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AIMS AND SCOPE

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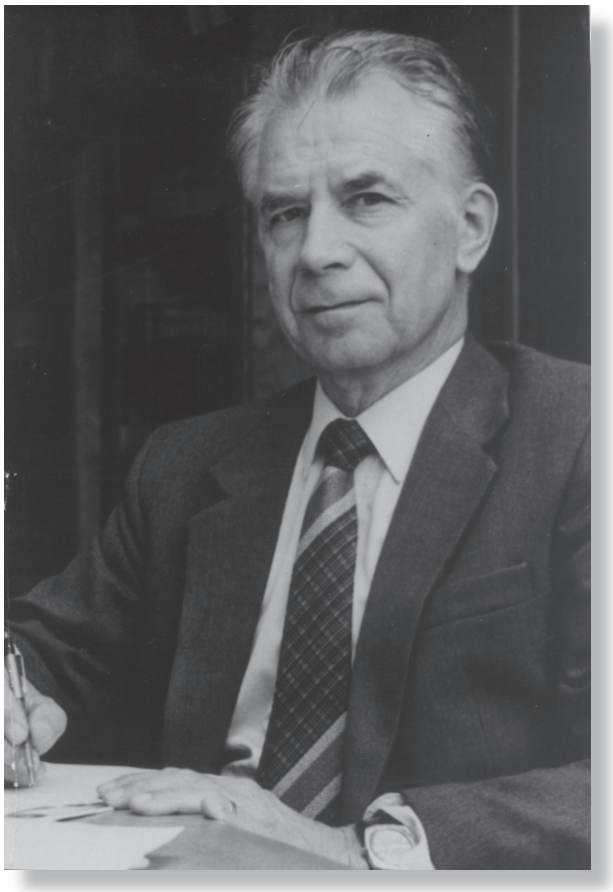
In memory of the prominent Slovak educator and scientist Prof. Ing. Pavol Majerčiak, DSc.

An outstanding representative of the Slovak agricultural and livestock sciences Prof. Ing. Pavol Majerčiak, DSc. would have lived 90 years on June 27, 2014. He was an extraordinary noble personality and outstanding expert with general encyclopaedic knowledge and truly exceptional teacher.

Prof. Majerčiak was born in Liptov on 27th June 1924 in Nemecká Ľupča (now Partizánska Ľupča) in a farmer's family. After passing the school-leaving exams at the grammar school of J. M. Hodža in Liptovský Mikuláš in 1945 he enrolled the subject of study agricultural engineering at the Technical University in Bratislava. He graduated in 1949 from the University of Agricultural and Forestry Engineering (UAFE) in Košice.

After graduating from the UAFE he worked for a short time in the Headquarters for Mechanization of Agriculture and from November 1952 became his occupation the scientific research. Since November 1952 he became a member of the staff in the Research Institute of Animal Production (RIAP) in Vígľaš and he continued his work in it after the institute moved to Nitra in 1961. Even after retirement in 1991 until the last days of his life he was still in active contact with the RIAP and contributed significantly to its professional and social profiling. Prof. Majerčiak died on 13th April 1998 at the age of 74 years.

Prof. Majerčiak devoted his whole fruitful professional life to research in pig breeding. Together with a team of researchers, biological



services, and affiliated centres in Slovakia and in the Czech Republic they addressed the topical questions of reproduction and rearing techniques of pigs with a view to economic impact on the production of high-quality pork.

Publishing portfolio of Prof. Ing. Pavol Majerčiak, DSc. contains hundreds of scientific and popular works, over 90 research reports and 40 books.

He was an external lecturer at the Faculty for Agronomy at the Slovak University for Agriculture in Nitra and a member of scientific councils of universities and research institutes not only in Slovakia but also in the Czech

Republic. He was member of editorial boards of three professional journals. For many years he was also chairman of the Central Committee of the Slovak Agricultural Scientific and Technological Society. He contributed to the promotion of the Slovak agricultural science within broader dimensions of the world with his contributions to the „Index of Current Research on Pigs and Pig News and Information“ for many years.

Prof. P. Majerčiak, DSc. passed his rich scientific knowledge and style of work to younger co-workers. He educated a number of scientific, pedagogical and professional staff, who successfully developed and implemented his ideas in scientific-research institutions, universities and agricultural enterprises in Slovakia and in the Czech Republic.

Professor Majerčiak´s life and work was characterised by harmony of humanity and professionalism. With respect and gratitude we took for granted his life‘s credo: „to work bravely and with pleasure; the good and the ethical behaviour should be regarded as a personal principle“.

With respect and gratitude,

Colleagues and Friends from the Research Institute for Animal Production Nitra

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EFFECT OF SEMEN COLLECTION FREQUENCY ON THE PROGRESS IN THE MOTILITY OF RABBIT SPERMATOZOA

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ABSTRACT

In the present study the effect of collection regime on rabbit semen development was analyzed. Eight seminal traits, such as overall ejaculate volume (VOL), spermatozoa concentration (CON), motility (MOT), progressive motility (PRO), percentage of progressive motility (% PRO), curvilinear velocity (VCL), beat-cross frequency (BCF) and amplitude of lateral head displacement (ALH) were analyzed. As the number of collections increased, more bucks were able to ejaculate. During collections the semen volume gradually increased from 0.4 ml to 1.0 ml with the average of 0.68 ± 0.34 ml. The average of MOT was 55.55 ± 26.63 % and the average of PRO reached 40.43 ± 27.35 %. The other assessed parameters were CON - 0.547 ± 0.165 x 10^9 ml^{-1}; ALH - 4.18 ± 1.18 µm; BCF - 27.42 ± 7.12 Hz; VCL - 104.04 ± 37.00 $µm.s^{-1}$. A positive correlation between ejaculate volume and the number of collections was found. MOT and PRO significantly correlated with the number of collections. In addition, positive correlation between % PRO and the number of collections was recorded. No correlation between the number of collections and VCL was detected. In the case of ALH, a moderate positive correlation between this parameter and the number of collections was observed. BCF slightly correlated with the number of collections. The results of present study indicate on possibility to increase the values of some seminal parameters, which opens new opportunities for raising the quality of semen directly *in vivo*.

Key words: rabbit; spermatozoa; motility; semen collection frequency; CASA; fertility

INTRODUCTION

In the past ten years, the development of biological sciences has been intensively connected to expansion of technologies, aimed at recognition of principles and control of biological processes of animal reproduction. One of the most important of them is assisted reproduction and artificial insemination. Artificial insemination has probably been the greatest technological advance in animal breeding and the main reasons for its success have been genetic gain, disease control, and the cost-effectiveness of insemination compared to natural mating (Laurinčík *et al.*, 2008). It has also been the most noteworthy example of the successful integration of both research and widespread application (Vishwanath, 2003). Moreover, biotechnological methods include *in vivo* and *in vitro* experiments, as well. In the *in vivo* experiments living animals are used. Conversely, *in vitro* cultivations are explicitly aimed at the work with cells out of organism in such conditions imitating the organism environment (Laurinčík *et al.*, 2008).

The commercial success of artificial insemination of any species depends on the extensive use of genetically superior males to impregnate a large group of females with relatively low doses of spermatozoa per insemination. This requires high semen quality which can be assessed by several parameters (Farrell *et al.*, 1993; Castellini *et al.*, 1996; Castellini *et al.*, 2000; Brun

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et al., 2002; Ducci *et al.*, 2002).

In order to predict fertility before insemination, different *in vitro* tests have been developed to determine spermatozoa quality. Computer-assisted semen analysis (CASA) provides a repeatable estimate of many spermatozoa movement criteria and allows determination of specific motion characteristics of spermatozoa. This objective semen assessment technique could be used to evaluate differences between bucks and attempt to predict the *in vivo* performance of semen. Among semen parameters, spermatozoa motility is believed to be the most important characteristics correlated to male fertility (Bostofte *et al*., 1990; Eimers *et al.*, 1994) because of its importance for spermatozoa migration through the female genital tract and for gamete interaction at fertilization (Robayo *et al*., 2008). Many authors observed, that some other CASA motility parameters, or a combination of more ejaculate traits (motility parameters, semen concentration, seminal plasma compounds, spermatozoa morphological parameters) often highly correlated with success of fertilization itself (Ellington *et al*., 1993; Farrell *et al*., 1998; Wood *et al*., 1986; Shibahara *et al*., 2004; Freour *et al.*, 2009; Fréour *et al*., 2010; Hirano *et al*., 2001; Macleod *et al*., 1995; Marshburn *et al*., 1992; Siduhu *et al*., 1997).

The aim of this study was to analyze the progress in spermatozoa motility in male rabbits in relation to the frequency of semen collection.

MATERIAL AND METHODS

As a biological material, the semen (ejaculates) of 34 sexually mature and healthy rabbits (4 - 5 months) of the HYLA breed were used. The males were housed in individual cages, under a photoperiod of 10 - 15 h of daylight. All animals were fed *ad libitum* with a commercial diet and water was provided *ad libitum* with nipple drinkers. The ejaculates were collected 18 times for 11 weeks using artificial vagina (Massányi *et al.*, 2008) from February to May. Each ejaculate was analyzed using the CASA technique with SpermVision 3.0 software using the Makler Countig Chamber (Sefi–Medical Instruments, Germany).

In total, the following sperm characteristics were assessed: motility (MOT), progressive motility (PRO), percentage of progressive motility (% PRO), beat cross frequency (BCF), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and spermatozoa concentration (CON). The average ejaculate volume (VOL) for every collection was evaluated, as well. The average value of each of these parameters was computed for every collection individually.

The percentage of PRO of MOT was computed as % PRO = (PRO/MOT) x 100 % for every collection. The correlation between MOT, PRO, % PRO, VOL, CON, VCL, ALH, BCF and the number of collections was assessed by a Spearman correlation coefficient (r_S).

Table 1: Percentage of collected ejaculates in relation to the number of collections

Collection	Number of rabbits	Collected	Percentage
1.	35	18	51 %
2.	35	28	80 %
3.	35	24	69 %
4.	35	32	91 %
5.	35	31	89 %
6.	35	29	83 %
7.	35	27	77 %
8.	35	32	91 %
9.	35	31	89 %
10.	35	32	91 %
11.	35	33	94 %
12.	35	33	94 %
13.	35	33	94 %
14.	35	34	97 %
15.	35	34	97 %
16.	35	34	97 %
17.	35	34	97 %
18.	34	33	97 %

Furthermore, correlation between % PRO, MOT, PRO and VOL was determined through a Pearson correlation coefficient (r_P). All the statistical tests were carried out with the SigmaPlot version 12.0 at the level of significance $\alpha = 0.05$.

RESULTS

Before every semen analysis, we attempted to collect the ejaculate from all 35 males. In the first collection, only 18 bucks (51 %) ejaculated. By further attempts more bucks were able to ejaculate and in the last collection 34 ejaculates were obtained (97 %). Detailed information is provided in Table 1.

A gradual increase in semen volume during performed collections was recorded. While in the beginning of the process the volume was equal 0.40 ± 0.25 ml, in the last collection the value reached 1.00 ± 0.30 ml (Fig. 1). Overall, the average volume was 0.68 ± 0.64 ml. The correlation between the ejaculate volume and the number of collections ($r_S = 0.964$) was statistically significant, and the dependence of ejaculate volume on the number of ejaculate collections was confirmed.

By assessing the spermatozoa movement, we detected a moderate increase in motility (MOT) from 74 % to 84 % and also a moderate increase in progressive motility (PRO), which started at 54 % and culminated at 72 %. The average MOT was 55.55 ± 26.63 % and the average PRO reached 40.43 ± 27.35 %. In the case of % PRO, a similar progress in its development was noted. Therefore, a significant positive correlation between MOT ($r_S = 0.581$), PRO ($r_S = 0.651$), % PRO and the number of collections was observed (Fig. 2).

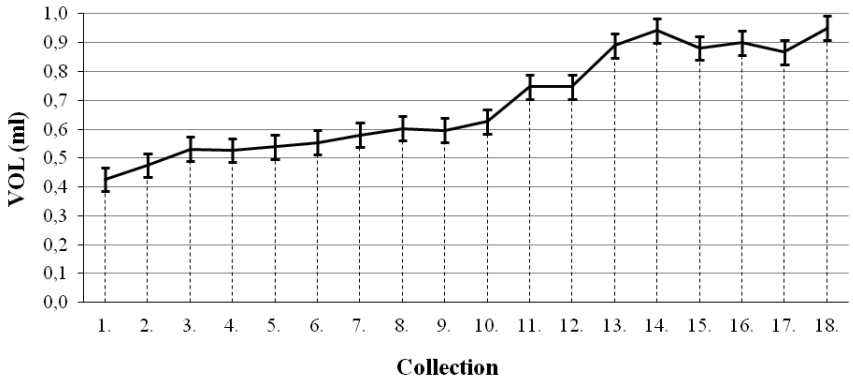

Fig. 1: Ejaculate volume dependence on the number of collections

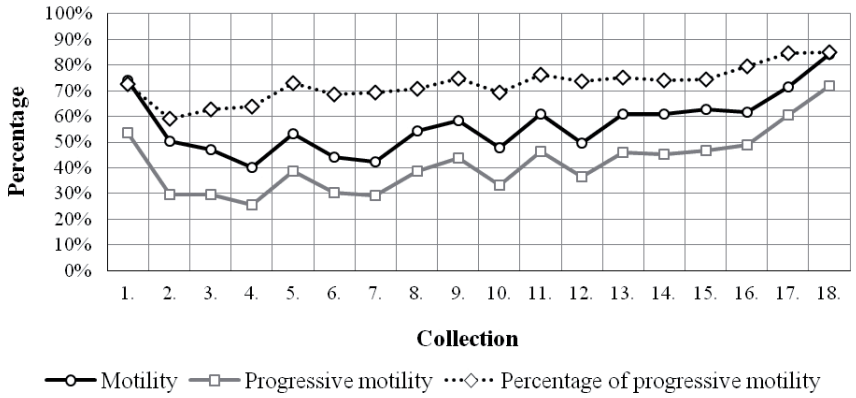

Fig. 2: Development of MOT, PRO and % PRO with regard to the number of collections

Likewise, a positive correlation between the number of collections and the spermatozoa concentration (CON) was observed (r_S = 0.789), whereas the value of spermatozoa concentration varied from 0.37 to 1.05 x 10^6 ml^{-1} (Fig. 3), with 0.55 ± 0.16 x 10^9 ml^{-1} in average.

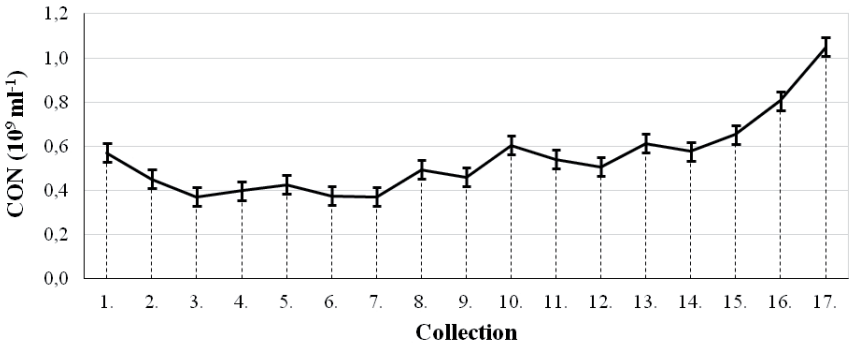

Fig. 3: Spermatozoa concentration development with regard to the number of collections

Motility (MOT) and progressive motility (PRO) correlated to the ejaculate volume (r_P = 0.548 and r_P = 0.640, respectively). In the case of the dependency of the ejaculate volume and spermatozoa motility, positive correlation (r_P = 0.751) between % PRO and the ejaculate volume was observed (Fig. 4).

The relationship between the number of collections and ALH, BCF, VCL was also studied. No correlation between the number of collections and the VCL (r_S = 0.267) value development was detected (Fig. 5). In the case of ALH, a moderate positive correlation (r_S = 0.478) between this parameter and the number of collections was observed (Fig. 6). BCF slightly correlated (r_S = 0.560) to the number of collections (Fig. 7). The average value of VCL was 104.04 ± 37.00 µm.s^{-1}. ALH was represented by 4.17 ± 0.28 µm and the average value of BCF reached 27.50 ± 1.96 Hz.

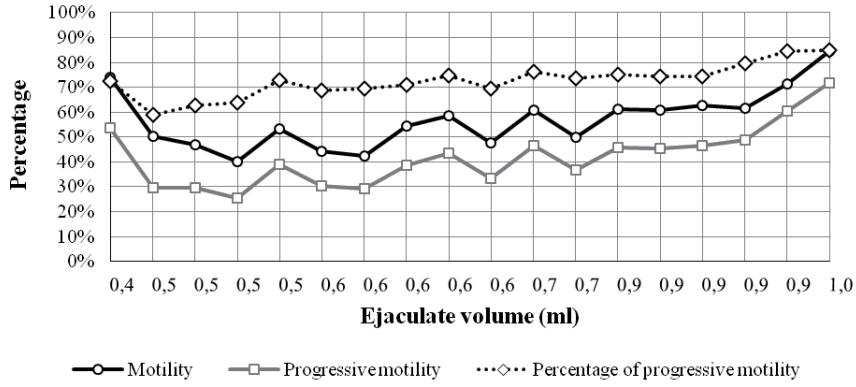

Fig. 4: Spermatozoa motility development with regard to the ejaculate volume

DISCUSSION

For artificial insemination a proper ejaculate with desired seminal parameters is required. Some seminal parameters could have a high relationship to the success of fertilization process. There are several publications, the aim of which lies on analyzing the development of selected seminal traits with the relationship to the regime of semen collection (Castellini *et al*., 2006; Nizza *et al*., 2002). Some of such traits, as shown in present study, may be influenced through regular semen collection. The results of this study indicate on possibility to increase the values of these parameters, which opens new opportunities for raising

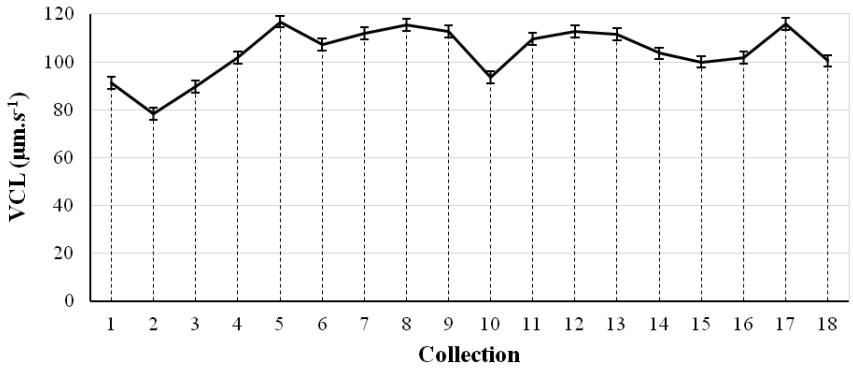

Fig. 5: VCL development with regard to the number of collections

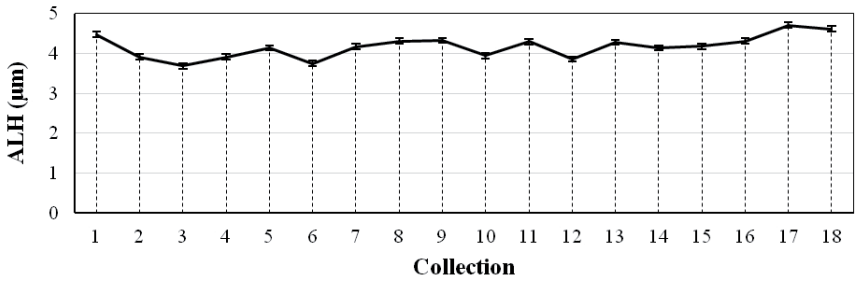

Fig. 6: ALH development with regard to the number of collections

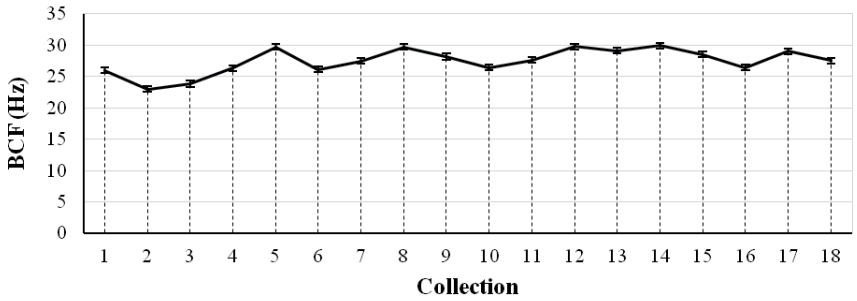

Fig. 7: BCF development with regard to the number of collections

the quality of ejaculates directly *in vivo*.

During continuous semen collections it came to a significant increase in the ejaculate volume. After the first ejaculate collection, its average volume was 0.43 ml and this value increased progressively up to 0.95 ml for the last collection. This increase is caused by the stimulation of the male accessory reproductive organs, which produce more secret as the glands get long-term stimulated. Schneidgenova *et al.* (2011) in their study report an average ejaculate volume 0.68 ± 0.25 ml in 25 rabbits in the spring season, which is similar to the values obtained in our work (0.68 ± 0.34 ml). The similar results (0.60 ± 0.05 ml) were obtained in the work of Castellini *et al.* (2006). Taking into consideration similarity of methods used for collection, it can be concluded that there is probably no significant influence of any other factors than the regime of semen collection on the ejaculate volume development.

Almost three-fold increase in spermatozoa concentration from the beginning (0.37 x 10^6 ml^{-1}) to the end of collections (1.05 x 10^6 ml^{-1}) was found. It can be concluded that the rising number of collections generally causes an increase in semen concentration. However, Nizza *et al.* (2002) recorded increase in semen concentration neither after 34 nor 68 semen collections, which were done from July to November. This can be a consequence of season influence on rabbit semen development. Chrenek *et al*. (2011) assessed ejaculates of 10 HYLA males, semen concentration of which reached 0.94 x 10^9 ml^{-1}. Similarly, in the study of Schneidgenova *et al*. (2011) the average semen concentration was measured as 1.18 x 10^9 ml^{-1}. Diametrically different results were obtained by Castellini *et al*. (2006), where the CON value was about 0.261 ± 0.127 x 10^9 ml^{-1}. Despite large differences in these values from that of present work (0.547 ± 0.165 x 10^9 ml^{-1}), it only indicates on individual variability in rabbit semen concentration.

With the number of collections it came to an increase of MOT (74 % - 84 %) and also a moderate raise of PRO (54 % - 72 %) were observed. The average MOT was 55.55 ± 26.63 % and the average PRO reached 40.43 ± 27.35 %. In the study of Schneidgenova *et al*. (2011) the average MOT was 69.94 ± 16.64 %. For the PRO parameter they reported the average value 52.23 ± 20.63 %. These present differences are probably due to individual variations among males. Moreover, it is necessary to take into consideration the occurrence of many factors that influence analyzed seminal traits, such as occurrence of urine in the ejaculate or environmental temperature.

By evaluation of the other motility parameters, we observed no relationship between the number of collections and VCL (r_S = 0.267). In contrary, ALH moderately positively correlated (r_S = 0.478) with the number of collections. The same applies to the BCF parameter (r_S = 0.560). The average value of VCL was 104.04 ± 37.00 $\mu m.s^{-1}$, for ALH it was 4.17 ± 0.28 μm and the average value of BCF reached 27.50 ± 1.96 Hz. For VCL Schneidgenova *et al.* (2011) reported an average value of 81.44 ± 24.21 $\mu m.s^{-1}$. In the same study the average ALH was 4.03 ± 0.82 μm, the result not very different from those reported in this study. The BCF value is lower as well, when compared to our results (22.81 ± 5.75 Hz). Explanation of this phenomenon lies on the fact that as the frequency of spermatozoa head movement decreases it comes to simultaneous reduction of the linear movement.

Our results suggest that some seminal traits and, thus, the semen quality can be improved by regular semen collection.

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LINEAR AND QUADRATIC EFFECTS OF INDIVIDUAL AND EWE INBREEDING ON GREASY FLEECE WEIGHT AND REPRODUCTIVE TRAITS OF MAKUIE SHEEP BREED

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ABSTRACT

A pedigree file consisting of 5860 individuals, 167 sires and 1582 dams collected at Makuie sheep breeding station (MSBS) during a period of 24 years (1990 to 2013) was used to calculate the inbreeding coefficients to reveal any probable effects of inbreeding (F) on the studied traits. The studied traits were: greasy fleece weight at 6 months of age, greasy fleece weight at 18 months of age, conception rate, gestation length, number of lambs born, number of lambs alive at weaning, litter mean weight per lamb born and litter mean weight per lamb weaned. The inbreeding coefficient among the individuals ranged from 0 to 25 % with an average of 0.33 %, and ranged from 0 to 25 % among the ewes with an average of 0.21 %. Fluctuations in individual and ewe inbreeding were observed in the period under study. The average generation interval was calculated as 3.6 years. The effective population size of the flock was 51.8 animals. The rate of inbreeding was 0.08 % per year and 0.53 % per generation. Six different models were applied and likelihood ratio test (LRT) was used to select the appropriate model. Based on the LRT, model II was selected as an appropriate model for greasy fleece weight at 6 months of age. Quadratic regression coefficients of greasy fleece weight at 6 months of age and greasy fleece weight at 18 months of age were determined as significant ($P<0.001$) - 0.007 and - 0.40, respectively per 0.01 change in the individual F. The reproductive traits were studied basing on the ewe inbreeding coefficient. Number of lambs born was affected negatively ($P<0.01$) by the linear effect of ewe F. The lambs produced by the Inbred ewes had significantly ($P<0.05$) more survival ability from birth to weaning than those produced by the non-inbred ones. The significant quadratic regression coefficient of conception rate was determined (- 0.22; $P<0.05$). Significant quadratic regression coefficient ($P<0.01$) of litter mean weight per lamb born was determined (- 0.63 per 0.01 change in the ewe inbreeding coefficient). Gestation length and litter mean weight per lamb weaned were not affected significantly by linear or quadratic effects of ewe F. Therefore, inbreeding should be avoided, except for purposes of genetic breeding, whose main objective is the fixation of certain alleles in the population.

Key words: passive inbreeding depression; active inbreeding depression; greasy fleece weight; reproductive traits

INTRODUCTION

More than 20 indigenous sheep breeds are reared in Iran. Makuie sheep is one of the famous breeds of the country which is reared in Azerbaijan province with an approximate population size of 2.7 million heads (Abbasi and Ghafouri, 2011). Makuie is a multipurpose sheep whose main products are meat, milk and wool.

In animal breeding, active inbreeding where animals are mated according to family relatedness (inbreeding coefficient > 6.25 %) can be distinguished from passive inbreeding, what is the result of small effective population size (inbreeding coefficient < 6.25 %). In the first case inbreeding accumulates at a faster rate and severe inbreeding depression is possible. In the second case, inbreeding accumulates at slower rate, and natural and/ or artificial selection eliminates most deleterious genes (Miglior, 2000).

In general, inbreeding impairs growth, production, health, fertility and survival (Falconer and Mackay,

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1996). In the closed flocks, intensive selection reduces the genetic variability and increases the rate of inbreeding as compared to crossbreeding. Some theories have been proposed to explain the undesirable effects of inbreeding on the mean phenotypic values of traits. According to Crow and Kimura (1970), the heterozygotes generally present higher phenotypic values than the homozygotes. In contrast, Lush (1945) proposed that the desirable genes tend to be dominant or partially dominant. On the basis of these two theories, inbreeding depression can be defined as a linear function of the inbreeding coefficient. However, according to Lynch and Walsh (1998), if epistatic interactions are considered as a mechanism to explain the genetic basis of inbreeding depression, the decline in the phenotypic mean can be defined as a nonlinear function of the inbreeding coefficient.

The rates of inbreeding must be limited to maintain diversity at an acceptable level so that genetic variation will ensure that future animals can respond to changes in environment (Van Wyk *et al*., 2009). Concerning the importance of the knowledge about the level of inbreeding, probable effects of inbreeding on the studied traits and its influences on breeding decisions, present study was aimed to determine the effect of inbreeding on fleece (greasy fleece weight at 6 months of age and greasy weight at 18 months of age) and reproductive traits (conception rate, gestation length, number of lambs born, number of lambs alive at weaning, litter mean weight per lamb born and litter mean weight per lamb weaned) in Makuie sheep.

MATERIAL AND METHODS

The breeding flock

Makuie sheep has been adapted to cold and highland environments. It is a medium-sized (ewe = 45 - 48 kg, ram = 51 - 53 kg), and fat-tailed sheep breed. The common color of its body is white and black rings are around its eyes, nose and knees. Because of the Makuie sheep importance in Azerbaijan region economy, in 1986 the Makuie sheep breeding station (MSBS) was established in the city of Maku, Western Azerbaijan, in Iran. The main goals of MSBS were protection and improvement of this sheep breed. Base animals of MSBS were provided from the flock holders of the region. All ewes were bred to rams for the first time at an average of 18 months. The age distribution of the ewes is 18 month (parity 1) to 84 months (parity 7). Averagely 16 rams and 181 ewes have been included in the breeding program per breeding year. Estrus synchronization was carried out in the flock with a progesterone-releasing intra vaginal sponge (CIDR). The ewes were then bred either by an artificial insemination in the first cycle of estrus or using controlled rams. Flushing and equine chorion gonadotrophin (ECG) injection at CIDR removal was applied since 2000 year to increase the litter size. The ewes were kept in the flock for a maximum of 7 lambings. The rams were kept for 5 breeding seasons. In a closed flock, such as MSBS, the excessive use of superior individuals, such as breeding rams or ewes, to make the faster genetic progress could quickly result in an increase in the level of inbreeding.

Data

The pedigree file consisting of 5860 individuals, 167 sires and 1582 dams collected at MSBS during 24 years (1990 to 2013) was used in the present study (Table 1). The studied traits were classified into two main groups. Greasy fleece weight traits were: greasy fleece weight at 6 months of age (GFW1) and greasy fleece weight at 18 months of age (GFW2). Reproductive traits were: conception rate (CR: with code of 1 or 0, that is whether a ewe exposed to a ram did or did not lamb), gestation length (GL: has continuous expression with low range), number of lambs born (NLB: the number of fully formed lambs born per ewe lambing), number of lambs alive at weaning (NLAW: the number of lambs alive at weaning, reared both by the ewe and in the nursery), litter mean weight per lamb born (LMWLB: the average weight of lambs at birth from the same parity), litter mean weight per lamb weaned (LMWLW: the average weight of lambs at weaning from the same parity). The inbreeding coefficients were categorized into five classes according to Queiroz *et al.* (2000).

Statistical analysis

All known relationships among the animals were used to compute inbreeding coefficients by using the Pedigree program (2000) according to Wright's formula:

$$F_X = \left[\left(\frac{1}{2}\right)^{n_1 + n_2 + 1} (1 + F_A)\right]$$

The rate of inbreeding (ΔF) was estimated as the difference between the inbreeding of the individual (F_t) and the average inbreeding of the parents (F_{t-1}) divided by ($1-F_{t-1}$) (Falconer and Mackay, 1996). The effective population size (N_e) for the flock was calculated basing on the sex ratio using $N_e = \frac{4N_mN_f}{N_m + N_f}$ (Falconer and Mackay, 1996), where N_m is the number of males and N_f is the number of females. The average generation interval was calculated as the mean age of the parents at the time their offspring were born. The linear and quadratic effects of individual and maternal inbreeding (F) on the studied traits were analyzed using the GLM procedure of the SAS program (2005), as well as statistical analysis to determine the significance of fixed effects on the traits.

Table 1: Pedigree structure of Makuie sheep breed

	No. of animals	% of total	Average F (%)	SD (%)	SE
Total number of animals	5860	100	0.332	1.83	0.02
Non inbred	5301	90.46	0.000	-	-
Inbred	559	9.54	4.610	4.87	0.21
Sires in total	167	2.87	-	-	-
Dams in total	1582	26.99	0.210	1.70	0.02
Animals with progeny	1749	29.85	-	-	-
Animals without progeny	4111	70.15	-	-	-
Base animals	545	9.30	-	-	-
Non base animals	5315	90.70	-	-	-
Number of years	24	-	0.033	-	-

SD, standard deviation; SE, standard error

Six different univariate models were fitted for each trait. They were different in the concept of random effect and their correlations. Maternal genetic or permanent environmental effects were taken into account by including them into appropriate models, as described by Meyer (1992).

The linear forms of six models were:

Model I: $Y_{ijklmn} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + b_{1xijklmn} + b_2x^2_{ijklm} + e_{ijklmn}$

Model II: $Y_{ijklmno} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + pe_n + b_1x_{ijklmno} + b_2x^2_{ijklmno} + e_{ijklmno}$

Model III: $Y_{ijklmno} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + m_n + b_1x_{ijklmno} + b_2x^2_{ijklmno} + e_{ijklmno}$
($r_{am} = 0$)

Model IV: $Y_{ijklmno} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + m_n + b_1x_{ijklmno} + b_2x^2_{ijklmno} + e_{ijklmno}$
($r_{am} \neq 0$)

Model V: $Y_{ijklmnop} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + pe_n + m_o + b_1x_{ijklmnop} + b_2x^2_{ijklmnop} + e_{ijklmnop}$
($r_{am} = 0$)

Model VI: $Y_{ijklmnop} = \mu + YR_i + SX_j + BT_k + AD_l + an_m + pe_n + m_o + b_1x_{ijklmnop} + b_2x^2_{ijklmnop} + e_{ijklmnop}$
($r_{am} \neq 0$),

where $Y_{ijkl....}$ each observation on traits under study; μ, overall mean of population; YR_i, 24 levels, fixed effect of the year of birth i (for reproductive traits fixed effect of year of breeding i); SX_j, 2 levels, fixed effect of sex of animal j (for reproductive traits this effect was omitted from the models); BT_k, 3 levels, fixed effect of birth type k; AD_l, 7 levels, fixed effect of age of dam l (for reproductive traits fixed effect of the parity of the ewe l); an_m, individual additive genetic effect of animal m; pe_n, random effect of permanent maternal environment in n levels (n = number of maternal levels for each trait); m_n, maternal genetic effect; $x_{ijkl...}$, individual or ewe inbreeding coefficient of *ijklmnop*-th individual included as co-variable ; b_1 and b_2 are linear and quadratic individual or ewe F regression coefficients, respectively; $e_{ijklmnop}$, random error associated with *ijklmnop*-th observation.

In model I, the direct additive genetic was considered as the random effects; in addition to fixed and random effects an individual or ewe inbreeding coefficient was fitted as linear or quadratic covariate. In model II, the maternal permanent environment was added to the model I as a random effect. Model III included the maternal genetic and those mentioned for model I. In model IV, the correlation between direct and maternal genetic effect was studied. In Model V, the direct additive genetic, maternal genetic and maternal permanent environments were considered as the random effects; in addition to fixed and random effects an individual or ewe inbreeding coefficient was fitted as linear or quadratic covariate. In model VI, the random effects in model V plus correlation between maternal and additive genetic were studied.

The model VI was the full model. The best model was selected based on the likelihood ratio test (LRT). In LRT, the log-likelihood value of alternative model was compared with log-likelihood values of null models. LRT supposed to be distributed as chi-square (x^2), then its degree of freedom is differentiation between number of parameters of alternative model and null models. Statistical significance for models set at 5 % probability level. If the LRT value was greater than a critical value from a x^2 distribution with an appropriate degree of freedom (*df*), it can be concluded that the additional random effect has significant effect in the model and null model was not a better model. When the differences

were not significant, the null model, which had fewer parameters, was chosen as an appropriate model. Some genetic parameters including direct heritability (h^2), maternal heritability (m^2) and variance ratio due to permanent environmental component (C^2) were done using DFREML program (Meyer, 1989).

RESULTS AND DISCUSSION

Inbreeding

A major part of inbreeding in the MSBS's flock was due to the small effective population size that can be considered as passive inbreeding coefficient. The animals with inbreeding coefficient lower than 6.25 were the main part of the inbred population (Table 2).

Descriptive statistics for individual inbreeding coefficients for the whole population and the inbred population is shown in Table 3. The mean of individual inbreeding coefficient in females and males was 0.33 and 0.29 %, respectively. The maximum value of inbreeding coefficient (25 %) indicated that some mating of close relatives occurred, but the number of these matings was low. The same results have been reported for Muzaffarnagari sheep (Mandal *et al*., 2005) and Moghani sheep (Dorostkar *et al*., 2012). The inbreeding coefficient calculated in the present study was lower than those reported by Swanepoel *et al*. (2007), Norberg and Sorensen, (2007) and Oravcová and Krupa (2011).

According to Figure 1, an increasing trend of the mean inbreeding (both individual and ewe) is observable over the 24 years. The maximum individual and ewe F were observed in 1999 and 2002, respectively. The individual F was peaked again in 2013. The mean individual F and ewe F were zero in the early years of the studied period. The increased values of inbreeding in some years may be due to the poor controlling of close relative matings and excessive using of some individuals as breeding rams.The zero values of individual F in the years of 2001, 2002, 2008 and 2009 indicated that the prevention of close matings has been occurred. Fluctuations in the individual and maternal F tendency indicated that the control of inbreeding in the flock has not been managed properly. Effective population size as a criterion of the size of ideal population was calculated in average as 51.8 animals vs. 0.33 % estimated for individual inbreeding coefficient (Table 4). The maximum value of N_e accompanied with the minimum value of F (Figure 1). This would indicate that as the effective population size decreased (decreasing the hetrozygosity of alleles) the cumulative homozygosity and inbreeding is increased. In a closed flock, such as Valachian sheep, N_e was estimated to be 20.6 animals vs. 1.69 % estimated for the individual inbreeding of the whole population

Table 2: Distribution of animals in different classes of individual F for studied traits

Traits	Groups of F									
	F = 0		0 < F < 6.25		6.25 ≤ F < 12.5		12.5 ≤ F < 18.75		F ≥ 25	
	% animal	Mean	% animal	Mean	% animal	Mean	% animal	Mean	% animal	Mean
GFW1 (kg)	87.83	0.44^{a}	10.00	0.46^{a}	1.19	0.44^{a}	0.70	0.42^{a}	0.28	0.30^{b}
GFW2 (kg)	89.13	1.19^{abc}	9.05	1.42^{a}	0.90	1.14^{bc}	0.55	1.25^{ab}	0.37	0.95^{c}
CR (%)	93.24	88.08^{a}	5.23	92.31^{a}	0.64	87.50^{a}	0.64	62.50^{b}	0.25	66.67^{b}
GL (day)	91.09	149.23^{a}	7.20	149.68^{a}	0.85	150.29^{a}	0.61	148.80^{a}	0.25	149.00^{a}
NLB	93.14	1.03^{a}	5.56	1.08^{a}	0.65	1.00^{a}	0.45	1.40^{b}	0.20	1.00^{a}
NLAW	93.14	0.97^{a}	5.56	1.05^{a}	0.65	1.00^{a}	0.45	1.20^{a}	0.20	1.00^{a}
LMLB (kg)	93.12	4.12^{a}	5.57	4.35^{a}	0.65	4.27^{a}	0.46	3.67^{a}	0.20	3.90^{a}
LMLW (kg)	92.84	19.22^{a}	5.85	19.23^{a}	0.71	19.59^{a}	0.40	18.60^{a}	0.20	18.25^{a}

Differences between two levels of the same factor with different letter are significant at P<0.05 based on Duncan's test; GFW1, greasy fleece weight at 6 months of age; GFW2, greasy fleece weight at 18 months of age; CR, conception rate; GL, gestation length; NLB, number of lambs born; NLAW, number of lambs alive at weaning; LMWLB, litter mean weight per lamb born; LMWLW, litter mean weight per lamb weaned

(Oravcová and Krupa, 2011). Therefore, the inbreeding can be avoided using an appropriate number of males and females in the breeding programs. This was in agreement with other studies (Falconer and Mackay 1996; Caballero and Toro, 2000), where an inverse relationship was observed between the effective population size and the inbreeding coefficient. Leroy *et al.* (2013) revealed that depending on breed, species and computation method, effective population size may vary quite widely.

The correlation between individual and ewe inbreeding coefficient was estimated as 0.15. The mean value of this correlation was reported to be 0.10 in Texel, Shropshire and Oxford Down sheep (Norberg and Sorensen, 2007). This value establishes a separation between the individual and ewe effects of inbreeding (Norberg and Sorensen, 2007).

In general, the differences in studied traits between the categorical inbreeding levels were almost significant (Table 2). However, the effects of inbreeding based merely on inbreeding levels cannot be estimated exactly. To properly recognize the amount of probable harmfulness and / or usefulness effects of inbreeding, the calculating of the numerical values of regression coefficients is essential. Along with the linear regression coefficient it is essential to consider the quadratic regression coefficient as well (Santana *et al.*, 2010). The quadratic regression coefficient clarifies the tail end of the linear regression of inbreeding coefficient on the traits. Generally, in the present study, the studied traits were affected negatively by the active inbreeding coefficients. The rate of inbreeding was 0.08 % for all animals per year during the 24 years of the period of the study. With an average generation interval of 3.6 years, it was calculated that the period from 1990 to 2013 involved approximately 6.6 generations. The rate of inbreeding, therefore, seemed to accrue at a rate of 0.53 % per generation. The rapid increase in inbreeding

Table 3: Descriptive statistics for inbreeding coefficients for the studied population of Makuie sheep

	All population			Inbred population		
	Female+ male	Female	Male	Female+ male	Female	Male
Animal, no	5860	3122	2738	559	299	260
Mean (%)	0.332	0.33	0.29	4.61	2.98	3.02
SD (%)	1.83	1.89	1.76	4.87	4.94	4.81
SE	0.02	0.0003	0.0003	0.21	0.003	0.003
Minimum (%)	0.00	0.00	0.00	0.003	0.012	0.003
Maximum (%)	25.00	25.00	25.00	25.00	25.00	25.00

Table 4: Number of lambs and distribution into inbreeding classes from 1990 to 2013

Year of birth	No.	F = 0	0 < F < 6.25	6.25 ≤ F < 12.5	12.5 ≤ F < 18.75	F ≥ 25	Ne	GI
1990-1992	696	100.0	0.00	0.00	0.00	0.00	52.29	3.44
1993-1995	825	99.50	0.00	0.25	0.25	0.00	49.55	2.91
1996-1998	781	98.00	0.37	0.63	0.75	0.25	48.14	3.96
1999-2001	611	91.64	4.60	0.98	1.63	1.15	37.93	3.75
2002-2004	608	87.33	9.38	1.98	1.15	0.16	54.10	3.66
2005-2007	601	69.88	27.12	2.50	0.50	0.00	48.43	4.30
2008-2010	629	99.20	0.32	0.00	0.00	0.48	55.34	3.41
2011-2013	576	60.94	34.55	2.08	2.43	0.00	68.43	3.58

Ne, effective population size; GI, generation interval

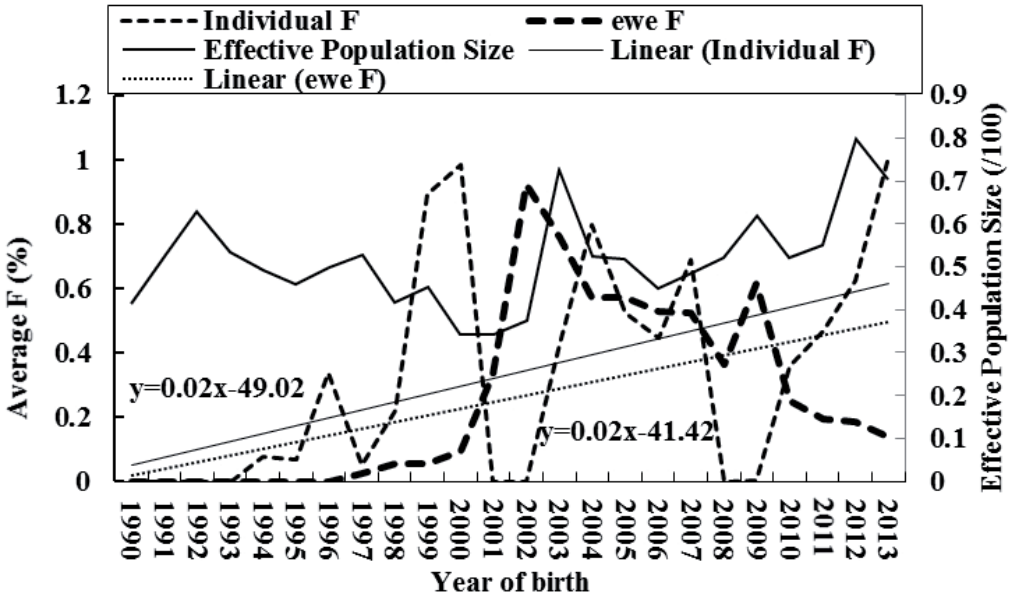

Fig. 1: Average individual and ewe F (%) of population and effective population size by year of birth

Table 5: Likelihood values of six different models for studied traits (the appropriate models are in bold faced)

Traits	MF	h^2	C^2	Log- likelihood values					
				Model I	Model II	Model III	Model IV	Model V	Model VI
GFW1 (kg)	II	0.20	0.06	1466.74	**1470.88**	1466.74	1466.74	1466.74	1466.74
GFW2 (kg)	I	0.22	-	**231.21**	231.21	231.21	231.21	231.21	231.21
CR (%)	I	0.07	-	**716.80**	716.80	716.80	716.80	716.80	716.80
GL (day)	I	0.08	-	**-998.62**	-998.62	-998.62	-998.62	-998.62	-998.62
NLB	I	0.03	-	**1199.97**	1200.78	1199.97	1199.97	1199.97	1199.97
NLAW	I	0.03	-	**676.51**	678.04	676.51	676.51	676.51	676.51
LMLB (kg)	I	0.20	-	**109.61**	109.61	109.61	109.61	109.61	109.61
LMLW (kg)	I	0.12	-	**-1550.32**	-1550.32	-1550.32	-1550.32	-1550.32	-1550.32

GFW1, greasy fleece weight at 6 months of age; GFW2, greasy fleece weight at 18 months of age; CR, conception rate; GL, gestation length; NLB, number of lambs born; NLAW, number of lambs alive at weaning; LMWLB, litter mean weight per lamb born; LMWLW, litter mean weight per lamb weaned; MF, model fitted; h^2, direct heritability; C^2, variance ratio due to permanent environmental component

levels could be attributed to the declining population size and the ratio of males to females over the studied period, especially in the later years. In animal breeding, it is recommended to maintain ΔF of at most 0.5 to 1.0 % per generation (Norberg and Sorensen, 2007). The annual inbreeding rate of this study was higher than the estimate of Dorostkar *et al.* (2012) and lower than those of Swanepoel *et al.* (2007) and Norberg and Sorensen (2007).

Model selection

Table 5 shows that the best model for GFW1 was the model II. Although the model I was selected for GFW2 and reproductive traits. Then the direct additive genetic variance, maternal permanent environmental variance (so-called dam-lamb association such as uterus environment, amount of milk production, milk composition and udder conditions), linear regression coefficient, quadratic regression coefficient and residual

Table 6: Linear and quadratic regression coefficients of individual and ewe F of studied traits for a 0.01 change in inbreeding

Traits	MF	Fixed effects (mean square)				Inbreeding coefficient				Random effects	
						Individual F		ewe F			
		YR	SX	BT	AD	b_1 (S.E)	b_2 (S.E)	b_1 (S.E)	b_2 (S.E)	h^2	C^2
GFW1 (kg)	II	29.77***	15.13***	0.15^{NS}	0.61*	0.06 (0.07)^{NS}	- 0.007 (0.03)***	- 0.12 (0.08)^{NS}	0.05 (0.03)^{NS}	0.20	0.06
GFW2 (kg)	I	0.80^{NS}	10.86***	0.32^{NS}	0.22^{NS}	1.14 (0.31)**	- 0.40 (0.14)***	0.82 (0.30)*	- 0.32 (0.14)*	0.22	-
CR (%)	I	0.30*	-	0.12^{NS}	0.14^{NS}	-	-	0.55 (0.40)^{NS}	- 0.22 (0.18)*	0.07	-
GL (day)	I	20.37*	-	0.05^{NS}	75.15***	-	-	1.46 (2.93)^{NS}	- 0.37 (1.32)^{NS}	0.08	-
NLB	I	0.21^{NS}	-	0.10^{NS}	3.92***	-	-	- 0.29 (0.27)**	0.16 (0.12)^{NS}	0.03	-
NLAW	I	0.21^{NS}	-	0.25^{NS}	4.05***	-	-	0.09 (0.44)*	0.005 (0.20)^{NS}	0.03	-
LMLB (kg)	I	8.44***	-	0.01^{NS}	477.92***	-	-	1.68 (0.78)^{NS}	- 0.63 (0.35)**	0.20	-
LMLW (kg)	I	150.58**	-	0.17^{NS}	450.12***	-	-	- 0.43 (5.14)^{NS}	0.27 (2.31)^{NS}	0.12	-

GFW1, greasy fleece weight at 6 months of age; GFW2, greasy fleece weight at 18 months of age; CR, conception rate; GL, gestation length; NLB, number of lambs born; NLAW, number of lambs alive at weaning; LMWLB, litter mean weight per lamb born; LMWLW, litter mean weight per lamb weaned; MF, model fitted based on the log-likelihood ratio test; YR, year; SX, sex; BT, birth type; AD; age of dam; b_1, linear regression coefficient; b_2, quadratic regression coefficient; h^2, direct heritability; C^2, variance ratio due to permanent environmental component; *, Significant at 0.05 probability level; **, significant at 0.01 probability level; ***, significant at 0.001 probability level

variance were the main sources of variation for traits which are recorded in the early stages of life. In other words the younger individuals in addition to the direct additive genetic and inbreeding effects were the direct object of maternal permanent environment (Safari *et al.*, 2005).

Greasy fleece weight traits

Table 2 shows that the individual inbreeding level at the value more than 25 % results in significant (P<0.05) loss of GFW1. Estimated quadratic regression coefficient of individual F for GFW1 was significantly different from zero (P<0.001; - 0.007), whilst linear regression coefficient of individual F did not significantly differ from zero. Due to the high value of wool production in the commercial aspects of a flock holder, high inbreeding coefficients may be resulted in the economic loses. GFW2 was affected positively by the passive individual F (<6.25) and negatively by the active individual F (>6.25). The linear and quadratic regression coefficients of individual F for GFW2 were estimated at 1.14 and - 0.40, respectively (Table 6). The results of the present study showed that the fleece weight of Makuie sheep at the mature age (18 months of age) was more influenced by the high levels of inbreeding than other breeds i.e. Elsenburg Dormer Sheep stud (Van Wyk *et al.*, 2009). This finding strengthened the advantages of the hypothesis of passive inbreeding coefficient. In the other words, individual F at the low ranges may be a useful approach to accumulate the beneficial genes resulting in the promotion of the population. In the report of Ercanbrack and Knight (1991) the linear individual F had a harmful effect on fleece weight, whereas the quadratic ones were estimated to be non-significant. In Hissardale sheep, the linear regression coefficient of pre-mature fleece weight was estimated to be - 0.0002 per 1 % increasing of individual inbreeding coefficient (Akhtar *et al*., 2000).

Reproductive traits

Conception rate

The mean values of studied traits in the inbred and non-inbred population are summarized in Table 2. Apparently the conception rate of inbred ewes with an inbreeding coefficient lower than 6.25 % was higher than that of non-inbred ones. Concerning a significance level of 5 % only the groups 4 (12.5≤F<18.75) and 5 (F≥25) were negatively differed from other groups (Table 2). Therefore, the lower inbreeding coefficients (or passive inbreeding) may not be considered as a deleterious effect on CR. The linear and quadratic regression coefficients of CR were estimated to be 0.55 and - 0.22 per 0.01 changes in ewe F, respectively (Table 6). The deleterious effects of inbreeding on the reproductive traits have been reported by Ercanbrack

and Knight, 1991; Van Wyk *et al.*, 1993; Boujenane and Chami, 1997; Akhtar *et al.*, 2000; Mandal *et al.*, 2005; Swanepoel *et al.*, 2007.

Ercanbrack and Knight (1991) demonstrated the non-significant effect of quadratic regression of CR, but the linear regression coefficients of CR for Rombouillet, Targhee and Columbia sheep were estimated to be - 0.23, - 0.01 and - 0.09, respectively.

Gestation period

In the present study regression coefficients for gestation period were not significantly differed from zero (Table 6). Also, table 2 shows that there were no significant differences between five ewe´s groups for F value. There are reports, which show the significant effect of inbreeding on the increase in the gestation period in other species i.e. cattle (Rollins *et al*., 1956) and pig (Farkas *et al.*, 2007).

Number of lambs born

NLB as a criterion of the litter size at birth was affected negatively (P<0.01) by the linear and positively (not significantly) by the quadratic effects of ewe´s F. The linear and quadratic regression coefficients of NLB were calculated to be - 0.29 and 0.16, respectively (Table 6). In Texel, Shropshire and Oxford Down the linear regression coefficients of NLB were reported to be - 0.032, - 0.019 and - 0.03, respectively (Norberg and Sorensen, 2007). Boujenane and Chami (1997) reported non-significant effect of inbreeding coefficient for litter size at birth. Neither linear regression nor quadratic regression coefficients were significant for NLB in Elsenburg Dormer sheep stud (Van Wyk *et al.*, 1993).

Number of lambs alive at weaning

For NLAW, however, table 2 demonstrates no significant differences between five groups studied. However, regarding table 6, inbred ewes produced lambs whose mortality increased linearly along with the increasing of the ewe‘s inbreeding. The results of present study in general were in agreement with those of Ercanbrack and Knight (1991), Van Wyk *et al.* (1993) and Boujenane and Chami (1997).

Litter mean weight per lamb born and weaned

LMWLB as a criterion of mass lambs weight produced by the ewe was decreased significantly (P<0.01) by 0.63 kg per 0.01 change in the ewe‘s inbreeding. The quadratic regression coefficient of ewe F for LMWLB was estimated as significant (P<0.01) - 0.63.

Table 2 and Table 6 coordinately show that LMWLW were not affected by different levels of ewe inbreeding coefficients. The linear and quadratic regression coefficient for litter mean weight at weaning (LMWLW) were calculated (not significantly) to be - 0.43 and 0.27, respectively. These findings were in agreement with those of Boujenane and Chami (1997). Linear regression coefficient of Sardi sheep for litter weight at 90 days of age was estimated at - 0.01 (Boujenane and Chami, 1997).

CONCLUSION

Despite the low level of inbreeding in Makuie sheep in this study, in general it was showed that inbreeding had significant effects on the studied traits. The results revealed that the low levels of individual or ewe F, (lower than 6.25 %) can be considered as a reliable measure in gathering the promoting genes in the studied population. Inbreeding level of the flock can be maintained in a non-harmful level by using an acceptable number of males and females and/or by preventing of close relative matings. Compared to quadratic regression results, estimation of inbreeding depression based on linear regression may lead to wrong decisions about the genetic structure of the flock. To have an appropriate estimate of the deleterious effects of the inbreeding, both the individual and ewe F should be evaluated in the population.

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PRINCIPAL COMPONENT ANALYSIS OF BODY MEASUREMENTS IN A POPULATION OF INDIGENOUS NIGERIAN CHICKENS RAISED UNDER EXTENSIVE MANAGEMENT SYSTEM

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ABSTRACT

This study was carried out to investigate the relationship among body measurements in indigenous Nigerian chickens sampled in Niger state using principal component analysis with the view of identifying components that best define body conformation in them. A total of 750 birds were used for the study. The parameters recorded were: body weight, body length, body girth, wing length, shank length and shank thickness. The descriptive statistics showed that the mean body weight was 1.69 kg while the body measurements were 38.77 cm, 25.30 cm, 22.23 cm, 11.01 cm and 1.10 mm for body length, body girth, wing length, shank length and shank thickness, respectively. The coefficients of correlation obtained were: r = 0.709 (between body weight and body length), r = 0.448 (between body weight and body girth), r = 0.667 (between body weight and wing length), r = 0.203 (between body weight and shank length) and, r = 0.499 (between body weight and shank thickness) respectively. Principal component analysis with variance maximizing orthogonal rotation was used to extract the components. Two principal components were extracted in the chickens explaining 66.4 % of the total variation in the original variables. Generally, the first principal component had the largest share of the total variance and correlated highly with body weight, body length and wing length while the second principal component had its loadings on shank length. These components could be used as selection criteria for improving body weight of indigenous Nigerian chickens.

Key words: indigenous chicken; body measurements; extensive management; principal component analysis

INTRODUCTION

The Nigerian indigenous chicken represents a large pool of untapped genetic resource. In spite of increase in the growth of the poultry industry in Nigeria (particularly with the introduction of exotic chicken breeds), the indigenous chicken breeds still remain the largest source of poultry meat and eggs. Although they are generally less productive when compared to the exotic species, indigenous chickens play a vital role in the socio-economic life of those keeping them (Alabi e*t al*., 2012). It is important to have knowledge of the variation of morphometric traits in local genetic resources as such measurements have been discovered to be very useful in comparing body size and by implication, shape of animals (Latshaw and Bishop, 2001). Such comparison could be used as basis for selection and improvement programmes.

Growth in the indigenous chicken like in all animals apart from relating to increase in body cells and volume is a complex process. It is controlled by both genetic and non-genetic factors (Kor *et al.*, 2006).

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Rosario *et al.* (2003) opined that the mechanisms involved in chicken growth are too multifaceted to be explained using univariate analysis. This according to them is because the traits are biologically linked due to linkage of gene loci and the effect of pleiotropy. Principal component analysis (PCA), a multivariate procedure could be a leeway to solving problems associated with univariate analysis of growth and related traits. This is due to its ability to reduce related variables into lesser number of uncorrelated variables called principal components. Jolliffe (2002) stated that the components will be arranged in such a way that the first few components will retain most of the variations existing in the original variables (Udeh and Ogbu, 2011). Multivariate analysis involving the use of principal components has been reported by Yakubu *et al.* (2009). The study was however carried out in a different location. In genetic terms, every ecological niche (i.e. ecological zone or environment) is governed by its own peculiar variability. The present study was carried out therefore to estimate body weight from body measurements of indigenous Nigerian chickens sampled in Niger state using orthogonal conformation traits derived from principal components.

MATERIAL AND METHODS

Study area

The study was carried out in Niger state, Nigeria. Niger state is located in the Southern guinea savannah area on longitude 30˚2′ North and latitude 11˚3′ East. The state has a land area of 80,000 square kilometres with maximum altitude at its highest point of 1475 m above sea level. The state experiences distinct dry and wet seasons with annual rainfall varying from 1100 mm in the north to 1600 mm in the south. The maximum temperature does not exceed 39˚C and is experienced between March and June. The minimal temperature (as low as 21˚C) is usually experienced between December and January.

Data collection

Seven hundred and fifty (750) indigenous chickens (male and female) between the ages of 5 to 6 months and above (i. e. 20 to 24 weeks) were sampled in the three (3) agricultural zones of the state. The birds were randomly sampled at Bida, Lavun and Badeggi (representing zone A), Minna, Paikoro and Gwada (representing Zone B) and at Kontagora, Tegina and Rijau (representing zone C).

Parameters measured

Body weight of individual birds was measured using a mechanical hanging balance of 2.5 kg with a precision of 20 g. The following metric measures were recorded using tape rule (cm): body length (BL), body girth (BG), wing length (WL) and shank length (SL). Shank thickness (ST) was determined using vernier calliper. The metric measurements were as described by Fayeye *et al*. (2006). The reference points were: body length (distance from the tip of the beak, through the body trunk to the tail), body girth (the circumference of the breast region), wing length (length of the wing from the scapula joint to the last digit of the wing), shank length (length of the tarso-metatarsus from the hock joint to the metatarsal pad) and shank thickness (diameter of the tarso-metatarsus just below the spur).

Data analysis

Means, standard errors and coefficient of variation of body weight and body measurements of the chickens were obtained using Microsoft Excel 2007. The data was pooled for both sexes. Pearson correlation coefficients among the body measurements were calculated and the correlation matrix was the primary data required for principal component analysis (PCA). Bartlett's test of sphericity was used to test if the correlation matrix was an identity matrix or a correlation matrix full of zeros. The suitability of the data set to carry out PCA was further tested using the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy. This tested whether the partial correlations among variables were small. A KMO measure of 0.60 and above was considered adequate (Eyduran *et al.*, 2010). The stepwise multiple regression procedure was used to obtain models for predicting body weight from body measurements (a) and from factor scores (b).

$$BWT = a + B1X1 + \ldots + BkXk \quad (a)$$

$$BWT = a + B1FS1 + \ldots + BkFSk \quad (b)$$

Where, *BWT* is the body weight, *a* is the regression intercept, *B1* is the i^{th} partial regression coefficient of the i^{th} linear body measurement (*X1*) or the i^{th} factor scores (*FS*). The principal components analyses and multiple regressions were carried out using the SPSS 16 (2007) statistical package.

RESULTS

Table 1 shows the descriptive statistics for body weight and body measurement traits of indigenous Nigerian chickens (pooled data because of non significant effect of sex, P>0.05). The mean body weight was 1.69 kg while the body measurements were 38.77 cm (BL), 25.30 cm (BG), 22.23 cm (WL), 11.01 cm (SL) and 1.10 mm (ST), respectively. Shank thickness varied more (coefficient of variation = 38.53 %) while body girth (coefficient of variation = 5.87 %) varied the least.

Table 1: Mean, standard deviation (SD) and coefficient of variation (CV %) for live body weight and body measurements of indigenous Nigerian chickens

Parameter	Mean	SD	CV
Body weight (kg)	1.69	0.35	20.95
Body length (cm)	38.77	7.14	18.42
Body girth (cm)	25.30	1.49	5.87
Wing length (cm)	22.23	1.56	7.00
Shank length (cm)	11.01	1.34	12.20
Shank thickness (mm)	1.10	0.42	38.53

Table 2: Correlation coefficients between live body weight and body measurements of indigenous Nigerian chicken

	BW	BL	BG	WL	SL	ST
Body weight (BW)	1					
Body length (BL)	0.709**	1				
Body girth (BG)	0.448**	0.351**	1			
Wing length (WL)	0.667**	0.696**	0.516**	1		
Shank length (SL)	0.203**	0.213**	0.212**	0.200**	1	
Shank thickness (ST)	0.499**	0.419**	0.359**	0.406**	0.124**	1

Correlation coefficients were all highly significant ($p<0.01$)

The skeletal dimensions (BL, SL and ST) were more variable (coefficient of variation ranged from 12.20 to 38.53 %) compared to the flesh dimension (BG).

The coefficient of correlation of body weight and body measurements of indigenous Nigerian chicken is presented in Table 2. The correlation ranged from r = 0.124 to r = 0.709. The relationships between body weight and all the body measurements were positive and highly significant ($P<0.01$). The highest correlation was obtained between body weight and body length while correlation between shank length and shank thickness was observed to be the least. Kaiser-Meyer Olkin (KMO) measure of sampling adequacy was 0.804 while results of the Bartlett test of sphericity was significant (chi-square 1473.00; P = 0.000).

The Eigen value of the total variance, the rotated component matrix and communalities of body weight and body measurements are presented in Table 3. The communalities ranged from 0.456 (BG) to 0.963 (SL). The Eigen value showed the amount of variance explained by each of the factors out of the total variance. Two common factors were identified with Eigen values of 3.049 (PC1) and 0.936 (PC2). The two factors combined accounted for 66.40 % of the total variability present in the parameters measured. PC1 had high loadings on wing length (0.840), body weight (0.826) and body length (0.814) while PC2 being orthogonal to PC1, loaded heavily on shank length (0.997). Negative loading was observed only for shank thickness (PC2).

The results of regression analysis for predicting live body weight from the five interdependent body measurements of indigenous Nigerian chicken showed that body length alone accounted for 38.3 % of the variability in live body weight. The proportion of explained variation gradually increased from 44.2 % when body girth was added, to 60.2 % when all the five body measurements (BL, BG, WL, SL and ST) were used in the equation. PC1 and PC2 together accounted for 68.4 % of the variation in live body weight of the indigenous Nigerian chickens.

DISCUSSION

The Information on body weight and body measurements of the birds showed that they were

Table 3: Explained variation linked to rotated component matrix, communalities, Eigen values and percentage of total variance of body measurements of indigenous Nigerian chicken

Parameter	PC1	PC2	Communalities	Unique factor
Body weight	0.826	0.049	0.684	0.316
Body length	0.814	0.125	0.678	0.322
Body girth	0.613	0.248	0.456	0.544
Wing length	0.840	0.152	0.728	0.272
Shank length	0.095	0.997	0.963	0.037
Shank thickness	0.689	-0.015	0.475	0.525
Eigen value	3.049	0.936		
% of total variance	50.81	15.59	66.40	
Description	General size	Shank length		

PC = principal component

Table 4: Multiple regression (stepwise) of live body weight (kg) on original body measurements and their orthogonal traits of indigenous Nigerian chicken

Step	Predictor	Intercept	Regression coefficient	SE	R^2
Original body measurements as independent variables					
1	Body length	- 1.555	0.074	0.46	0.383
2	Body length	- 2.419	0.065	0.45	0.442
	Body girth		0.048		
3	Body length	- 2.641	0.046	0.43	0.454
	Body girth		0.029		
	Wing length		0.066		
4	Body length	- 2.181	0.050	0.33	0.577
	Body girth		0.028		
	Wing length		0.054		
	Shank length		0.003		
5	Body length	- 2.082	0.044	0.32	0.602
	Body girth		0.021		
	Wing length		0.050		
	Shank length		0.003		
	Shank thickness		0.351		
Orthogonal traits as independent variables					
1	FC1	1.327	0.484	0.33	0.682
2	FC1	1.327	0.484	0.33	0.684
	FC2		0.029		

SE: standard error of estimate; FC = factor; R^2 = coefficient of determination

heavier than those reported by Yakubu *et al.* (2009) in normal feathered, naked neck and frizzled indigenous Nigerian chickens. The values for body length, body girth and shank length were also superior to the values reported by Peters *et al.* (2010) in Nigerian native chickens. These differences could be due to environmental factors, differences in the genetic makeup of the birds and feed availability in the ecological niches where the birds are reared.

Body weight related highly and positively with all the original body measurements of the chickens. This is suggestive of their possible usage (i. e. the body measurements) in the prediction of body weight in the indigenous chickens. This is because an increase in any of the body measurement will invariably lead to a corresponding increase in the body weight of the chickens (Ajayi *et al*., 2008). Shank length had the lowest correlation coefficient with body weight. Shank length is a non economic part of the chicken; so even if it does not grow proportionately as the chicken grows, it might not necessarily be a bother to farmers. The strong relationship existing between body weight and body measurements may be useful as a selection criterion. This is because correlated traits are more likely to be governed by the same gene action. Yakubu *et al*. (2009) reported that this could be the basis for genetic manipulation and upgrading of the native chicken stock.

The observed high value of Kaiser-Meyer-Olkin measure of sampling adequacy (0.804) means that correlations between the variables were not unique, that is not related to the remaining variables outside each sample correlation. Kaiser (1960) reported a measure of sampling adequacy above 0.80 to be meritorious. The significance of the correlation matrices tested with Bartlett's Test of Sphericity for the body measurements of indigenous chicken provided ample support for the authenticity of using factor analysis for the data set.

The high communalities gave further credence to the appropriateness of the principal component analysis. According to Wuensch (2012), communalities represents the amount of the variable that is accounted for by the components (since the loadings are correlations between variables and components and the components are orthogonal, a variable's communality is the coefficient of determination of the variable predicted from the components). Similarly high communalities have been reported in Nigerian indigenous chicken by Yakubu *et al*. (2009), in different breeds of broiler chickens (Mendes, 2011; Udeh and Ogbu, 2011; Ajayi *et al*., 2012) and in indigenous turkey (Ogah, 2011a). The low contribution of shank length to PC1 was not too surprising as the trait equally had the lowest correlation with body weight. This is a clear indication of its weakness in explaining the total variation in the body measurement of the indigenous chickens. Body girth had the lowest communality with about 45.6 % of the variation accounted for by common factors and 54.4 % of the variation accounted for by unique factors related to it alone. The communalities for the skeletal dimensions (body length, wing length and shank length) were higher than for the flesh dimension (body girth). Ogah (2011b) reported similar findings working with adult Muscovy ducks.

The first principal component (PC1) had the highest variability correlating very much with body weight, body length and wing length. This has been the trend in most studies (Kashimawura *et al*., 2001; Salako, 2006; Sadek *et al*., 2006). Body length alone accounted for 38.3 % of the variation in body weight of the indigenous Nigerian chickens. This is much lower than the 83.0 % reported by Yakubu *et al.* (2009) for Nigerian indigenous chickens managed extensively. The amount of variation was however enhanced with the inclusion of more independent variables in the equation similar to the findings of Ajayi *et al*. (2012). The use of PC1 as a single predictor in the present study explained 68.2 % of the total variability in the body weight of indigenous Nigerian chickens. Combination of PC1 and PC2 only led to a 0.29 % improvement in the amount of variance explained in the chickens. Orthogonal variables gave a better and more dependable estimation of body weight than the use of the original independent variables. This is because of the problem of multicollinearity commonly connected with the use of interdependent original body dimensions. According to Malau-Aduli *et al.* (2004), multicollinearity is associated with unstable estimates of regression coefficients.

CONCLUSION

This study revealed the interdependency of the five original body measurement characters on each other. This interdependency was explored by analysing them at the same time using principal component analysis rather than by analysing them separately. Also, orthogonal body measurements obtained from the analysis was discovered to be a more appropriate means of predicting live body weight in indigenous Nigerian chickens than the use of the original interrelated traits measured.

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INFLUENCE OF COMPLETE FEED MIXTURE CONTAINING NAKED OAT OF THE TATRAN VARIETY ON PARAMETERS OF UTILITY AND QUALITY OF EGGS OF LAYING HENS

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ABSTRACT

The aim of this experiment was to define the nutritional response of laying hens (Hy-Line Brown hybrid) receiving feed mixture consisting partly of Tatran naked oat on the utility parameters and egg quality in comparison with control group, which was fed a complete feed mixture based on the maize-wheat-soybean extract meal. Totally 72 hens were divided into two groups of 36 animals per each group. In the experimental group, hens were fed with a feed mixture based on 60 % of naked oat of the Tatran variety, maize and soybean extract meal. In the control group, the laying hens were fed with feed mixture based on maize, wheat, soya extract meal and the sunflower oil. Hens of experimental group had statistically significantly higher average of egg production (276.07 ± 0.05 pieces, 16.96 ± 0.004 kg) per 1 hen against control group (267.97 ± 0.062 pieces, 16.11 ± 0.005 kg) ($P<0.001$). The average weight of eggs was significantly higher ($P<0.001$) in the experimental group (61.42 ± 3.160 g) compared to the control group (60.13 ± 3.412 g). The number of non-standard eggs per hen was significantly lower (13.79 ± 0.041) in the experimental group in comparison with the control group (15.89 ± 0.045) ($P<0.05$). Likewise, significantly higher average number of double-yolk eggs (2.09 ± 0.018) per hen was recorded in the experimental group. Only the colour of egg yolk was significantly higher ($P<0.01$) in the control group in comparison with the experimental group. For a whole season we recorded significantly higher daily consumption of feed calculated per 1 egg (125.95 ± 17.828 g) and per 1 kg of the egg mass (2.094 ± 0.311 kg) in the control group ($P<0.001$) in comparison with the experimental group (121.38 ± 14.664 g) per 1 egg and (1.976 ± 0.257 kg) per 1 kg of the egg mass. The results suggest that the naked oat-based feed mixture for laying hens might improve intensity of the egg production, increase the weight of eggs, reduce the number of non-standard eggs and consequently reduce cost of the egg production.

Key words: laying hens; naked oat; diet; egg quality

INTRODUCTION

Naked oat is phenotypically characterized by non-lignified husk, which becomes detached during harvesting and this leads to increased metabolisable energy in feed mixture for poultry. Their potential in poultry nutrition has been increased by selection for high oil content. High-oil naked oat lines yielded 12 % more metabolizable energy than wheat. Naked oats, excluding the experimental high-oil lines, yielded 8.5 % more energy than simultaneously assayed wheat samples. The addition of β-glucanase produced an increase of about 4 % in the apparent metabolisable energy of oats for broiler chickens (MacLeod *et al*., 2008).

Naked oats has higher proportion of essential amino-acids than wheat or barley. This offers the possibility of replacing some imported soya and animal proteins by valuable energy of oats. Further advantages of naked oats include a high concentration of polyunsaturated oils (40 % of oil is monounsaturated,

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40 % polyunsaturated) and significant antioxidant activity. The latter two characteristics confer benefits on egg and meat quality. The health benefits of polyunsaturated oils could make eggs and meat from oat-fed hens more attractive to consumers. The effects on meat quality have been shown to include enhanced sensory evaluation and properties such as low drip loss and longer shelf life (Lopez-Bote *et al.*, 1998a, b).

Research shows that naked oat supplemented with feed-grade lysine and methionine or with canola meal, to the exclusion of soybean meal, dietary levels with content of 70 or 88 % naked oat supported egg laying equally to the corn-soy control. By the 88 % level of naked oat, egg size was significantly increased. This is of benefit to younger laying flocks, giving better egg grades and higher monetary returns (Burrows *et al*., 1993).

Naked oats provide a successful alternative to corn as the staple grain of poultry layer and breeder diets, and can replace a large part of soybean meal traditionally used in such diets. When supplemented with lysine plus methionine or with canola meal, no soybean meal is required in the naked oat diet. While egg yield was satisfactory, egg weight was increased from levels of 300 $g.kg^{-1}$ and feed utilization was decreased at levels of 600 to 874 $g.kg^{-1}$ (Cave *et al.*, 1989).

Naked oats may be used by 40 % inclusion in broiler diets with no adverse effect on growth, feed efficiency, dressing percentage or bone strength (Maurice *et al*., 1985).

MATERIAL AND METHODS

The aim of this experiment was to verify the effect of inclusion of naked oat of the Tatran variety into complete feed mixture for high-production laying hens, on egg performance and quality of eggs, in comparison with control group, which was fed with a complete feed mixture based on maize, wheat, soya extract meal and the sunflower oil.

The Tatran variety is medium to early maturing of naked oat variety. This variety has good resistance to powdery mildew, stem rust, crown rust, leaf stripe. Resistance to lodging is consistent with the Avenuda variety. The average of weight of thousand grains is 29.14 g, volume weight is 64.0 $kg.hl^{-1}$ and low percentage of husk corns (3.1 %). The Tatran variety overcame the control Avenuda variety in pure grain yield by 5 % for 2 years (MPSR, 2010).

Totally, 72 laying hens were divided into the experimental group (36 pieces of hens fed with feed mixture based on 60 % of naked oat of the Tatran variety, maize and soybean extract meal) and the control group (36 pieces of hens fed with feed mixture based on maize-wheat-soybean extract meal and the sunflower oil). The mineral feed and premix of the supplement mass were used in the same production batch. This experiment was performed on laying hens with brown egg shell production of hybrid Hy-Line Brown starting since 140 days of age. Hen's condition was verified by

Table 1: Composition and nutritional value of feed mixtures

Components (%)	Mixture		Nutritional value ($g.kg^{-1}$)	Mixture	
	CFM-E	CFM-C		CFM-E	CFM-C
Ground naked oat „Tatran"	60.00	-	Dry matter	893.0	887.0
Ground maize	15.20	38.00	Nitrogenous compounds	163.0	162.0
Ground wheat	-	30.00	Fat	62.7	45.0
Soybean extract meal	15.40	21.10	Fiber	18.4	22.3
Sunflower oil	-	1.50	Ash	107.0	105.0
Calcium carbonate	7.80	7.80	NFE	541.9	552.7
Monocalcium phosphate	0.70	0.70	Carbohydrates	33.6	36.0
Sodium chloride	0.30	0.30	Starch	427.0	424.0
Vitamin-mineral premix	0.50	0.50	Ca	33.6	30.6
Methionine 99 %	0.10	0.10	P	5.70	5.26
Totally	100.00	100.00	Na	1.38	1.45

NFE- nitrogen-free extract
CFM-E - complete feed mixture in the experimental group
CFM-C - complete feed mixture in the control group

individual weighing before starting the experiment.

The production test was divided in two phases. The first phase of the egg production lasted since 20th to 46th weeks of age and the second phase since 47th to the first day of 62nd weeks of hen´s age. Pullets were randomly divided into pairs and placed to the cages for laying hens. Microclimatic condition and light regime were regulated on the principles of valid technological condition for hybrid production. The experiment was finished by the second day after 62nd weeks of hen´s age.

The live weight of hens was detected by individual weighing three times during the experiment: first time in 20 weeks of hen´s age, second time in 46 weeks of age and third time in 62 weeks of hen´s age.

Complete feed mixtures were fed *ad libitum*. Fresh clean water was always at disposal from automatic poultry drinker.

To ensure the correct microclimate housing conditions, temperature and humidity were monitored three times a day (Table 2).

An essential part of the investigation of the actual development of the animals were individual weighing of all laying hens included to the experiment with the exactness of 5 gramm (at the beginning of the experiment, at the end of the first phase of production, in hens of 47th week of age, at the end of the experiment). The egg production was monitored once per day and collectively.

The level of utility was monitored with precise control of feed consumption of (by group). The average consumption of feed and nutritions was calculated per 1 egg and per 1 kg of eggs. The number of non-standard eggs was recorded daily in both groups. The number of eggs with cracked shell, broke shell, double-yolk, shell-free egg or other non-standards were recorded individually. The weight of eggs was recorded daily in both groups.

From each group 10 pieces of eggs were evaluated, which were randomly selected in monitoring of quality indicators of eggs. For each of four weeks the following parameters were evaluated: egg-yolk index, egg-white index, compactness of shell (the weight and surface of the egg). Egg quality was determined using the Haugh units (Haugh, 1937), colour of raw egg yolk – by a scale of Hoffmann La Roche (Weis *et al.*, 2002). Sensory quality of eggs, such as taste and smell of boiled eggs were evaluated twice during the experiment (each time on 5 pieces of random selected eggs from each group on 25th and 45th weeks of age of laying hens).

The cost of consumed feed was evaluated every day per 1 hen, per 1 egg, per 1 kg of eggs. Results of experiment were statistically evaluated by Student‘s t-test.

Table 2: Climatic and technological indicators from 20th to 62nd weeks of hen´s age

Temperature in hall (˚C)			
Age of laying hens	Min	Max	$\bar{x}$
20th – 46th week	10	18	14,55
47th – 62nd week	14	26	20,15
20th – 62nd week	10	26	16,57
Relative humidity in hall (%)			
Age of laying hens	Min	Max	$\bar{x}$
20th – 46th week	29	82	53,53
47th – 62nd week	35	86	62,82
20th – 62nd week	29	86	56,88

RESULTS AND DISCUSSION

At the first weighing we recorded higher average live weight of hens in the control group. At the second and last weighing we recorded the higher average live weight of hens in the experimental group. However, these differences were not statistically significant.

Hartini *et al.* (2003) confirmed highly statistically significant weight of the gizzard ($P<0.01$) in laying hens, which were fed with oats and statistically highly significant total weight of juvenile hens fed oats. Positive effect on the weight of laying hens was significantly confirmed ($P<0.05$) in the previous study of Brenesl *et al.* (1993).

The whole season of egg production lasted from the 22nd to the first day of the 62nd weeks of hen´s age. During the whole season all evaluated indicators of egg production were better parameters of

Table 3: Egg production

Group	$\bar{x}$	s	T test (significance)
The average egg production per 1 hen (piece)			
1.	276.07	0.050	***
2.	267.97	0.062	5.689E-09
The average egg production per 1 hen (kg)			
1.	16.96	0.004	***
2.	16.11	0.005	5.503E-14
The average weight of eggs (g)			
1.	61.42	3.160	***
2.	60.13	3.412	2.045E-06
The average number of non-standard eggs per 1 hen			
1.	13.79	0.041	*
2.	15.89	0.45	0.0481
The average number of double-yolk eggs per 1 hen			
1.	2.09	0.018	**
2.	0.94	0.011	0.0018

1. Experimental group (complete feed mixture based on 60 % of naked oat, maize and soybean); 2. Control group (complete feed mixture based on the maize, wheat, soybean, and sunflowers oil); N eggs = 295; N hens in the experimental group = 36; N hens in the control group = 36; *P<0.05; **P<0.01; ***P<0.001

the utility in the experimental group in comparison to the control group. Animals of experimental group had statistically significantly higher average egg production (276.07 ± 0.050 pieces, 16.96 ± 0.004 kg) per 1 hen compared to the control group (267.97 ± 0.062 pieces, 16.11 ± 0.005 kg) (P<0.001). The average weight of eggs was significantly higher (P<0.001) in the experimental group (61.42 ± 3.160 g) in comparison with the control group (60.13 ± 3.412 g). The significantly lower (P<0.05) number of non-standard eggs per hen (13.79 ± 0.041) was recorded in the experimental group in comparison with the control group (15.89 ± 0.045). We recorded significantly higher average number of double-yolk eggs (2.09 ± 0.018) per 1 hen only in the experimental group in comparison with control group (15.89 ± 0.045) (Table 3).

Our results suggest that naked oat is a valuable feedstuff with positive influence on the egg production, which is related to nutrition profile of naked oat. In the experimental group of hens we detected higher intensity of egg production P<0.001 in comparison with the control group. Recorded results are in agreement with the previous finding of MacLeod (2010), who confirmed a trend to raise the average of laying hens fed with oats.

Table 4: Feed consumption

Group	$\bar{x}$	s	T test (significance)
The average feed consumption per 1 egg			
1.	121.38	14.664	***
2.	125.95	17.828	3.872E-04
The average feed consumption per 1 kg of egg mass			
1.	1.976	0.257	***
2.	2.094	0.311	2.159E-07

1. Experimental group (complete feed mixture based on 60 % of naked oat, maize and soybean); 2. Control group (complete feed mixture based on the maize, wheat, soybean, and sunflowers oil); N eggs = 295; *P<0.05; **P<0.01; ***P<0.001

Increasing in the egg production of laying hens fed with oats was confirmed in the study of Bennett and Classen (2003). Shafey *et al.* (1999) detected significantly higher weight of yolk and higher share of unsaturated fatty acids (oleic, linoleic and linolenic) in the yolk mass in hens, which were fed with naked oat. Hsun and Maurice (1992) stated that the naked oats can be used to replace all or part of the maize meal and part of the soyabean meal without any reduction in performance.

For the whole season we detected daily significantly higher consumption of feed per 1 egg and per 1 kg of the egg mass in the control group (P<0.001) in comparison to the experimental group (Table 4). These results related to the high available energy content of a naked oat.

According to Scheideler *et al.* (1998) oats increased the weight of eggs and potential nutrient digestibility in laying hens. It is also worth noting that oats, a high-fibre cereal, is being used in north-western Europe for feeding poultry, especially during the moulting, and obviously with positive results (Pottgüter and Tierzucht, 2008). Sokol *et al*. (2004) confirmed that the feed consumption does not decrease when naked oat is added into feed mixtures.

In laying hens, Krimpen (2008) observed that the nutrient dilution and addition of (coarse) insoluble non-starch polysaccharides increases feeding related behavior, as expressed by prolonged eating time and decreased eating rate. Supplementation with 15 % of diluted diets to rearing hen resulted in less damage of feather during the laying period.

Qualitative properties of eggs involve the evaluation of Haugh units, yolk colour, egg white index, yolk index and egg soundness. The results of evaluation are shown in table 5. The colour of egg yolk was different between groups in relative terms 15.25 % for the control group. These differences were also statistically very highly significant (P <0.001) for control group. Other qualitative characteristics of eggs were not statistically significant.

For the whole season of the experiment we found an average egg yolk index in the control group at 37.16 and 36.95 in the experimental group. The Haugh units were different between groups in relative units 0.18 % for the experimental group.

The naked oat does not contain carotenoids, which influence the final concentration of pigments in the yolk, for that reason the intensity of yolk colour was significantly lower. Oat has lack of carotene pigment and yolk colour intensity decreases with increasing content of oat (Burrows *et al*. 1993). According to Hammershøj and Steenfeldt (2005), yolk colour became significantly lighter and more yellow with lupin content, but darker

Table 5: Qualitative evaluation of eggs (average of the two ratings)

Group	n	$\bar{x}$	s	T test (significance)
Color of the egg yolk				
1.	100	4.50	0.249	**
2.	100	5.31	0.401	0.000037
The Haugh units				
1.	60	105.57	4.803	-
2.	60	105.38	6.066	0.851089
Egg yolk index				
1.	100	36.95	1.756	-
2.	100	37.16	1.547	0.774654
Egg white index				
1.	100	168.30	11.764	-
2.	100	167.91	11.722	0.941817
Egg soundness				
1.	100	1.00	0.083	-
2.	100	0.98	0.098	0.784885

1. Experimental group (complete feed mixture based on 60 % of naked oat, maize and soybean); 2. Control group (complete feed mixture based on the maize, wheat, soybean, and sunflowers oil); 1.*P<0.05; **P<0.01; ***P<0.001

and less greenish with foraging material. The decisive factor for obtaining the desired colour is the content of egg yolk carotenoids in feed mixture for laying hens. Factors responsible for the colour of yolk are: feed mixture, physiological factors, health status of laying hens, feed production, and properties of carotenoid premix (Baker and Günther, 2004).

CONCLUSION

Through interventions in hen's nutrition it is possible to influence the health of laying hens, utility and also enrich the final product for important substances for human nutritional needs. In the experiment of hybrid Hy-Line Brown laying hens we tested the variety of naked oat called Tatran. The live weight of laying hens was not affected by the experimental intervention. The average parameters of the egg production were higher in the experimental group, which was fed with a feed mixture based on 60 % of naked oat of the Tatran variety, maize and soybean extract meal. The number of non-standard eggs and share of the egg production was lower in the experimental group. Using qualitative evaluation of eggs, we found statistically very significant differences in yolk color in comparison to control group. In others qualitative evaluations there differences between the groups were slight and statistically insignificant. At the sensory evaluation of boiled eggs we did not find significant differences. The taste and smell of eggs were found as a typical for eggs. For a whole experimental season, significantly lower ($P<0.001$) cost of consumed feed mixture in the experimental group per 1 egg and per 1 kg of the egg mass was recorded in comparison with the control group. Significantly higher average egg production per 1 laying hen was recorded in the animals of the experimental group in comparison with the control group. Significantly higher number of egg masses, as well as the average weight of eggs was obtained in the experimental group.

On the basis of the obtained results we can recommend for practice to use the experimental complete feed mixture based on 60 % of naked oat of the Tatran variety, maize and soybean extract meal, which significantly improved the egg production and the weight of eggs, whilest the feed consumption was reduced compared to the control complete feed mixture used in this experiment.

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QUALITATIVE CHARACTERISTICS, MICROBIAL POPULATIONS AND NUTRITIVE VALUES OF ORANGE PULP ENSILED WITH NITROGEN SUPPLEMENTATION

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ABSTRACT

The aim of this study was to evaluate the qualitative characteristics, microbial profile and nutritive value of high-moisture orange pulp co-ensiled with poultry by-product meal (PBM) or urea as additional nitrogen (N) source. The optimum dry matter (DM) content of each experimental silage was achieved by adding the appropriate proportion of wheat straw. A control silage was also prepared with orange pulp and wheat straw, but without any supplemental nitrogen. Each experimental silage was ensiled in three replicates of 12 kg mini-silos and left intact for 90 days. The evaluated traits were: silage pH, microbial population, lactate, acetate, butyrate, crude protein (CP), true protein and NH_3-N contents. In addition to these, *in situ* dry matter and CP degradability as well as ruminal and post-ruminal CP disappearance rates were also measured. Both N-supplemented silages showed higher pH values and CP contents compared to the control silage ($P<0.05$). With regard to NH_3-N, the lowest values were detected in silage supplemented with PBM ($P<0.05$). The highest acetate production and total bacteria (TB) count ($P<0.05$) and the lowest lactic acid bacteria count ($P<0.05$) were observed in silage supplemented with urea. Control silage and urea supplemented silage had the highest and lowest lactate contents, respectively. Addition of PBM and urea did not alter degradation rates (c) of DM and CP, however, the latter caused a significantly increased ($P<0.05$) potentially degradable CP fraction (b). The highest ($P<0.05$) post-ruminal CP disappearance rate, gas production rate and ME value were observed in silage supplemented with PBM ($P<0.05$). In conclusion, both nitrogen sources used in this study enhanced nutritive value of orange pulp silage. Ensiling may be applied as a practical approach for long-term preservation of fresh orange pulp.

Key words: orange pulp; silage quality; *in situ* degradability

INTRODUCTION

Growing up feeds cost values in many parts of the world have increased attending in utilization of citrus by-product feedstuffs as specific feeds for ruminants. One of the citrus by-products that produced exceedingly is orange pulp and its cost is partly low compared to its nutritive value. Citrus pulp is by-product derived from the citrus juice industry and includes mixture of citrus peel, pulp and seeds (Lashkari and Taghizadeh, 2011). Citrus pulp is a suitable energy supplement, but is low in CP and neutral detergent fiber (NDF). Citrus pulp also has high potential rumen degradability, high apparent digestibility and considered as pectin-rich foods (Lashkari and Taghizadeh, 2012). It contains little starch and an excellent high-fiber energy source. High moisture content is main problem in conserving of this feeds and utilizing it with high moisture and high sugar content because of spoiling, fungi and mold exposed at risk human and animal health. More than 30 % of fresh citrus pulp was wasted by feeding wet citrus pulp (Arthington and Pate, 2001).

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Therefore, it is used in animal feeding after dehydration or ensiling processes and most of research related to citrus pulp focus on dried products (Fegeros *et al*., 1995). The process of drying is costly and often inconvenient, but using the ensiling citrus pulp is cheaper than dry processing and can be easily accomplished by the farmer. Ensiling losses due to the high moisture content of citrus pulp are high and sticky nature makes it difficult to storage in sheds, bunkers or silos (Bampidis and Robinson, 2005) and high moisture silages promote seepage losses from the silo. To avoid these problems and prepare the appropriate dry matter for ensiling, citrus pulp was ensiled with high dry matter feeds such as chopped wheat straw which limits ensiling losses and gives to the silage the characteristics of a suitable and cheap substitute for farm forages (Scerra *et al.*, 2001). Nutritive value of ensiled feed such as crop residues and low quality feeds can be improved with such additive as non-protein nitrogen and animal protein source (Schingoethe *et al.*, 1980). Also, in order to increase the protein content in control silage (citrus pulp plus straw) poultry by-product meal and urea was added.

The objectives of this research were to evaluate the orange pulp silage quality and microbial contents, *in situ* degradability of DM and CP, *in vitro* gas production and fermentation characteristics, with the addition of straw and different nitrogen supplementation.

MATERIAL AND METHODS

Silage preparation and treatment

Fresh orange pulp without further processing after juicing was coarsely chopped to 5 cm pieces using a machine meant for chopping whole plant maize. Due to the high moisture content and the physical property (after chopping) of orange pulp, wheat straw was added as an absorbent. The PBM samples were randomly collected from rendering unit of industrial poultry slaughter-houses in the east Azerbaijan Province, Iran. Poultry by-product meal included the following ground, cleaned, and rendered carcass parts of poultry including heads, feet, viscera and trace amounts of feathers and blood. PBM was processed at approximately 142 °C and at approximately 380 kPas. Then PBM were dried at 110 °C. Silage masses were mixed after addition with protein additives, and the compositions of silages were as follows: 1) 73 % orange pulp + 27 % straw (control), 2) 74 % orange pulp + 12 % straw + 14 % poultry by-product meal (OSP) and 3) 63 % orange pulp + 25 % straw + 12 % urea solution (3 %). Silages were ensiled for 90 days in 12-kg plastic buckets (triplicate per treatment) and their compositions are listed in Table 1.

Silage analyses

Silage samples were obtained from each silo after opening and dried in a forced air oven at 60 °C for 48 h. Dried samples were ground using a grinder with a 1-mm sieve and analyzed for ash, ether extract and crude protein as described by AOAC (2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using an ANKOM$^{200/220}$ Fibre Analyser (Ankom Technology Corporation, Macedon, NY) according to the manufacturer's instructions without sodium sulphite. In addition, NDF was analyzed without amylase with ash included. For measurement of pH, silage (15 g) was blended with 135 ml of deionized water for 30 s. The homogenate was filtered through two layers of cheesecloth and pH was immediately measured (Zahiroddini *et al*., 2004). The filtrate was used for the determination of volatile fatty acids (VFA) and NH_3-N. Subsamples of filtrate were prepared for analysis of VFA by adding 1 ml of 25 % (wt/vol) meta-phosphoric acid to 5 ml of filtrate. Lactic acid in water extracts of the silages was determined by spectrophotometry according to Barker and Summerson (1941). Another 5 ml of filtrate were combined with 0.4 ml of 65 % trichloric acid for analysis of NH_3-N, as described by Markham (1942). In order to analyze VFA and NH_3-N, samples were stored on ice and then stored at - 40 °C. Before analysis, the samples were thawed overnight at 4 °C. Silage VFAs were quantified using gas chromatography (WCOT Fused Silica Capillary,

Table 1: Chemical composition of silage ingredient (g/kg)

Type of silage	DM[1]	CP[2]	OM[3]	NDF[4]	ADF[5]
Orange pulp	130	78	912	234	164
Wheat straw	870	40	940	884	590
Poultry-by product meal	900	560	900	15	0

1 - DM = Dry matter, 2 - OM= Organic matter (g.kg^{-1} DM), 3 - CP = Crude protein (g.kg^{-1} DM), 4 - EE = Ether extract (g.kg^{-1} DM), 5 - NDF = Neutral detergent fiber (g.kg^{-1} DM), 6 - ADF = Acid detergent fiber (g.kg^{-1} DM)

chrompack CP 9002), flame ionization detection and crotonic acid was used as the internal standard.

Crude protein fraction

The true protein, neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) fractions were determined according to standardization and recommendations published by Licitra *et al.* (1996). True protein was calculated as nitrogen precipitated with 100 $g.l^{-1}$ (w/v) of trichloroacetic acid. The procedure used here is based on NDF and ADF prepared. After that, association nitrogen into NDF and ADF measured as NDIN and ADIN, respectively.

Enumeration of microorganisms

For isolation and enumeration of microorganisms, 11 g of fresh silage were added to 99 ml of sterile 70mM potassium phosphate buffer (pH 7) and agitated for 60 s (Zahiroddini *et al.* 2004). Extracts required for enumeration of microorganisms were prepared from fresh silage as described by Zahiroddini *et al.* (2004). A semi-selective lactobacilli medium (MRS) and the nutrient agar (NA) were used for the isolation of lactic acid bacteria (LAB) and total bacteria (TB), respectively. Sabouraud's dextrose agar (SDA; Difco) was used for the isolation of yeasts and moulds. Serial dilutions (10^{-2} to 10^{-7}) of the suspension were prepared and 100 µl aliquots of three consecutive dilutions were plated onto a medium in triplicates. Lactobacilli MRS agar and nutrient agar contained 200 µg/ml of cycloheximide (Sigma, Mississauga) and SDA contained 100 µg/ml each of tetracycline and chloramphenicol. Lactobacilli MRS agar and NA plates were placed in an incubator at 37 °C for 24-48 h and SDA plates were incubated at 25 °C for 48-72 h. Colonies were counted from the plates at appropriate dilutions containing a minimum of 30 and a maximum of 300 colonies per plate and the number of colony forming units (cfu) was expressed per gram of fresh silages.

***In situ* study**

Ruminal disappearance of DM and CP of silages were determined using a nylon bag technique (Ørskov and McDonald, 1979). Silage samples were dried at 60 °C in a forced air oven for 48 h and ground through a 2 mm screen and ground samples (5 g) were placed in Dacron bags (12 cm × 6 cm with 50 µm pore size). Each feed sample was incubated in 6 replicates (2 replicates) in the rumen of three wethers (mean weight of 43.9 ± 4 kg). The incubation times for samples were 0, 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 h. Nylon bags were suspended in the rumen in a polyester mesh bag (25 × 40 cm, 3 mm pore size) and were removed from the rumen at the same time, so that all bags could be washed simultaneously. The nylon bags were then removed from the mesh bag and washed with a washing machine until the rinse water remained clear. Bags were then dried in forced air oven at 60 °C until a constant weight was achieved before determination of DM disappearance and CP analysis. The kinetics of *in situ* DM and CP disappearance were estimated using a non-linear procedure of SAS (1991). The model of McDonald (1979) was fitted to the percentage of DM and CP disappearance as:

$Y = a + b\,(1 - e^{-ct})$

Where, "*a*" - is the soluble fraction, "*b*" - the slowly disappearing fraction, "*c*" - the fractional rate of disappearance (per h) and t - is the incubation time (h). Effective ruminal disappearance was estimated using the following model:

$Y = a + bc/(c + k)$,

where "*k*" is the fractional rate of particulate passage, assumed to be 0.03 and 0.05/h (Ørskov and McDonald, 1979).

Modified three-step procedure

This part of the experiment followed the procedure of Gargallo *et al.* (2006). Approximately, 5 g of a sample were weighed and placed into a 5 cm × 10 cm nylon bags, 50 µm pore size dacron polyester bag (four bags per sample) and suspended in the rumen of three wethers (mean weight of 43.9 ± 2.4 kg) fitted with permanent rumen cannulae for 12 h. Bags were then removed and washed with washing machine and dried in a forced-air oven at 55°C for 48 h and weighed. Samples from each bag were taken for N analysis using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator). After weighing, the residue (0.5 g) was put in *in situ* bag and placed into an ANKOM Daisy incubator for determination of post ruminal digestibility (Gargallo *et al*., 2006). Briefly, samples were incubated in a pepsin/HCl solution for 1 h in a Daisy incubator, followed by the incubation in a pancreatin/KH_2PO_4 solution for 24 h. After 24 h, the liquid was drained from the bottles, and bags were again rinsed until the runoff was clear. Bags were allowed to drain and were dried in a forced hot air oven at 55 °C for 48 h. The dry weights of the samples and bags were recorded, and bags were opened and pooled by the sample for CP analysis.

***In vitro* gas production**

Dried samples (300 mg) were placed into the vial with 100 ml of serum and each sample was incubated in 6 replicates with 20 ml of rumen liquor and buffer solution (1:2). McDougall (1948) buffer solution was prepared and placed into a water bath at 39 °C. Rumen liquor samples were obtained from three wethers

(mean weight of 43.9 ± 2.4 kg) fitted with permanent rumen cannulae and fed on a diet comprising (DM basis) of 550 $g.kg^{-1}$ alfalfa hay, 400 $g.kg^{-1}$ barely grain, 48 $g.kg^{-1}$ wheat bran and 2 $g.kg^{-1}$ lime stone at maintenance level. Rumen fluid was collected after the morning feeding and pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in six replicates with 20 ml of rumen liquor and buffer solution (1:2). Six vials were used as blank samples. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc. Melors dark, USA) set at 120 rpm and housed in an incubator. Amounts of cumulative gas production were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72 and 96 h of incubation using a water displacement apparatus (Fedorak and Hurdy, 1983).

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979):

$P = A\ (1\text{-}e^{-ct})$

where p is the gas production at time t, "*A*" is the gas production of soluble fraction and potentially degradable fraction, and "c" is the gas production rate.

The metabolizable energy, short chain fatty acids and digestible organic matter in dry matter of silages were calculated using equations of Menke and Steingass (1988) and Getachew *et al.* (2002) as follows:

DOM (% DM) = 9.00 + 0.9991 GP + 0.0595 CP + 0.0181 ash ($n = 200$, $r^2 = 0.92$)

ME (MJ/kg DM) = 0.016 DOMD

SCFA (mmol/200 mg DM) = 0.0222 GP - 0.00425

where: DOMD is digestible organic matter in a dry matter; GP is 24 h net gas production (ml /200mg DM); CP and ash (% DM).

Statistical analysis

Experimental data were performed using GLM procedure of SAS (1991) for completely randomized design. Data of each mini-silo of the three individual silos within each treatment were averaged. Mean values of each individual silo within each treatment (three silos of each) were used as the experimental unit, and the statistical model was:

$Y_{ij} = \mu + T + e_{ij}$

Dependent variable representing the response for i treatment; μ = mean; T = treatment and e_{ij} = residual. The means were compared using Duncan's test used for the multiple comparisons among mean values for the treatments.

RESULTS AND DISCUSSION

Silage characteristics

Chemical composition and microbial counts ($log10.cfu.g^{-1}$ fresh silage) of silages after 90 days of ensiling are outlined in Table 2. The higher protein content of silages supplemented with urea and PBM in comparison with control silage was undoubtedly due to the addition of urea and PBM. The pH value of the control and PBM silages was lower ($P<0.05$) than urea silage. Fegeros *et al.* (1994) reported that citrus pulp contains high amount of pectin and soluble carbohydrates. As a result, the fermentation of high soluble carbohydrates in this by-product leads to low final pH in the both control silage and silage supplemented with PBM (McDonald *et al.*, 1991). Its high soluble carbohydrates lead to a rapid production of alcohol as well as of VFA's and lactic acid. In our study, control silage and silage supplemented with PBM had considerable amounts of lactic acid (Table 2). Low pH and high lactic acid in the citrus pulp silages has been reported by Gado *et al.* (2011). Nevertheless, pH values of control silage and silage supplemented with PBM were near to required value for acceptable preservation of silage containing such high DM content (McDonald *et al*., 1991). Increased pH of silage supplemented with urea may be due to the extensive conversion of urea to ammonia-N resulting in high ammonia-N concentration (McDonald *et al*., 1991). In addition, CP content of silage supplemented with PBM was higher than in the control silage but ammonia-N was not affected by the addition of PBM to silage. Reduced protein degradation in silage with PBM was demonstrated by lower NH_3-N in silage supplemented with PBM versus silage supplemented with urea. This result indicated that the silage microorganisms were not capable to break down and convert the PBM protein to ammonia-N.

Highest mean value of total bacteria (TB) population was observed in silage supplemented with urea ($P<0.05$), whereas lactic acid bacteria (LAB) population was the lowest ($P<0.05$) in the silage supplemented with urea. Yeast counts were not affected ($P = 0.89$) by different N-supplements. It is noticeable that no yeasts and moulds were observed in silage supplemented with urea supplementation cultures. Highest mean value of the TB population in silage supplemented with urea with lower lactic acid bacteria (LAB) population could increase the silage pH and preservation of silage from spoilage (Table 2). The sufficient amount of lactic acid indicates the proper silage quality in this experiment. Holzer *et al*. (2003) suggested that lactic acid fermentation is a suitable method to preserve silage from spoilage

and pathogenic organisms, such as yeasts, moulds, enterobacteria and clostridia. The preservative effect is chiefly due to acid production and pH decline but is also a result of diminishing the oxidation-reduction potential and competition for essential nutrients. It is also possibly caused by the production of inhibitory compounds (Bonestroo *et al.*, 1993). High pH observed in silage supplemented with urea in our study was similar to the results of Sinclair *et al.* (2004), who added urea (20 g/kg DM) to crop silage and found that the final pH increased up to 8. Lactic acid bacteria were effective bacteria on the final fermentation products of silage. These bacteria produce more lactic acid and less acetic acid and butyric acid, resulting in low final pH of silage (Keles and Demirci, 2010).

Lower LAB and TB counts in control silage and silage supplemented with PBM compared to silage supplemented with urea might be due to microbial cell death (Inglis *et al*., 1999), also LAB contains an inhibitory effect on various gram-negative and gram-positive bacteria by their production of hydrogen peroxide and bacteriocins (Chateau *et al*., 1993). Danner *et al.* (2003) suggested that lactate and acetate in the silage have antimicrobial effects and proposed that lactate and acetate have a lipophilic character; that results in agglutination of acid molecule which penetrates the bacterial plasma membrane.

Yeast counts were not affected (P = 0.89) by introducing different N-supplements. High numbers of yeasts and molds in control silage and silage supplemented with PBM compared to silage with urea supplementation may be due to higher level of

Table 2: Chemical and microbial composition of orange pulp[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with Urea	SEM
Dry matter (g/kg)	276. 5^{b}	311. 2^{a}	270^{b}	5.45
pH	4.14^{c}	4.29^{b}	8.13^{a}	0.70
Analysis (g/kg DM)				
Organic matter	898.61^{a}	904.42^{a}	844.38^{b}	7.60
Crude protein	63.00^{c}	196.40^{a}	148.00^{b}	1.97
Ether extract	10.39^{c}	36.38^{a}	18.51^{b}	3.84
Neutral detergent fiber	558.46^{a}	357.85^{b}	589.89^{a}	38.10
Acid detergent fiber	311.74^{a}	217.46^{b}	336.15^{a}	20.10
Total protein (g/kg total N)	540.46^{b}	824.92^{a}	464.54^{c}	54.90
NDIN (g/kg total N)	34.80^{b}	9.40^{a}	19.59^{c}	11.54
ADIN (g/kg total N)	16.82^{a}	6.64^{c}	10.68^{b}	1.49
NH_3-N (g/kg total N)	48.20^{b}	16.41^{c}	256.75^{a}	37.08
Lactic acid	37.20^{a}	30.00^{b}	12.46^{c}	3.68
Acetic acid	5.71^{c}	7.53^{b}	10.20^{a}	0.68
Propionic acid	2.77^{b}	5.53^{a}	5.51^{a}	0.48
Butyric acid	2.25^{b}	3.81^{a}	3.53^{a}	0.31
Total Fatty acid	47.61^{a}	47.19^{a}	30.06^{b}	3.01
Lactic acid : Acetic acid ratio	6.53^{a}	3.99^{b}	1.22^{c}	0.78
Enumerations (log cfu/g fresh silage)				
Total bacteria	3.48^{b}	3.50^{b}	3.65^{a}	0.02
Lactic acid bacteria	3.45^{a}	3.47^{a}	3.38^{b}	0.01
Yeasts	2.33	2.39	no	0.21
Molds	4.00	2.30	no	0.51

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with Urea, orange pulp plus straw plus urea.
NDIN = Neutral detergent insoluble nitrogen, ADIN = Acid detergent insoluble nitrogen; [4]Standard error of the mean; no: not observed.

lactic acid in these silages. Kung *et al.* (2003) suggested that lactic acid exhibited lower activity for inhibition of growth the yeasts and moulds. It is noticeable that no yeasts and moulds were observed in silage with urea (Table 2), and it is likely due to the higher concentration of acetic acid and lower concentration of lactic acid in the silage supplemented with urea. Acetic acid is a known fraction inhibitor of both yeasts and moulds (Woolford, 1975), as well as it has been reported that acetic acid is one of the most effective substances for inhibition of spoilage microorganisms. Acetic acid in silages is necessary to successfull preservation the silages after the silos when they opened (Danner *et al.*, 2003). Inoculants containing *Lactobacillus buchneri*, a heterofermentative LAB aim to promote in acetic acid are specifically designed to reduce aerobic deterioration of silage by yeasts and moulds (Kung and Ranjit, 2001).

***In situ* DM and CP disappearance**

Soluble (*a*) and insoluble (*b*) fractions of DM were affected by N-supplementation (P<0.05, Table 3). Dry matter degradation rate (*c*) of the b fraction for all silages showed similar values (P = 0.10). The effective degradability of DM (EDDM) was highest for silage supplemented with PBM at two rumen flow rates (P<0.05). The results of DM degradability show that true protein content of the silages increased the EDDM and positively affected by PBM, however, in silage supplemented with urea true protein content did not increase, therefore EDDM was not influenced by increased CP content. These results are conflicting with those of Ramírez *et al.* (2004), who reported that CP content of grasses positively influenced EDDM, because this investigator reported, that with increasing of CP the EDDM content was also increased. Likewise, the high EDDM in silage supplemented with PBM may be related to lower NDF and ADF content in comparison to other silages. This result was in agreement with Woods *et al.* (2003), who found that there was high negative correlation between EDDM and the ADF and NDF components of the diet.

At two outflow rates the effective degradability of CP (EDCP) silage supplemented with urea was higher (P<0.05) than the other silages. According to this result, the "*c*" values were not affected by N-supplementation (P = 0.12). Woods *et al*. (2003) suggested that the "*c*" value of the "*b*" fraction has

Table 3: Dry matter and crude protein disappearance (g/g incubated) and estimated parameters of silage[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with Urea	SEM
DM degradation[2]				
a	0.146[b]	0.152[b]	0.172[a]	0.0410
b	0.472[a]	0.450[a]	0.386[b]	0.0178
c (per h)	0.038[a]	0.031[a]	0.037[a]	0.0050
a + *b*	0.618[b]	0.651[a]	0.558[c]	0.0143
EDDM (0.03/h)	0.417[b]	0.437[a]	0.382[c]	0.0820
EDDM (0.05/h)	0.360[b]	0.384[a]	0.326[c]	0.0871
CP degradation[2]				
a	0.196[b]	0.050[c]	0.474	0.062
b	0.431[a]	0.444[a]	0.296[b]	0.023
c (per h)	0.041	0.043	0.048	0.061
a + *b*	0.496[c]	0.496[b]	0.770[a]	0.039
EDCP (0.03/h)	0.446[b]	0.313[c]	0.684[a]	0.005
EDCP (0.05/h)	0.391[b]	0.256[c]	0.65[a]	0.059

Means within a row with different subscripts differ (P<0.05).
[1]Control, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
[2]a = rapidly degraded fraction, b = slowly degraded fraction, a + b = maximum potential of degradability in the rumen. c = rate of degradation (per h), EDDM (0.03/h) = effective degradability of DM with passage rate of 0.03/h, EDDM (0.05/h) = effective degradability of DM with passage rate of 0.05/h. EDCP (0.03/h) = effective degradability of CP with passage rate of 0.03/h, EDCP (0.05/h) = effective degradability of CP with passage rate of 0.05/h. [4]Standard error of the mean.

a dramatic role in the determination of effective degradability. In the present study degradation rates were not influenced by N-supplementation, but the "*b*" values have significant difference between the silages; so that the "*b*" values showed a major role in determination of EDCP. Silage supplemented with urea had a higher "*a*" fraction and lower "*b*" value than the other silages and the EDCP value was higher for silage supplemented with urea, resulting in similar "*c*" values; "*a*" fraction had significant effect on EDCP. Also this fact was observed for silage supplemented with PBM and control, where the "*b*" value was low and resulted in decreased EDCP at two outflow rates.

Ruminal, post-ruminal and total tract protein disappearance

The results indicated an effect (P<0.05) of N-supplementation on ruminal, post-ruminal and total tract protein disappearance of silages (Tables 4). Silage supplemented with urea had the highest value for ruminal CP disappearance (P<0.05), whereas the highest (P<0.05) post-ruminal protein disappearance of ruminal-undegraded protein was observed in silage supplemented with PBM. Highest post-ruminal protein disappearance in silage supplemented with PBM supplementation may be due to the low NDF and ADIN. Van Soest (1994) suggested that the differences in ruminal and post-ruminal protein disappearance of the tropical feed sources may be due to differences in NDF and ADIN between these feeds. Low ruminal CP disappearance was compensated by digestion in post ruminal; resulting in a higher total tract disappearance in silage supplemented with PBM. Lashkari and Taghizadeh (2012) reported that in the citrus by-product such as sweet lemon pulp with low ruminal CP disappearance, the post ruminal disappearance has been high and this result may be due to the compensatory digestion in small intestine. For silage supplemented with PBM, higher values for post ruminal (P<0.05) showed that this silage can escape the rumen fermentation and supply the rumen undegradable protein requirement. The highest total tract CP disappearance in silage supplemented with urea was consistent with the result of Danesh Mesgaran and Stern (2005), who found that in maize silage treated with 24 g urea kg^{-1} DM had higher total tract protein disappearance compared with the other treatment.

***In vitro* gas production**

Potential (*A*) and rates (*c*) of gas production differed (P<0.05) among the silage treatments (Table 5). The "*A*" and "*c*" values were lowest (P<0.05) for silage supplemented with urea. Short chain fatty acid (SCFA) and ME in control silage and silage supplemented with PBM was higher (P<0.05) than silage supplemented with urea (P<0.05). Silage supplemented with PBM provides more potential gas production which is fermentable energy source. As an expected CP contents had an effect on gas production after 24 h of incubation. It has been reported that protein degradation influences gas production (Cone and Van Gelder, 1999). The CP content may have effect on gas production after 12 or 24 h of incubation that was also reported by Chenost *et al.* (2001). Differences in total gas production between silages could be explained by the differences in SCFA production and molar proportion of SCFA (Beuvink and Spoelstra, 1992). Likewise, because of the low contents of NDF and ADF the silage supplemented with PBM has high "*A*" value. This result was supported by the result of Gurbuz (2007) that estimated parameters and ME were negatively correlated with NDF and ADF, which is slowly fermented by microorganisms. In addition, it can be clearly observed that NDF and ADF in control silage and silage supplemented with urea were higher than silage supplemented with PBM. An increase in NDF and ADF resulted in the low gas production rate (*c*) in control silage and silage supplemented with urea. This result

Table 4: Ruminal, post-ruminal and total tract protein disappearance (g/g incubated) of silage[1]

Item	Control silage	Silage supplemented with PBM	Silage supplemented with urea	SEM
Ruminal[2]	0.487[b]	0.208[c]	0.621[a]	0.0609
Post-ruminal[3]	0.389[b]	0.619[a]	0.277[c]	0.0502
Total tract protein disappearance[4]	0.827[b]	0.876[a]	0.899[a]	0.0110

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
[2]CP disappearance after 12 h incubation in the rumen using a nylon bag technique.
[3]Rumen- undegradable protein digestibility determined by the three-step procedure.
[4]Summation of ruminal and post ruminal.

Table 5: Estimated parameters and fermentation characteristics using *in vitro* gas production of silage[1]

	Control silage	Silage supplemented with PBM	Silage supplemented with urea	SEM
A	300.93[b]	322.25[a]	300.06[b]	3.97
c (per h)	2.90[b]	3.00[a]	2.00[c]	0.16
GP	182.16[a]	181.71[a]	96.16[b]	9.89
DOMD	459.78[a]	467.88[a]	294.15[b]	19.59
ME	7.35[a]	7.48[a]	4.70[b]	0.31
SCFA	0.80[a]	0.80[a]	0.42[b]	0.04

Means within a row with different subscripts differ (P<0.05).
[1]Control silage, orange pulp plus straw; silage supplemented with PBM, orange pulp plus straw plus poultry by-product meal; silage supplemented with urea, orange pulp plus straw plus urea.
A = potential gas production (ml/g DM); *c* = fractional rate of gas production (per h); GP = 24 h cumulative gas production (ml/g DM); DOMD: digestible organic matter in dry matter (g/kg DM); ME = metabolizable energy (MJ/kg DM); SCFA short chain fatty acid (mmol /200 mg DM)

was consistent with the findings of Kamalak (2006) who found that "*c*" value was negatively correlated with NDF and ADF. This suppressing effect probably results in the attachment of ruminal microorganisms to feed particles (McAllister *et al.*, 1994). Control silage and silage supplemented with PBM possess high DOM amounts; the resulted silage with higher gas production contains higher DOM.

CONCLUSION

The nutritive value of orange pulp was improved by the addition of PBM and urea, whereas control silage and silage with PBM were preserved, what can be illustrated by the proper fermentation characteristics, such as low pH, acetic, butyric acids and high lactic acid. Result indicated that LAB and TB counts were not affected by adding the PBM. Various microorganisms present in silage may affect the nutritive value of silages. LAB was probably mainly responsible for lowering the pH during ensiling. Fermentative products and microbiological assessments of silage can help us to define the type of fermentation that occurred in the silo.

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THE EFFECTS OF BENZOIC ACID AND PROTEIN LEVEL ON URINE PH AND AMMONIA EMISSION OF PIGS

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ABSTRACT

Twelve hybrids gilts (initial BW 29.9 ± 1.7 kg) were used for evaluation to identify the effect of benzoic acid and dietary protein in the diets on urine pH and ammonia of the slurry by growing piglets. We used two protein concentrations - high protein 18.8 % (HP) and low protein 14.0 % (LP) and two benzoic acid levels 0.0 % and 1.0 %. The same energy level (13.3 MJ. kg) in the diets was maintained by supplementation with rapeseed oil. The piglets were housed in metabolic cages and fed with two equal doses at 7 a.m. and 5 p.m. at a daily rate of 90 g. $kg^{0.75}$. Water was offered *ad libitum*. Each experimental period consisted of a 6-d adapted and was followed by a 4-d collection phase. During the collection phase faces and urine (using bladder catheters) were collected. Experimental data were subjected to ANOVA and when significant value was observed for treatment effect, the differences between means were assessed using Fisher's LSD procedure. Nitrogen and dry matter intake was significantly affected in diets. The numbers of N balance was a significant increase of N uptake only in pigs fed the diet with benzoic acid and HP. We found a significant reduction of urine pH, specifically pH (0.7 to 0.9) by both experimental groups fed with benzoic acid diets, regardless of the nitrogen content in the diet. The coefficients of excretion determination between hippuric acid and urine pH were $R^2 = 0.57$, the same for HP and LP diets. The higher decrease of ammonia nitrogen was observed in experimental LP groups, but it was not statistically significant.

Key words: ammonia excretion; benzoic acid; urine pH; piglets

INTRODUCTION

The excretion of minerals from pig farms on the environment is of general interest in most countries. Likewise, emissions of ammonia and odder emissions from pig production sites and manure handling are in the spotlight. The main source of ammonia emissions is a mixture of urea and excreted in the urine. Urea is converted to ammonia and carbon dioxide by urease present in faeces. The most important factors that affect the process are the urea concentration in urine and the pH and temperature of the slurry (Sommer and Husted, 1995). Other factors affecting the production of NH_3 is a kind of floor covering system for manure removal, climatic conditions inside the building, diet composition and feed efficiency of animals. One of the possibilities to reduce ammonia emissions is through dietary manipulation (Gatel and Grosjean, 1992). Organic acids such as formic acid, lactic acid are added in the diet of pigs (Jongbloed *et al*., 2000), are readily metabolized in the liver, and do not cause significant urine acidification. Benzoic acid (BA) may be one of the promising candidates to replace organic acids, nutritional use of antibiotics and affect growth performance and also reduce ammonia emissions from excrements.

The objective of the experiment described herein was to study pH changes in urine and ammonia emissions of pigs which were fed with different nitrogen level diets supplemented with benzoic acid.

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MATERIAL AND METHODS

Eight crossbred gilts, progeny of Large White sows and Landrace boars (initial BW 29.9 ± 1.7 kg) were allotted to a 2 x (4 × 4) Latin square design (n = 8). Pigs were individually housed in galvanized steel metabolism cages in an environmentally controlled room (20 ± 1.3°C). The dietary treatments included: diet LP with lower CP content (14 %), supplemented with isoleucine, lysine, methionine, threonine, tryptophan and valine; diet LPBA was similar to diet LP with 10 g.kg^{-1} benzoic acid; diet HP contained 18.8 % CP and diet HPBP was similar to diet HP with 10 g.kg^{-1} benzoic acid. The diets were based on wheat, maize and soybean meal, diets 3 and 4 were supplemented only with lysine and threonine. Diets were formulated to an equal ME content (13.3 MJ.kg) by means of supplementation with rapeseed oil (Table 1). The pigs were fed in two equal feed doses of 07:00 and 17:00 at a daily rate of 90 g.kg$^{0.75}$.

After a 5-days preliminary period, during which the pigs were adapted to cages and the new environment, four consecutive 10-days experimental periods followed. Each experimental period consisted of a 6-days adaptation period, within which the animals were adapted to the experimental diet and followed by a 2 x 48 hour collection period (7 to 8 day and 9 to 10 day). During the collection period, samples of urine and faeces were separately collected. Urine was collected via catheters, without addition of sulphuric acid and stored in ice-cooled containers. Urine pH was measured before freezing each day. 10 % aliquot was stored at - 20°C. Faeces were collected, pooled, and stored at - 20°C until analysis.

Briefly, 2 kg of fresh slurries prepared maintaining the respective proportion of urine and faeces in the excreta, and placed in a 10 L bowl, 280 mm high and 230 mm in diameter, covered by a lid connected to a tube system. Air entered the bowl

Table 1: Composition of diets and analysed content of nutrients

	Diet[1]			
Ingredients *(g.kg^{-1} diet)*	LP	LPBA	HP	HPBA
Maize	571.80	552.00	445.60	425.50
Wheat	300.00	300.00	300.00	300.00
Soybean meal	83.00	86.90	218.8	222.7
Beet pulp	-	-		
Rapeseed oil	2.00	8.30	3.30	9.60
L-isoleucine	0.87	0.90	-	-
L-lysine.HCl	5.85	5.80	1.74	1.66
DL-methionine	0.85	0.90	-	-
L-threonine	2.20	2.20	0.33	0.32
L-tryptophan	0.44	0.40	-	-
L-valine	0.86	0.90	-	-
Monocalcium phosphate	14.10	14.10	12.70	12.70
Limestone	11.00	11.00	10.70	10.70
Salt	3.90	3.90	3.80	3.80
Vit.-min. premix[2]	3.00	3.00	3.00	3.00
Benzoic acid	-	10.00	-	10.00
Analysed nutrient contents	*(g.kg^{-1} air-dried)*			
Dry matter	884.4	883.5	889.6	886.3
Crude protein	137.1	139.2	182.6	183.8
Crude fibre	30.6	30.4	31.5	34.6

[1]LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.
[2]Supplied per kg of diet: vit. A 9 000 IU. vit. D3 1 500 IU. α– tocopherol 35.0 mg. vit. B1 1.7 mg. vit. B2 6.0 mg. vit. B6 2.5 mg. Ca-panthothenate 15.0 mg. niacin 38.0 mg. vit. K3 2.0 mg. biotin 0.12 mg. cyanocobalamin 0.03 mg. choline 156 mg. Fe 103.0 mg. Zn 116.5 mg. Mn 49.0 mg. Cu 40.0 mg. I 1.2 mg. Co 0.4 mg. Se 0.3 mg.

through a small hole at the edge of the lid and left the bowl from the centre. Ammonia was removed from the air by passing through 2 flasks, each containing 100 mL 1N H_2SO_4. The air left the system after passing through a water trap, a flow controller at the rate of 2.3 L.min^{-1}, and a vacuum pump. The first flask was replaced daily whereas the second was replaced after 7 days. The concentration of ammonia in the liquid was determined using titration with 0.2 N NaOH. Nitrogen emission was determined daily for 7 consecutive days.

Analyses of diet, urine and faeces samples for dry matter, total N and fibre were performed in accordance with standard methods of AOAC (1998). Feeds and faeces sample were analysed for dry matter (DM) after drying at 105 °C for 8 hours. Crude protein (N x 6.25) was determined by Kjeldahl method using a Kjell-Foss 16200 auto analyser (method 978.02). Chemical analyses were conducted in duplicate.

The data were subjected to one-way ANOVA using Statgraphic Plus package (version 3.1., Statistical Graphics Corp., Rockville, MD, USA). In statistical significance testing, the differences between means were assessed using Fisher's LSD procedure.

RESULTS AND DISCUSSION

The analysed amount of crude protein in the diet was slightly lower than would be expected from the proposed formulation components in Table 1. Treatments with 1 % benzoic acid in the diet did not significantly influence nitrogen intake or dry matter intake (1.22 vs. 1.23, and 1.16 vs. 1.12 kg.d^{-1}; $P = 0.34$).

Reduction in dry matter intake of 4 % and 9 % was found in a diet with higher nitrogen vs. diets with low nitrogen content. Nitrogen balance (Table 2) showed adoption amount of nitrogen intake, which correspond to the dry matter intake and is higher by 17 to 27 %, despite the fact that the nitrogen content on the HP diet was higher by 32-33 %.

The different nitrogen content of diet caused variability in the content of nitrogen in the faces (- 3 to + 3 %), but the differences were not statistically significant. The diets with the same nitrogen content, with different contents of benzoic acid was found to increase nitrogen in the faces of + 1 to + 6 %, but without being significant statistically. However, the nitrogen content of the urine was about 73-86 % higher than for lower nitrogen diet (33 % different of the nitrogen in the diet HP). In all groups there was a switch from urinary to faecal excretion, the largest but not significant in the group LPBA ($P = 0.19$), although not statistically significant. Faecal N excretion increased by + 7.35 % (LP vs. LPBA). The reduction for urinary nitrogen by - 5.5 % was in the HPBA group ($P = 0.28$). Retention of nitrogen was affected in both experimental groups. The retention calculated as a percentage of nitrogen intake was to improve the impact of benzoic acid 3.2 % ($P = 0.06$), and retention calculated as a percentage of the absorbed nitrogen was up to 4.6 % ($P = 0.04$). The numerically higher N retention is consistent with the trend for a better growth performance observed by Bühler *et al*. (2006) using diets supplemented with 1 % benzoic acid in the grower and the finisher phases.

The value of benzoic-, hippuric acids, pH of urea and the ammonia release from the slurries are reported in Table 3. Intake of benzoic acid was methodically conducted and was not statistically evaluated.

Table 2: Effects of dietary protein and benzoic acid level on nitrogen (N) balance

Items	Diet[1]				pooled SEM	*P*-value[a]	
	LP	LPBA	HP	HPBA		1	2
DM intake, *[kg.d^{-1}]*	1.22	1.23	1.16	1.12	0.22	0.34	0.34
N intake *[g.d^{-1}]*	30.16	31.07	38.21	36.58	6.52	0.27	0.33
Faecal N *[g.d^{-1}]*	4.31	4.63	4.46	4.52	1.10	0.19	0.47
Urinary N *[g.d^{-1}]*	7.92	8.02	14.76	13.94	2.19	0.40	0.28
Absorbed N of intake *[%]*	85.21	84.74	88.17	87.27	3.76	0.36	0.26
Retained N of intake *[%]*	57.81	59.01	49.43	52.66	6.52	0.36	0.06
Retained N of absorbed *[%]*	67.76	69.41	55.98	60.56	6.79	0.29	0.04

[1]LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.
[a]Contrast: 1 = LP vs. LPBA; 2 = HP vs. HPBA.

Table 3: Effects of crude protein and benzoic acid intake on urine pH and emission from slurry

Items	Diet[1]				pooled SEM	*P*-value[a]			
	LP	LPBA	HP	HPBA		1	2	3	4
Benzoic acid intake *[g.d^{-1}]*	0.00	13.96	0.00	13.23	1.96	0.00	0.00	-	0.25
Hippuric acid excretion *[g.d^{-1}]*	0.83	16.34	0.90	17.54	3.75	0.00	0.00	0.28	0.31
Initial urine pH	5.73	5.06	6.82	5.94	0.35	0.00	0.00	0.00	0.00
NH_3-N emission *[g.d^{-1}]* *	0.94	0.82	1.21	1.15	0.22	0.12	0.36	0.06	0.01

[1]LP- control low protein diet + 0 % benzoic acid; LPBA- low protein + 1 % benzoic acid; HP- control high protein diet + 0 % benzoic acid; HPBA - high protein + 1 % benzoic acid.
[a]Contrast: 1 = LP vs. LPBA; 2 = HP vs. HPBA; 3 = LP vs. HP; 4 = LPBA vs. HPBA
*average per day of total emission from 2 kg fresh slurry during 7 days' determination

Hippuric acid is a direct metabolite of benzoic acid. The addition of benzoic acid to the diet determined a numerical decrease of the urinary pH due to the conversion of benzoic acid into hippuric acid in the liver. Hippuric acid is then excreted with urine (Bridges *et al*., 1970). The concentration of hippuric acid in urine of pigs treated with benzoic acid was significantly higher ($P < 0.001$) as compared to the control animals.

Regression analysis showed that content of hippuric acid (y) was a linear function of urinary pH (x) (Figure 1 and 2). Their negative relationship was almost identical as described with an equation for both levels of nitrogen. The constant in the equation is calculated as pH value for zero contents of hippuric acid by 5.95 and 6.79 for LP, HP respectively. The slope of the regression equation showed that each g.day^{-1} hippuric acid in urine reduces the pH of 0.018 to 0.02 point.

Significantly reduced pH in urine was detected in both groups ($P < 0.01$): in the group with lower nitrogen content by 0.67 (LP vs. LPBA 12 %) and in the group with higher nitrogen content by 0.88 (HP vs. HPBA 13 %). The difference between the groups with the addition of benzoic acid and different nitrogen content was as point of pH 0.88, which means up to 17 % (LPBA vs. HPBA). The decrease in urine pH (0.67 - 0.88 points of pH) is much lower than that (two points of pH decrease) obtained by Kristensen *et al.* (2009) in pigs of 63 ± 1 kg body weight. But Guingand *et al*. (2005) noted a decrease of 0.9 points as a result of adding 1 % benzoic acid to the feed.

The trend for a NH_3-N emission was from the diets LP diet 0.94 g.d^{-1} to the diet with containing benzoic acid LPBA was 0.82 g.d^{-1} (- 13 %), however because of the high residual variation of the values no significant differences were observed among

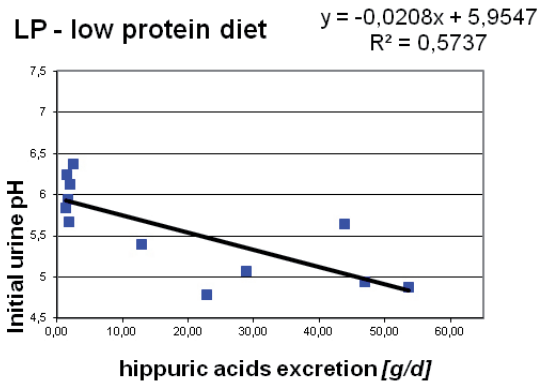

Fig. 1: Relationship between hippuric acid and urine pH of pig with low protein diets

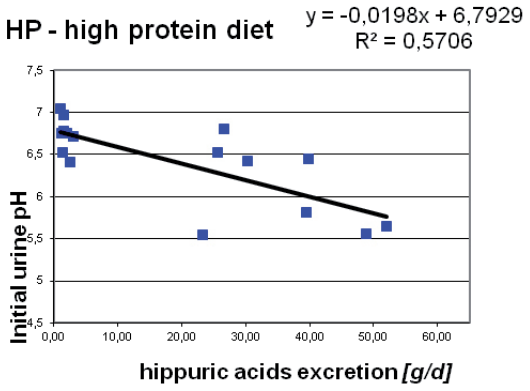

Fig. 2: Relationship between hippuric acid and urine pH of pig with high protein diets

treatments ($P < 0.12$). Slightly insignificant decrease of 0.7 $g.d^{-1}$ (- 5 %) ammonia emissions from the manure intended for diets with a higher nitrogen content ($P = 0.36$) was noted. Emission reduction was more pronounced with reduction of the nitrogen content in the diet. The diet free benzoic acid was 0.27 $g.d^{-1}$ (27 %; $P = 0.06$) which corresponds approximately to the amount of reduced nitrogen in the feed. However, the addition of benzoic acid to the diet resulted in loss of emission of 0.33 $g.d^{-1}$ (40 %; $P < 0.01$). Hansen *et al.* (2007) measured concentrations of NH_3 in the exhaust air found in the reduction of dietary crude protein (from 14 % to 16 %), and with the addition of 3 % benzoic ammonia emission reduced by 30 % and 57 %, respectively. In our experiment, we observed a greater effect of the nitrogen content as benzoic acid content. Philippe *et al.* (2011) concluded that diets with reduced crude protein content are highly effective in reducing the emissions with almost a 10 % reduction for every 10 $g.kg^{-1}$ reduction in dietary crude protein. That argument is confirmed by our experiment, the nitrogen content where reduced by 10 $g.kg^{-1}$ and reduction of emissions decreased by 40 %.

CONCLUSION

In conclusion, it may be stated that a strong interaction between the total quantity of nitrogen in the diet and its emissions provides the benefit to the environment. It‘s a close match between dietary intake and requirement of pigs under physiological and growth stages when there is limited manipulation. A more favourable impact in the experiment was that the addition of benzoic acid in the diet of the pigs resulted in a numerical decrease of the urinary pH and is beneficial to the environment without effects on N balances.

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EVALUATION OF QUALITY ACCEPTANCE OF COW´S MEAT IN RESPECT TO CERTAIN SOCIAL ASPECTS OF CONSUMERS

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ABSTRACT

The aim of the research study was to determine the attitudes of consumers towards sensory properties of beef (cow's meat) with regard to gender, age, education and preference of beef consumption under conditions of Slovakia. Sensory properties were analysed on 199 samples of cow's meat of various breeds and crosses. Cows were kept on the farms in different regions of Slovakia and slaughtered during 2006-2011. The samples (approx. 800 g) of *musculus longissimus lumborum et thoracis* between 9th to 11th ribs from the right side of the carcass were taken 24 h post mortem. Seven days after slaughter a consumer panel test for determination of sensory traits of meat was performed. The samples were sliced into 2.0 cm thick steaks and grilled for four minutes. Sensory traits (odour, taste, tenderness, juiciness) were evaluated by 5-point scale (1 - the worst, 5 - the best). The effect of gender (men - women), age (≤ 30 years; 31-50 years; ≥ 51 years), education (high-school, university) and preference of beef consumption (liking, disliking, indifferent) were determined. Overall, the sensory properties were evaluated by 612 randomly selected consumers.

Men evaluated better odour, juiciness and taste in comparison with women. The difference for odour was significant (3.66 and 3.64, respectively, $P<0.05$). Age of consumers had significant influence on tenderness and taste of cow's meat where both traits were assessed more positive by a group of consumers aged 31-50 years than by the older group (3.31 vs. 3.10 and 3.64 vs. 3.40). High-school educated consumers evaluated better than university educated ones for all the traits except for odour. The difference for tenderness was significant between both groups (3.38 and 3.16, respectively). Preference of beef consumption had significant impact on juiciness and taste where, paradoxically, the highest assessment was conceded by consumers disliking beef.

Key words: beef; sensory properties; panel test, consumer preferences

INTRODUCTION

Currently cows are slaughtered at slaughterhouses in Slovakia in growing numbers. In the past, cow's meat was used more for processing into products, however it is being offered to consumers also as retail beef cuts. In some states cow's meat is not accepted as retail beef cuts, as a matter of principle (Benes, 1994). On the other hand, meat of beef cows under age of 6 years is considered as very valuable in France. It is often discussed if cow's meat should not be used to produce meat products only; there is also opinion that it should be prohibited as retail beef cuts (Steinhauser, 2001) or be limited by the age of 3 years, i.e. selected primiparous cows (Franc and Herrmann, 1994). The cow's meat does not fulfil higher demands for retail meat, mainly because of its less tender consistency after heat treatment (Benes, 1994). The price of cow's meat is lower compared to young bull's meat, which can positively influence the customer at purchase of beef in favour of the cow's meat (Gondeková, 2011). Consumer demand for beef is highly influenced by

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consumer concerns about beef quality, health issues, nutritional value and safety, environment and animal welfare requirements (Xue *et al.,* 2010), as well as by the product origin (Banović *et al.,* 2010). Nevertheless, sensory characteristics still remain the main purchasing and repeated purchasing criteria (Calkins and Hodgen, 2007). Sensory traits, such as juiciness and tenderness, are known to be important to the consumer and thus influence their consumption of meat, especially beef. These traits are difficult to measure and often require the use of taste panels to assess the complex parameters involved in the eating experience (Gill *et al.,* 2010). A number of studies dealt with the sensory evaluation of cow's meat (Hoffman, 2006; Juie *et al.,* 2007; Koucký and Kudrna, 2006; Mojto *et al*., 2009; Moon *et al*., 2006; Stelzleni, 2007; Stelzleni and Johnson, 2008; Raines *et al.,* 2009; Stika *et al.,* 2007). The social aspect of consumers (age, sex, region, education level, income, preference of beef) was given less attention. Mennecke *et al.* (2007) studied the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. Consumers' preferences for quality grade and degree of doneness were solved in study of McKenna *et al*. (2003). Evaluation of beef quality by consumers from the viewpoint of various social aspects (age, sex, consumption of beef) were analysed by Oliver *et al.* (2006). Branscheid *et al.* (2006) reported consumer acceptability of beef and lamb in respect of certain social aspects of consumers (age, education, region). The acceptability of cow's meat by customer remains doubtful. According to our knowledge the acceptance of cow's meat by customers from the viewpoint of various social aspects has not been evaluated in Slovakia till now. The objective of this work was to evaluate acceptance of cow's meat by customers considering their sex, age, education and preference for beef consumption.

MATERIAL AND METHODS

Animals

Samples of meat from the carcasses of 199 cows of various breeds were collected randomly from different farms in Slovakia. Breeds composition copied herd structure in Slovakia and was as follows: Black and White Holstein, Red and White Holstein, Simmental, crossbreds of Black and White Holstein and Simmental crossbreds. Cows were slaughtered at abattoirs in Slovak Republic during 2006-2011 at the average age of 59 months. The oldest cow from evaluated group was 167.9 months old and the youngest was 19 months old. The animals whose meat samples were used to determine meat quality parameters were subjected to stressful situations, or otherwise handled to improve meat quality.

Sensory indicators

Meat samples from *musculus longissimus thoracis et lumborum* for tasting the meat were taken from the right carcass halves between 9th to 11th ribs. Sensory characteristics of meat were determined on 7th day after the slaughter of animals by a consumer test. Samples of meat were sliced into 2.0 cm pieces and then were grilled for 4 min at 200°C using electrical contact grill. Odour, taste, tenderness and juiciness were assessed. To evaluate the sensory characteristics, 5 - point scale (Jedlička, 1988) (1st degree - very bad, 5th degree - very good) was used. The effects of gender [men (M), women (F)], age of respondents [≤ 30 (AGE 1); 31-50 (AGE 2); ≥ 51 years (AGE 3)], education [high-school (EDH), university (EDU)] and preference of beef consumption [liking (LI+), disliking (LI-), indifferent (LI0)] were determined. Totally, the sensory properties were evaluated by 612 randomly selected consumers.

Statistical analysis

Statistical package SAS/STAT (2002-2003, v. 9.2) was used in the analysis. Basic statistics was done using MEANS procedure. The effect of gender, age, education and beef preference was investigated using GLM procedure.

RESULTS AND DISCUSSION

The results observed in the study showed the effect of gender. Differences between men and women only when odour of grilled meat was taken into account (Table 1). Rhodes *et al.* (1955), Van Syckle and Bruog (1985) and Ramsey *et al.* (1963) pointed out that odour is dominant for the customer at sensory evaluation. This statement was confirmed also by Koch *et al.* (1982), McKeith *et al.* (1985) and Galli *et al.* (2008) in their works. Men evaluated better the odour, juiciness and taste of meat compared to women. Women evaluated grilled meat as follows: odour was evaluated the best, followed by taste, juiciness and tenderness of meat. The lowest obtained value was tenderness evaluated by men, and the highest value was odour also evaluated by men. Men overall gave more points to the evaluated samples of grilled cow's meat. It can be caused by the fact that at home women prepare meat more often than men, and so they were stricter as far as the studied parameters of grilled meat were concerned. On the contrary, men are more active at "grilling at home". Brandscheid *et al.* (2006) found no influence of consumer's gender when evaluating beef; Farmer *et al.* (2006). Mojto *et al.* (2009b) reported statistically significant differences between sex when women evaluated more

Table 1: Organoleptic quality of cow's meat with respect to gender of tasters

Indicator	M (n = 270)		F (n = 342)		t-test
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	
Tenderness	3.20	0.06	3.25	0.06	-
Odour	3.66	0.04	3.64	0.04	*
Juiciness	3.49	0.06	3.46	0.04	-
Taste	3.64	0.05	3.48	0.05	-

**P < 0.05*
Scale: 1 - without tenderness, odour, juiciness, taste; 5 - very high tenderness, odour, juiciness, taste

Table 2: Organoleptic quality of cow's meat with respect to the age of tasters

Indicator	AGE1 (n = 85)		AGE2 (n = 349)		AGE3 (n = 178)		f-test	t-test
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		
Tenderness	3.20	0.09	3.31	0.05	3.10	0.08	*	2:3
Odour	3.67	0.07	3.67	0.04	3.60	0.06	-	-
Juiciness	3.34	0.10	3.52	0.05	3.46	0.07	-	-
Taste	3.48	0.09	3.64	0.04	3.40	0.07	*	2:3

**P < 0.05,*
Scale: 1 - without tenderness, odour, juiciness, taste; 5 - very high tenderness, odour, juiciness, taste

favourable not only tenderness but also juiciness of meat. Men evaluated better taste and odour of meat only.

When analysing data according to age, we noted statistically significant differences in taste and tenderness of meat between groups AGE2 and AGE3. Group AGE2 gave the most points to all properties of grilled meat, i.e. the consumers evaluated the meat samples as the best ones. The group AGE1 evaluated all parameters, better than group AGE3 with the exception of juiciness. The respondent's group AGE3 evaluated the organoleptic properties of grilled cow's meat as the worst, and group AGE2 evaluated them as the best. Farmer *et al. (*2006) also noticed the influence of the panel's age when evaluating the organoleptic characteristics of meat.

Significant effect of education of consumers was found when tenderness of grilled cow's meat was evaluated. The group EDH gave more points to all parameters except for odour. Branscheid *et al.* (2006) studied the influence of education in two different regions. The university graduates found tenderness and odour of grilled meat better in one region, whereas the graduates of high-school found them better in the other region.

The effect of preference for beef consumption is shown in the table 4. We noted statistically significant differences between group LI+ and group LI- as well as between group LI- and group LI0 in juiciness. Similar statistically significant differences among the above mentioned groups of consumers were found also when taste was evaluated. Consumers in group LI- gave the most points (the best evaluation) to all parameters. Group LI+ gave the least points, except for odour. Overall we obtained the best evaluation from consumers who do not prefer beef. The consumers who like beef were the strictest evaluators. It is necessary to remember that in this case the consumers who do not like beef did not have acquired experiences with the evaluated meat properties compared with those preferring and consuming beef. Therefore they were more benevolent;

Table 3: Organoleptic quality of cow's meat with respect to education level of tasters

Indicator	EDH (n = 197)		EDU (n = 415)		t-test
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	
Tenderness	3.38	0.07	3.16	0.05	*
Odour	3.62	0.06	3.66	0.04	-
Juiciness	3.53	0.06	3.45	0.04	-
Taste	3.57	0.06	3.54	0.04	-

**P < 0.05*
Scale: 1 - without tenderness, odour, juiciness, taste; 5 - very high tenderness, odour, juiciness, taste

Table 4: Organoleptic quality of cow's meat whit respect to the preference of beef consumption of tasters

Indicator	LI+ (n = 464)		LI- (n = 9)		LI0 (n = 139)		f-test	t-test	
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		1:2	2:3
Tenderness	3.18	0.05	4.00	0.29	3.36	0.08	-	-	-
Odour	3.66	0.04	3.67	0.37	3.63	0.06	-	-	-
Juiciness	3.43	0.04	4.44	0.18	3.58	0.07	*	***	**
Taste	3.54	0.04	3.89	0.31	3.55	0.06	*	*	*

**P < 0.05, **P<0.01, ***P<0,001*
Scale: 1 - without tenderness, odour, juiciness, taste; 5 - very high tenderness, odour, juiciness, taste

it means they gave more points to the samples at degustation and subsequent evaluation.

A comparison of our results with other studies is problematic due to a lack of research on cow's meat. It does not attract such attention as the beef – meat from bulls. Since the share of cow's meat has been still growing in commercial sale in Slovakia, further research including other social aspects (e.g. region, income, effect of advertising) of consumers will be needed.

CONCLUSION

The effect of gender, age, education level and beef preference of consumers on sensory properties of cow's meat was observed. Men, consumers aged 31-50, high-school educated and not preferring beef evaluated cow's meat more positive than women, younger and/or older, university-educated and beef preferring respondents.

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Review

EFFECT OF NOISE ON PERFORMANCE, STRESS, AND BEHAVIOUR OF ANIMALS

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ABSTRACT

The paper deals with findings in animal's response to noise. Factors including species, gender, age, and length of exposure on metabolism, performance, health, reproduction, and behaviour are discussed. The review covers research carried out on farming animals mainly, but contains also general literary sources on response from laboratory animals. This paper summarises the auditory range and some typical levels of sound that have been recorded for farm animals inside and outside housing, during transport and lairage stay. Effects of continuous and sudden noise on animals are also presented in detail. More physiological and behavioural responses have been described as increased hormonal production, increased heart rate, and reduction in production. Animal species exhibit a wide variety of responses to noise. Some animal species are more sensitive than others, because they may exhibit different forms or strengths of responses.

Key words: noise; animal; housing; performance; stress; behaviour

INTRODUCTION

In current animal husbandry noise has become an increasingly great but little noticed problem. Noise produced in intensive animal housing by ventilation system, feeding and excrement removal lines and by animals themselves is a potential stressor and affects not only animals but also the tending personnel.

The purpose of the current study was to determine effects of noise on animals, especially farming animals, and compare the results of previous studies on noise assessment in particular housing situations to demonstrate the impact and significance of the noise problem for design of housing, and management practices. This topic is also relevant to the welfare of animals, because high-frequency noise and intermittent sounds are generally perceived as the most alarming.

Sensitivity to noise

Noise is described as unwanted sound, either chronic or intermittent, and can be described in terms including its frequency, intensity, frequency spectrum, and shape of sound pressure through time (Burn, 2008). Decibel (dB) is the unit for measuring the intensity of sound. It is equal to ten times the logarithm to the base ten of the ratio of the intensity of the sound to be measured to the intensity level of sounds of some reference sound, usually the lowest audible note of the same frequency (B = log 10 (P 1/P 2), where B = Bel, and P 1 and P 2 are power levels. 1 Bel is equal to 10 decibels). Frequency means the number of vibrations per second of the air in which the sound is propagating and it is measured in Hertz (Hz) (Berglung *et al*., 1999).

The interpretation of noise assessment in animal housing is difficult as goals and methodology differ

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substantially between studies (Schäffer *et al.*, 1997). Whether or not a sound is to be described as noise therefore depends on the subjective notion as to whether listening brings about agreeable or disagreeable feelings. The condition under which a recipient is subjected to noise is important (Algers *et al*., 1978). The effects of noise on animal productivity and behaviour depend not only on its intensity or loudness (dB), its frequency (Hz), and its duration and pattern (including vibration potential), but also on the hearing ability of the animal species and breeds, the age and physiological state of the animal at the time of exposure. It also depends on the experience of the animal what sounds the animal has been exposed to during its lifetime (noise exposure history of the animal) and to the predictability of the acoustic stimulus (Castelhano-Carlos and Baumans, 2009).

How animals perceive noise? The effect of noise on the central nervous system is dependent on the state of the brain. In an exhausted individual the compensatory mechanisms are more vulnerable than in a rested individual. Intense noise exposure can damage the cochlea and inner ear and lead to a cascade of auditory effects along the entire central auditory cascade (Castelhano-Carlos and Baumans, 2009). A sudden and unexpected noise gives a dilatation of the pupil (Algers *et al*., 1978). Sound levels of approximately 40 dB are suggested as the appropriate level during the night. Sound levels above this have been shown to decrease the quality of sleep (Algers *et al.*, 1978). However, the noise levels in most husbandry buildings are considerably greater than 40 dB.

Susceptibility to noise hearing is species dependent, and it has been shown to be genetically determined (Henry, 1992; Lanier *et al.*, 2000). Animals have a different spectrum of audible sounds with maximum sensitivity at frequencies that are inaudible to humans (Voipio, 1997). The rat´s peak sensitivity lies between about 8 and 50 kHz (Burn, 2008). The lowest frequency rats have been reported to hear is 0.25 kHz and the highest is 80 kHz. When comparing rat and human hearing sensitivity, human values were about 10-20 dB higher than rat´s. Humans are most sensitive to noise in the range of 500 Hz to 4 kHz which includes the range of normal speech (i.e. within this range we can hear quieter sounds) (Castelhano-Carlos and Baumans, 2009).

The sensitivity of cattle, sheep and pigs to sound, and the levels to which they are exposed, has been reviewed by more authors. Cattle hear high-frequency sounds much better than humans, their high-frequency hearing limit being 37 kHz, compared with only 18 kHz for humans (Heffner, 1998). Their best audible sound is also at a higher frequency, at about 8 kHz, compared with 4 kHz for humans (Phillips, 2009). However, thresholds for discomfort for cattle was noted at 90-100 dB, with physical damage to the ear occurring at 110 dB (Phillips, 2009). Indeed, cattle, with an auditory range between 25 Hz and 35 kHz, can detect lower pitched sounds than other farm species (Heffner and Heffner 1993). Dairy breeds are more sensitive to noise than beef breeds (Lanier *et al*., 2000).

The auditory range of sheep is 125 Hz to 40 kHz with the most sensitive frequency a little higher than cattle and pigs at 10 kHz (Heffner, 1998). Sheep are most sensitive at 7 kHz (Ames and Arehart, 1972).

Pigs' hearing range is similar to that of humans, but with a shift toward ultrasound (Kittawornrat and Zimmerman, 2011). The auditory range of pigs is between 55 Hz and 40 kHz and their sense of hearing is more sensitive in the range 500 Hz to 16 kHz, particularly acute around 8 kHz (Heffner and Heffner, 1993). The frequency range for reasonable detection varies between 42 Hz and 40.5 kHz, with a region of best sensitivity from 250 Hz to 16 kHz (Heffner, 1998). Heffner and Heffner (1993) showed that the frequency range of pigs was an octave higher than the human frequency range, 40 Hz - 40 kHz. In addition, they found that the quietest sound which pigs can hear is 8 dB louder than the minimum sound level which humans can hear. However, the sound pressure level at which sound becomes painful to pigs is unknown.

Marler *et al.* (1973, cit. Algers *et al.*, 1978) observed that birds had the greatest increase of auditory threshold in the higher frequency ranges after exposure to noise of 95-100 dB.

Animals not only have to accept the noise, but they also emit (Manteuffel *et al*., 2004; Brumm *et al.*, 2009). Rodents not only produce sounds that we can hear, but also produce and hear frequencies that are inaudible to humans (above 20 kHz), perceiving sounds up to 80 kHz stimulus (Castelhano-Carlos and Baumans, 2009). Vocalisations of animals are important for communication and they also respond to the vocalisations of other species (Phillips, 2009) as might be expected in herd animals that evolved in multi-species grazing environments and which can be prey of carnivores. Previous research with cattle and pigs has indicated that vocalizations are an indicator of stress.

Cattle vocalisations generally range between 50 and 1,250 Hz (Kiley, 1972; cit. Watts and Stookey, 2000). Vocalisations of newly-weaned calves with fundamental frequencies as low as 31 Hz have been recorded (Watts and Stookey, 2000). Weeks *et al.* (2009) recorded mean levels of vocalisations from cattle in the range 80-90 dB and sheep vocalisations at around 70 dB. The use of high-frequency vocalisation was a powerful indicator of behavioural thermoregulation in pigs (Hillmann *et al.*, 2004; Malmkvist *et al*., 2004).

Noise and its source

Several studies have been published showing

the different sounds that can occur inside the animal facility (Castelhano-Carlos and Baumans, 2009). Husbandry procedures cause the loudest sounds, especially if metallic equipment is involved or if the work is performed in a hurried manner (Burn, 2008). Noise experienced during housing of farm animals can be short-term or chronic (Clough, 1999). The sources of noise can be technical devices, routine works (opening and closing doors, changing pens, washers, push carts, workers' speech, feed dispensing), basal sound levels caused by mechanical ventilation, animals activities (climbing and chewing on fences), and by their vocalizations (Žitňák *et al*., 2011; Mihina *et al.*, 2012). Sound pressure levels exceeding 75 dB have also been reported at frequencies in excess of 60 kHz in some laboratory animal housing (Morgan and Tromborg, 2007). A background sound level of 50 dB has been suggested to avoid disturbance to animals or personnel (Clough, 1999). According to Venglovský *et al.* (2007), short-lasting but intensive noise can have harmful effect not only on animals but also on personnel. This issue requires further monitoring and attention. Although differences exist in perceived loudness of the same sound, occupational noise limitations have been established for workers, and employees should be provided appropriate hearing protection and monitored for their effects (Mc Bride *et al.*, 2003; Lendelová *et al.*, 2013). The noise contributes to the development of some diseases and disorders caused by stressful conditions such as high blood pressure and other psychosomatic diseases (Šístková and Peterka, 2009).

Weeks *et al.* (2009) measured 75-90 (mean 84) dB in cattle barn, while Algers *et al.* (1978) found the noise range from 61-73 dB. Weeks (2008) noted typical values for grazing cattle at 35 dB. The noise environment in animal production contributes not only means of mechanization end equipment, but also the noise emission emerging manifestation of living animals (biological noise). The background noise level (biological noise) emerging from the biological manifestations of dairy cows ranged from 72.7-83.8 dB (Šístková *et al.*, 2010).

The average levels of noise measured by Algers *et al.* (1978) were 58.6 dB for fattening pigs. They reported that pigs in mechanically ventilated buildings were frequently exposed to noise levels greater than 70 dB. In another study the noise recorded in fattening units of pig farms ranging from 69-78 dB. Talling *et al.* (1998) recorded noise at six farms, on five transporters and at four abattoirs. The average sound pressure level measured in mechanically ventilated pig buildings was 73 dB, naturally ventilated buildings were on an average 10 dB quieter. The frequency content of the sound present on farms ranged from 20-6.3 kHz. Peaks in frequency, probably caused by fans, were identified in the mechanically ventilated buildings but not in the naturally ventilated buildings. A diurnal variation in overall sound pressure level was noted in naturally ventilated buildings but not in mechanically ventilated ones. Weeks *et al.* (2009) measured the sound from gates clanging at a consistent 85 dB. The sound levels varied between 85-138 dB in pig fattening halls and included the vocalisations of the pigs as well as background noise (Weeks *et al*., 2009).

Observations from 13 laying hen farms showed that most noise was produced by feed supplier and distribution systems (Oh *et al*., 2011). During the period 2008-2010 noise measurement was carried out on two poultry farms accounting for a total capacity of 650,000 heads of poultry (broilers, layers, and pullets). Measured values of equivalent sound level in the surroundings of farms were low (38.1-43.8 dB), the high value of noise on the farm has been caused by the handling of feedstuffs (79.3 dB, when the measurement performed 6 m from the containers) and the ventilation systems (67.1 dB, when the measurement performed 3 m from the suction-fan) (Šístková, 2011).

Generally, the sources of harmful noise in animal housings are various: feeding 104-115 dB, mating 94-115 dB, high-pressure cleaning 105 dB, feed mixing 88-93 dB (Venglovský *et al*., 2007). According to Šístková *et al*. (2010), hygienic limits are exceeded only during distribution of feed and bedding and thus only for a short time.

Studies reviewed the claims by farmers linking adverse effects of aircraft or helicopter noise on livestock. Farm owners concluded that aircraft overflights can affect feed intake, growth, or production rates in domestic animals (Cottereau, 1978). Helicopters are commonly used for managing wildlife populations, but their effect on wildlife behaviour is often ignored. The severity of response to disturbance may vary with species, group size, social groups, sex, age, vegetation cover, season, terrain, and distance from the aircraft or helicopter (Gladwin *et al*., 1988).

The exposure of farm animals to noise has been identified as a potential stressor not only in housing (Talling *et al*., 1998; Schäffer *et al*., 2001; Correa *et al.*, 2010) but also during the transport and at the abattoir (Agnes *et al*., 1990; Geverink *et al*., 1998; De la Fuente *et al.*, 2007). The average noise level measured during transport was 91 dB at the frequency range 20 - 16 kHz (Talling *et al.*, 1998). Animals are often exposed to acute noise levels before slaughter in lairages where noise is caused by ventilation fans and operational equipment. The negative impact of noise on animal welfare in lairages has also been reported by Grandin (1998) and Geverink *et al*. (1998).

Noise developed during transport was shown to increase the heart rates of free-ranging cattle (Albright and Arave, 1997), while cattle habituated to the sounds of cars and trucks will readily graze along

highways and seldom react (Grandin, 1997). Sheep appear to adapt to increased noise levels, particularly when these are relatively continuous, such as the noise of transport vehicles at around 60-90 dB, although they may show an initial rise in heart rate. Sheep in lairage appeared more responsive to mechanical noise such as metal banging and hosing than to noises of animal origin. Weeks *et al.* (2009) found mean sound levels from clanging gates and other fittings in 11 sheep lairages to be 76 dB and they recorded sheep vocalisations at around 70 dB. One study that measured sound levels during the transport of lambs found that the sound pressure level was continuously above 90 dB (Algers *et al*., 1978).

Other experiments have shown that pigs are exposed to higher sound pressure levels during transport and at the abattoir with different intensity, frequency and duration (Correa *et al*., 2010). Talling *et al.* (1998) noted that noise recorded in fattening units of pig farms ranges from 69 to 78 dB, from 88 to 96 dB at below 16 kHz during transport and between 85 and 97 dB at the abattoir. In four lairages they measured noise levels between 76 and 86 dB, with up to 97 dB in the prestunning pens. The movement of machinery as well as pig vocalisations was found to be a major source of noise and it was concluded that the sound levels and types of sound pigs were exposed to in transit and in lairage were likely to be aversive, and should therefore be regulated to improve welfare (Talling *et al*., 1998). Rabaste *et al.* (2007) recently measured sound levels in Canadian lairages in the range 82-108 dB.

The noise intensity to which poultry is exposed in slaughterhouses during slaughter is relatively high, varying in the range of 80-100 dB (Chloupek *et al*., 2009).

Health and performance

Scientific sources indicate that noise in farm animal environments is a detrimental factor to animal health. Especially longer lasting sounds can affect the health of animals. Noise directly affects reproductive physiology or energy consumption (Escribano *et al*., 2013). Noise may also have indirect effects on population dynamics through changes in habitat use, courtship and mating, reproduction and parental care (Rabin *et al.*, 2003). Male rats exposed to noise showed oligospermia and modifications of the testicle structure. The ovaries and the uterus diminished significantly in female rats after a noise exposure of 110 dB for five minutes 15 times per day for 11 days at 375-500 Hz. Remaining estrus occurs after noise exposure as well. Increase in abortion frequency and fetus resorption, or reduction of fetus weight have been also registered (Algers *et a*l., 1978).

According to Geber (1966) noise is received by the mother‘s ear, the different brain cells integrate the signals. The hypothalamus and the hypophysis are activated; the adrenal cortex and medulla are stimulated and secrete their respective hormones. The uterine blood flow, gas-interchange, nutrition and interchange of waste products between fetus and mother are decreased. The reproductive function of rats can also be affected by sounds. Zondek (1964, cit Castelhano-Carlos and Baumans, 2009) showed that exposure of rats to noise of 50-80 kHz at 80-90 dB in the four days during the mating period reduced fertility by 73.2 %. Exposure to 100 dB of 3-12 kHz for one minute during the four days of copulation reduced fertility by 70-80 %.

Zondek *et al.* (1964, cit Castelhano-Carlos and Baumans, 2009) also showed the influence of sound on the gonadotropic functions in mature rabbits. Losses have been reported from mink farms in the form of premature births and insufficient lactation in connection with exposure to sonic booms. There are reports that the females kill their own offspring (Algers *et al*., 1978).

Neural and neuroendocrine systems are possible mechanisms for the effects of noise on feed efficiency. Sound emission at the frequency of 2 kHz in noise of 75 dB, 85 dB, and 95 dB was found to contribute to appetite reduction of animals (Cwynar and Kolacz, 2011). Algers and Jensen (1991) found reduced milk yield in dairy cows exposed to 1.4 h of 80-100 dB of noise twice daily. A three weeks study found no differences between intensities of 70 dB abd 80 dB noise when produced in an autotandem milking parlour (Kauke and Savary, 2010). Sudden noise of 105 dB could, however, decrease the quantity of milk at the next milking. An ejection in progress might even be interrupted (Algers *et al*., 1978). According to Kovalčík and Šottník (1971), noise as high as 80 dB had no negative effect on dairy cows. Feed intake was increased, milk yield was unchanged, and indices of the rate of milk-releasing were improved. However, immediate exposure to a high-intensity noise (105 dB) resulted in decreased feed consumption, milk yield, and intensity of milk release. Gradual increase of noise to 105 dB resulted in a less-negative response. Gygax and Nosal (2006) investigated on 50 dairy farms the effect of vibration and noise on somatic cell counts in milk. Somatic cell counts increased with an increasing intensity of vibration but not with acoustic noise.

Unexpected high intensity noise (above 110 dB), such as low altitude jet aircraft overflights at milking time could reduce effectiveness of the milk ejection reflex, decrease efficiency of milk removal, increase residual milk, and lead to overall reduction in milk yield. However, a majority of the studies reviewed suggests that there is little or no effect of aircraft noise on cattle. Adverse effects of low-altitude flights have been noted in some studies but have not been uniformly reproduced in other reports (Manci *et al.*, 1988). A number of studies investigated the effects of aircraft noise and sonic

booms on the milk production of dairy cows. Milk yields were not affected. Beyer (1983) found that helicopters caused worse reaction than other low-aircraft overflights. However, helicopters at 9 to 18 m overhead did not affect milk production and abortion rates of cows and heifers (Dufour, 1980; Gladwin *et al*., 1988). Cows exposed to recorded jet noise just before milking showed no behavioural or productivity responses during 21days treatment periods (Head *et al*., 1993).

Noise at 75 dB increased average daily weight gain of lambs and improved their feed efficiency compared to control and the 100 dB groups. Acclimatization to sound was evident (Ames, 1978).

Pigs exposed to 90 dB prolonged or intermittent noise decreased growth (Otten *et al*., 2004). The number of pigs farrowed and the number of survivors were not influenced by exposure of the parents to loud sound during mating, or exposure of sows to reproduced sounds at 120 dB for 12 hours daily beginning 3 days before farrowing and continuing until their piglets were weaned (Bond, 1971). Studies using simulated aircraft noise at levels of 100 dB to 135 dB found only minor effects on rate of feed utilization, weight gain, food intake, and reproduction rates of boars and sows (Manci *et al*., 1988; Dufour, 1980).

According to Campo *et al*. (2005) noise seems to affect adversely the productive performance of the birds.

When poultry are transported to intermittent loud noise, rate of laying eggs and growth rate were decreased and mortality increased (Oh *et al*., 2011). Broiler chickens exposed to 110 dB aircraft noise for five minutes every 20 minutes each day and every three nights for nine weeks from birth showed no difference in growth compared to a control group. Egg productivity was affected at exposure levels as high as 120 to 130 dB. Noise at 90 dB seemed not to affect productivity and egg quality of laying hens (Oh *et al*., 2011).

Generally, exposure to sudden, intense noise cause reduced egg production in fowls. Pheasants are reported to have broken their eggs, while suffocation in panic-struck was observed in fowls (Algers *et al*., 1978).

Exposition to 120 dB for 84 days showed no significant influence in quantitative and differential sperm counts of roosters, but these sperms used on insemination worsened the hatchability of eggs (Algers *et al*., 1978). Noise acting for a long time reduces productivity of eggs. More severe responses are possible depending on the number of birds, frequency of noise exposure, environmental conditions, and on experience of animals (Gladwin *et al*., 1988). Study involving turkeys examined the differences between simulated versus actual over flight aircraft noise (Bowles *et al.,* 1990). Findings suggested that turkeys habituated to noise quickly and no growth rate differences between the experimental and control groups were noted while there was increased difficulty for handling individuals.

Metabolism and stress

Noise has been demonstrated to induce a variety of physiological changes in mammals, such as changes in the cardiovascular homeostasis and in the secretion of hormones. Through hearing impulses are given to the brain stem and the hypothalamus. From formatio reticularis the sympathetic nervous system is influenced. Via the hypophysis, adrenocorticotropic hormone (ACTH), and thyroid-stimulating hormone (TSH) the hypothalamus gives signals to the adrenal medulla and the thyroid gland. The parasympathetic nervous system is also influenced and has a mainly reversed effect compared to the sympathetic nervous system (Algers *et al*., 1978; Manteuffel, 2002).

Noise may be a potential stressor causing the organism to react in farm animal husbandry. High noise exposure has also been reported to cause cellular effects. Ultrastructural alterations in myocardium and adrenal glands have been shown in rats exposed to noise of 100 dB for 12 h. DNA damage was also found to be associated with noise exposure (Castelhano-Carlos and Baumans, 2009). Loud sound is well known for adverse effects on blood pressure and heart rate in humans and animals (Geverink *et al.*, 1998; Morgan, Tromborg, 2007). The most obvious effect is a general stress reaction with higher secretion of ACTH giving an increase of adrenocortical hormones in the blood (Burrow *et al*., 2005). Reactions occur in the circulatory system and in the gastrointestinal motility via the sympathetic nervous system. Other effects are sleep disturbances, changes in the glucose metabolism of the liver, changes in the enzymatic activity of the kidneys, and an increase of eosinophils percentage in blood, and immunosuppression (Algers *et al*., 1978).

Noise research has been carried out mainly on man and laboratory animals. These investigations have shown that noise causes a general stress reaction influencing most organs. Stress reaction causes short-term effects and also partly long-term effects. Physiologically, prolonged exposure to intense noise is associated with increased activity of the autonomic nervous system. Its prolonged activation is correlated with increased activity in the hypothalamic-pituitary-adrenal system, elevated metabolic rates, increased blood pressure, and tachycardia (Ames, 1978; Morgan and Tromborg, 2007). According to Weeks (2009) loud noise can cause disturbance of sleep. Long-term noise exposure caused a decrease in plasma glucocorticoids and an increase in plasma catecholamines, ACTH and cortisol concentrations (Otten *et al*., 2004; Kanitz *et al*., 2005). However, not only prolonged stress but also repeated distress is dangerous. Kanitz *et al.* (2005) indicated that exposure of domestic pigs to repeat noise stress causes

changes in neuroendocrine regulations.

The physiological responses of dairy cows to noise were reported by Broucek *et al*. (1983). The sound of a tractor engine (97 dB) significantly increased glucose concentration and leucocyte counts and markedly reduced the level of hemoglobin in the blood. The same authors treated primiparous cows individually by the 30 min noise of 110 dB, frequency 1 kHz in an open-field arena. Highly significant increase of glycemia, non-esterified fatty acids content and creatinine under the effect of accoustic exposition were recorded. Haemoglobin level dropped highly significantly. After the repetition of stress after the 2nd calving similar trend was recorded, but the changes were smaller. It refers to the habituation (Broucek *et al.*, 1988a). In another study by Broucek *et al.* (1988b), cows were divided into three groups: mothers and daughters, sisters after mothers and sisters after bulls. The reaction of daughters was, in contrast to mothers, less pronounced. In increasing glucose and creatinine, a highly significant relationship ($r = 0.659^{***}$; $r = 0.549^{**}$) was noted between mothers and their daughters. A non-significant correlation was found in the elevation of non-esterified fatty acids ($r = 0.568$) and creatinine ($r = 0.492$) between older and younger sisters. The reactions of primiparous cows on noise load were influenced by their fathers. We found the differences in frequency of heart rate, haemoglobin, non-esterified fatty acids, glycaemia, and thyroxine contents.

In another trial, pure-tone sound (1 kHz, 110 dB) increased blood glucose, nonesterified fatty acids and creatinin values in blood serum, and decreased the level of hemoglobin, with a slight decrease in thyroxin in plasma. Waynert *et al.* (1999) reported that beef cattle subjected to noise exposure for 1 min per day over 5 days displayed an overall steady reduction in heart rate.

Sounds produced by humans might also be stressful for farm animals. Loud cry causes stress responses in farm animals (Hemsworth *et al.*, 2003). Shouting on dairy cows appears to be very aversive (Pajor *et al.*, 2000). Noise made by humans shouting and slamming of metal gates increases heart rate and activity in cattle (Waynert *et al*., 1999). Lanier *et al.* (2000) also noted that cattle appeared more stressed by intermittent loud human vocalisation, particularly when high-pitched like a child's. Unexpected high intensity noise, such as low altitude jet aircraft overflights with more than 110 dB at milking time could provoke increase peripheral or mammary release of catecholamines (Albright and Arave, 1997).

Arehart and Ames (1972) observed that the adrenal and pituitary weights declined in sheep after noise exposure. Prolonged exposure to loud noise of 100 dB for 8 h increased their respiration rate. Lambs which were not previously exposed to loud noise had elevated heart rates when exposed to 100 dB. It was found that in comparison to a control group carried out in a 65 dB, the increased intensity of sound emission causes stress in experimental animals (Cwynar and Kolacz, 2011).

Ames (1971) published trial with growing lambs. Each animal was exposed to a control period (63 dB background), followed by 3 weeks treatment periods of 75 and 90 dB. Noise intensities of 90 dB noise inhibited the release of thyroxine and triiodthyronine. Significant decrease in the lymphatic tissue of the thymus was recorded in guinea-pigs intermittantly exposed to 139-144 dB noise for 8 hours a day for six weeks (Algers *et al*., 1978). Manci *et al.* (1988) and Gladwin *et al.* (1988) demonstrated no adverse effects on the thyroid and adrenal gland condition of pigs subjected to observed aircraft noise.

Physiological and behavioural studies have identified noise stress during housing (Schäffer *et al*., 2001). Pigs exposed to 90 dB prolonged or intermittent noise increased cortisol, ACTH, noradrenaline to adrenaline ratios (Otten *et al*., 2004). Acute sound exposure was found to increase heart rate (Talling *et al*., 1996). This response was stronger for a frequency of 8 kHz than for 500 Hz and for an intensity of 97 dB than for 85 dB. The heart rate of piglets increased more in response to high frequency sounds (Talling *et al*., 1996; Kittawornrat and Zimmerman, 2011). Trials showed that pigs respond with an increase in heart rate and plasma glucocorticoids when exposed to a short-term noise stress (Talling *et al*., 1998). A single and shortterm noise exposure of pigs at 120 dB was found to increase plasma glucocorticoid concentrations, but had no effect on plasma catecholamines (Kemper *et al.*, 1976; cit. Venglovský *et al.*, 2007). In another study, Kanitz *et al.* (2005) exposed pigs to daily or three times weekly noise at 90 dB for two hours. This caused both short-term adrenocortical and long-term stress effects.

Cannulated pigs were exposed to either a daily stimulation with noise (2 h, 90 dB), or to the same stimulus three times a week. Noise exposure caused an increase of corticosteroid binding globulin, ACTH and cortisol levels in daily stimulated pigs in first week followed by a subsequent decrease until week 4. The ACTH and cortisol response of the second group increased after week 1 and was significantly elevated in week 4. There were also significant structural modifications in the adrenal gland of first group of pigs resulting in differentiated effects on the adrenal cortex and medulla (Kanitz *et al.*, 2005). These findings show that pigs are very sensitive to noise and they should not be exposed to constant or sudden noise. Therefore, noise levels above 85 dB must be avoided in that part of any building where pigs are kept (Fottrell, 2009).

Noise intensities of 115 dB were effective in

interrupting brooding in hens (Gross, 1990). Acute noise exposures at 80 dB and 100 dB in broilers increased corticosterone level after 10 min of exposure (Chloupek *et al*., 2009). The chickens were exposed to sound of 95 dB which lasted 120 min every day during different age periods. This chronic stress caused significant changes in histological structure of their adrenal glands (Žikić *et al*., 2011).

Noise treatment of 80 dB resulted in a significant elevation of heterophil to lymphocyte ratio indicating stress response of the broilers. Noise treatment of both 70 and 80 dB intensities also resulted in a significant elevation of basophil granulocytes (Bedanova *et al*., 2010). Chloupek *et al*. (2009) simulated slaughterhouse sounds to which broilers were exposed for 10 min in the test room. Noise stimuli of both 80 dB and 100 dB intensities induced a highly significant elevation in the plasma corticosterone level in broilers when compared to the control birds. McFarlane and Curtis (1989) reported that continuous noise for seven days at the level 80 or 95 dB did not have a significant effect on the plasma corticosterone concentration of broiler chickens. The noise during transport increased heart and breathing rates, and secretion of stress hormone of poultry (Oh e*t al.*, 2011).

Although the current legislation requires that the noise level be minimised, the noise intensity to which poultry is exposed during production life is relatively high, varying in the range of 80-90 dB.

Behaviour

It has been stated in literature that excessive noise has an influence on behaviour and coordination. Mammals in particular appear to react to sudden higher intensity noise, with responses including the startle response, freezing, and fleeing from the sound source. Compared with chronic background or repetitive noise, this aperiodic or unpredictable noise is especially effective for provoking distress responses. Most animals become less responsive to sounds emitted for long periods or at regular intervals.

The degree of animal reaction varied with species of animal, age and individual. The character of the behaviour reactions observed that domestic animals experience from excessive noise is disturbing their well being (McAdie *et al*., 1993; McGlone and Swanson, 2010). Animal activity may be increased at background noise. Particular states of emotion may thus be accompanied by specific behaviours. Animals were also reported to tend to be more active in the morning periods than in the afternoon periods tested, which might be related to the arrival of staff and beginning of the working day with a general increase in noise levels (Castelhano-Carlos and Baumans, 2009).

An understanding of animal response to helicopters or aircrafts is important in predicting the consequences of the disturbance on the ecology, welfare and behaviour of exposed free kept farming or wildlife animals (Tracey and Flem, 2007). Although some studies reported the effects of aircraft noise on domestic animals as inconclusive, a majority of the literature reviewed indicates that domestic animals exhibit behavioural responses to overflights but generally seem to habituate to the disturbances over a period of time.

Exposure of laboratory animals to noise induced increased abnormal behaviour, suppressed exploratory behaviour, and impaired learning. It has been reported that guinea pigs will jump when exposed to sudden very loud noise (139-143 dB), reduce activity and remain huddled together for up to 30 minutes afterwards. Tooth grinding was also observed in male guinea pigs subjected to frequent loud noise over a period of 6 weeks (Johnson, 2006).

Different levels of background noise were shown to influence learning and behaviour in rats (Castelhano-Carlos and Baumans, 2009). Guinea-pigs showed the most marked reduction in activity when exposed as compared to rats which appeared least affected by noise exposure. After the noise was turned off there was an increase in the general activity of mice as well as of rats. At the beginning of the exposure all the animals huddled in a group. Another reaction of mice and rats was freezing into a motionless stance. When rats were exposed to 95 dB at 0.5-5 kHz for two 5-minutes‘ periods per day for 28 weeks, their behaviour changed and above all they became aggressive (Algers *et al*., 1978). Sudden sounds are probably also more startling than those with gradual onsets (Burn, 2008).

In open field behaviour, continuous noise of 85 dB was shown to increase defecation and reduce both social activities and non-social activities (sniffing, grooming or crawling) of rats when compared with 50 dB, 65 dB or 75 dB. Although noise of moderate intensity is commonly present during experiments on animal learning and memory, its impact has not been explored fully (Prior, 2006). Noise-exposed rats made fewer errors, explored less and finished their trials sooner. Results show that the acoustic environment is an important variable in studies with animal models of learning and memory.

Many studies indicate that sudden, novel sounds seem to affect cattle behaviour more than continuous high noise (Head *et al*., 1993; Grandin, 1998; Arnold *et al.*, 2007). When the aircraft was 152 m above ground level, the cattle ran for less than 10 meters and resumed normal activity within one minute. Unexpected high intensity noise, such as low altitude jet aircraft overflights (above 110 dB), at milking parlour could provoke adverse behaviour, such as kicking or stomping (Morgan and Tromborg, 2007).

The noise threshold expected to cause a behavioural response by cattle is 85 to 90 dB (Manci *et al.*, 1988). Noises greater than threshold have provoked retreat, freezing, or strong startle response (Morgan and Tromborg, 2007). When the transmitter of ultrasound was switched on at a distance of 1 m, calves got up and orientated towards the sound source. After 30 s, all calves had their ears directed away from the sound source. After 10 min, some calves started to scratch their ears repeatedly. During the 10 minutes period of exposure, none of the calves would lay down again (Algers, 1984).

Arnold *et al.* (2008) examined the effect of noise on the choice behaviour of dairy heifers in a maze. The percentage of heifers that chose the quiet side of maze was increasing as the experiment progessed. Heifers exposed to the noise from milking parlour show escape-type behaviours, consistent with a fear response. They learned to avoid the noise. Pajor *et al.* (2000) assessed responses of dairy cows to various handling treatments. Exposure to noise increased avoidance behaviour, as indicated by increases in stopping and amount of required handler intervention. Broucek *et al.* (1988b) observed the effect of sire lineage on movement activity in dairy cows tested during noise at open-field arena.

Noise in the milking facility has direct implications for on-farm efficiency related to improving cow behaviour and human-animal interactions. Faster movement in response to noise persisted for the first 4 days of the treatment phase, with some evidence of habituation of this response on the fifth day (Waynert *et al.*, 1999). Responses to noise in commercial milking facilities may be influenced by processes of habituation. As dairy cows are regularly exposed to the milking environment, there is opportunity for reduction of any fear responses arising from exposure to noise.

Dairy heifers were exposed to the noise of 85 dB during the 23 m long transfer test raceway. Exposure to noise resulted in increases in heart rate and faster transit times. There were no significant effects of noise on latency to enter the raceway, or animal handling parameters (Arnold *et al.*, 2007). These data indicate that anthropogenic noise generated in the course of routine human activity may have adverse effects on cattle welfare.

The 90 dB of noise level in the short term has caused in sheep a departure from the source of the sound and accumulation of a cohesive group of individuals in the lying position (Algers, 1984). Responses to helicopter over-flights have the potential to alter the time budget of behaviour activities. Bighorn sheep responded to helicopter flights by decreasing their time spent foraging and they were the most sensitive to disturbance during winter (43 % reduction in foraging efficiency). Further analyses indicated a disturbance distance threshold of 250-450 m (Stockwell *et al*., 1991). Caribous (*Rangifer tarandus*) respond to the sound produced by aircraft flyovers with increased activity, although the degree of reaction varies with time of year (Maier *et al.*, 1998). Similar effects of aircraft noise have been found in mountain sheep (Bleich *et al*., 1994; Weisenberger *et al*., 1996).

Changes in behaviour can adversely affect wildlife and reduce the effectiveness of management operations. Bleich *et al*. (1994) suggested that frequent disturbance by aircraft could cause animals to vacate their home territory. The distance from the source of disturbance is an important indicator of alert behaviour. Goats displayed alert behaviour when the helicopter was directly overhead and alert response decreased exponentially with horizontal distance from the helicopter. The distance moved decreased sharply when the helicopter was further than 150 m away. Goats were often disoriented and ran away to a distance up to 1.5 km in response to helicopter over-flights. However, Tracey and Flem (2007) found that helicopter flights did not cause mothers to abandon their young, nor adversely affect their immediate or long-term welfare. Feral goats displayed aversion and learnt to respond to helicopter disturbance (Tracey and Flem, 2007).

Horses are also very sensitive to noise. Algers (1984) wrote that after the start of noise stimul horses turned their heads and directed their ears towards the source and then immediately turned their ears away. At a new tone, the horses reacted with attention for a very short time and then turned their ears away again. All horses showed marked attention for the whole 10-min experimental period. The author recorded flight reactions when the noise source was switched on (Algers, 1984).

Several reviews presented a varied response of horses to low-altitude aircraft overflights. Observations noted that horses galloped in response to jet flyovers (Gladwin *et al.*, 1988). Intensive flight reactions, random movements, and biting/kicking behaviour were displayed. However, no injuries or abortions occurred, and evidence suggested that the mares adapted to the flyovers over the course of a month (Manci *et al*., 1988).

Auditory stimuli are used by pigs as a means of communication in all social activities (Gonyou, 2001). Pigs exposed to 90 dB prolonged or intermittent noise increased time lying down and decreased social interactions (Otten *et al*., 2004). According to Talling *et al.* (1996) pigs show an aversion to sudden loud noise during tested in an open-field. This response was stronger for a frequency of 8 kHz than for 500 Hz and for an intensity of 97 dB than for 85 dB, although habituation occurred relatively quickly (Kittawornrat and Zimmerman, 2011). Repeated exposure of pigs to noise levels of 90 dB has negative implications on their welfare (Kanitz *et al*., 2005).

Longer lasting sounds, for example continuous fan noise, can also affect pigs. Behaviour of piglets and sows during suckling in relation to sound levels were investigated by Algers and Jensen (1985). Sows were exposed to a relatively silent background noise of 59 dB or exposed to fan noise at a level of 85 dB. In the noise-exposed environment, the piglets failed to respond to the gruntings of the sow, which led to a disrupted pattern. Significantly decreased massaging of the udder and hence reduced milk production were recorded. Authors concluded that the noise-exposed piglets gained less milk than the ones in the silent environment. In the study of Algers (1984) with sudden noise exposure 10-day-old pigs an immediate attention and orientation reaction for about 10 s was noted. The 6-week-old pigs were immediately activated and started to orientate themselves towards the sound source. An intensive searching behaviour by all pigs started and continued for the whole 10 min period. Attention and waving of the ears were recorded initially in all sows (Algers, 1984). When suckling piglets were subjected to continuous loud noise, they were to a lesser extent attracted to the front teats and more frequently used the teats at the rear part of the udder (Algers and Jensen, 1991).

The intensity of 90 dB prolonged or intermittent noise increased time of lying down, and decreased social interactions (Otten *et al.*, 2004). Some trials showed that pigs respond with an increase in ambulation score when exposed to a short-term noise stress (Talling *et al*., 1996, 1998; Kanitz *et al*., 2005). Talling *et al*. (1996) found that within a continual 15 min exposure to noise, initial differences between treatment and pre-treatment locomotion in pigs decreased over the course of the trial. Habituation to noise has also been observed over repeated exposure on separate occasions.

Drastic effects have been noticed connected with sonic bangs caused by low crossing aircraft, mink and rabbits killing their young (Algers *et al*., 1978). Laboratory rabbits alter their behaviour when exposed to normal laboratory sounds in nonsound isolated housing (Jildge, 1991). Effect of noise on rabbits causes adverse effects including nervous and behavioural abnormalities and can cause a startled response and traumatic injuries to limbs and back (Marai and Rashwan, 2004). Particularly, most concerns about noise effects have traditionally focused on impairment of reproductive and maternal behaviours, although a few controlled studies have been done to support the observations of animal caretakers that noise inhibits production. With regard to the noise, threshold areas in the sensitive range of rabbits lie between zero and 20 dB sound pressure, which means a sensitive hearing.

The typical reaction of domestic fowls after exposure to sudden, intense noise is a short-term startle response. The reaction ceases as soon as the stimulus is ended, and within a few minutes all activity returns to normal. This suggests that the birds habituate relatively quickly (Gladwin *et al*., 1988). A significant negative effect of acute noise exposure at 80 dB and 100 dB on stressfulness and fearfulness in broilers was observed by Chloupek *et al*. (2009). Campo *et al*. (2005) found that laying hens exposed to noise at 90 dB (truck, train and aircraft noises) for 60 minutes were more fearful than control hens kept at 65 dB caused by bird vocalizations and fans. Algers *et al*. (1978) noted characteristic reactions to long lasting sound (95 dB, 500 Hz) as startle response, latent period, running, total immobility, small jerky head movements, and sleep-like behaviour in chickens. The reaction varied in form and strength according to the age of the experimental birds and was strongest in about 26-day old chickens. Sudden loud noises have also been reported to cause hysteria in various strains of chickens (Mitloehner *et al*., 2010). Book and Bradley (1990) reported higher panic and aggression in turkeys in response to noise stimuli simulating aircraft overflights. Wild birds have been reported to react with disrupted sitting (Algers *et al.*, 1978). Bright (2008) recorded noise (background machinery and hen vocalisations) in 21 commercial free-range laying hen flocks aged 35 weeks. Ten of the flocks were classified as feather pecking and 11 as non-feather pecking. For the acoustic parameters measured, there were no differences between the general flock noise of feather and non-feather pecking flocks.

Behaviour of adult animals in captivity is also affected by noise. In zoos and aquaria, noise from visitors increases as visitor numbers increase. Loud sound has been shown to increase vigilance and activity and agitation behaviours in pandas (Morgan and Tromborg, 2007).

CONCLUSION

The intention of this review is to document and compare the results of previous studies on noise assessment, in particular housing situations and to demonstrate the impact and significance of the noise problem for farm animal welfare, housing, design and management. Environmental and communication noises are present in animal housings. Although the majority of the literature suggests that farming animals and wildlife species exhibit adaptation after repeated exposure to noise, careful planning should be made before construction of the animal building, in order to avoid stressful environmental sounds both for the animal and personnel.

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Invitation to the 30th International Film Festival AGROFILM

Dear executive producers, filmmakers, specialists in the sphere of agriculture, research workers, pedagogues, representatives of mass media, and friends of the film festival Agrofilm!

We take pleasure in informing you that the jubilee 30th International Film Festival AGROFILM will take place from 29th September to 3rd October 2014. The festival is traditionally held in Nitra, Slovakia and this year will be also the town Zvolen the venue of festival's events.

Agrofilm is a festival of films and video-programmes aimed especially at themes from agriculture and rural development. The objective of the festival is to inform public about the latest findings in the sphere of agriculture, food-production, nutrition of population, problems in rural areas and their inhabitants, conservation of natural resources and improvement of life quality. The festival films shall show problems and try to bring good examples, innovation solutions, they promote results of research.

Last year we changed the dramaturgy of the festival with the aim to bring the festival nearer to the public. Special events, screening for students of secondary schools and universities followed by special lectures and discussions as well as screening of films connected with promotion of high quality foodstuffs and lectures in the shopping centre met positive acceptance of the public and massmedia. Visitors of the IFF Agrofilm 2013 saw 152 films from 24 countries. The jubilee 30th Agrofilm will therefore continue these activities. We will not omit the traditional cultural concomitant festival events such as the art exhibition.

Agrofilm is arranged by the Ministry of Agriculture and Rural Development of the Slovak Republic. The festival is organized by the Research Institute for Animal Production Nitra that is a part of the National Agricultural and Food Centre since January 2014. Partners of the festival are the town Nitra, the Nitra Self-governing Region, universities, the Centre of Scientific-Technical Information, the Food and Agriculture Organization and other international and Slovak institutions.

We sincerely hope that you will incorporate Agrofilm into your business calendar this year also. The festival offers you the opportunity to present your work to public. We will welcome recordings of professional as well as beginning film makers. For the latest information about the festival, statute of the festival including the festival electronic application form please go to www.agrofilm.sk. Novelty is also the fact that we brought this year's festival even nearer to the young and it is on social networks.

We are looking forward to cooperation and meeting during the 30th International Film Festival Agrofilm.

Prof. Štefan Mihina, PhD.
President of Agrofilm 2014

Contacts: Research Institute for Animal Production Nitra - National Agricultural and Food Centre, Address: Hlohovecká 2, 951 41 Lužianky, Slovak Republic, e-mail: agrofilm@vuzv.sk, web: www.agrofilm.sk, phone: ++421 37 6546 126, ++421 37 6546 387

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