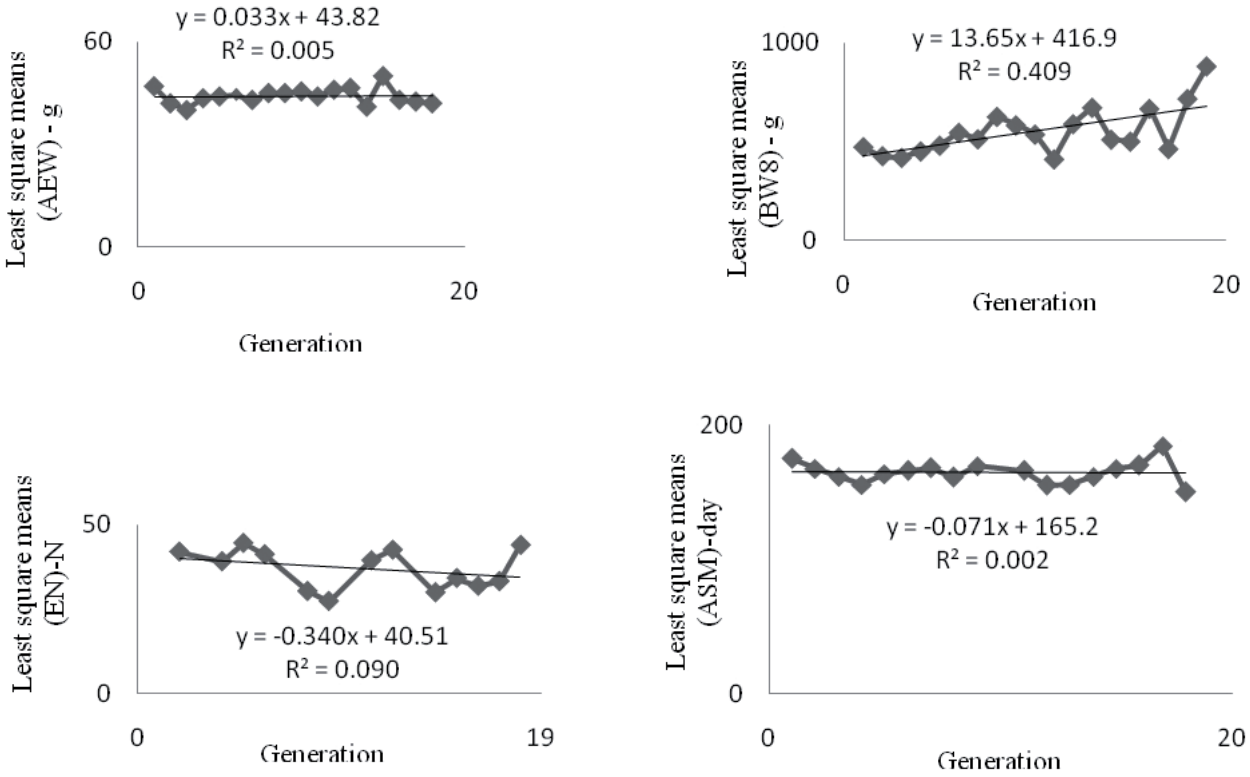

Fig. 1: Phenotypic trend of : (a) average egg weight (AEW), (b) body weight at 8 weeks of age (BW8), (c) egg number (EN) and (d) age at sexual maturity in 19 generations of selection

0.045 respectively. According to this model the estimated values for (c^2), (r_{am}) and (h_t^2) were 0.054, -0.37 and 0.34 respectively. The estimated genetic parameters of ASM using Bayesian methods are shown in Table 4, direct heritability of ASM based on model 4 as a better model was 0.41 and the estimated maternal heritability of this trait was 0.048. The correlation between direct additive genetic effect and maternal additive genetic effects (r_{am}) and total heritability were estimated to be -0.38 and 0.36 respectively. As shown in Figure 1, (a) to (d), the phenotypic trends were calculated considering studying traits. The least square means on generation number were 0.33 (g), 13.65 (g), -0.34 (num) and -0.71 (day) for AEW, BW8, EN and ASM respectively. As reported in Figure 2, (a) to (d), the genetic trend estimation of AEW, BW8, EN and ASM were -0.100, 5.65, 0.72, and- 1.558, respectively. The genetic trend estimation showed a significant and positive improvement in trait BW8 and EN and the genetic trend of AEW trait proved to be negative due to a negative correlation present between EN and AEW. According to negative genetic trend of ASM, the age of maturation in this breed decreased as well during the 19 generation selection. Considering these traits genetic trends indicate that selection would be effective. The means of the posterior distribution of additive genetic variance of average egg weight, body weight at 8 weeks, egg number and age at sexual maturity are shown in Figure 3 (a) to (d).

In the studies of Chamber *et al.* (1990) and Akbas *et al.* (2002), the maternal genetic effects were evaluated using different models with considering paternal and maternal half-sib and full-sib progeny. According to their reports, the highest value of direct heritability in maternal half-sib progeny was estimated by maternal effects. In

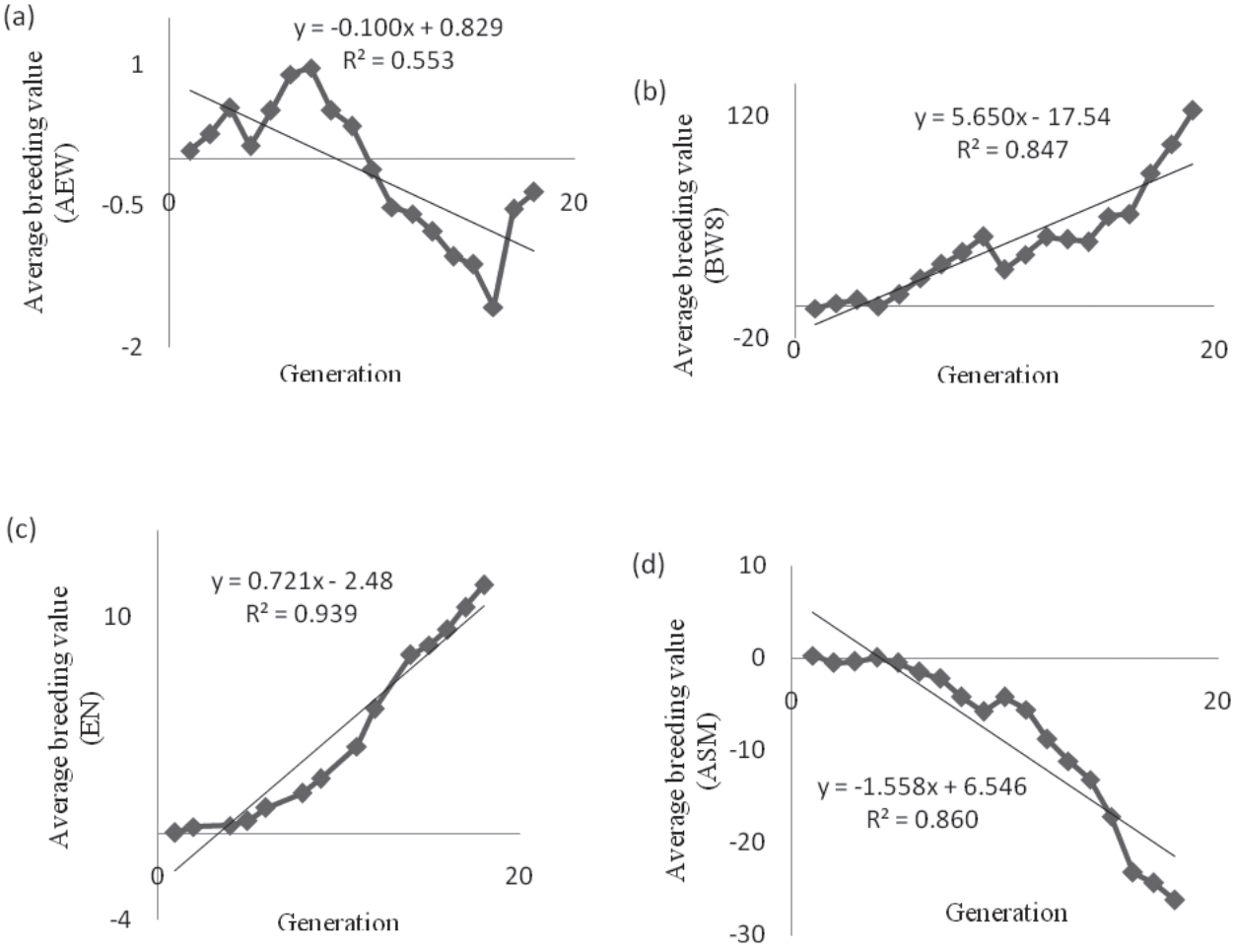

Fig. 2: Genetic trend of : (a) average egg weight (AEW), (b) body weight at 8 weeks of age (BW8), (c) egg number (EN) and (d) age at sexual maturity in 19 generations of selection

another study on Iranian native chicken by Seraj *et al.* (2007) it was revealed that maternal genetic and environmental effects considering the covariance of maternal and direct genetic effects proved to be important for body weight at 8 weeks, which are similar to our results. Fathi (2003) reported that estimated genetic parameters for six-week-old body weight using an animal model results in an overestimation of direct additive genetic variance and heritability if maternal additive genetic and environmental effects were ignored in the models. Our results indicate that estimated heritability decreased in complete model as compared with other simple models due to the negative covariance of maternal and direct genetic effects in studied traits. Consistent with the present finding, Rahman *et al.* (2010) reported that the analysis of day-old chick body weight and six-week-old body weight records on commercial broiler line and the direct maternal genetic effect observed for weight at six weeks of age might be a factor transferred from genes influencing weight at hatch to weight at six-week-old. Koerhuis *et al.* (1996) allocated 2, 2, 3, and 4 % of observed phenotypic variance from egg weight, age at sexual maturity, egg number and six-week weight of a broiler line trait to maternal environmental effects, which are inconsistent with the results of the current research. In a study by Koerhuis *et al.* (1997) maternal heritability and maternal permanent environment as a proportional of phenotypic variance on six-week-old body weight of the chicks was estimated to be 2 - 4 and 5 - 6 percentages, respectively. The results of this study indicated that ignoring maternal effects in the model resulted in overestimating of additive genetic variance and direct heritability considering a body weight at 8 weeks-old traits. Regarding age at sexual maturity, average egg

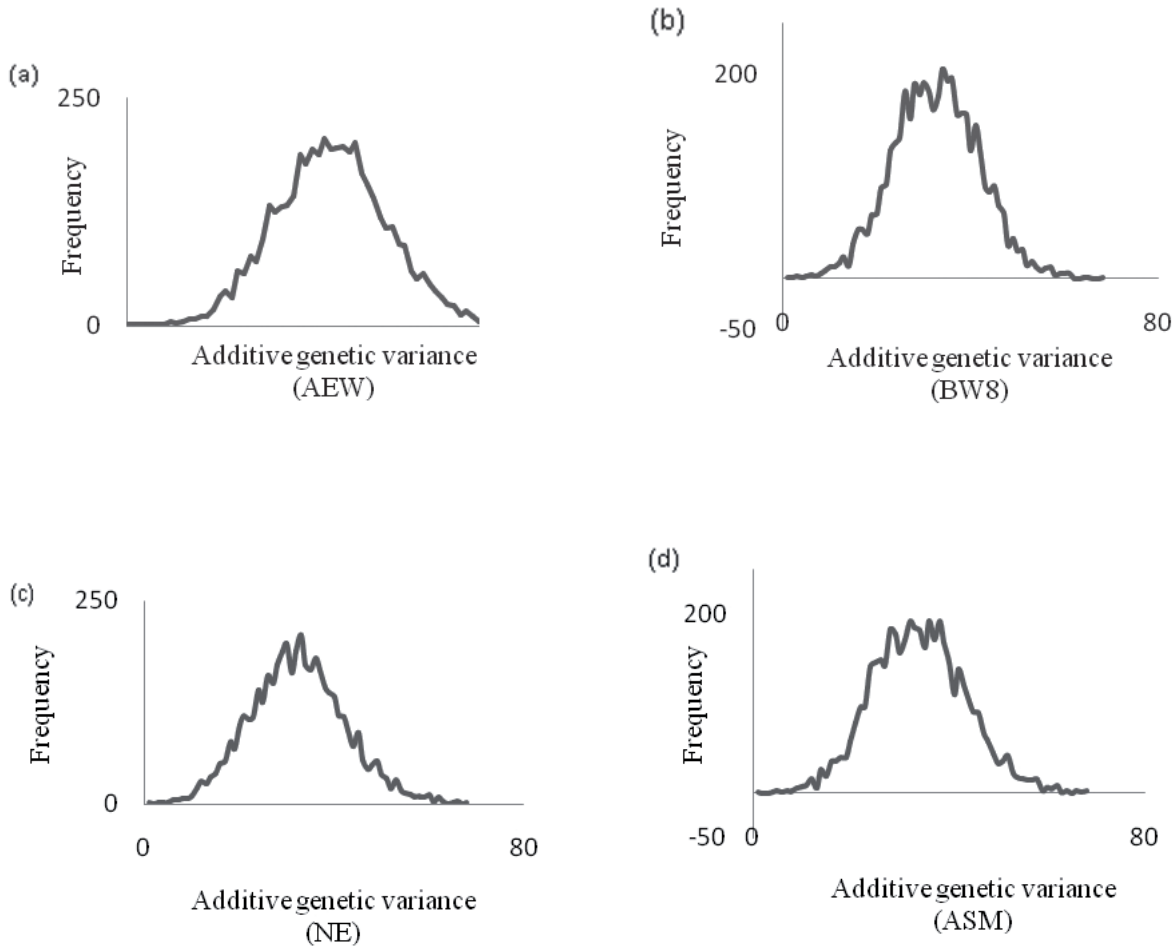

Fig. 3: The posterior distribution of additive genetic variance for (a) average egg weight AEW), (b) body weight at 8 weeks of age (BW8), (c) egg number (EN) and (d) age at sexual maturity

Table 3: Estimation of (co) variance component and derived parameters for average egg weight and body weight at 8 weeks old

Traits	Method	M	$h^2_a \pm$ S.E.	$c^2 \pm$ S.E.	$h^2_m \pm$ S.E.	r_{am}	h^2_t	*-2LogL , DIC*
AEW	REML	1	0.51 ± 0.01	-	-	-	-	258188
		2	0.50 ± 0.02	0.02 ± 0.004	-	-	-	229500
		3	0.49 ± 0.01	-	0.02 ± 0.005	-	0.50	204108
		4	0.54 ± 0.02	-	0.03 ± 0.013	-0.36	0.48	204078
		5	0.49 ± 0.02	0.01 ± 0.005	0.01± 0.005	-	0.50	204082
		6*	0.55 ± 0.03	0.03 ± 0.005	0.01 ± 0.001	-0.53	0.48	204040
	Bayesian	1	0.50 ± 0.02	-	-	-	-	220675
		2	0.48 ± 0.02	0.03 ± 0.007	-	-	-	224878
		3	0.49 ± 0.02	-	0.02 ± 0.009	-	0.50	220092
		4*	0.54 ± 0.022	-	0.034 ± 0.008	-0.37	0.47	217927
		5	0.48 ± 0.02	0.02 ± 0.005	0.01 ± 0.007	-	0.49	220442
		6	0.55 ± 0.01	0.03 ± 0.006	0.01 ± 0.007	-0.50	0.49	219147
BW8	REML	1	0.27 ± 0.023	-	-	-	-	899994
		2	0.24 ± 0.019	0.04± 0.003	-	-	-	813195
		3	0.22 ± 0.022	-	0.037 ± 0.008	-	0.24	813228
		4	0.23 ± 0.020	-	0.044 ± 0.008	-0.17	0.23	813222
		5	0.19 ± 0.023	0.040 ± 0.005	0.003 ± 0.006	-	0.20	813087
		6*	0.22 ± 0.024	0.041 ± 0.005	0.009 ± 0.007	-0.52	0.19	813070
	Bayesian	1	0.27 ± 0.018	-	-	-	-	845754.4
		2	0.25 ± 0.022	0.044 ± 0.008	-	-	-	848357.0
		3	0.22 ± 0.022	-	0.037 ± 0.006	-	0.24	847529.2
		4*	0.23 ± 0.019	-	0.044 ± 0.009	-0.17	0.23	846372.4
		5	0.19 ± 0.017	0.038 ± 0.009	0.007 ± 0.009	-	0.20	846784.7
		6	0.22 ± 0.021	0.040 ± 0.007	0.011 ± 0.008	-0.52	0.19	847482.7

h^2_a = direct heritability; c^2 = proportion of maternal environmental variance to phenotypic variance; h^2_m = maternal heritability ; r_{am} = correlation between direct additive genetic effect and maternal additive genetic effects; h^2_t = total heritability; *2LogL* = log likelihood (in REML method); (M = models). *DIC* = deviance information criterion (in Bayesian method); (*) = best model

weight and egg number considering maternal effects in models without inclusion of covariance of maternal and direct genetic effects resulted in lower estimation of additive genetic variance and consequently direct heritability. Results obtained from univariate parameter estimation showed that in all of the studied traits there is a negative correlation between maternal and direct genetic effects. In the study of Robinson *et al.* (1993) the negative correlation between maternal genetic and direct effects were observed for body weight trait. They reported that this negative correlation can be resulted from ignoring the maternal effects during last generations and selection which is only based on direct additive animal genetics.

CONCLUSION

(Co) variance components, estimated using two Bayesian and of REML methods, were almost the same. In best model selection using Bayesian method the best model resulted in the simple models with fewer effects. We assume that including maternal effect in statistical models is essential for estimation of genetic parameters; the models with covariance between the direct and maternal effects give more accurate result in most of the traits.

Table 4: Estimation of (co) variance component and derived parameters for egg number and age of sexual maturity

Traits	Method	M	h^2_a ± S.E.	c^2 ± S.E.	h^2_m ± S.E.	r_{am}	h^2_t	*-2LogL , DIC*
EN	REML	1	0.16 ± 0.011	-	-	-	-	283602
		2	0.13 ± 0.014	0.023 ± 0.00	-	-	-	242487
		3	0.12 ± 0.011	-	0.01 ± 0.007	-	0.13	224161
		4	0.15 ± 0.012	-	0.03 ± 0.009	-0.58	0.11	224135
		5	0.11 ± 0.014	0.02 ± 0.006	0.003 ± 0.008	-	0.12	224150
		6*	0.15 ± 0.013	0.02 ± 0.005	0.02 ± 0.008	-0.74	0.10	224119
	Bayesian	1	0.16 ± 0.012	-	-	-	-	257119.8
		2	0.13 ± 0.014	0.025 ± 0.00	-	-	-	228671.1
		3	0.12 ± 0.012	-	0.01 ± 0.005	-	0.13	241563.4
		4	0.16 ± 0.015	-	0.04 ± 0.001	-0.58	0.11	241389.0
		5	0.11 ± 0.014	0.02 ± 0.009	0.002 ± 0.006	-	0.12	241719.3
		6*	0.15± 0.013	0.02 ± 0.007	0.02 ± 0.003	-0.77	0.10	241012.5
ASM	REML	1	0.43 ± 0.014	-	-	-	-	271589
		2	0.38 ± 0.018	0.051 ± 0.008	-	-	-	229500
		3	0.38 ± 0.020	-	0.035 ± 0.006	-	0.40	216275
		4	0.42 ± 0.019	-	0.046 ± 0.006	-0.33	0.37	216271
		5	0.35 ± 0.016	0.042 ± 0.005	0.031 ± 0.007	-	0.37	216173
		6*	0.39 ± 0.021	0.055 ± 0.005	0.041 ± 0.005	-0.43	0.33	160538
	Bayesian	1	0.44 ± 0.018	-	-	-	-	233820.1
		2	0.36 ± 0.019	0.057 ± 0.008	-	-	-	233814.1
		3	0.37 ± 0.027	-	0.063 ± 0.008	-	0.40	234559.7
		4*	0.41 ± 0.024	-	0.048 ± 0.007	-0.38	0.36	229611.4
		5	0.35 ± 0.018	0.046 ± 0.009	0.031 ± 0.006	-	0.37	233494.7
		6	0.39 ± 0.021	0.054 ± 0.007	0.045 ± 0.005	-0.37	0.34	231385.7

h^2_a = direct heritability; c^2 = proportion of maternal environmental variance to phenotypic variance; h^2_m = maternal heritability; r_{am} = correlation between direct additive genetic effect and maternal additive genetic effects; h^2_t = total heritability; *2LogL* = log likelihood (in REML method); (M = models). *DIC* = deviance information criterion (in Bayesian method); (*) = best model

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DEGRADATION OF STARCH AND CRUDE PROTEIN IN DENT AND DENT X FLINT MAIZE HYBRIDS IN DIFFERENT STAGES OF MATURITY

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ABSTRACT

In situ experiment was conducted to determine degradation of starch and crude protein in maize hybrids with dent and dent x flint type of grains. The samples of grains were harvested in four stages of maturity. The first harvesting was done in milk-waxy stage and than fornightly at intervals until full grain ripeness. Used dent x flint hybrids were Mesnil, Chambord, Queen, while Aude, Meridien, KX 13 93, Omero belonged to dent.
In sacco experiment was carried out on three rumen canullated cows of live weight 630 kg. The cows were fed a ration at maintenance level twice daily.
The effective degradability of crude protein and starch of maize grains have decreasing tendency with increasing maturity stage. The maize hybrids of dent type had higher effective starch degradability (60.2 %) than hybrids dent x flint (59.6 %). The degree of maturity had a significant ($P < 0.01$) effect on effective degradability of starch and crude protein. The maize hybrids KX 1393 and Meridien had the highest rate of starch degradability (parameter c: 0.075 $\%.h^{-1}$ and $0.074.h^{-1}$, respectively). Protein and starch in the hybrids dent x flint were less rapidly degraded than dent hybrids. This means that the starch and crude protein of these hybrids are more efficiently used by ruminants.

Key words: dent; dent x flint hybrids; maize grain; starch; crude protein; rumen degradability; *in sacco* method

INTRODUCTION

Maize grain is a primary energy supplement in dairy diets and can contribute up to 30, 60, and 98 % of the diet's protein, net energy, and starch, respectively. Specialty maize hybrids are one result of efforts to select corn based on nutrient content (Dado, 1999). The default form for farming based on kernel characteristics, maize are divided into five types: flint, popcorn, floury, dent and sweet corn (Corona *et al*., 2006). The endosperm of flint corn consists of hard-textured vitreous starch and has a greater proportion of vitreous endosperm than dent maize (Philippeau *et al.*, 1999, Kotarski *et al*, 1992). With advancing maturity, kernel vitreousness and density increases while ruminal starch availability and total tract starch digestibility decreases (Philippeau and Michalet-Doureau, 1997; Correa *et al.*, 2002; Johnson *et al.*, 2002; Pereira *et al*., 2004; Szasz *et al.*, 2007). Starch in vitreous dry corn is more extensively encapsulated by prolamins and is less degradable in the rumen as compared to floury or opaque maize grains (Kotarski *et al*, 1992). Phillipeau *et al.* (1999) compared 8 dent and 6 flint varieties and reported that dent maize averaged 51 % vitreousness vs. 72 % for flint maize and effective ruminal degradability was on average 62 % for dent and 46 % for flint maize varietiess.

Since maize grain often accounts for a large part of the diet of beef cattle, information on the pattern of degradation of maize protein in rumen contents is important in order to improve the precision with which

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diets are compounded (Níkolić and Filipović, 1981).

In Slovakia maize hybrids are nowadays imported from other countries and it is important to determine their nutritional quality. The rate and extent of degradability of crude protein and starch are important characteristics for their effective utilization by ruminants. Therefore, the aim of our study was to determine the effect of grain type and maturity stage on ruminal crude protein and starch degradability *in situ* from selected corn hybrids of dent and flint x dent types.

MATERIAL AND METHODS

The grains of maize hybrids of the type dent (Aude, Meridien, KX 1393, Omero) and dent x flint (Mesnil, Chambord, Queen) were used in the experiment.

The maize grains were sampled at fornightly intervals, first at the time of milk-waxy maturity (13.8., at harvest maize for silage), until full ripeness, i.e. 26.8, 10.9 and 23.9. Maize grains were shelled from collected cobs, than dried at room temperature, and ground to pass through a 3 mm sieve.

Starch and crude protein degradability in the maize grains were determined *in sacco* (Harazim and Pavelek, 1999). *In sacco* experiments were carried out in three nonlactating cows with large rumen cannulae (an average of 10 cm). The animals were fed twice a day with a diet consisting of 70 % forage and 30 % concentrate on a dry matter basis at maintenance level. The ration consisted of maize silage, alfalfa hay, wheat, barley meal (1:1) and Vitamix S1. Access to water was *ad libitum.*

Ground samples were weighed (approx. 2.50 g dry matter) into bags made of Uhelon 130T (HEDVA, "Moravská Třebová", the Czech Republic) with pore size of 47 μm. Minimum of three separate bags for hybrids, incubation time and animals were used. The bags with samples were incubated for 2, 3, 4, 6, 9, 16, 24, and 48 hours. The 0 h time bags were only washed in water to determine washing losses.

The bags containing 15 mg samples were inserted into the rumen just before morning feeding. The content of starch and crude protein was determined in the maize hybrids and in the residues after all incubation times. The content of starch was determined acoording to modified enzymatic method (Salomonsson *et al.*, 1984) and crude protein according to Decree MP 2145/2004-100. The parameters of degradability (a, b, c, and "effective degradability") were calculated using the equations by Ørskov and McDonald (1979) with outflow rate of $0.06.h^{-1}$.

The obtained data on nutrients and degradability of crude protein and starch in maize hybrids were evaluated statistically using models in statistical package Statistix 8.0.

RESULTS AND DISCUSSION

Due to lack of information on the grain quality of maize hybrids grown in Slovakia this study emphasized on chemical composition and in situ ruminal degradability of crude protein and starch in dent and dent x flint hybrids. Dent x flint maize (*Zea mays L.*) hybrids are commonly planted in the early maize growing regions of Europe (Moreno-Gonzales *et al*., 1997; Moreno-Gonzales *et al*., 2000). Table 1 shows the results of starch and protein content, on the grounds that these two nutrients could most affect grain degradability in the rumen. The differences among maize hybrids as well as between the stages of maturity in concentration of nutrients and extent of degradability were observed in many studies (Lebzien *et al*., 1997; Loose *et al*., 1998; Pereira *et al.*, 2004). The endosperm contains primarily starch and abundant storage proteins. The main maize proteins are prolamins, named zein, that comprise 50-60 % of total protein in maize grain.

Results from the tested hybrids showed that the content of starch depends on the type of grain (Table 1). The mean starch content was higher in dent hybrids, and kept on changing with ripening of grain. The maximum starch concentration was determined in hybrid KX 1393 (716.6 $g.kg^{-1}$ DM) and the lowest in hybrid Queen (669.3 $g.kg^{-1}$ DM). The differences between individual hybrids were significant ($P<0.05$), and while between KX1393 and Queen were highly significant ($P<0.01$). The results also confirm the changes in the starch content in corn grain during the ripening process. Highly significant differences were noted between 1st and 2nd, 3rd and 4th samplings ($P<0.01$) and between 3rd and 4th samplings ($P<0.05$) respectively in the content of crude protein (CP), which was also different among hybrids and kept on changing with the date of sampling. Average (average of all hybrids and harvestings by type of grain) concentration was lower (102.8 $g.kg^{-1}$ DM) in the dent x flint hybrids than in dent hybrids (108.4 $g.kg^{-1}$ DM). Among the hybrids differences were statistically significant, although dent hybrids had 2 % higher content.

Maize grain differs from other cereals in terms of degradation in the rumen and has high content of starch and unique structure and characteristics of grain (Kopčeková *et al.*, 2008). Degradation of maize starch is about 60 %, while for barley and wheat it is more than 90 %. Kopčeková *et al.* (2010) and Šimko *et al.* (2006) showed resistance to degraded maize starch in the rumen. Similar results were also documents by Lebzien *et al.* (1997), who found the effective degradability of

Table 1: Content of starch and crude protein in grains of maize hybrids (g.kg^{-1} DM)

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
Crude protein	1.	98.3	97.3	112.4	115.7	108.2	117.5	115.2
	2.	106.6	88.9	107.4	101.9	102.6	101.5	114.6
	3.	109.4	94.9	116.5	96.2	109.5	106.9	102.7
	4.	107.3	90.7	108.5	112.0	108.9	106.7	112.5
	$\bar{x}$	102.4a	93.0a,b	109.6a,b	106.2b,c	106.0b,c	108.0a,b	111.2a,b,c
	SEM	6.73	3.96	2.39	8.90	8.56	2.11	5.48
Starch	1.	689.4	680.8	676.5	651.2	726.2	671.8	673,6
	2.	693.9	715.7	689.7	718.7	742.7	717.9	679.4
	3.	658.3	659.6	632.8	692.3	708.4	681.2	675.8
	4.	702.4	722.5	678.3	694.3	688.9	722.7	721.5
	$\bar{x}$	686.0a*	694.7	669.3a,b	689.1a	716.6a*,	698.4b	687.6a
	SEM	17.80	27.47	23.18	25.93	21.41	23.74	21.05

Means with the same letters in the same row are significantly different at P<0.01 for CP content
Means with the same letters in the same row are significantly different at P<0.05 and P<0.01*
SEM = Standard Error of Mean

maize starch at 55 % and wheat starch at 95 %.

According to Kotarski *et al.* (1992) better understanding of chemical factors that potentially influence starch digestibility in ruminants required understanding of anatomy and physiology of maize seed. From three basic morphological parts, the endosperm represents approximately 75 % to 80 % of the corn kernel by weight and is the morphological structure which contains starch. Prolamin proteins cross-link encapsulating starch into a water tight (hydrophobic) matrix. Starch kernels in vitreous maize are more extensively encapsulated by prolamins (zein) and therfore are less degraded in the rumen as compared to floury maize.

Also, according to Michalet-Doreau *et al*. (1995) differences in the degradation of starch in maize grain among hybrids are primarily linked to the structure of the endosperm. The vitreous endosperm is hard, and abundant in protein matrix, with larger and more numerous protein bodies, and compact and polygonal starch granules. In the floury endosperm, the protein matrix is discontinuous and has few protein bodies, and the starch granules are spherical, larger, less aggregated and surrounded by air spaces (Robutti *et al*., 1974; Pratt *et al.*, 1995; Gibbon *et al.*, 2003).

Proportions of vitreous and floury endosperm vary among corn hybrids and maturity at harvest. Corn hybrids with kernels containing high proportions of vitreous (or horney) endosperm are flinty and those containing high proportions of floury endosperm are called floury, or dent (Kotarski *et al.*, 1992).

Dent type hybrids (Aude, KX 1393, Meridien and Omero) showed higher effective degradability of starch than type dent x flint in all harvestings. Averages of degradability parameters for individual hybrids from all four harvesting times confirmed lower starch degradation rate in dent x flint maize grains than dent maize grains (Table 2). Effective degradation of starch (EDS) was lower for crossbred dent x flint varieties with properties closer to dent than for flint hybrids. The differences between them were not high even though differences among hybrids were significant. The hybrids Mesnil (62.7 %) and Aude (57.7 %) were beyond the average. The rate of starch degradability was the highest in dent hybrids KX 1393 (0.075 %.h^{-1}) and Meridien (0.074 %.h^{-1}). Philipeau *et al.* (1999) reported a significant effect of the properties of the protein matrix of endosperm, especially on the rate of starch and crude protein degradation. Flint corn has a greater proportion of vitreous endosperm than dent corn. Philippeau and Michalet-Doureau (1997) observed that increased kernel vitreousness was associated with decreased ruminal starch degradation.

Average effective degradability of starch declined from 64.5 % (1st sampling) to 55.9 % (3rd sampling) but in the last sampling it increased again (60.1 %).

Table 2: Parameters of effective degradability of starch in grains of dent x flint and dent type maize hybrids

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
a (%)	1.	21.2	19.0	22.6	18.0	33.0	31.1	33.0
	2.	22.4	22.8	23.0	18.8	17.0	18.2	17.0
	3.	30.7	22.4	27.2	23.4	14.4	15.3	14.4
	4.	27.1	27.2	21.6	22.4	26.9	27.3	26.9
	$\bar{x}$	26.9	22.8	23.6	20.7	26.2	23.0	22.8
	SEM	4.01	4.17	3.14	2.40	4.45	6.93	8.64
b (%)	1.	72.5	81.0	77.4	82.0	66.9	67.5	66.9
	2.	76.8	77.2	77.0	81.2	83.0	81.8	83.0
	3.	65.7	74.1	70.4	76.6	85.6	84.7	85.6
	4.	72.9	27.2	77.7	77.6	73.1	71.1	73.1
	$\bar{x}$	72.0	75.6	75.6	79.4	73.2	76.3	77.1
	SEM	4.75	6.41	3.78	2.40	4.47	7.86	8.75
c (% h^{-1})	1.	0.082	0.058	0.072	0.069	0.061	0.091	0.061
	2.	0.071	0.037	0.046	0.053	0.065	0.068	0.065
	3.	0.071	0.052	0.052	0.044	0.063	0.064	0.063
	4.	0.038	0.054	0.058	0.056	0.059	0.072	0.059
	$\bar{x}$	0.066g	0.051cfg	0.057be	0.056ad	0.074def	0.075abc	0.062
	SEM	0.020	0.015	0.016	0.014	0.021	0.015	0.014
ED starch (%)	1.	69.4	60.0	63.2	60.9	58.7	70.5	58.7
	2.	63.2	56.4	58.0	57.5	60.4	58.7	60.4
	3.	59.8	49.9	56.5	53.4	53.9	47.4	53.9
	4.	58.4	60.8	59.7	59.2	62.2	61.0	62.2
	$\bar{x}$	62.7f	56.8ef	59.3b	57.7a	65.0abcde	59.4d	58.8e
	SEM	4.77	5.05	3.96	3.61	5.57	9.27	4.86

Means with the same letters in the same row are significantly different at $P<0.05$ and $P<0.01$
SEM = Standard Error of Mean

The parameters of degradation (a, b, c) varied with grain ripening (Table 2). It was found that the effective degradability of starch as well as the rate of degradation of fraction „b“ (parameter „c“) had decreasing trend with the stage of maturity. The rate of degradation in the hybrid Mesnil dropped from 0.082 %.h^{-1} in 1st sampling to 0.038 %.h^{-1} in 4th sampling. The decline the parameter “c” was more marked in dent x flint hybrids than in dent type hybrids. The parameter “a“ was the highest in dent type hybrids (Meridien and Omero 33.0 %) from the 1st harvesting. Dent grain is not so hard as compared to dent x flint type hybrids. The starch in dent grain is more soluble. Similar results were obtained by Bal *et al.* (2000). According to Philippeau and Michalet-Doreau (1997) increasing maturity at harvest resulted in increased vitreousness and decreased *in situ* ruminal starch degradation for both flint and dent hybrids. Starch degradability by ruminal microbes was much greater for dent hybrids compared to the flint hybrid at similar DM concentrations from ~ 30 % to ~ 40 % for whole plant DM.

Prolamins define differences in the chemical composition between vitreous dry corn (glassy, translucent) and floury or opaque corns although the relationship is not absolute. Prolamins are characterised by a highly hydrophobic glutamine and proline contents that develop tertiary structure localized on exterior of starch granules Philippeau and Michalet-Doreau (1997).

Table 3: Parameters of effective degradability of crude protein in grains of dent x flint and dent type maize hybrids

Nutrients	Sampling	Hybrids						
		Dent x flint			Dent			
		Mesnil	Chambord	Queen	Aude	KX 1393	Meridien	Omero
a (%)	1.	24.7	34.4	30.0	27.2	26.9	23.4	25.5
	2.	21.4	24.3	20.3	23.6	20.4	19.6	21.3
	3.	24.8	13.5	21.1	15.8	23.6	23.6	15.1
	4.	13.1	14.4	15.7	13.2	16.9	19.3	19.9
	$\bar{x}$	21.0	21.7	21.8	20.0	22.0	21.5	20.5
	SEM	4.99	8.89	5.87	5.92	4.82	2.30	4.66
b (%)	1.	74.8	65.6	70.0	73.4	73.1	76.1	73.0
	2.	78.6	74.2	78.4	76.4	79.3	80.4	78.7
	3.	75.2	83.0	72.4	84.2	76.4	76.4	84.9
	4.	86.9	79.7	84.3	86.8	77.8	80.7	80.1
	$\bar{x}$	78.9	75.6	76.3	80.2	76.6	78.4	79.2
	SEM	5.14	8.21	6.03	5.73	3.96	2.58	5.79
c (% h^{-1})	1.	0.064	0.035	0.039	0.040	0.038	0.057	0.029
	2.	0.034	0.033	0.030	0.027	0.041	0.035	0.040
	3.	0.029	0.026	0.036	0.030	0.035	0.037	0.040
	4.	0.014	0.031	0.023	0.018	0.040	0.027	0.019
	$\bar{x}$	0.035	0.031	0.032	0.029	0.039	0.039	0.032
	SEM	0.022	0.018	0.016	0.013	0.020	0.019	0.019
EDCP starch (%)	1.	61.2	61.1	59.4	57.8	58.3	59.0	50.0
	2.	52.0	53.9	49.9	51.8	55.1	52.0	54.6
	3.	52.3	43.1	49.8	45.3	53.2	53.2	48.6
	4.	42.2	43.3	45.5	41.4	48.3	49.1	47.9
	$\bar{x}$	51.9	50.3	51.1	49.0a	53.7a	53.3	50.2
	SEM	7.53	8.83	6.26	7.72	5.57	5.79	4.65

Means with the same letters in the same row are significantly different at $P<0.05$
SEM = Standard Error of Mean

Potentially, starch digestion requires rumen bacteria to first degrade prolamin-zein before amylolitic activity in the rumen to actively hydrolyze starch (Cotta, 1988).

Starch in vitreous dry corn is more extensively encapsulated by prolamins and is less degradable in the rumen as compared to floury or opaque corns Philippeau and Michalet-Doreau (1997).

In individual stage of maturity small differences in degradability of CP (Table 3) was noted among hybrids. The degradability was higher in the dent type than dent x flint hybrids but differences were not signifficant. Effective degradability of CP was statistically different ($P <0.05$) only between hybrids Meridien and Aude both of which are dent type. The effective degradability is affected by the rate of degradation "c" of the insoluble fraction "b". This parameter showed a decreasing tendency with ripening of grain (for Aude form 0.040 $\%.h^{-1}$ in the 1st sampling to 0.018 $\%.h^{-1}$ in the 4th sampling, Mesnil from 0.064 $\%.h^{-1}$ to 0.014 $\%.h^{-1}$). Not every hybrid had such a sharp decline rate with maturity.

According to Pereira *et al.* (2004) vitreousness of the hard and soft texture grain increased linearly with advancing maturity. It is important that effective crude protein degradability (ECPD) declined with grain ripening grain (Table 3) in both types of grains. The most significant changes were in hybrids Mesnil and Chambord, where the differences between the first and fourth samplings in EDCP were until

18 % units. For hybrid Omero (dent) a slight decline in degradation rate was noted with ripening (from 50.0 % to 47.9 %). Comparing the degradation of crude protein in all four samplings, it was found that the parameters degradability and ECPD were highly statistically significant (P <0.01). By reducing the degradation of the protein degradability of starch was also reduced.

Resistance to degradation by ruminal microbes for starch in vitreous endosperm compared with floury endosperm is primarily because of the distribution of proteins in the endosperm. Concentrations of zein proteins increase and glutelin proteins decrease with increasing vitreousness (Philippeau *et al.*, 2000). The insoluble zein proteins limit accessibility of the starch granules to ruminal microbes compared with the soluble glutelin proteins (Philippeau *et al*., 2000). The protein matrix seems to limit the enzymatic digestion of starch in cereals (Kotarski *et al*., 1992) and is responsible for differences in ruminal degradability of grains (McAllister *et al*., 1993; Rooney and Pflugfelder, 1986). There is evidence that hard-textured maize grain is less degraded in the rumen than soft grains (Philippeau and Michalet-Doreau, 1997).

CONCLUSION

There were differences in starch content between dent and dent x flint maize hybrids during ripening process. Dent type maize hybrids are higher in starch content. With advancing maturity in maize grain of dent x flint type hybrids starch and CP degradability decrease was more than in dent hybrids. The effective degradability of crude protein and starch in dent type hybrids was higher than in dent x flint type hybrids. In practical terms this means that the use of protein and starch as a source of energy from maize grains of dent x flint type hybrids is more effective in ruminants as starch and CP are less extensively degraded in the rumen to glucose and ammonia in comparison to dent type hybrids grains.

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PREFERENCE OF MUTURU CATTLE TO EITHER FRESH FORAGE OR PELLETED HAY OF *PANICUM MAXIMUM* AND *PENNISETUM PURPUREUM* CUT AT FOUR AND EIGHT WEEKS

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ABSTRACT

Short-term preference studies were carried out with growing Muturu calves based on diets of local grass forages found in South – Western Nigeria. Twelve intact pure bred muturu calves aged 9 - 12 months were used. Two grass species - guinea grass (*Panicum maximum*) and elephant grass (*Pennisetum purpureum*) at four and eight weeks of regrowth were harvested when needed either for pelleting or for fresh green chop. The diets were served to animals individually and later in group. Feed preference was assessed from the total intake at the end of 15 min cafeteria study while the chemical composition of each diet was also assessed. The CP content of the grasses ranged from 105 to 133 g/kg DM with pelleted *Panicum* at 8 weeks old having the highest CP. Pelleted grasses of 4 week old had the lowest ($P<0.05$) NDF in the trial. Green chopped *P. maximum* of 4 weeks old was most preferred by the calves. Age at harvest influenced preference as forages harvested at 4 weeks old had higher intake. Forage preference considered in terms of intake rate indicated that growing calves preferred fresh *P. maximum* of 4 week old to the other samples used in this study. Group feeding also influenced forage preference.
It is concluded that in order to optimize DM intake farmers should consider the type of grasses and their age at harvest particularly for Muturu. Pelleting improves acceptability of forages when rejected by animals in fresh forage form due to advanced age.

Key words: *Panicum*; *Pennisetum*; age at harvest; pelleting; Muturu calves

INTRODUCTION

The Muturu cattle breed is a variety of West African Shorthorn, which appears to have evolved through adaptation to the humid forest environment. Most of the Muturu cattle found in Nigeria are spread over the Benue plateau and the Southwest. This breed is also found in Southeastern coastal area of Ghana, eastern coastal areas of Maryland and Sinoe counties of southeastern Liberia (Rege *et al*., 1994). The management level where these cattle are kept is low in spite of which they maintain good body condition by grazing and browsing throughout the year. The Muturu is found in areas heavily infested with tsetse, as a result of which this breed has adapted and naturally selected to be tolerant to trypanosomosis, ticks and tick-borne diseases although it is susceptible to rinder-pest (Adeniji, 1983). The productivity of Nigerian livestock, Muturu in particular, is below its genetic potential principally due to poor nutrition and inadequacy of good quality feed produced from forages (Lamidi *et al.*, 2005).

Due to various degrees of constraints involved in the production of ruminants in Africa, such as the seasonality of pastures, this is brought about by climatic changes from season to season. Low nutritive pastures are unable to meet the nutritional requirement of these

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animals and also high capital involved in establishment of intensive systems of ruminant production. Ajayi *et al.* (2008) reported that if grass of any age is effectively managed, it can strategically be exploited to ameliorate forage scarcity in the off season. An example of these is processing it into pelleted forms, hay and silage which can be stored for feeding during the dry season. Therefore, since ruminants according to Babayemi and Bamikole (2006) are the best assessors of the nutritive value of any feed, as they always consume more of the forages that are high in protein than the high lignin containing grasses, a knowledge of the selectivity of the available forage will go a long way in increasing production and also the establishment of pasture and its conservation through various means such as silage, hay and pellet production to meet the nutritive needs of animals in periods when there is low availability of forage.

This trial is therefore aimed at evaluating the selective preference in Muturu cattle for two common tropical grasses at different ages served fresh and to evaluate their acceptability when processed (i.e. pelleted). Also, another objective was to evaluate the influence of group or individual feeding on forage acceptability by animals. Such information can aid in identifying key species, explain shifts in diet quality and animal performance (Holechek *et al*., 1981), and assist in developing grazing programmes (Gordon, 1988).

MATERIAL AND METHODS

The experiment was carried out at the research paddock and laboratory of the Department of Pasture and Range Management, College of Animal Science and Livestock Production, University of Agriculture, Abeokuta. The research site was located in the derived savanna zone of Southwest Nigeria with monthly rainfall which ranged from 120 mm in May to195 mm in September and mean monthly temperature ranging from 22.5º to 33.7ºC. The relative humidity in the rainy (late March-October) and dry (November-early March) season ranged between 63-96 % and 55-84 %, respectively.

Guinea grass (*Panicum maximum*) and elephant grass (*Pennisetum purpureum*) at four and eight weeks of regrowth prior to flowering collected in triplicates were staged to provide forage regrowth either 4 or 8 weeks at the time needed for pelleting or for fresh green chop. The forages for pelleting were harvested with a flail cut and were chopped into 50-60 mm using a reel cutter. They were immediately dehydrated at 120ºC (*Panicum*) and 150ºC (*Pennisetum*). The temperature difference was to achieve approximately similar moisture since *Pennisetum* has larger stems than *Panicum*. After dehydration the dried forage was milled with a hammer mill fitted with 3 mm sieve and pelleted using a 6 mm mesh size to produce pelleted hay of average length of 40 mm. Water was used as the binding agent.

Cafeteria feeding study

A total of 12 pure bred Muturu calves of ages ranging from 9-12 months old were used. The cattle pens were thoroughly washed and disinfected. The animals were dewormed (using Albendazole) and dipped to destroy both internal and external parasites before the experiment. All the animals were housed in a roofed shed in well ventilated individual pens (1.5 x 3 m) for the duration of the experiment. Each pen had water and a feed trough. An initial adaptation period of 7 days was used to accustom the animals to the pen conditions as the animals were fed with *Andropogon gayanus* grass and a commercial concentrate containing crude protein (18 %), cottonseed cake (30 %), maize (16 %), common salt (2 %) and oyster shell (2 %).

Preference for forages fed individually

One kg of each sample of green chopped forage were measured and served each animal daily. Feed was delivered into cafeteria style feeders placed in a feed pen that consisted of four containers (53 dm^3) with the containers placed at each end of the pen being empty to nullify any border bias. Calves were fed at same time with containers positioned randomly in the manger at each feed delivery. At the end of 15 min, the feeding bowls were withdrawn and the left-over in the plastic feeding bowls weighed and recorded to determine the intake per 15 min. This trial was recorded for 7 days. Each day the position and order of placement of the forages before the animals was rotated throughout the trial to avoid conditioning and learning effects. After collection of the leftovers, the animals were allowed to graze. At the end of the experiment, animal were allowed to graze for a period of two weeks after which the same forage preference cafeteria method was repeated with the pelleted forms of both the grasses at 4 and 8 weeks after regrowth.

Preference for forages offered in group

In a second feeding experiment, all the animals were released in same holding yard. The animals were trained for 4 days to eat from four food boxes at equidistance in the yard using a small amount of *Andropogon gayanus* in each food box. After training (i.e., all animals learned to eat from all boxes), a 7 day trial was conducted. The four forages of known weights were placed in heaps in an enclosed yard into which animals were released. At the end of 15 min, the amount of grass remaining was weighed and recorded. The order of the presentation of the feed was altered in the following days of the trial to avoid any form of biasness. At the end of the experiment,

animals were allowed to graze for a period of two weeks after which the same forage preference cafeteria method was repeated with the pelleted forms of both grasses at 4 and 8 weeks after regrowth.

Feed preferred were assessed from the total dry matter intake throughout the experiment. A forage type was said to be preferred by animals to the others when its intake is more than others.

Laboratory Analysis

At the end of each collection, triplicate samples of green chopped forages and the pelleted forages were dried at 60ºC for 3 days, they were then milled in a Thomas-Wiley mill (2 mm sieve) and composited for subsequent analysis. The DM content was determined by drying the sample at 105ºC overnight and ash by igniting the samples in muffle furnace at 525ºC for 8 h, ether extract (EE), crude fibre (CF) and CP (N x 6.25) were determined (AOAC, 1990). Neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) were also done (Robertson and Van Soest, 1981). The samples were also digested by nitric and perchloric acids mixture (ratio = 4.1, v/v) and the concentrations of the minerals - Calcium (Ca), Potassium (K), Phosphorus (P) and Magnesium (Mg) in the samples were determined by an Atomic Absorption Spectrophotometer (Buck scientific model 200a; Buck Scientific, East Norwalk, CT 06855, USA).

The trials were arranged in 2 x 2 x 2 factorial completely randomized design. The statistical analysis was completed using all the data generated from the experiment in analysis of variance (ANOVA) using General Linear Model (GLM) procedures of SAS 2001 and separation of treatment means was done using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

The chemical composition of either chopped or pelleted *Panicum* and *Pennisetum* harvested at 4 or 8 weeks regrowth is presented in table 1. There was a significant (P<0.05) three-way interaction among grass species, age at harvest and processing methods on the DM, CP, CF, EE, Ash, P and Ca content of the grasses.

The CP content of the grasses ranged from 105 to 133 g.kg^{-1} DM with pelleted *Panicum* at 8 weeks regrowth having the highest CP. The interaction effect of

Table 1: Chemical and mineral composition (g.kg^{-1} DM) of both green chopped and pelleted *P. maximum* and *P. purpureum* harvested at two stages of growth

Interaction	DM	CP	CF	EE	ASH	K	P	Mg	Ca
P. maximum									
4 Pelleted	947a	105b	160c	24a	171a	33.4	4.09a	1.67	14.7 b
8 Pelleted	936a	147a	150c	20ab	110bc	31.6	3.25bc	1.50	23.0a
4 green chopped	722b	129ab	330b	24a	125b	30.0	2.69c	1.58	16.5b
8 green chopped	722b	115ab	400a	12c	95bc	26.5	2.79	1.33	13.3c
P. purpureum									
4 Pelleted	951a	129ab	150c	16bc	90bc	27.6	1.82b	1.38	13.4c
8 Pelleted	932a	115ab	160c	18abc	80c	26.5	3.94a	1.33	17.7b
4 green chopped	702c	133ab	380ab	21ab	125b	26.5	3.93ab	1.33	17.5b
8 green chopped	707c	115ab	370ab	12c	110bc	26.8	2.56ab	1.29	17.2b
SEM	1.88	0.41	2.36	0.37	0.65	1.64	3.29	0.08	1.21
Interaction									
S	*	**	**	**	NS	*	**	NS	**
A	NS	NS	NS	*	**	*	*	**	**
P	**	NS	*	NS	NS	NS	**	NS	NS
S x A	NS	NS	NS	**	**	NS	*	**	**
S x P	**	NS	**	NS	**	NS	NS	NS	**
A x P	**	NS	**	**	NS	NS	NS	NS	**

abc: means in the same column with different superscripts are significantly different (P < 0.05), ** *P* < 0.01, * *P* < 0.05
DM: Dry matter; CP: Crude protein; CF: Crude fibre; EE: Ether extract; K: Potassium; P: Phosphorus; Mg: Magnesium; Ca: Calcium
S: Species; A: Age at harvest; P: Processing

species and age at harvest, species and processing was not significant (P>0.01) on the CP content of the grasses, however, the main effect of species showed that *Panicum* had higher (P<0.01) CP than *Pennisetum* in this trial.

DM values were higher (P<0.01) in *Panicum* when green chopped at both 4 and 8 weeks than in *Pennisetum* of similar ages, while the DM remained similar when the forages were pelleted at both ages.

The CF contents of the grasses were influenced by the interaction of species, processing and age at harvest of the forages. Among the treatments, green chopped *Panicum* at 8 weeks regrowth had greater (P<0.05) CF content compared to others. The main effect of processing and species significantly (P<0.01) reduced the values of CF in both species.

The EE contents of the grasses were influenced by the interaction of species, processing and age at harvest of the forages. The effect of age and processing was similar (P>0.05) within each species of grasses while the 8 weeks old green chopped forages recorded the lowest EE content.

Interaction of grass species, processing and age at harvest influenced the ash content of the grasses. The ash content ranged from 90 and 171 g.kg^{-1} DM with 4 weeks old pelleted *Panicum* having the highest ash content. Species interaction with age, harvest and processing influenced (P<0.01) the ash content of both grasses while age at harvest with processing did not.

The Phosphorus (P) content of *Panicum* and *Pennisetum* were dependent on the interaction between species and age at harvest, and the main effect of each of the factors.

Interaction effect between species and processing resulted in higher (P<0.01) Ca content in pelleted *Panicum* compared with *Pennisetum* but lower (P<0.05) Ca content in green chopped *Panicum* compared to *Pennisetum*.

The Magnesium (Mg) content of *Panicum* and *Pennisetum* were dependent on the interaction between species and age at harvest, and the main effect of age at harvest.

The neutral detergent fibre (NDF) values, which constitute the cell wall of the grasses, were influenced by the interaction of grass species, processing and age at harvest. The NDF values ranged between 400 and 590 g.kg^{-1} DM. Pelleted grasses of 4 week old had the lowest (P<0.05) NDF in the trial. The main effect of species and processing as well as their interaction

Table 2: Fibre fraction (g.kg^{-1} DM) of both green chopped and pelleted *P. maximum* and *P. purpureum* harvested at two stages of growth

Treatment	ADF	NDF	ADL	HEM	CELL
P. maximum					
4 Pelleted	130e	420c	105	211c	198d
8 Pelleted	260d	527ab	120	272a	238c
4 green chopped	310c	530ab	100	227b	303b
8 green chopped	410a	560b	91.0	227b	433a
P. purpureum					
4 Pelleted	130e	400c	105	131b	340abc
8 Pelleted	270d	550b	101	239b	351ab
4 green chopped	380b	590a	75.1	236b	419a
8 green chopped	380b	590a	115	216c	419a
SEM	2.13	1.89	1.03	1.06	4.68
Interactions					
S	NS	NS	NS	**	NS
A	**	**	NS	NS	**
P	**	**	NS	NS	**
S x A	NS	NS	NS	**	**
S x P	NS	NS	NS	NS	NS
A x P	NS	**	NS	NS	NS

abc: means in the same column with different superscripts are significantly different (P < 0.05), ** P < 0.01, * P < 0.05
ADF: Acid detergent fibre; NDF: Neutral detergent fibre; ADL: Acid detergent lignin; HEM: Hemicellulose; CELL: Cellulose
S: Species; A: Age at harvest; P: Processing

influenced the NDF content of the grasses. The NDF content of both species pelleted at 4 weeks regrowth were lower than those pelleted at 8 weeks regrowth.

Interaction of grass species, processing and age at harvest affected the ADF content of the grasses as well as their single effect. Pelleted *Panicum* of 8 weeks age had greater (P<0.05) ADF content compared to others. The interaction of species and age at harvest on the ADF of the grasses were similar (P>0.01). The ADF content of both species pelleted at 4 weeks age were lower than those pelleted at 8 weeks age while pelleting also lowered (P<0.01) the ADF content of each species.

Preference was observed using intake from the cafeteria method as recorded in figure 1. Green chopped *P. maximum* of 4 weeks age was most preferred by the calves. *P. maximum* was more preferred than *P. purpureus* either green chopped or pelleted at both ages. Intake was influenced most by individual feeding method for all categories except green chopped *P. purpureum* of 8 weeks age where intake increased in group feeding than individual feeding method. Group feeding method increased preference for pelleted samples than that served individually. When served in group calves preferred green chopped to pelleted forages except in 4 weeks old *P. purpureum* where pelleted forage had similar intake with green chopped ones. Age at harvest influenced preference as forages harvested at 4 weeks age had higher intake when served individually than forages harvested at 8 weeks age. However, when served in group, calves' preference for fresh forages at 8 weeks old was higher than forages at 4 weeks old.

The assessed quality parameters of the grasses were dependent on the interaction of species, processing and age at harvest. The CP content of the grasses ranged from 105 to 133 g.kg^{-1} DM and these values fall within the range reported for tropical grasses (Topps and Oliver, 1993) and well above 8 % suggested by Norton (1994) for ruminal function. The interactions of species, processing and age at harvest, CP content of the grasses were similar irrespective of method of processing.

The neutral detergent fibre (NDF) values, which constitute the cell wall of the grasses, were influenced by the interaction of grass species, processing and age at harvest. The NDF values ranged between 400 and 590 g.kg^{-1} DM. The values were comparable to values reported by Minson (1990) as typical of tropical grasses. In all the treatments, the NDF content increased with increasing plant maturity irrespective of the species difference. Mtui (2009) reported no difference in NDF content between *Panicum*, *Pennisetum* and certain forage grass species and the type of season. The increase in NDF with age of regrowth is related to physiological changes that occur as plant ages, that lead to a decrease in cell cytoplasm's highly soluble components (cell contents), accompanied by an increase in cell wall fibre components (Nogueira *et al*., 2000). Pelleting resulted in decreased NDF content within species and age with greater reduction in the NDF content of *Pennisetum* than *Panicum*. Since reduction in forage particle size greatly influences the effectiveness of fibre (Allen, 1995), this implied that intake of *Pennisetum* with high NDF content could be improved through pelleting. West (1998) reported that a major factor which could enhance intake of forages by cattle is to simply lower the cell wall content.

A similar trend to that of NDF was observed with the ADF content of the grasses with advancing maturity. These changes are due to increased secondary thickening in cells associated with plant support and water transport (Buxton, 1989). ADF contents of pelleted *Pennisetum* were comparable with *Panicum* at similar age but were higher than ADL content of *Panicum* in

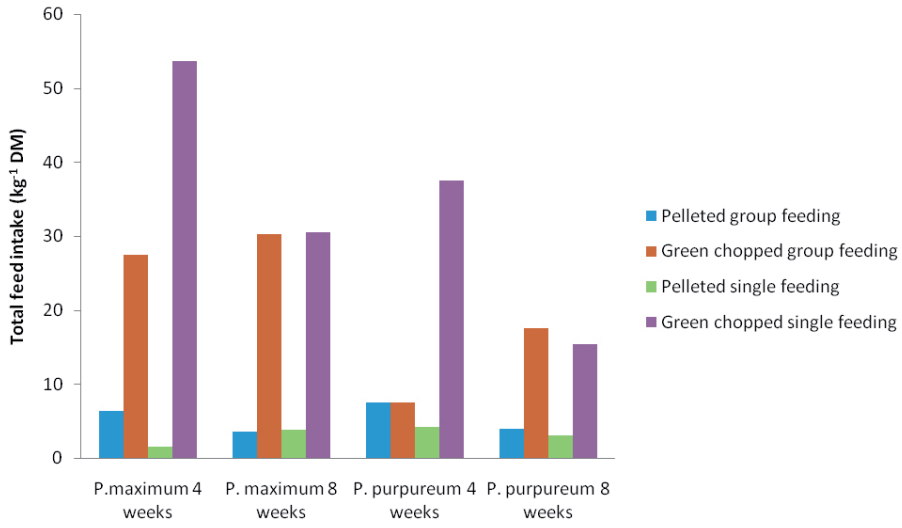

Fig. 1: Feed preference as assessed from the total intake

green chopped samples. Thus pelleting reduced the ADF content.

The effect of species on ether extract was pronounced with *Panicum* having higher content of EE than *Pennisetum*. Preference can be calculated either as the proportion of time spent grazing each species or as the proportion of total intake derived from each species (Parsons *et al*., 1994). Given that total intake is more important than grazing time in terms of the animal's energy balance (and, therefore, growth and production), preference should ideally be calculated using total intake, especially when there are significant differences in the rate at which the animals eat the different herbage species.

Forage preference considered in terms of intake rate indicated that growing calves preferred fresh *P. maximum* at 4 week old to the other samples used in this study. For a given plant, ingestibility, like digestibility, is dependent on the vegetation stage and the number of the vegetation cycle (Demarquilly *et al*., 1981). The decrease in ingestibility with age of forage is the consequence of the increase in its fill effect. As the plant ages, its morphological and histological development decreases the amount of cell content, which is soluble, rapidly degraded and has almost no fill effect, and increases the amount of cell walls. Consequently, forage retention time in the rumen and thus fill effect increases. In addition, tissue lignification increases the undegradable fraction of the cell walls and decreases the degradation rate of the degradable fraction (Grenet and Demarquilly, 1987).

The DM content of forages at the time of feeding may have had an influence on the relatively higher preference of fresh *P. maximum* both singly or in group. This is in agreement with Gibb *et al*. (1998) and Vollborn (1998) who reported that DM content and surface moisture content of grazed forages raise bite rates and bite mass on fresh weight basis though on DM basis are the lowest.

The CP and NDF concentrations of diets are the most important factors affecting DMI. Intake of grass species declines when the NDF concentration of the consumed forage increases. It was reported by Wandera (1996) that *Pennisetum* species were more palatable than *Panicum*, *Cynodon* and other grass species. Similarly, earlier work in the SPD system by Turiani (Komwihangilo *et al.*, 2007) indicated that farmers consider *P. maximum*, *P. purpureum* and *R. cochinchinensis* to be eagerly eaten by animals in a similar manner or vice versa.

However, Muturu calves showed more preference for fresh *P. maximum* than *P. purpureum* in this trial. This could be as a result of the coarse and hairy morphology of *P. purpureum* because the sense of touch plays a role in the response of the animal to the feed. Physical characteristics of the forage such as hair, thorns, coarseness and resistance to fracture are known to affect ease of apprehension and thus intake rate (Inoue *et al*., 1994). Higher leaf to stem ratio in guinea grass may also have influenced its preference over elephant grass. Digestibility had been indicated to influence forage preference such that the highly digestible forages would be more favoured (Lu, 1988). However, in such short-term trials like those in the present study, it is unlikely that digestibility of materials would have influenced preferences. On the other hand, tastes and odour of the feeds could also have applied in the observed situation, as was the case in studies of De Rosa *et al*. (1997).

Group feeding tended to show increased intake as preference for pelleted samples increased than when served individually. Mustafa *et al.* (2009) reported that cafeteria calves show more eating behaviour and less idle standing, licking object and drinking behaviour which led to increased ruminating and intake in comparison to individually fed calves The instinct for completion invariably decreased idle standing which could probably result to increased intake of fresh forages at 8 weeks old as observed in the present trial than forages at 4 weeks old when served in group.

CONCLUSION

Intake rates observed in the present study represent some of the key factors in understanding palatability and voluntary feed intake. The high intake rate for any forage may have significant implication to a small-holder farmer who harvests forages on daily basis or one planning to establish one pasture species from among the choices available.

Forage acceptability by animals on pasture or under zero grazing conditions is a function of forage, inherent chemical traits, forage morphology as was ascertained by the observed intake and also the influence of group feeding. Therefore, effort should encourage farmers to establish and maintain the forage species which are locally available that are also adapted to social and environmental conditions of respective areas.

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EFFECTS OF FATTENING BULLS REGROUPING ON THEIR MAINTENANCE AND ABNORMAL BEHAVIOUR

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ABSTRACT

The aim was to test the hypotheses that the behaviour of bulls after regrouping is influenced by the day of observation and sire. From 32 Holstein bulls kept in the loose-housing 5 were selected, originating from 3 fathers, having shown the fight, mounting, licking genitals, and pawing. These bulls were subject to another ethological observation in a different pen during three consecutive days. We tested if their behaviour varies depending on the day of the observation and sire lineage. Following treatments were evaluated: the new group formatting on the 1st day, coping on the conditions created during the previous day on the 2nd day, and re-littering the pen on the 3rd day. No significant differences between bulls were found in observed maintenance behaviours except of the time of feeding only ($P<0.01$). Between observed days, statistically significant differences were noted in frequencies of aggressiveness and playing behaviour ($P<0.05$; $P<0.001$). Effect of sire was manifested in the frequency of licking ($P<0.05$), aggressiveness ($P<0.01$), and playing ($P<0.05$).

Key words: bull; sire; maintenance behaviour; abnormal behaviour

INTRODUCTION

Animal husbandry is often affected by various factors, which has a negative impact especially on animal welfare, health, and production. The focus should be on behavioural abnormalities that indicate a problem in the herd. Sometimes is appears to be closely related to adverse situation, where animals are frustrated or restricted, while in other cases does not occur, or persists even when the environment is improved (Appleby and Hughes, 1997). An abnormal behaviour pattern may be a novel behaviour or an otherwise normal behaviour that is exaggerated in terms of frequency or intensity, disoriented in relation to the stimulus, or occurring in the absence of normal eliciting stimuli (Houpt, 1987).

Typical behavioural abnormalities in a herd are fighting between bulls, mounting on other bulls, licking genitals of other bulls, screaming and pawing the litter as expression of the most aggressive bulls (Albright and Arave, 1997; Laister *et al*., 2011). Abnormal behaviours are really components of normal behaviours directed toward inappropriate stimuli. Social aggression or territorial behaviour is typical for animals housed in groups. Unusually high levels of aggression are typically observed when unfamiliar animals are placed together for the first time (Houpt, 1991). Pawing behaviour by bulls creates bare patches of earth, and these patches located through his territory are clearly a claim to possession of given area (Albright and Arave, 1997). Two factors seemed to affect the establishment of social dominance and a hierarchy: adjusting to a new environment and the presence of unknown animals (Kilgour, 2012). Dominance in cattle depends on age, weight, sex, breed and presence of horns (Brouček *et al.,* 2008). Ishiwata *et al*. (2007, 2008) found that the proportion of walking was much lower in the pen condition compared to pasture conditions, but grooming, investigating, tongue playing, and licking objects were higher. Some bulls kept in intensive housing are prone to mount herdmates (Albright and Arave, 1997). Kooijman

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et al. (1990) reported that deprivation of roughage can increase an excessive manipulation of pen equipment and playing behaviour by the young cattle. The other behavioural disorder in fattening bulls is licking the genitals of other bulls. Sucking the penile sheath can stimulate urination which, if ingested, can depress the animal's appetite (Houpt, 1991).

Abnormal behaviour activities can often result in stereotypes, behaviours tied to a psychological disorder believed to be caused by the cattle frustration. Prepuce or scrotum sucking, and urine drinking are behaviours commonly referred to as stereotypes in bulls (Lawrence and Rushen, 1993; Albright and Arave, 1997).

The aim of the work was to test the hypotheses that the maintenance, comfort, playing, and abnormal behaviour of fattening Holstein bulls are influenced by sire and day of observation.

MATERIAL AND METHODS

A group of 32 Holstein bulls weighing 524.17 ± 44.31 kg on average 24.5 months ± 1.5 months were examined. The bulls were weighed once a month and during every change of the housing. Bulls were kept in the loose-housing barn, in 2 pens measuring 15 x 9 m, without outside yard 2 weeks before measurement. Floor space per bull was 8.4 m^2. Each pen was for accommodating 16 bulls. Both groups had the same age and similar live body weights.

Behaviour was monitored for 10 hours daily (from 8:00 am to 18:00 pm) during five consecutive days. The time of daily activities of bulls (lying, standing, moving, eating and ruminating) was recorded in 15 minute intervals. The frequency of following abnormal activities was recorded continually - fight (combat encounters between two or more bulls), licking (licking genitals of itself and other bulls), mounting (jumping on the other bulls in the group), pawing (pawing litter with screaming and mooing), and aggressiveness (jostling horns to other bulls, head movements, that indicated - wants of combat contact, inverted protruding eyes and finally also fight) as a manifestation of the most aggressive forms of behaviour. Also, play (running, blowing the straw, jumping on the spot) and comfortable behaviour (licking himself, throw straw on their backs, rubbing the head of straw - cleaning himself) were noted.

Only five bulls (15.6 %) were selected having shown the abnormal behaviour (an excessive and disproportionate fight, mounting, licking genitals, and also pawing with screaming). Comparison of the bulls with the regime of the group, it was found that they differ in almost all daily activities. These bulls descended from 3 bulls (Sire 1, n=3; Sire 2, n=1; Sire 3, n=1). Other manifestations of 27 remaining animals showed no abnormal activities. The origin of the father of these remaining bulls were as follows - Sire 1, n=11; Sire 2, n=7; Sire 3, n=9.

Selected bulls were subjected to another ethological observation in a different pen with the same size of 15 x 9 m. We tested if their behaviour varies depending on the day of the observation and sire lineage. Observation lasted 3 consecutive days, animals were affected by external environmental factors like formation of new group related with stocking on the 1st day, coping on the conditions created during the previous day, and re-littering the pen at 7.50 a.m. on the third day, which was typical for the farm (littering every other day). Weight of straw was 24 kg per pen (4.8 kg per animal). Behaviours were recorded for 10 hours daily (from 8:00 am to 18:00 pm).

Descriptive methods were used for statistical evaluation. The data were analyzed using a General Linear Model ANOVA (2 ways with the interactions) by the statistical package STATISTIX, Version 9.0.:

$$Yijk = \mu + \alpha i + \beta j + \gamma ij + \varepsilon ijk$$

where $Yijk$ is a dependent variable, μ is the overall mean, αi is the effect of factor A (day) on the level i, βj is the effect of factor B (sire) on the level j, γij is the interaction between factor A on the level i and factor B on the level j, and εijk is the residual error.

The dependent variables were ethological parameters and the independent variables were factors such as the day of observation, and the sire lineage. Values are expressed in minutes (min.) as means ± SE. The normality of data distribution was evaluated by the Wilk-Shapiro/Rankin Plot procedure. Significant differences between groups were tested by Comparisons of Mean Ranks through Tukey's test.

RESULTS AND DISCUSSION

No significant differences were noted between bulls in observed behaviours. Times of moving were gradually from the first to the third day and it did not increase significantly, while significant differences were recorded at the time of feeding only ($P<0.01$) (Table 1). Between observed days, no statistically significant differences were noted in frequencies of aggressiveness and playing behaviour (7.20 ± 1.06, 8.60 ± 0.75, and 10.20 ± 1.02, $P<0.05$; 1.00 ± 0.63, 1.80 ± 0.37, and 4.40 ± 0.68, $P<0.001$) (Table 2).

Also, no significant differences were found among sires in maintenance behaviour (Table 3). Effect of sire was manifested in the frequency of licking ($P<0.05$), aggressiveness ($P<0.01$), and playing ($P<0.05$). Descendants of sire 3 showed the highest aggressive expression compared with the descendants of the sires

Table 1: Times of maintenance behaviour according to days (mean ± SE)

	Day				
	1	2	3	P	
Lying	87.00 ± 17.36	87.00 ± 14.54	63.00 ± 12.00	0.4280	
Standing	280.00 ± 19.62	258.00 ± 15.29	273.00 ± 3.01	0.6092	
Ruminating	210.33 ± 18.37	192.00 ± 12.90	171.00 ± 10.17	0.1538	
Moving	213.00 ± 25.81	255.00 ± 25.10	286.00 ± 12.59	0.0871	
Feeding	171.00 ± 25.81	117.00 ± 11.02	117.03 ± 9.94	0.0071**	1:2,3*

Eating: Day*Sire = 0.0297*
N = 5; *P<0.05; **P<0.01
SE = standard error of mean

Table 2: Frequency of behaviours according to days (mean ± SE)

	Day				
	1	2	3	P	
Fight	15.20 ± 2.15	17.20 ± 1.56	19.60 ± 2.87	0.4779	
Lick	7.00 ± 2.86	6.80 ± 2.80	6.40 ± 2.64	0.9814	
Mount	22.40 ± 3.29	16.40 ± 0.87	15.80 ± 2.51	0.1152	
Paw	4.80 ± 3.50	4.40 ± 3.29	4.40 ± 3.69	0.9950	
Aggressiveness	7.20 ± 1.06	8.60 ± 0.75	10.20 ± 1.02	0.0241*	1:3*
Comfort	1.00 ± 0.45	1.40 ± 0.40	2.20 ± 0.20	0.1160	
Play	1.00 ± 0.63	1.80 ± 0.37	4.40 ± 0.68	0.0005***	1:3***, 2:3**

N = 5; *P<0.05; **P<0.01, ***P<0.001
SE = standard error of mean

Table 3: Times of maintenance behaviour according to sire (mean ± SE)

	Sire			
	1	2	3	P
Lying	90.00 ± 12.74	60.00 ± 8.66	65.00 ± 5.00	0.3076
Standing	273.89 ± 12.29	270.00 ± 25.98	260.00 ± 5.50	0.8386
Ruminating	203.33 ± 13.02	165.00 ± 0.03	180.00 ± 0.09	0.1570
Moving	237.22 ± 21.34	270.00 ± 17.32	275.00 ± 5.76	0.3827
Feeding	130.00 ± 7.90	150.00 ± 60.00	135.00 ± 8.66	0.4989

n = 5 (Sire 1, n=3; Sire 2, n=1; Sire 3, n=1); SE = standard error of mean

1 and 2 (7.78 ± 0.52, 8.33 ± 1.85, 11.67 ± 0.66, P<0.01) (Table 4). Bulls after Sire 1 had the least expressed licking behaviour and the most playing activities (3.67 ± 1.84, 11.33 ± 0.66, and 11.33 ± 0.67, P<0.05; 3.11 ± 0.65, 1.33 ± 0.88, and 1.33 ± 0.88, P<0.05).

Behaviour of an animal is an essential reflection of its well-being (Lidfors, 2005). The results of Fraser and Broom (1997), Broom and Fraser (2007), and Brouček *et al.* (2012) indicate that a well-balanced proportion of behaviours for cattle can be assumed by making

Table 4: Frequency of behaviours according to sire (mean ± SE)

	Sire				
	1	2	3	P	
Fight	17.22 ± 1.79	17.00 ± 4.16	18.00 ± 1.73	0.9714	
Lick	3.67 ± 1.84	11.33 ± 0.66	11.33 ± 0.67	0.0459*	
Mount	17.44 ± 1.46	19.33 ± 4.33	19.33 ± 4.33	0.7756	
Paw	7.55 ± 2.71	0.00 ± 0.00	0.00 ± 0.00	0.1941	
Aggressiveness	7.78 ± 0.52	8.33 ± 1.85	11.67 ± 0.66	0.0071**	1:3**, 2:3*
Comfort	1.67 ± 0.33	1.67 ± 0.37	1.00 ± 0.57	0.4889	
Play	3.11 ± 0.65	1.33 ± 0.88	1.33 ± 0.88	0.0149*	1:2,3*

n = 5 (Sire 1, n=3; Sire 2, n=1; Sire 3, n=1); *P<0.05; **P<0.01
SE = standard error of mean

comparisons of time budget of behaviours and activity pattern between various rearing conditions. Broom (1996) described a variety of behaviours expressed as one measure in a list of good animal welfare indicators.

In the present study, times of movement gradually increased from the first to the third day; however, feeding time was the longest on the first day. This seems to defy logic, as animals were expected to move the most and eat the least on their first day in an unfamiliar pen, where they were mixed with other animals. Dairy cattle must learn to cope with environments vastly different from the habitats to which their ancestors were adapted (Brouček *et al.*, 2011).

Behavioural disorder is any deviation from the normal natural behaviour of animals. However, not all the behaviour that is abnormal in the first sense is pathological (Appleby and Hughes, 1997; Fraser and Broom, 1997). Abnormal behaviours are considered behavioural response of the organism to unfavourable conditions in the life of animals. When interpreting abnormal behaviour it is often found to be similar to a natural behaviour that has not been fulfilled (Lidfors, 2005). Krohn (1994) argues that constantly tethered housing changes normal behaviour s and increases incidence of abnormal behaviour. Animals used in the current study were not tethered, but they had a limited area without outside yard. Since the animals did not run, nor had enough space to perform certain activities (comfort behaviour, playing) they could have expressed behavioural disorders. Bulls in a pen environment under a restricted feeding period might compensate for a lower amount of time spent feeding by performing non-nutritive oral behaviour. However, the level of non-nutritive oral behaviours was enough to compensate for the lack of feeding behaviour (Ishiwata *et al.*, 2007).

In present study, the addition of fresh straw litter on the third day was a huge intervention in the behaviour of the animals. That‘s what caused the increase in movement and the decrease in the length of eating. This explanation is also corroborated by the enormous increase in playing on the third day (P<0.001).

Statistically significant differences in frequencies of aggressiveness and playing behaviour were found during the observation days. This could be attributed to the enrichment of the animal surroundings on the third day. The pen was bedded, which triggered the animals' playing with straw and their throwing it in all directions. The movement of animals increased at the same time as their competitiveness, which may have caused an increased aggression in their interaction. However, the frequency of aggressive interactions declined rapidly as dominant-subordinate relationships were determined. Because this phenomenon is relatively common in nature as well as on farms and ranches, it should probably not be considered abnormal (Houpt, 1987).

Lawrence and Rushen (1993) and Mason and Rushen (2006) showed that mounting is abnormal sexual behaviour in fattening bulls. We recorded this disorder in all five bulls (from the group of 32 bulls it is 15.6 %). The level of sexual behaviour displayed is determined by genetics, physiological factors, environmental factors, health and previous experience. Bulls of dairy breeds are generally more sexually active than those of the beef breeds (Bouissou *et al*., 2001; Kilgour, 2012).

Our results showed that cattle under any environmental conditions engage in some sort of oral behaviours for a certain proportion of the daytime. Especially in an intensive pen environment, cattle might perform more oral behaviours other than eating to compensate for the lack of occurrence of feeding behaviour. In addition, it is indicated that cattle in pen conditions under a restricted feeding period might compensate for a lower time spent feeding by performing other oral behaviour. The lack of oral behaviours caused

by the loss of eating might be compensated by oral behaviours except for eating as compensatory behaviours. In cattle in an intensive environment, the ingestion of the concentrate diet should stimulate oral behaviours and increase the motivation to perform oral behaviours (Phillips and Rind, 2001; Ishiwata *et al*., 2008).

The aggressive behaviour (fighting and butting) did not seem to be very serious, because it most often occurred with low intensity. This supports the fact that in well-established groups of cows, threats and passive avoidance are the main patterns of agonistic behaviour used to maintain social rank (Krohn, 1994). The findings of Laister *et al.* (2011) suggest that relaxation effects induced by social licking differ between performers and receivers and are affected by the bull's basic activity. In receivers, there were clear indications of a calming effect implying the experience of positive affective states. In performers, such calming effects during social licking were not identified. Mixing-induced aggression is inevitable in group-housed animals, regardless of space allowance, group size, bedding, pen design, or feeding regimens. High frequencies of social disturbances are observed when the animals are mixed. Mixing leads to an increase in aggression as a new hierarchy has to be established (Bouissou *et al*., 2001; Laister *et al*., 2011).

In bulls, the increased aggression is accompanied by an increase in homosexual mounting. The most aggressive animals mount more often than the others and the rate of mounting increases dramatically when new steers are introduced in the group. The syndrome is seen most frequently when groups of animals are mixed, especially in crowded conditions (Laister *et al*., 2011).

Cattle appear to have the fewest stereotyped behaviour of the various species of farm animals. Possible explanations are that animals are often kept in less confined environments and they spend a large portion of their time ruminating, a behaviour that may impede the development of stereotyped behaviours (Houpt, 1987).

The genetic influences on behaviour can be clearly manifested by the study of influence of sires. The sire lineage influences a large part of the population and hence its genetic qualities are effective as a stabilization factor. The sire is effective in the herd during a relatively short period, so the complex of factors to which its daughters are exposed during rearing should not be of such variability. In the present study, effect of the sire was manifested in the frequency of licking, aggressiveness, and playing. It appears that activities, aggressiveness and playing are easily influenced by the mood and welfare of the animals. The reason as to why licking is affected by the father is puzzling. In the present study mother effects were not evaluated.

CONCLUSIONS

The results of this study revealed that among observed days with different treatments differences were noted in frequencies of aggressiveness and playing behaviour. Effect of sire was manifested in the frequency of licking, aggressiveness, and playing behaviour.

Further experimental investigations are needed to better understand the abnormal behaviour development in progressed management.

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Short communication

EFFECT OF *YUCCA SCHIDIGERA* HERBAL EXTRACT IN DIET ON WEIGHT GAINS OF RABBIT DOES (PRELIMINARY RESULTS)

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ABSTRACT

The aim of the study was to evaluate the effect of the addition of different concentrations of yucca herbal powder additive to the diet on weight gain of rabbit does.
Three months old rabbit does (n=45) of New Zealand White line were used in the experiment. The does in the control group (C; n=15) were fed with commercially available diet and in the experimental groups the diet was enriched with 5 g (E1; n=15) or 20 g (E2; n=15) of yucca (*Yucca schidigera*) powder extract added to the 100 kg of the diet.
We found out statistically significant difference ($p<0.05$) in total average weight gains in the experimental group 1 (E1; 1427.9±51.02 g) compared to the control group (C; 1285.9±35.09 g) and experimental group 2 (E2; 1252±46.24 g).
The highest (but not significant) total weight gains per week were observed in the experimental group 1 (E1; 203.99±40.62 g). Lower total weight gains per week were found in the experimental group 2 (E2) compared to the control group (C) (178.85±38.40 and 183.8±40.6 g respectively).
In conclusion, addition of lower (5 g/100 kg diet) concentration of *Yucca schidigera* plant extract to the commercially used diet had positive effects on rabbit doe weight gains, but more experiments are needed to prove this assumption.

Key words: rabbit does; yucca; weight gains

INTRODUCTION

Rabbit is considered a temporary biological model among laboratory and domestic animals. It is the most fertile mammal which is able of reproduction at the age of 17-19 weeks of life. Many environmental factors can influence quality of rabbit life (Krohn *et al.*, 1999).

Farmed rabbits have to be supplied sufficiently with all life-essentials (feedstuffs, fluids, fresh air and hygienic unobjectionable quantity and quality). The health and uninjuriousness of animals has to be promoted in every suitable way and controlled regularly. The housing systems for rabbits must guarantee their protection against climatic adversities and natural enemies, but also exclude behavioural damages of body and body parts (Löliger, 1996).

There are countless of feed additives that are added to food or water in various forms. These additives are intended for livestock but also for humans. Their role is to enrich feed and supply missing components in the feed mixture. Several teams of scientists studied the effect of herbal plants on animal organism. Their effect on the organism of consumer is considerably scientifically proven and these extracts are already a common part of daily or intermittent feed consumption. Authors examined effect of these additives on weigh gains, reproduction, growth, health and other (Durmic and Blache, 2012; Kumar *et al.*, 2012; Krishnaiah *et al.*, 2011). However,

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their results are controversial.

Mojave yucca (*Yucca schidigera*) is a flowering plant native to Mojave, Sonoran and Great Basin deserts that are found in the south-western USA and north-western Mexico. *Yucca schidigera* extract contains a number of steroidal saponins that, because of their biological activity, have attracted attention from the livestock industry for many years. Saponins in general are common in a large number of plants and are identified by their ability to form stable soap-like foams (Francis *et al*., 2002). Some of the physical and chemical properties of these compounds (e.g., surface active properties and ammonia binding capacity) have sparked research into their use in livestock production applications (Hristov *et al*., 1999). The plant contains several physiologically active phytochemicals too. For example, yucca is a rich source of polyphenols, including resveratrol and a number of other stilbenes (yuccaols A-E) (Cheeke *et al*., 2006). It also improves performance and health of the livestock in addition to feed in various concentrations. Two versions of yucca preparations are available on the market - yucca powder and yucca extract (Piacente *et al*., 2005).

This product is on the list of certified biotechnological preparations used for reduction of ammonia emissions and odour and it serves the same purpose when applied to feed, to deep litter, screens, dump excrement etc. (Tyl *et al*., 2010).

The effect of yucca or sarsaponin preparations has been studied on different species of farm animals such as ruminants (Goodall *et al*., 1982; Hristov *et al*., 1999, Liu *et al*., 2009), pigs (Duffy and Brooks, 2007) and rabbits (Amber *et al*., 2004; Chrenková *et al*., 2012) too.

The objective of this study was to evaluate the effect of different concentration of herbal additive yucca powder to the diet on rabbit does weight gains.

MATERIALS AND METHODS

Three months old clinically health rabbit does of the New Zealand White line were used in our experiments. The animals were housed in individual cages, under a constant photoperiod of 14 hours of light day. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at 60±5 % and 17±3ºC). The rabbits were fed *ad libitum* and water was provided *ad libitum* with nipple drinkers.

Rabbit does (n=45) were divided into three groups: control (C; n=15) and two experimental groups (E1; n=15 and E2; n=15). The does in the control group were fed with a commercially available diet. In the experimental group E1 5 g of a powder of plant *Yucca schidigera* was added to the 100 kg of the diet. In the second experimental group (E2) 20 g of a powder of *Yucca schidigera* was added to the 100 kg of the diet. The animals were fed for 50 days and weighed weekly.

The data were analyzed using t-test using SigmaPlot statistical package (Systat Software Inc., Germany).

RESULTS AND DISCUSSION

The highest average weight gain per week was in the first experimental group (E1; 203.99±40.62 g) compared to the control (C; 183.8±40.6 g) and the second experimental group (E2; 178.85±38.40 g) (Table 1).

The highest total average increases of weight were observed in the first experimental group (E1; 1427.9±51.02 g). Weight gains obtained in the other two groups (C, E2), had statistically significant ($p<0.05$) lower values (1285.9±35.09 g and 1252±46.24 g respectively) compared to the experimental group E1 (Figure 1).

The positive effect of the addition of *Yucca schidigera* on growth, health and reproductive activity of animals was pointed out by several authors (Anthony *et al.*, 1994; Amber *et al.*, 2004; Duffy and Brooks, 2007).

Anthony *et al.* (2004) examined effect of feeding diets containing yucca extract and probiotics on growth, digestibility, nitrogen balance and caecal microbial activity of growing New Zealand White rabbits. The authors observed that the supplementation of the diet with yucca extract or probiotics significantly ($p<0.05$) affected the growth performance. Average daily weight gain increased ($p<0.05$) by 12.1 and 9.6 % for rabbits feed with diets enriched with yucca extract or probiotics respectively, when compared to the control diet.

In our study, we found out that weight gains were increased when feed mixture enriched with the lower concentration of 5 g of *Yucca schidigera* plant was fed. Higher concentration (20 g) of *Yucca schidigera* plant extract in the experimental group 2 has no positive effect.

It was proposed that the *Yucca schidigera* plant extract has the ability to increase performance of animals. Feeding of young chickens with plant preparation containing *Yucca schidigera* extract increased ($p<0.05$) the average weight gains of chicks (Giffard *et al*., 2001). In our experiments with rabbits we determined statistically significant ($p<0.05$) increase in total weight gains in the experimental group 1 (E1). Positive influence in the case of the highest apparent protein and fat digestibility coefficient was also observed by Chrenková *et al*., (2012) when 5 g of yucca extract was added to the 100 kg of rabbit feed mixture.

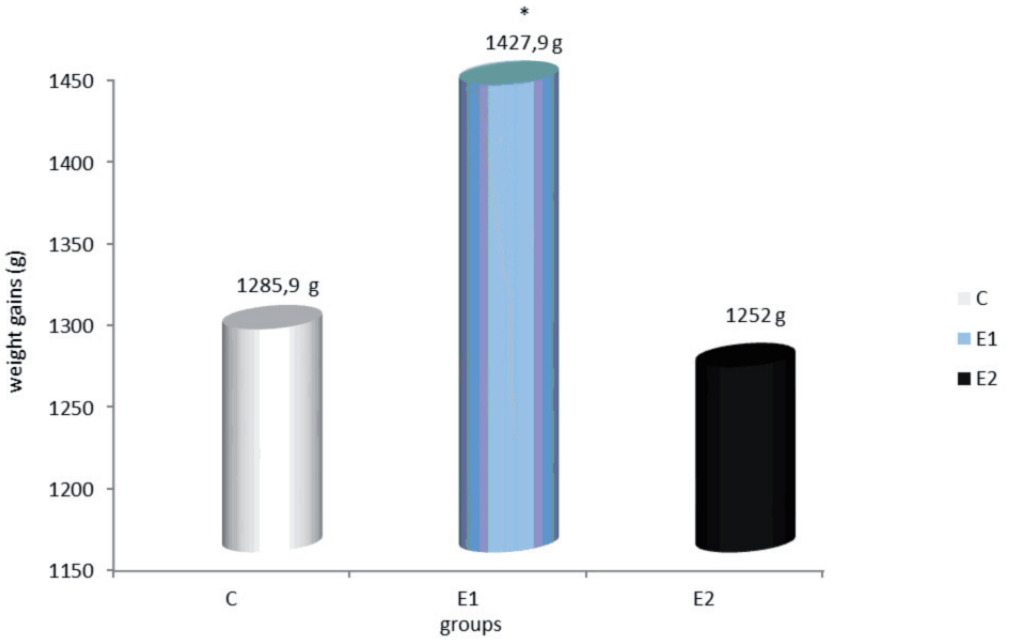
Positive effect of *Yucca schidigera* extract addition to the diet was documented also in pigs. Daily average weight gains in the group fed with diet enriched with *Yucca schidigera* were significantly higher when compared to the control group and the number of days required for supplemented animals to reach slaughter weight was reduced by 7 days when compared to control animals (Duffy and Brooks, 2007).

On the other hand, Ocak *et al*. (2008) published no differences in growth performance in broilers who were fed with herbal feed containing peppermint and thyme, but peppermint leaves had a higher growth promoter effect compared to the thyme at an early stage of the broilers.

Table 1: Weight gains (g) per week of analyzed rabbit does fed with *Yucca schidigera* plant extract from the 1st to the 49th experimental day

Groups	Weight gains per week (g)						
	1. week	2. week	3. week	4. week	5. week	6. week	7. week
C (n=15)	72.0±23.1	170.6±17.0	208.0±12.8	116.7±14.0	152.0±21.4	403.3±37.0	163.3±17.0
E1 (n=15)	129.3±16.1	210.0±16.9	148.0±17.8	191.3±10.2	144.0±15.5	439.3±40.8	166.0±21.1
E2 (n=15)	82.7±19.6	182.0±8.0	257.3±69.3	92.0±64.8	175.3±38.3	360.7±45.3	102.0±23.5

C – control group; normal diet, E1 – 5 g of *Yucca schidigera* added to 100 kg of normal diet, E2 – 20 g of *Yucca schidigera* added to 100 kg of normal diet



C – control group; normal diet, E1 - 5 g of *Yucca schidigera* added to 100 kg of normal diet, E2 - 20 g of Y*ucca schidigera* added to 100 kg of normal diet
- Differences in values are statistically significant at $p<0.05$.

Fig. 1: Total average weight gains (g) of analyzed rabbit does fed with *Yucca schidigera* plant extract from the 1st to the 49th experimental day

Also Hernández *et al*., (2004) reported that *Labiatae* extract (LE) from sage, thyme and rosemary had no positive effects on weight gains as well. No differences in feed intake or feed conversion were observed. Only from 14 to 21 days of age, broilers fed with the LE diet grew faster than the broilers fed with the control or EOE (essential oil extract) feeds (68.8 vs. 63.9 and 61.6 g/d, respectively).

Based on our preliminary results, we can agree with the conclusions of the authors mentioned above that the feed with the addition of *Yucca schidigera* plant extract significantly affected weight gains in the experimental animals. In case of the total average weight gains, the differences between the control group (C), the experimental group 1 (E1) and the experimental group 2 (E2) were statistically significant and the best results were obtained in the experimental group 1 (E1).

CONCLUSION

The addition of the lower concentration (approximately 5 g) of a dry powder of *Yucca schidigera* plant into the normal feed for small livestock animals, rabbits, had a positive effect on their weight gains.

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