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POST-THAW CHARACTERISTICS OF PINZGAU BULL SEMEN FOLLOWING LONG-TERM AND SHORT-TERM STORAGE

E. ŠPALEKOVÁ[1]*, B. KULÍKOVÁ[1, 2], A. BALÁŽI[1], A. MAKAREVICH[1], P. CHRENEK[1, 3]

[1]NAFC - Research Institute for Animal Production Nitra, Slovak Republic
[2]Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovak Republic
[3]Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic

ABSTRACT

The aim of the study was to examine sperm viability of Pinzgau bull insemination doses following long- or short-term storage. Insemination doses, provided by Slovak Biological Services Inc. (Lužianky, Slovak Republic), were slow-frozen and stored in containers with liquid nitrogen for 1 - 18 years. The sperm samples were divided, according to the length of storage, to the short-term (≤ 15 years) and long-term (> 15 years) groups. Post-thaw sperm assessment included total motility (CASA), dead/necrotic sperm occurrence (propidium iodide) and sperm morphology examination. No significant influence of storage length on the spermatozoa characteristics of Pinzgau bulls was noted. However, high inter-male variability in the susceptibility of Pinzgau bull sperm to cryodamages was found ($P \leq 0.05$). Our results suggest that bull´ individual variability should be taken into account when semen doses are to be stored as genetic resources for a future use.

Key words: Pinzgau cattle; sperm; storage; viability

INTRODUCTION

Preservation of genetic diversity of domestic animals is a global issue, which is important from a biological, economical and ethical standpoint (Prentice and Anzar, 2011). Intensive genetic selection, close range of production and reproduction traits of animals results in serious genetic diversity decline. Nowadays, number of farm animal breeds became extinct as a consequence of unilateral selection (Buerkle, 2007). Pinzgau breed is registered by the UN FAO (Food and Agriculture Organization, OSN) as threatened breed and it is classified as Animal Genetic Resource – AnGR since 1994 (Krupa *et al*., 2011). It represents dual purpose cattle, and is preferentially kept (bred) in the mountain regions of Slovakia (Kadlecik *et al*., 2004). The long-term national program for animal genetic resources protection can ensure minimization of extinction risk and support for sustainable utilization of local breeds (Tomka *et al.* 2013).

Cryopreservation of livestock semen has been used to improve the breeding of animals of genetic importance, and has contributed to the conservation of endangered species (Holt, 2000; Johnson *et al.*, 2000). There has been a growing interest in the understanding of long-term storage effects on post-thaw survival of mammalian sperm (Yogev *et al*., 2010; Fraser *et al*., 2014). The issue is of practical importance for the establishment of cryobanks and its operation. However, freezing-thawing process and storage of samples may lead to decreased viability and fertilization ability of frozen insemination doses. Most of the damage to spermatozoa brought by cryopreservation is caused by production of reactive oxygen species (ROS) during freezing, that might alter

***Correspondence:** E-mail: spalekova@vuzv.sk
Eliška Špaleková, NAFC - Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 196 Fax: +421 37 6546 189

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sperm membrane fluidity and decrease the sperm function following cryopreservation (Chatterjee and Gagnon, 2001). There is also evidence that long period of storage may result in a loss of sperm surface proteins associated with bull fertility (Lessard *et al*., 2000).

Therefore, the aim of our study was to examine sperm viability of Pinzgau bull insemination doses following long- or short-term storage. The viability characteristics, including sperm morphology, motility and occurrence of dead/necrotic sperm were analyzed in post-thaw sperm samples.

MATERIAL AND METHODS

Semen collection and cryopreservation process

Commercially available sperm insemination doses from nine healthy Pinzgau bulls, used in this study, were supplied by the Slovak Biological Services Inc., Lužianky, Slovak Republic. Semen was collected using an artificial vagina. Only fresh semen with required quality (min 70 % of motile sperm and concentration 0.7 x 10^6 ml^{-1}) was used for insemination dose production. The semen samples were diluted in a Triladyl extender, loaded onto 0.25 ml straws and slowly frozen using a programmable freezing device. The straws were stored in containers with liquid nitrogen for 1 - 18 years. The 'long-term storage' (LT) group included a total of 8 samples (4 bulls, 2 samples each), which were thawed for analysis after 16 -18 years of storage. A second group designated as the 'short-term storage' group (ST), was composed of 10 samples that were donated by 5 bulls (2 samples each). These specimens were kept in the bank for routine inseminations and were cryostored for 1 - 15 years. The definition for long and short-term storage was decided arbitrarily, by splitting the accessible specimens into two groups with a comparable number of individuals.

Semen thawing and analysis

The straws were thawed in a water bath at 37 ± 1 °C for 1 min. For sperm total motility measure, computer assisted semen analysis (CASA; Sperm Vision™ 3.5) was used. Sperm samples were diluted in a saline. Each sample was analyzed at the time intervals of 0, 0.5 h or 2 h following thawing and incubation at 37 °C.

Fluorescence assay was performed immediately after thawing. Occurrence of dead/ necrotic sperm cells was detected with nuclear stain - propidium iodide (PI). Samples were incubated in a staining solution (5 $\mu g \cdot ml^{-1}$ of PI in saline) for 20 min in the dark and washed in a saline. Four µl of sperm suspension were mixed gently on a microslide with 4 µl of Vectashield mounting medium containing DAPI (H-1200, Vector Laboratories, Burlingame, CA, USA), a blue-fluorescent DNA stain, which marks nucleoplasma of all the sperm in sample. Samples were immediately observed under a Leica fluorescent microscope (Mikro Ltd, Bratislava, Slovak Republic) with respective bandwidth filters for red and blue fluorescence.

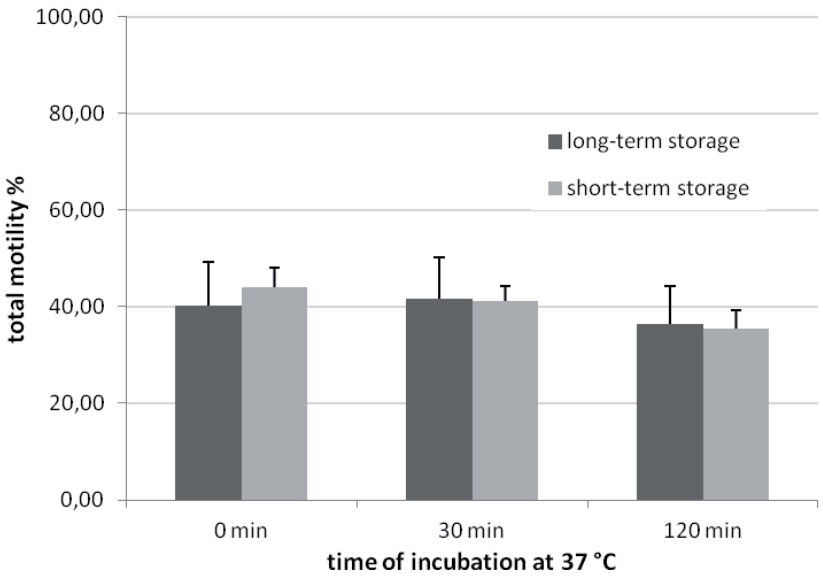

Fig. 1: Sperm total motility of frozen-thawed bull semen following long-term or short-term storage in liquid nitrogen

Assessment of pathological spermatozoa was performed by examining spermatozoa under a light microscope at 500x magnification, following sperm dilution/immobilization in distilled water. For each male a minimum of 400 spermatozoa were evaluated. The following changes in spermatozoa morphology were considered as pathological: separated tail (ST), knob-twisted tail (KT), torso tail (TT), rounded tail (RT), retention of cytoplasmic drop (RCD), broken tail (BT), small head (SH), large head (LH), acrosomal changes (ACH), other forms (OF) of pathological spermatozoa (teratogenic changes, club bag tumour, tail ball, etc.).

Statistical analysis

Statistical analysis was performed by One-Way ANOVA (Tukey test) for comparison of mean values using the SigmaPlot software. Differences at $P \leq 0.05$ were considered as statistically significant.

RESULTS

Cryopreserved semen used in this study was collected from sexually mature bulls that were of proven fertility and were undergoing regular semen collection for commercial artificial inseminations. The post-thaw motilities recorded for the males of LT or ST group were expressed as the means ± SE (standard errors of the mean). No significant difference in total motility between LT and ST groups measured at different time points after thawing and incubation at 37 °C was found (Fig. 1).

In both groups, post-thaw total motility was about 40 % and sperm were able to maintain this level around 30 % after 2h of incubation at 37 °C. The relatively large SEs in LT and ST groups (Fig. 1) may be explained by a high variability in mean values of post-thaw motility among the individual bulls (Tab. 1).

High level of inter-male variability was also found in percentage of necrotic sperm (Fig. 2). When the bulls were grouped according to length of storage, no difference in mean values between the LT and ST groups (31.16 ± 7.69 vs. 29.08 ± 3.96), respectively, was found. The percentage of morphologically abnormal sperm ranged from 10 to 33 % with no difference between the examined groups (17.21 ± 1.91 vs. 19.61 ± 3.99 %).

DISCUSSION

Long-term storage of cryopreserved sperm in LN_2 is of high importance for livestock breeding programs, gene bank establishment and maintenance of genetic diversity (Jalme *et al.*, 2003). Pinzgau cattle belong to the endangered breeds, due to radical decreasing of its population in Slovakia (Kadlecik *et al*., 2004). Cryopreservation and long-term storage of gametes is potential option for genetic diversity preservation of Pinzgau breed. However, knowledge about the effect of long-term storage in liquid nitrogen on sperm functionality is insufficient. Therefore, this study examined differences in post-thaw viability characteristics of long- and short-term stored bull sperm. Generally sperm cryopreservation and thawing process can lead to sperm membrane structures damage, motility deterioration (Bailey *et al.*, 2000) and decline in sperm forward progression in the female reproductive tract, that might cause reduction in fertilization ability (Salamon and Maxwell, 2000). Usually, normalized percentage of motile sperm in frozen-thawed livestock semen is about 50 % of those in the fresh counterparts (Barbas and Mascarenhas, 2009). All the samples used in our study fulfilled the recommended criterion of at least 30 % motility for the production of insemination

Table 1: Frozen-thawed sperm total motility (TM) of individual bulls (n = 9) observed immediately after thawing

Total motility % (mean ± SE)			
long-term storage (> 15years)		short-term storage (≤ 15 years)	
LT1	40.00 ± 1.53^{b}	ST1	56.01 ± 1.36^{a}
LT2	43.42 ± 2.38^{b}	ST2	50.50 ± 1.52^{a}
LT3	60.82 ± 1.61^{a}	ST3	42.28 ± 1.61^{b}
LT4	16.65 ± 0.74^{d}	ST4	36.82 ± 2.59^{bc}
		ST5	34.39 ± 1.22^{c}

Different superscripts indicate significant differences ($P < 0.05$).

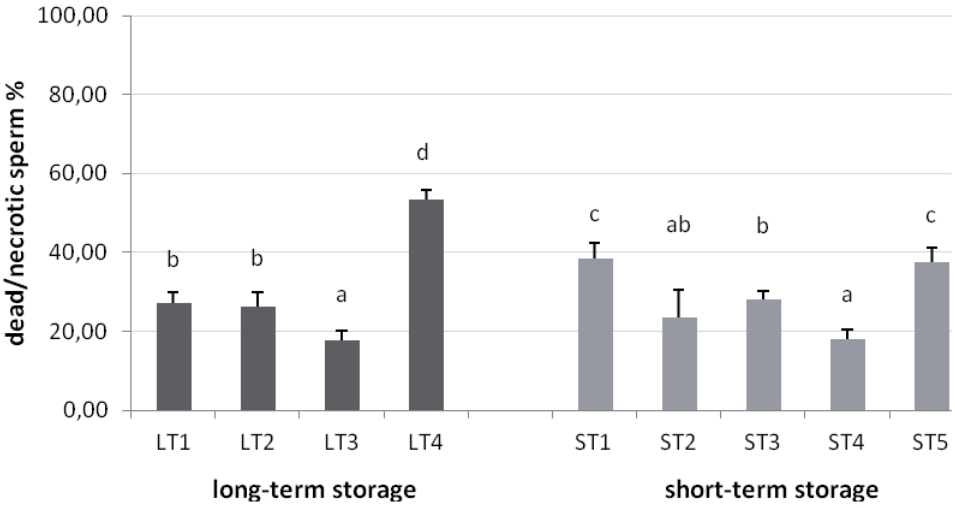

Fig. 2: Inter-male differences in the occurrence of dead/necrotic sperm (PI, %) in frozen-thawed bull semen after long-term or short-term storage. Different superscripts indicate significant differences (P < 0.05)

doses. No difference in total sperm motility either immediately after thawing or after incubation at 37 °C was found between long- and short-term stored groups. This finding is in accordance to the currently accepted cryobiological viewpoint, that there is no functional loss in case of proper storage at −196 °C in liquid nitrogen for indefinite periods of time (Clarke *et al*., 2006).

However, this argument might be discrepant with the results of Haugan *et al.* (2007), that dairy cow conception rates are negatively affected after semen prolonged cryostorage. The long-term storage was reported to be unfavourable compared to short-term storage concerning human sperm motility (Edelstein *et al.*, 2008), mitochondrial function and plasma membrane integrity of cryopreserved boar sperm (Fraser *et al*., 2014).

Our results did not show any substantial difference between sperm viability characteristics of long- or short-term stored bull sperm. The percentage of dead/necrotic sperm was almost similar in each group. Similarly, Edelstein *et al*. (2008) confirmed no difference in incidence of sperm with DNA damage between long- and short-term stored human sperm. In light of these contrary findings, there are still misdoubts in the effect of long-term storage in LN_2 on sperm viability and function. Our results have shown rather high inter-individual bull variability in motility and dead sperm occurrence. It was already stated, that differences in the ability of bull sperm to withstand the stresses of standard cryopreservation protocols and sperm viability are markedly depended on the impact of individual bulls (Kreysing *et al.*, 1997; Loomis and Graham, 2008).

In terms of morphological changes, most of the individuals examined demonstrated morphology in accordance to the commercial insemination dose standards (malformation rate ≤ 20 %). For two males this value was higher than 20 %. Deleterious effect of freezing-thawing procedure on sperm morphology, especially in the head and tail region, was confirmed by several authors in boars, ruminant and human (Garcıa-Herreros *et al.*, 2008; Hidalgo *et al.*, 2005; Connell *et al.*, 2002; Donnelly *et al.*, 2001; Hammadeh *et al*., 1999). Abnormal bull sperm represented one of the more significant effects on bull fertility (Freneau *et al*., 2010). Such spermatozoa caused decrease in embryonic development when were used for IVF (Walters *et al*., 2005). Therefore, morphometry measurements seem to be sensitive biomarker related to sperm fertilization ability (Sailer *et al*., 1996). In our study, abnormalities in long- and short-term stored sperm were localized mainly in the tail region, represented by minor defects like knob-twisted tail, coiled and rounded tail, and were generally regarded as a tertiary (post-ejaculation) defects, which usually occur after osmotic changes. Tail defects after cryopreservation have been previously reported in human, and plasma membrane destruction in this region has been suggested as the probable reason for these defects (Ozkavukcu *et al*., 2008).

No effect of storage time on occurrence of sperm morphology abnormalities was proved in short-term and long-term groups, however high inter-male variability was observed. This might be related to season of semen collection or age of bulls (Soderquist *et al*., 1996; Brito *et al*., 2002).

CONCLUSION

In conclusion, our results show no difference in the effect of storage time on the Pinzgau bull spermatozoa characteristics. On the other hand, we have observed the high inter-male variability in the susceptibility of bull sperm to cryoinduced damage. Although, our study was performed on a limited number of animals, it can be suggested that individual differences are an important factor that should be taken into account when semen from individual bulls is to be stored for a long time period as a genetic resource.

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GENETIC ANALYSIS OF EWE PRODUCTIVITY TRAITS IN GHEZEL SHEEP USING LINEAR AND THRESHOLD MODELS

R. NABAVI[1]*, S. ALIJANI[1], S. A. RAFAT[1], M. BOHLOULI[2]

[1]Department of Animal Science, University of Tabriz, Iran
[2]Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

ABSTRACT

In this study, the genetic parameters of ewe productivity (reproductive parameter) in Iranian native Ghezel sheep were estimated using six different linear and threshold univariate animal models. The data set consisted of 4173 records from 2420 ewes that were collected since 1992 to 2010 in the breeding centre of Ghezel sheep Station of Miandoab, Western-Azerbaijan province. Based on Akaike's Information Criteria and Deviance Information Criterion, the most appropriate linear and threshold model for each trait was the fourth model (including direct genetics of animal and maternal genetics with non-zero covariance between them). The direct heritability estimates (± standard errors) with linear analysis for conception rate, number of lambs born, number of lambs born alive, number of lambs at weaning, number of lambs born per ewe exposed, number of lambs at weaning per ewe exposed, total litter weight at birth per ewe lambing and total litter weight at weaning per ewe lambing were as 0.077 ± 0.02, 0.074 ± 0.01, 0.081 ± 0.01, 0.088 ± 0.02, 0.028 ± 0.01, 0.026 ± 0.01, 0.195 ± 0.02, 0.193 ± 0.01, respectively. But the estimates resulted from threshold analysis were as 0.080 ± 0.02, 0.079 ± 0.01, 0.084 ± 0.01, 0.088 ± 0.02, 0.035 ± 0.01, 0.032 ± 0.01, 0.196 ± 0.01, 0.195 ± 0.02, respectively. The results showed that the model with genetic correlation between direct and maternal effects seems to be reliable, and also demonstrated the possibility of application of the threshold model for routine genetic evaluation of reproductive traits in Ghezel sheep.

Key words: heritability; non-linear models; genetic parameter; reproductive traits; animal model

INTRODUCTION

One of the Iranian native fat-tailed and medium-sized sheep breed which is distributed in mountainous areas of Iran North-West, especially in Western and Eastern Azerbaijan provinces, is Ghezel sheep. Valuable products of this sheep are meat, milk, wool and skin (meat and milk are mostly focused). Growth rate of this sheep is high (200 g.day^{-1}) (Izadifard and Zamiri, 2007). This sheep's color usually varies from light brown to dark brown (legs wool is usually darker). A sidewise looking at the tail of this sheep represent 'S' shape in which the sheep popularity decreases when the tail is less S-shaped. Both rams and ewes are without horns and most of them have knot in front of their neck.

The Lighvan cheese, a traditional and delicious kind of Iranian cheese, is basically made from Ghezel sheep milk in the area of Sahand mountainside, located in the North-West of Iran. It is the most popular traditional and expensive cheese made from raw sheep's milk in Eastern-Azerbaijan province. The Lighvan cheese is characterized by unique hardness (semi-hard), saltiness and spiciness (Rasouli Pirouzian *et al.*, 2012).

The most important part of the sheep farming income is derived from lamb production. The efficiency of lamb production is influenced by reproduction, mothering ability, milk production of ewe, growth rate and lamb survival (Rao and Notter, 2000). Reproductive traits are the most important factors affecting the profitability of sheep farming (Matos *et al*., 1997).

***Correspondence:** E-mail: reza.nabavi2006@gmail.com
Reza Nabavi, M. Sc. Graduate of Genetics and Animal Breeding, Department of Animal Science, University of Tabriz, Tabriz, Iran
Tel.: +989352132467 Fax: +984133356004

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Increase in the number or total weight of lambs weaned per ewe can be achieved by increasing the number and the weight of lambs produced per ewe within a year (Duguma *et al*., 2002). Within-breed selection of animals from native breeds is an appropriate methodology for genetic improvement of traditional low-input production systems of small ruminants in the tropics (Kosgey *et al*., 2006).

In the last decade, to analyze discrete traits non-linear methods, resulting in more accurate estimation, are proposed in animal breeding. Generally, linear models consider only the direct genetic variance as an important factor, but others (maternal, environmental) as unimportant ones. Threshold model methods are based on the assumption of an underlying unobservable continuous response variable that follows the assumptions of a mixed linear model (Gianola and Foulley, 1983). Heritability of number of born lambs and number of weaned lambs in Turkish Sakiz sheep were 0.03 and 0.18 (Ceylan *et al.*, 2009) and in Moghani sheep were 0.11 and 0.02 (Rashidi *et al*., 2011), respectively. Estimates of heritability of genetic effects for reproductive traits were low due to the typical strong influence of environmental factors on reproductive traits. Although estimated heritability values by linear and threshold models are low and response to selection is slow, using threshold model will speed up the response to selection (Mohammadi *et al.*, 2012a). Thus, selection of the most appropriate and accurate model and method for improving this native sheep in case of these traits can speed up the response to selection. Consequently, products like milk, meat, wool, skin and Lighvan cheese will improve the efficacy of this farming branch.

Therefore, this study was carried out to estimate genetic parameters of reproductive traits for native Ghezel sheep using the better and the best method and model, based on the accuracy and Information Criterion (AIC and DIC) that are necessary to develop efficient selection programs to improve reproduction.

MATERIAL AND METHODS

Data and management

The data set used in this study included reproductive traits of Ghezel ewes, collected during 1992-2010 in the breeding centre of Ghezel sheep (Miandoab) located in Western-Azerbaijan province of Iran. The aim of this centre is to establish a nucleus source for genetic improvement of other herds in the region. Management system of the flock was semi-migratory. Mating season commences in the late of August to October. First mating of animals was at 18-24 months of age. Artificial insemination (AI) was done during the breeding season. The ewes used in this research were from one to seven parities. In the mating strategy controlled AI was done, where mating between very close animals was avoided. In every breeding year maximum number of allocated ewes per each AI ram was not more than 25 animals. Animals that could not conceive by AI were subjected to natural servicing, where the ewes were assigned to ram breeding groups with an average mating rate of 10-15 ewes per ram. Lambing season starts on January and continues until April. At the birth, all lambs were identified and birth weight, birth type, sex and pedigree information were recorded. The food of lambs was their mother‘s milk, and since 15th day of age it was also dry alfalfa hay. Weaning of lambs usually occurs at three months of age (90 days). The flock (ewes and weaned lambs) usually grazes in pasture during the day and penned at nights and winter with supplemental feeding consisting alfalfa, wheat straw and barley grain.

Studied traits

Studied traits can be classified into two main categories: basic and composite traits. The basic traits were conception rate (CR with measure of one or zero, meaning whether ewe was exposed to ram or not), total number of lambs born (NLB, with measures of zero, one, or two, which was the number of lambs born per ewe lambing), number of live born lambs (NLBA, with measures of one or two, which was number of lambs alive at 24 hours of age), number of live born lambs at weaning (NLAW, with measures of one or two, which was number of lambs weaned alive). Conception rate is a binary random variable based on continuous variation on the underlying liability scale expressed when a certain threshold is obtained and all other basic traits have discrete numerical observation.

Composite traits with discrete numerical observation were number of lambs born per ewe exposed (NLBEE = CR × NLB) and number of lambs weaned per ewe exposed (NLWEE = CR × NLAW). The composite traits with continuous expression were total litter weight at birth (TLBW), total litter weight at weaning per ewe lambing (TLWW). Table 1 represents the number of records per each trait.

Statistical analysis

Significant effects which should be stated in a final model were preliminarily determined by Logistic and GLM procedure of SAS software (SAS Institute, 2002) for discrete and continuous traits, respectively. The fixed effects of the final statistical model were: lambing year with 18 classes (1992-2010), herd of ewe with six classes, age of ewe with seven classes, and random parts were: additive genetics of animal, maternal genetics and permanent environmental

Table 1: Descriptive statistics of data sets

Traits*	No. of records	No. of ewes	No. of sires	Mean	S.D	C.V. (%)	range
CR	4173	2420	175	0.89	0.30	33.72	0-1
NLB	3673	1906	163	1.116	0.31	28.49	0-2
NLBA	3669	1906	163	1.112	0.31	28.41	1-2
NLAW	3405	1761	163	1.10	0.31	28.36	1-2
NLBEE	4173	2420	175	0.99	0.44	44.44	0-2
NLWEE	4173	2420	175	0.99	0.43	43.43	0-2
TLBW	3669	1906	163	4.60	1.43	31.08	1.9-7.1
TLWW	3405	1906	163	24.12	2.79	11.56	14.71-29.8

*CR: conception rate; NLB: number of lambs born per ewe lambing; NLBA: number of lambs born alive per ewe lambing; NLAW: number of lambs alive at weaning; NLBEE: number of lambs born per ewe exposed; NLWEE: number of lambs weaned per ewe exposed; TLBW: total litter weight at birth; TLWW: total litter weight at weaning; S.D.: standard deviation and C.V.: coefficient of variation

of ewe. The variance components for studied traits were estimated with six different univariate animal models,

1) $y = Xb + Z_1a + e$
2) $y = Xb + Z_1 a + Wpe + e$
3) $y = Xb + Z_1a + Z_2m + e$ Cov (a, m) = 0
4) $y = Xb + Z_1a + Z_2m + e$ Cov (a, m) ≠ 0
5) $y = Xb + Z_1a + Z_2m + Wpe + e$ Cov (a, m) = 0
6) $y = Xb + Z_1a + Z_2m + Wpe + e$ Cov (a, m) ≠ 0

where **y** is vector of records of reproductive traits; **a**, **b**, **m**, **pe** and **e** are direct additive genetic, fixed effects, maternal effects, permanent environmental and residual effects, respectively.
X, $\mathbf{Z}_1$, $\mathbf{Z}_2$ and **W** are the design matrices associating the corresponding effects with elements of **y**.
The (co)variance structure for random effects was:

$$var \begin{bmatrix} a \\ m \\ pe \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & A\,\sigma_{am} & 0 & 0 \\ A\sigma_{am} & A\,\sigma_m^2 & 0 & 0 \\ 0 & 0 & I_d\sigma_{pe}^2 & 0 \\ 0 & 0 & 0 & I_n\sigma_e^2 \end{bmatrix}$$

where:
a = direct additive genetic effect;
pe = permanent environmental effect related to repeated records of ewes;
m = maternal genetic effects;
e = residual effects;
σ^2_a = direct additive genetic variance;
σ^2_{pe} = permanent environmental variance for repeated records of ewes;
σ^2_m = maternal genetic variance;
σ^2_e = residual variance;
A = additive numerator relationship matrix;
$\mathbf{I}_d$, $\mathbf{I}_n$ = identity matrices with order equal to the number of ewes (d) and records (n), respectively.

Based on Akaike's Information Criteria (AIC) (Akaike, 1974) and Deviance Information Criterion (DIC), the most appropriate linear and threshold model for each trait was determined, respectively.

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

where $\log L_i$ is the maximized Log-likelihood of model i at convergence and pi is the number of parameters obtained from each model.

$DIC = \bar{D}(\theta) + p_D = 2\bar{D}(\theta) - D(\bar{\theta})$

where $\bar{\mathbf{D}}(\theta)$ is the posterior expectation of the Bayesian deviance represented a measure of the fit of the model, and θ is the vector of parameters of the model; $\mathbf{p_D}$ is the effective number of parameters representing penalty for increasing model complexity; $\mathbf{D}(\bar{\theta})$ is the Bayesian deviance evaluated at the posterior mean of the parameters. Smaller values of AIC and DIC indicate better model fit.

The (co)variance components were estimated using AIREMLF90 for linear model and THRGIBBS1F90 software with Gibbs sampling methodology of Bayesian inference for threshold model (Misztal, 2002). Number of samples, length of burn-in and sampling interval in Gibbs sampling methodology of Bayesian inference were 200000, 10000 and 100, respectively.

RESULTS AND DISCUSSION

Fixed effects

Herd, year of lambing and age of ewe were fixed significant effects ($P < 0.01$) for all traits. Data set recorded in years 1992, 1993 and 1996 for basic traits; NLBEE and NLWEE had the lowest performance and were mostly records for two year old ewes. But usually by increasing age of the ewe it was improved up to seven years of age and then decreased again. For both TLBW and TLWW, records of 1998-2001 had the lowest performance and were improved by increasing the age of ewe. Coefficient of variation of a trait is a criterion for determining the trait variation. This statistics for the studied traits ranged from 11.56 % for TLWW to 44.44 for NLBEE. Since some part of the recorded data sets of the station was from flocks of people in the region, significant effect of herd can be arisen due to different management system in herds. Climatic changes and its influence on pasture of cultivated plants, different management system and nutrition over the years can cause significant effect of year of lambing (Vatankhah *et al*., 2008; Bromley *et al.*, 2001; Ekiz *et al*., 2005). Significant effects of year of lambing on reproductive traits in different sheep breeds have been reported by several authors as well (Mohammadi *et al.*, 2012a; 2012b; Ceylan *et al*., 2009). Significant effects of ewe age may be due to nursing and maternal behavior of ewe at different ages, as well as maternal effect differences (Ekiz *et al*., 2005; Rosati *et al*., 2002; Afolayan *et al*., 2008). Other authors (Rashidi *et al*., 2011; Ceylan *et al.*, 2009, Poortahmasb *et al*., 2007) have reported the significant effect of ewe age on reproductive traits, while other researchers (Mokhtari *et al*., 2010) reported an insignificant influence of ewe age on NLB and NLAW of Kermani sheep. The reported coefficients of variations in Sabi sheep for CR, NLB, NLW, NLBEE, NLWEE and TLWW were 35.9, 30.5, 48.9, 47.8, 62.9 and 28.00, respectively (Matika *et al*., 2003).

(Co)variance components and genetic parameters

All traits were analyzed using six different univariate linear and threshold animal models and basing on their AIC and DIC estimates, the fourth model was the most appropriate (including direct additive genetics of animal and maternal genetics with non-zero covariance between them). Estimates of (co)variance components (direct additive, maternal, residual and phenotype), heritabilities (direct additive and maternal) and correlations (additive genetics and maternal genetics) are listed in Table 2.

The direct heritability estimates with linear model for CR, NLB, NLBA, NLAW, NLBEE, NLWEE, TLBW and TLWW were 0.077 ± 0.02, 0.074 ± 0.01, 0.081 ± 0.01, 0.088 ± 0.02, 0.028 ± 0.01, 0.026 ± 0.01, 0.195 ± 0.02, 0.193 ± 0.01, respectively; and the estimates resulting from threshold model were 0.080 ± 0.02, 0.079 ± 0.01, 0.084 ± 0.01, 0.088 ± 0.02, 0.035 ± 0.01, 0.032 ± 0.01, 0.196 ± 0.01, 0.195 ± 0.02, respectively.

The estimates of maternal genetic heritability with linear model for CR, NLB, NLBA, NLAW, NLBEE, NLWEE, TLBW and TLWW were 0.04 ± 0.02, 0.017 ± 0.01, 0.020 ± 0.01, 0.016 ± 0.01, 0.013 ± 0.01, 0.012 ± 0.01, 0.054 ± 0.02, 0.071 ± 0.01, respectively; using threshold model were 0.047 ± 0.02, 0.032 ± 0.01, 0.034 ± 0.01, 0.032 ± 0.01, 0.025 ± 0.01, 0.023 ± 0.01, 0.060 ± 0.01, 0.074 ± 0.02, respectively.

The estimates for direct heritability of CR, reported by other authors (Mohammadi *et al*., 2012a, b; Rosati *et al*. 2002; Safari *et al*. 2005), were consistent with the results of this study. The low value of heritability estimate of CR may be due to random environmental effects on variability and categorical expression of trait (Falconer, 1989). Although CR is economically important, genetic improvement of this trait by selection is difficult (Rosati *et al*., 2002). Observed negative correlations between direct and maternal genetics in Table 2 can be due to differences between direct and maternal genetic effects influencing the trait. Negative covariance between direct and maternal genetic effects indicate that antagonistic pleiotropy (between additive and maternal genetic effects) may maintain genetic variance and limit responses to selection (Wilson and Réale, 2006). Although there is high correlation between direct and maternal genetics, it cannot be considered important due to the low estimates of genetic variance for both of them (Rosati *et al*., 2002).

Differences between NLBA and NLB may probably be due to influences of environmental effects, e.g. neo-natal diseases, on lamb mortality at the first 24 hours of life and of dead-born lambs (Rosati *et al*., 2002). Heritability estimate for NLB was reported as 0.11 ± 0.01 for Makooei sheep (Mohammadi *et al.*, 2012b); 0.053 and 0.059 for Turkish Merino and Dormer sheep (Ekiz *et al*., 2005; van Wyk *et al*., 2003), respectively. The obtained results for maternal heritability estimates represent little evidence of maternal genetic effects on NLB and NLBA that is due to low estimates of maternal heritability (Rosati *et al*., 2002).

Lower maternal heritability estimate of NLAW in comparison with direct heritability estimate can indicate that model could not consider whether lambs were artificially or naturally nursed and because the ewe effect probably diminished from birth to weaning (Rosati *et al*., 2002). Reported heritability estimates in different studies for Makooei and Zandi sheep were 0.06 ± 0.01 (Mohammadi *et al*., 2012a) and 0.16 ± 0.01 (Mohammadi *et al.*, 2012b), respectively; and other heritability estimate was reported (van Wyk *et al*., 2003)

Table 2: Estimates of variance components and genetic parameters from univariate analysis of reproductive traits

Traits	σ^2_a	σ^2_m	σ^2_e	σ^2_p	$h^2_d \pm$ S.E.	$h^2_m \pm$ S.E.	ram
Linear							
CR	0.007	0.003	0.075	0.085	0.077 ± 0.02	0.034 ± 0.02	-0.78
NLB	0.007	0.002	0.091	0.101	0.074 ± 0.01	0.017 ± 0.01	0.87
NLBA	0.008	0.002	0.090	0.100	0.081 ± 0.01	0.020 ± 0.01	0.89
NLAW	0.009	0.002	0.086	0.097	0.088 ± 0.02	0.016 ± 0.01	0.85
NLBEE	0.005	0.002	0.170	0.178	0.028 ± 0.01	0.013 ± 0.01	0.82
NLWEE	0.005	0.002	0.167	0.174	0.026 ± 0.01	0.012 ± 0.01	0.82
TLBW	0.398	0.112	1.526	2.040	0.195 ± 0.02	0.054 ± 0.02	-0.78
TLWW	1.619	0.595	6.168	8.381	0.193 ± 0.01	0.071 ± 0.01	0.72
Threshold							
CR	0.007	0.004	0.075	0.086	0.080 ± 0.02	0.047 ± 0.02	-0.81
NLB	0.008	0.003	0.091	0.102	0.079 ± 0.01	0.032 ± 0.01	0.90
NLBA	0.008	0.003	0.089	0.100	0.084 ± 0.01	0.034 ± 0.01	0.91
NLAW	0.009	0.003	0.086	0.098	0.088 ± 0.02	0.032 ± 0.01	0.87
NLBEE	0.006	0.005	0.169	0.180	0.035 ± 0.01	0.025 ± 0.01	0.88
NLWEE	0.006	0.004	0.166	0.177	0.032 ± 0.01	0.023 ± 0.01	0.85
TLBW	0.404	0.123	1.528	2.060	0.196 ± 0.01	0.060 ± 0.01	-0.80
TLWW	1.646	0.631	6.177	8.450	0.195 ± 0.02	0.074 ± 0.02	0.75

σ^2_a: direct genetic variance; σ^2_m: maternal genetic variance; σ^2_e: residual variance; σ^2_p: phenotypic variance; h^2_d: direct heritability; h^2_m: maternal heritability; r_{am}: correlation of direct and maternal genetics; S.E.: standard error

for NLAW in Dormer (0.026), what is in consistence with this study. Poortahmasb *et al*. (2007) reported the heritability estimate for NLW as 0.06 ± 0.02 by linear model and 0.23 by threshold model. Estimated values in this study were comparable with the reported values.

Lower heritability estimates of NLWEE attributed to NLBEE may be probably due to loss of lambs during suckling period which is more related to lamb genotype than to ewe genotype (Mohammadi *et al.*, 2012a; 2012b; Rosati *et al*., 2002). Previous studies reported direct heritability of NLBEE in Makoeei and Zandi sheep of 0.08 ± 0.02 (Mohammdi *et al*., 2012b) and 0.12 ± 0.01 (Mohammadi *et al*., 2012a), respectively, and heritability of NLWEE of 0.04 ± 0.02 and 0.11 ± 0.01, respectively. Estimated values for NLBEE and NLWEE in this study were lower than CR, NLBA and NLAW, respectively and in consistence with weighted mean values reported previously (Safari *et al*., 2005; Fogarty, 1995).

Total litter weight at birth per ewe lambing indicates the ewe capacity to produce lamb weight at birth without considering the number of lambs born. Observations of this trait are continuous and can be regarded as normally distributed, although skewed to the right (Mohammadi *et al*., 2012b). Achieved values in this study are in consistence with the results of Mohammadi *et al*. (2012b) who reported the value 0.17 ± 0.03 for Makoeei sheep. Reported estimates are consistent with the estimates measured in this study of Safari *et al*. (2005) and Fogarty (1995). This large estimate shows that it is possible to select for total litter weight at birth per ewe lambing (Mohammadi *et al*., 2012b). If out-of-season breeding was successful, selection intensity would be larger. Actually, it might cause reduction of generation interval for TLBW observations obtained at birth. Thus, genetic trends would be available more, when generation intervals are larger reduced (Mohammadi *et al*., 2012b; Rosati *et al*., 2002). There are evidences that reported estimates (Mohammadi *et al*., 2012b; Rosati *et al*., 2002) are in consistence with estimates of this study.

Due to permanent environmental effects, phenotypic variances for basic traits were lower than the composite ones. Increasing the heritability estimate of NLAW attributed to NLBA and NLB may be due to increasing of variation between ewes and increasing similarity within ewes. Estimated (co)variance components by linear model were usually

lower than threshold model. This may be due to nature of threshold model in which a normal distribution for discrete trait is considered and sampling is carried out. In some traits like NLAW both linear and threshold models have the same direct heritability estimate. This may be due to nature and number of data sets and pedigree records.

The results obtained in this study showed that the model with genetic correlation between direct and maternal effects seems to be reliable for genetic evaluation of reproductive traits in Ghezel sheep. This means that the most appropriate model in both linear and threshold models are the same. Although heritability estimate of reproductive traits with both linear and threshold models and response to selection are low, applying the threshold model for categorical traits would increase the accuracy and consequently speed up the response to selection. It should be noted that there is a considerable variation for ewe productivity traits, especially reproductive ones. Despite large phenotypic variations for reproductive traits, heritability estimates for these traits were low. This means that genetic changes by direct selection for these traits would be difficult and non-genetic factors improvement in flocks such as nutrition of ewe before mating (flushing) and late pregnancy and controlling rams fertility can lead to the improvement of these traits.

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DETECTION OF MAJOR GENES AFFECTING GROWTH-RELATED TRAITS IN A BROILER CHICKEN LINE

F. SARBAZSHENDI[1], A. PAKDEL[2], H. MEHRABANI YEGANEH[1], S. ALIJANI[3]*

[1]Department of Animal Sciences, University of Tehran, Karaj, Iran
[2]Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
[3]Department of Animal Sciences, University of Tabriz, Tabriz, Iran

ABSTRACT

In this study the body weight at birthday (BW) and at six weeks of age (BW6) in a commercial broiler chicken line, including 1555 roosters and 12142 hens, were analyzed to detect a single locus affecting growth-related traits by using the Major Gene Index (MGI) method. Based on the selection index method, the commercial broiler line was selected for 19 consecutive years in order to achieve gain in weight in the paternal line and reproductive traits in the maternal line. The goal was to investigate the deviation of offspring-predicted breeding values from parents-predicted breeding values using the MGI method. Trait means were 42.93 and 1861.5 g for BW and BW6, respectively. The MGI values for the entire population of the commercial broiler line at three levels of α (0.5, 1 and 2) were less than 1 (0.8, 0.72 and 0.77 for BW and 0.91, 0.78 and 0.85 for BW6). The MGI values for candidate individuals were greater than 1, and this index was also increased by the change of α (0.5-2). The results indicated that 8 of 65 roosters and 115 of 314 hens for BW trait, from 58 roosters and 714 hens as candidates for BW6 trait, 9 roosters and 216 hens were identified as major gene carriers. In conclusion, the MGI approach is suggested to be a useful preliminary step to detect major genes.

Key words: body weight; commercial broiler line; major gene index

INTRODUCTION

The polygenic model of inheritance is the basis of traditional animal breeding for quantitative traits. This model assumes that a quantitative trait is controlled by many genes with small effects (Falconer and Mackay 1996; Cemal and Karaca, 2005). The great advances are achieved in animal and plant breeding relying on the classical theory. However, in recent decades several genes with major effect on economic traits have been detected in domestic animals. Such loci are called as QTL (Quantitative Trait Loci) or major loci (Cemal and Karaca, 2005). Some of them in poultry are avian dwarf and naked neck genes, which affect body size and heat resistance, respectively (Leroy *et al.*, 1989; Merat, 1990). The maximum likelihood, complex segregation analysis and mixed model methodologies are statistical methods to detect major genes and to estimate their effects and frequencies using distribution of phenotypes (Ochial *et al*., 2005). Among these methods segregation analysis is the most powerful method for major gene detection, due to the fact that the whole information about data is considered in the data analysis. This method requires complex calculations for large population. The Major Gene Index (MGI) method is an easier method that has been offered by Karlin *et al*., (1977) to prevent complex computing. In this method, it is only required to calculate the predicted breeding values of individuals and then to check the deviation between parents and offspring to determine if the candidate individual is a carrier of the major gene (Ochial *et al*., 2005). In fact, the MGI is a ratio which is a measure of the deviation of the offspring breeding value from the mid-parental breeding value and its deviation from

***Correspondence:** E-mail: sad-ali@tabrizu.ac.ir
Sadegh Alijani, Department of Animal Sciences,
University of Tabriz, 5166614766, Tabriz, Iran
Tel.: 009841113392062 Fax: 009841113356004

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each parental breeding value. This method first was used to analyze human blood pressure data using phenotypic records (Karlin *et al*., 1979). In later years, the predicted breeding values with Best Linear Unbiased Prediction (BLUP) were used to calculate the index (Famula, 1986). Indeed, the calculated index by BLUP is considered to be more reliable than that by phenotypic data (Ochial *et al.*, 2005). Estimated additive heritability for Body Weights at birthday (BW) and at six weeks of age (BW6) traits of the commercial broiler line were 0.02 and 0.21, respectively (Seraj *et al*., 2010; Salimi *et al*., 2011). No any research was conducted for detecting major genes at BW and BW6 traits in the commercial broiler line in spite of the fact, that in the world, investigations were carried out in order to detect major genes for body weight trait at various ages. The aim of this study was to investigate the presence of major genes that affected production traits by the MGI method.

MATERIAL AND METHODS

Animals and records

Commercial broiler line was selecting for productive traits over 19 years (1992-2011). The data used in the present study were related to BW and BW6. Records from 14 and 3 generations were applied for the former and the latter case, respectively. Records of three generations were used for BW6, because the number of three generation data at BW6 was approximately the same as the number of 14 generations data at BW. The examined birds included 1555 roosters and 12142 hens. Selection in the paternal line was based on growth-related traits and desired gain selection index scheme. The purpose of selection index in this line was to increase higher growth-related trait.

Statistical method for detecting major gene

Major Gene Index method

Two formulas were used for calculation of the MGI. The MGI was calculated as the below formula for a whole population of the commercial broiler line:

$$\text{MGI}(\alpha) = \frac{\Sigma^{n}_{i=1}\left[\,|O_i - 0.5(S_i + D_i)|^{\alpha}\right]}{\Sigma^{n}_{i=1}\left[\,|O_i - S_i|^{\frac{\alpha}{2}}\,|O_i + D_i|^{\frac{\alpha}{2}}\right]}$$ (Famula,1986)

where O_i, S_i, D_i, n and k – show the offspring breeding value, the rooster breeding value, the hen breeding value, the offspring's number of parents and the known parameter (0.5, 1 or 2), respectively.

The MGI was calculated as the below formula for individual as a candidate of the commercial broiler line:

$$\text{MGI}(P, \alpha) = \frac{\sum_{i=1}^{n}\frac{1}{k_i}\sum_{j=1}^{k_i}\left[\left|a(O_{ij}) - \frac{a(P) + a(M_i)}{2}\right|\right]^{\alpha}}{\sum_{i=1}^{n}\frac{1}{k_i}\sum_{j=1}^{k_i}\left[\left|a(O_{ij}) - a(P)\right|^{\frac{\alpha}{2}}\left|a(O_{ij}) - a(M_i)\right|^{\frac{\alpha}{2}}\right]}$$

(Ochial *et al.*, 2005)

where P, M_i, O_{ij} indicate individual as a candidate, the ith mate and the jth offspring from parents P and M_i respectively, a – is an indicator of a predicted breeding value, n is the number of mates, k_i is the number of offspring from parents P and Mi and α is the known parameter (0.5, 1 or 2). Values of α were recommended as three levels for evaluation of the MGI to emphasize small (α = 0.5), moderate (α = 1) and large (α = 2) deviations by Karlin *et al*. (1979).

In this study, 65 roosters with 30-40 offspring and 394 hens with 6-12 offspring for BW, 58 roosters with 40-60 offspring and 714 hens with 6-16 offspring for BW6 were chosen as a candidate P. Polygenic model assumes that the deviation of offspring from the mid-parental average is smaller than the deviation from each parent value. The MGI calculation is based on the assumption of polygenic model. Indeed, the MGI is the ratio that its numerator is the deviation of offspring from the mid-parental average and its denominator is the deviation from each parent value. Therefore, if the trait is under polygenic inheritance, the MGI value must be smaller than 1. When the MGI value is greater than 1, it might be expected that major gene is affecting the trait. It should be noted that the MGI value increases by increasing α (0.5-2).

Prediction of breeding values

Breeding values for all the birds in the line were predicted using BLUP animal model. Breeding values were calculated by using Bayesian method with Gibbs 3f90 software (Misztal, 1999). The three parameters of Gibbs sampling were: total sampling period of 100000, burn-in period of 5000 and sampling interval of 50. The used animal model was as following:

$$y = X\,b + Z_1a + Z_2m + Z_3c + e \quad \sigma_{am} \neq 0$$

where y – is a vector of observations, b – is vector of fixed effects (Generation-hatch, Sex, hen age effects for two traits and Age at recording effect for BW6), a – is an unknown random vector of direct additive genetic effect, m – is an unknown random vector of maternal genetic effect, c – is an unknown random vector of maternal permanent environmental effect and

e – is an unknown random vector of residuals. The X, Z_1, Z_2 and Z_3 are design matrices relating observation to the corresponding effects. More information about Bayesian estimation procedure can be found in Blasco (2001). The software programs SAS/STAT 9.2 (2002-2008), FoxPro (Microsoft Visual FoxPro 9.0) and Excel (Microsoft Excel 2013) were used in this study.

RESULTS AND DISCUSSION

Descriptive statistics of the studied traits was included into the Table 1. The MGI for a whole population at three levels of α (0.5, 1 or 2) is shown in the table 2. The MGI values in Table 2 were less than 1; the whole population was under polygenic inheritance. However, the calculation of the MGI for individuals, as candidate for presence of a major gene, confirmed some of them as a carrier of a major gene. Descriptive details of predicted breeding values and the MGI for candidate roosters carrying major gene are shown in Table 3, and for candidate hens carrying major gene are shown in Table 4. Only hens as carriers of major gene with more than 10 offspring for BW and hens as carriers of a major gene with more than 14 offspring for BW6 are shown in Table 4 due to the high number of hens' carrying major gene. As mentioned before, when the MGI value is greater than 1, regardless of value of α, and when the index increases by increasing of α (0.5-2), the candidate individual can be considered as a carrier of the major gene. Accordingly, among 65 roosters for BW and 58 roosters for BW6, the individuals shown in Table 3 were considered to be carriers of a major gene. Also, of the hens tested in this investigation, 115 of 314 hens were identified to be carriers of major genes for BW, and 216 out of 714 hens were identified to be carriers of major genes for BW6 (all of them are not shown in Table 4). The rooster number 73904 and his offspring have desirable predicted breeding values for BW. Additionally, the rooster number 115386, 117544, 119758 and 121040 and their offspring have desirable predicted breeding values for BW6. The hen number 75910, 65823, 65372 and 91263 and their offspring have desirable predicted breeding values for BW and the hen number 116441, 115385, 120313 and 119763 and their offspring have the same feature for BW6 (not shown in Table 4). According to these results, these roosters and hens could have segregation of a major gene with favorable effects on each trait.

There is number of researchers, who have reported about major genes in poultry. Navarro *et al.* (2006) found segregation of a major gene in the genetic control blood oxygen saturation in a commercial broiler line using segregation analysis. Ochial *et al*. (2005) showed impact of a major gene on age at sexual maturity and egg production traits in a selected laying line by using the MGI method. Alijani *et al*. (2010) investigated major gene affecting the age at first laying, body weights at the end of eight weeks and 12 weeks, average egg weight during 84 days of laying and number of eggs laid during egg production period traits in Mazandaran and Azerbaijan rural poultry. They found segregation of a major gene for all traits in Mazandaran population and for average egg weight trait

Table 1: Descriptive statistics of studied traits in the commercial broiler line

Trait	Number of records	Trait means (g)	Standard deviation (g)	Maximum value (g)	Minimum value (g)	Coefficient of variation (%)
BW	7441	42.93	4.14	58	28	9.63
BW6	8478	1861.56	355.81	2861	542	19.11

Table 2: MGI values for the entire population of the commercial broiler line

Trait	MGI (0.5)	MGI (1)	MGI (2)
BW	0.883	0.728	0.774
BW6	0.913	0.789	0.856

Table 3: Descriptive details of Predicted Breeding Values (PBV) and Major Gene Index (MGI) for roosters carrying major gene

P	N.	BW6					P	N.	BW				
Rooster	Offspring	PBV	PBV of offspring	MGI (0.5)	MGI (1)	MGI (2)	Rooster	Offspring	PBV	PBV of offspring	MGI (0.5)	MGI (1)	MGI (2)
115140	44	-181.69	-108.50	1.01	1.02	1.07	54211	30	-0.62	0.23	1.07	1.14	1.20
115386	55	68.85	47.19	1.01	1.04	1.14	65231	35	-0.67	-0.50	1.03	1.08	1.17
117544	59	49.48	33.05	1.00	1.02	1.06	73125	30	-0.26	-0.14	1.05	1.11	1.25
118236	42	-79.37	-11.51	1.03	1.19	1.37	73904	35	0.53	0.23	1.10	1.12	1.14
119758	48	131.05	71.41	1.01	1.04	1.20	78199	32	-0.22	-0.32	1.00	1.06	1.29
120263	54	-52.75	36.31	1.03	1.08	1.17	87732	32	-0.14	-0.14	1.03	1.06	1.31
121040	49	137.47	124.19	1.06	1.12	1.19	108670	31	0.03	-0.19	1.07	1.16	1.33
122519	59	-78.36	-53.57	1.02	1.05	1.06	-	-	-	-	-	-	-

Table 4: Descriptive details of Predicted Breeding Values (PBV) and Major Gene Index (MGI) for hens carrying major gene (Only hens as carriers of major gene with more than 10 offspring for BW and hens as carriers of major gene with more than 14 offspring for BW6)

P	N.	BW6					P	N.	BW				
Hen	Offspring	PBV	PBV of offspring	MGI (0.5)	MGI (1)	MGI (2)	Hen	Offspring	PBV	PBV of offspring	MGI (0.5)	MGI (1)	MGI (2)
122776	14	34.48	112.65	1.13	1.17	1.71	60123	11	0.13	-0.17	1.04	1.10	1.22
116451	15	22.56	57.32	1.02	1.07	1.20	60723	10	-0.21	-0.30	1.02	1.06	1.12
116547	15	105.51	-5.56	1.08	1.20	1.57	65006	10	-0.12	-0.62	1.06	1.15	1.31
116913	14	-205.49	-126.10	1.04	1.12	1.32	65052	10	-1.27	-0.55	1.05	1.07	1.11
117038	14	1.08	53.05	1.07	1.09	1.10	73846	12	0.65	0.59	1.02	1.08	1.21
117466	14	59.04	127.36	1.02	1.04	1.05	77475	12	0.13	0.02	1.07	1.14	1.29
117781	14	16.96	49.08	1.00	1.01	1.02	93324	10	-0.43	-0.64	1.09	1.13	1.14
118444	16	72.50	111.69	1.00	1.03	1.09	-	-	-	-	-	-	-
118731	15	55.04	7.72	1.00	1.01	1.02	-	-	-	-	-	-	-
120456	14	-23.37	-25.05	1.02	1.05	1.08	-	-	-	-	-	-	-
120848	16	-47.23	-50.10	1.02	1.03	1.04	-	-	-	-	-	-	-
121974	14	-139.77	-36.87	1.02	1.07	1.27	-	-	-	-	-	-	-

in the Azerbaijan population using segregation analysis. The results of present study are consistent with the data of Alijani *et al*. (2010) for body weight trait. Szawczkowski *et al*. (2001) suggested that existence of a major gene in egg weight and body weight traits, age at the first egg and egg production were caused by polygenic inheritance model in the Polish Rode-Island Red layer line. In this study, BW and BW6 traits were found to be influenced by a major gene, which was consistent with the results of Szawczkowski *et al*. (2001). The MGI method could be used for detecting major genes that contributed more than 20 % of phenotypic variation of a particular trait, but it cannot estimate the gene and genotypic frequency. However,

its simplicity, low cost and less time-consuming make the MGI method to be suitable as a preliminary step for major gene detecting before applying advanced Bayesian methods or molecular techniques. Detection of a major gene has several useful applications like its great impact on improvement of the efficiency of animal breeding programs by their positive effects on economical traits (Argente *et al.*, 2003), especially on low heritability traits or the traits that can only be measured in one sex (Falconer and Mackay, 1996) and their effect in our understanding of the biology of economical traits (Jennen *et al.*, 2004). Therefore, as the segregation of major gene using the MGI method for two traits was verified, the segregation of major gene in the line, investigated by the Bayesian marker-free segregation analysis methods, might be considered as the most powerful statistical method. This method is also able to estimate the effect of a major gene on interested traits as well as allelic frequency.

CONCLUSION

The purpose of this study was to investigate the presence of major genes affecting BW and BW6 traits related to a commercial broiler line in Iran by the MGI method as a simple, low cost and less time-consuming one. Analysis of the phenotypes of BW and BW6 for a commercial broiler chicken line using the MGI method suggested that the major gene can significantly affect BW and BW6 traits. The MGI values at three levels of α (0.5, 1 or 2) for some of candidate roosters and hens were greater than 1 (shown in Tables 3 and 4) and the index was increased by increasing α (0.5-2) in the individuals that were carriers of a major gene. Nowadays, despite an increase of genomic selection application in genetic improvement of economically important animal, identification of major genes is also important issue because of their great impact on improvement of animal breeding programs, on our understanding of the biology of traits and functional genomics step. Results of this paper provide a basis to support further molecular genetic studies about the genetic effects on BW and BW6 traits.

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GROWTH PERFORMANCE AND CARCASS QUALITY OF ENTIRE MALES, SURGICAL CASTRATES AND GILTS

I. BAHELKA[1]*, J. TOMKA[1], O. BUČKO[2], E. HANUSOVÁ[1]

[1]NAFC - Research Institute for Animal Production Nitra, Slovak Republic
[2]Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic

ABSTRACT

The present study evaluates the growth performance and carcass traits of entire male pigs, castrates and gilts. Pigs were crosses of Landrace sows and YxL boars. Entire males (EM), surgical castrates (SC) and gilts (G) were housed in pens (each of 2 pigs) according to sex. Entire males grew faster (EM: 974 vs. SC: 890 and G: 854 g.day^{-1}) and had better feed conversion ratio (EM: 2.71 vs. SC: 2.86 and G: 2.93 kg.kg^{-1}) than castrates and gilts, as differences compared to gilts were significant ($P < 0.01$ and $P < 0.05$, resp.). Slaughter and carcass weights of the three groups of pigs were not significantly different. Compared to SC and G, entire males had lower backfat thickness (SC: 26.71, G: 25.38 vs. EM: 20.90 mm, $P < 0.001$). Percentage of valuable meat cuts and lean meat content measured using TP (Two Point) method were the highest in EM (53.11 and 59.03 %) and were statistically significant (50.92 and 55.67 %, $P < 0.05$) in relation to C. The values of G were intermediate (52.80 and 57.87 %) and non-significant in comparison to EM ($P > 0.05$). Percentage of fatty cuts was the lowest in EM and significantly different to that of SC (EM: 11.27 vs. SC: 13.84 and G: 12.55 %, $P < 0.001$). Gilts achieved the lowest percentage of less valuable cuts than other two groups (G: 13.94 vs. EM: 15.41 and SC: 14.64 %, $P < 0.001$ and $P < 0.05$, resp.).

Key words: pigs; entire males; growth performance; carcass

INTRODUCTION

Surgical castration of male piglets is a common practice in the pig breeding industry used to prevent a development of unpleasure odour – boar taint occurring in meat of sexually mature boars. This smell is perceived negatively and such meat is rejected by most of the consumers (Font i Furnols *et al*., 2003; Bonneau and Squires, 2004). In recent years, surgical castration without anaesthesia has been criticised from the animal welfare point of view (EFSA, 2004; Prunier *et al*., 2006). Several european countries have already prohibited surgical castration without anaesthesia and EU envisages to stop surgical castration of piglets in the member states by the year 2018 (EC declaration, 2010). In the view of these changes, stakeholders involved in piglets castration have been looking for the alternatives to surgical castration. At present, one of them is rearing of entire male pigs.

The aim of this study was to evaluate the growth performance and carcass yield of entire males, surgical castrates and gilts of commercially produced hybrid pigs in Slovakia.

MATERIAL AND METHODS

Forty-two pigs, entire males (EM), surgical castrates (SC, castrated until 7 days after birth) and gilts (G), each of 14, was randomly selected for the experiment. Pigs were crosses of Landrace sows and YxL boars. From seven litters 6 sibs were selected each time

***Correspondence:** E-mail: bahelka@vuzv.sk
Ivan Bahelka, NAFC - Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 188

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(2 EM, 2 SC and 2 G). They were housed in a test station at 22-26 kg live weight because of acclimation to new space and feed. Pigs were housed in pairs in pens according to gender. They were fed by commercial diet (Table 1) according to nutrient requirements for growing-finishing pigs (Šimeček *et al*., 1995) and had free access to water.

Table 1: Composition and nutrient content of the diet

Item	%
Ingredient	
Barley	33.0
Corn	15.0
Wheat	12.0
Wheat bran	8.0
Rapeseed meal	6.0
Soybean meal	8.0
Animal fat	0.5
Premix VUL	1.0
Ground limestone	1.2
Feed salt	0.4
Monocalcium phosphate	0.8
Analyzed composition	
DM	89.92
CP	14.98
Crude fibre	4.77
Crude fat	2.35
Ash	4.17
Lysine (in DM)	7.03
Methionine + Cysteine (in DM)	5.34

DM = dry matter, CP = crude protein

Experiment started at 30 kg live weight. Pigs were weighed at the beginning, then once a month and at the end of the experiment once a week for information on growth intensity – average daily gain (ADG). Feed conversion ratio (FCR) per 1 kg of body gain was calculated per pen. After reaching the average slaughter weight of 105 kg*, pigs were slaughtered at the experimental slaughter house of the Research Institute for Animal Production situated approximately 200 m from the test stable. Age of pigs at slaughter was calculated. During the experiment, two pigs (1 EM and 1 G) were excluded because of health reasons.

A slaughter was done according to standard procedure e.g. electrical stunning, vertical exsanguination, vapour scalding and evisceration. Carcasses were measured for information on carcass length, backfat thickness and lean meat content using TP (Two Point) method. After that, carcasses were chilled 24 hours at air temperature of 2 ºC to 4 ºC, air velocity 0.5 to 1.0 m/s started approximately 60 min post mortem. The second day after slaughter, the dissection of the right half of carcass was done. Weight of shoulder, neck, loin, and ham (meat with bone) was recorded and percentages of ham, valuable meat cuts, fatty- and less valuable cuts were calculated.

Statistical package SAS (2009) was employed in the analyses. Basic statistics was done using MEANS procedure. The differences between sexes were analysed using ANOVA:

$$y_i = \mu + B_i + e_i$$

where y_i – characteristics of trait selected
μ – intercept
B_i – effect of sex (i = EM, SC, G)
e_i – random error

**A note: Entire males were slaughtered at two different slaughter weights – 105 kg and 80 kg, respectively. For this study, the results of growth performance and carcass yield of „lighter" entire males (n = 6) slaughtered at 80 kg live weight were not be taken into account.*

RESULTS

Growth performance of tested pigs is shown in Table 2. Entire males had the highest growth rates when difference compared to gilts was significant ($P < 0.01$). Similarly, boars had improved feed conversion ratio (FCR) when difference between them and gilts was also significant ($P < 0.05$). The values of castrates were intermediate. Higher growth performance of entire male pigs resulted in lower age at slaughter by 12 – 13 days compared to castrates and gilts, respectively ($P < 0.05$).

Carcass traits of entire males, castrates and gilts are presented in Table 3. Pigs were slaughtered at average slaughter weight of 104.62 to 106.07 kg. Differences between sexes were not significant. Also, any effect of sex was not observed in carcass weight and carcass length. However, entire males had significantly lower backfat thickness than gilts and castrates ($P < 0.001$). Carcasses from boars had the highest lean meat content measured by TP-method while castrates reached the lowest value. Difference between these two groups was significant ($P < 0.05$). Lean meat content of gilts had intermediate value. Weights of shoulder, neck, loin and ham did not show any effect of sex.

Table 2: Growth performance of entire males, castrates and gilts

Item	EM	SC	G
ADG in test, g	974.00 ± 40.0[a]	890.00 ± 86.0	854.00 ± 80.0[b]
FCR, kg.kg^{-1}	2.71 ± 0.22[a]	2.86 ± 0.24	2.93 ± 0.31[b]
Age at slaughter, day	159.00 ± 5.00[a]	171.31 ± 10.70[b]	172.38 ± 9.62[b]

EM = entire males, SC = surgical castrates, G = gilts, ADG = average daily gain, FCR = Feed conversion ratio
Values with different letters within rows are significantly different (min P < 0.05)

Also, percentage of ham between three groups of pigs was not statistically significant. On the other hand, entire males and gilts had significantly higher percentage of valuable meat cuts (P < 0.05) and lower percentage of fatty cuts than castrates (P < 0.001 and P < 0.05, respectively.). Percentage of less valuable cuts of gilts was significantly lower than that of entire males (P < 0.001) and castrates (P < 0.05).

DISCUSSION

Entire males in this study grew faster than castrates and gilts. It is in agreement with Blanchard *et al.* (1999) reporting higher daily live-weight gain in boars than gilts. Higher ADG in entire males than gilts, gilts and castrated or castrated males is presented also in other studies (Sather *et al*., 1991; Weatherup *et al.*, 1998; Dostálová and Koucký, 2008; Škrlep *et al*., 2012). On the other hand, some studies on growth performance of boars relative to castrates did not observe a difference between both groups (Knudson *et al*., 1985; Friend *et al.,* 1989) or some observed better growth intensity in castrates than entire males (Squires *et al*., 1993; Xue *et al.*, 1995; Dunshea *et al*., 2001; D´Souza and Mullan, 2002; Pauly *et al*., 2008). The discrepancy in these findings in the literature may be due to several factors such as dietary levels of proteins and amino acids, energy intake, age at castration, conditions of housing, slaughter weight etc. Several authors (Giersing *et al*., 2000;

Table 3: Carcass traits of entire males, castrates and gilts

Item	EM	SC	G
Slaughter weight, kg	105.57 ± 1.90	106.07 ± 2.59	104.62 ± 2.40
Carcass weight, kg	84.71 ± 1.78	86.96 ± 3.48	85.96 ± 2.85
Carcass length, cm	85.29 ± 2.87	83.29 ± 2.84	84.31 ± 1.65
Backfat thickness, mm	20.90 ± 2.21[a]	26.71 ± 1.18[b]	25.38 ± 2.30[b]
Lean meat – TP, %	59.03 ± 1.83[a]	55.67 ± 2.82[b]	57.87 ± 1.85
Weight of			
shoulder, kg	5.08 ± 0.28	4.98 ± 0.24	4.92 ± 0.18
neck, kg	3.08 ± 0.28	3.04 ± 0.18	3.03 ± 0.22
loin, kg	5.10 ± 0.44	4.81 ± 0.31	5.08 ± 0.33
ham, kg	8.80 ± 0.68	8.88 ± 0.55	9.24 ± 0.69
Percentage of			
valuable meat cuts, %	53.11 ± 1.74[a]	50.92 ± 1.13[b]	52.80 ± 2.03[a]
ham, %	21.20 ± 1.37	20.83 ± 0.96	21.89 ± 1.56
fatty cuts, %	11.27 ± 1.33[a]	13.84 ± 0.78[b]	12.55 ± 1.68[a]
less valuable cuts, %	15.41 ± 0.80[a]	14.64 ± 0.54[a]	13.94 ± 0.62[b]

EM = entire males, SC = surgical castrates, G = gilts
Values with different letters within rows are significantly different (min P < 0.05)

Cronin *et al*., 2003; Rydhmer *et al.*, 2006; Pauly *et al*., 2008) reported that entire males (group-housed) spent less time eating and more time mounting and other sexual activity. Such behaviour can induce social stress (Suster *et al*., 2006) which stimulates production of cortisol. It has been documented that higher cortisol level reduces feed intake, production of growth hormone and IGF-1 (Black *et al*., 2001). In our study, EM were housed by pairs in pens and grew up together from birth (litters were not mixed). These facts could reduce sexual behaviour and consequently the stress level and contributed to better growth rate of EM than castrates.

Feed efficiency of EM in our study was better than those of castrates and gilts. This fact is, generally, observed in all studies including those when castrates had higher growth intensity than entire males (Squires *et al*., 1993; Xue *et al*., 1995; Weatherup *et al*., 1998; Blanchard *et al*., 1999; Pauly *et al*., 2008; Dostálová and Koucký, 2008; Škrlep *et al*., 2011). It has been documented (Xue *et al*., 1995; Pauly *et al*., 2008; Škrlep *et al*., 2011) that castrates had greater appetite and different metabolism (mainly by the time of 55 kg) than boars. This difference is probably due to anabolic effect of gonadal steroids in uncastrated boars since castrates treated with testosterone or estradiol had reduced daily feed intake (Claus and Weiler, 1994). The improved feed efficiency of EM in this study is apparently related to their carcass composition. Higher lean meat content and less fat tissue (*e.g.* backfat thickness) of boars compared to castrates has been observed. While 75 % of lean tissue is water, content of water in fat tissue is only 25 %. It means that production of fat is much more energy (feed) requiring than lean tissue. Thus, entire males having more lean and less fat tissue have better FCR than castrates (Xue *et al*., 1997).

A higher ADG of entire males than castrates and gilts in our study resulted in earlier age at slaughter. However, Pauly *et al.* (2008) suggested higher age at slaughter in entire males than castrates. It has most likely been due to group-penned system of EM in the experiment.

Boars in our study had lower backfat thickness than castrates and gilts. The same results have been found in several studies (Sather *et al*., 1991; Xue *et al*., 1997; Nold *et al*., 1997; Pauly *et al*., 2008). Significantly lower deposition of subcutaneous fat tissue in EM with comparison to castrates resulted in reducing percentage of fatty cuts which corresponds with findings of Squires *et al*. (1993), Dostálová and Koucký (2008) and Pauly *et al*. (2008). No effect of sex observed in carcass length and percentage of ham is in agreement with results of Sather *et al*. (1991) and Škrlep *et al*. (2012) as well. However, another study showed significantly greater percentage of ham of entire males than castrates (Pauly *et al*., 2008).

Several studies showed an advantage of entire males in lean meat deposition related to castrates (Squires *et al*., 1993; Xue *et al*., 1997; Dostálová and Koucký, 2008; Pauly *et al*., 2008; Škrlep *et al*., 2012), gilts (Sather *et al*., 1991) or both (Nold *et al*., 1997). As mentioned above, higher lean meat content and less fat tissue in the carcasses of entire males compared to castrates (and partly to gilts) are due to differences in metabolism of energy and nutrients between boars and two other groups of pigs.

CONCLUSION

Entire male pigs have presented several advantages as compared to surgical castrates. They grew faster and improved feed efficiency than castrated male pigs. Moreover, entire males had higher proportion of lean meat and less fatty tissue in carcasses than barrows. All these findings may bring a higher financial benefit for pig producers from rearing entire males than from castrates provided they both will sell at the same price.

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NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

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METHANE YIELD FROM CATTLE, SHEEP, AND GOATS HOUSING WITH EMPHASIS ON EMISSION FACTORS: A REVIEW

J. BROUČEK

NAFC - Research Institute for Animal Production Nitra, Slovak Republic

ABSTRACT

Global methane (CH_4) concentrations are increasing in all parts of the world. This review study intends to provide an integrative approach to the complex relationships between environmental systems of farm animals. It reveals that more data are needed to better quantify CH_4 emissions from farms. Methanogenic microbial functional groups play an important role in total methane flux from agroecosystems. The factors that regulate the activity of these organisms (temperature, diet composition, feeding technique, manure management) have been documented. The research based on the literature available presented was conducted under extensive and intensive management conditions. In principle, the approaches discussed can be applied to any dairy, beef or sheep production system because their aim is increasing productivity at the herd level. Recent studies on the effects of environmental temperature, feeding, internal and genetic factors, and emission from excrements on CH_4 production are discussed. Finally, emission factors for dairy and beef cattle, as well as goats and sheep, are listed in tables.

Key words: methane; dairy cattle; beef cattle; goat; sheep; emission; manure

INTRODUCTION

Greenhouse gas emissions (GGE) from livestock and their impact on climate changes are a major concern worldwide. Enteric CH_4 production from ruminant livestock accounts for 17 – 37 % of global anthropogenic CH_4 (Lassey, 2008; Pedreira *et al.*, 2009; Alemu *et al.*, 2011; Cottle *et al.*, 2011; Knapp *et al.*, 2014).With regard to CH_4, the global livestock sector is responsible for 37 % of all human-induced CH_4 emissions, with 89 % of these livestock-derived emissions arising from enteric fermentation (Steinfeld and Wassenaar, 2007; Jiao *et al*., 2014).

Methane emissions from ruminants are the focus of scientists (Sejian *et al*., 2011; Ramin and Huhtanen, 2013; St-Pierre and Wright, 2013). With the relative global warming potential of 25 compared with CO_2, CH_4 is one of the most important GGE (Pinares-Patiño *et al*., 2007; Sejian *et al*., 2011). Decreasing methane emissions by livestock has therefore become a priority and an integral part of climate control (Martin *et al*., 2010). The leading role of livestock in methane emission has long been established (Charmley *et al*., 2008; Chagunda *et al*., 2009; Mihina *et al*., 2012).

In ruminant production systems, enteric CH_4 production is the largest contributor to GGE followed by CH_4 from manure systems, main emission sources are enteric fermentation, feed fertilization, and land application (Hensen *et al*., 2006; Klevenhusen *et al*., 2011; Hristov *et al.*, 2013; Montes *et al.*, 2013). Dairy cattle and beef cattle generate similar amounts of GGE, but on the basis of the numbers of animals beef production contributes 41 % of total sector emissions while emissions from milk production amount to 20 % of total sector emissions (Gerber *et al.*, 2013a). Methane emissions from grazing cattle are a significant source

Correspondence: E-mail: broucek@vuzv.sk
Jan Brouček, NAFC - Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 280 Fax: +421 37 6546 361

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of agricultural GGE, however, these emissions are difficult to quantify because of the sparse and roving nature of the source (Huarte *et al.*, 2010; McGinn *et al*., 2011).

Methane creation

Ruminant animals are the principal source of emissions because they produce the most CH_4 per unit of feed consumed. Ruminal gases, generated during the fermentative process in rumen, represent a partial loss of feed energy and are also pointed to as important factors in greenhouse effect (Cottle *et al*., 2011). Around 90 % of the enteric CH_4 produced by ruminants has its origin in the rumen (McAllister and Newbold, 2008; Eckard *et al.*, 2010; Dini *et al*., 2012).

The rumen is characterized as a large fermentation vat. Ruminant animals have coevolved with a complex gut microbiota in a manner that has mutually improved the efficiency of digestion of complex plant polymers. In ruminants, microbial fermentation primarily takes place in the pre-gastric reticulum and rumen, where fluid mixes freely through the reticulo-rumen fold in adult ruminants. The development of a multi-chambered fore-stomach allows for increased retention time of ingested plant biomass and therefore a greater degree of microbial fermentation of non-labile C in the form of lignin, cellulose and hemicellulose (Finn *et al*., 2015).

The total number of rumen archaeal species is unknown (Janssen and Kirs, 2008), but has been estimated to be approximately 360 to 1,000 on an operational taxonomic unit basis (Kim *et al.*, 2011; Kong *et al*., 2013). These complex anaerobic microbial communities consist of many species from divergent groups such as protozoa, fungi, bacteria and archaea (St-Pierre and Wright, 2013). The microbes ferment the plant material consumed by the animal through a process known as enteric fermentation (Cassandro *et al*., 2013). Representatives from the following orders of methanogens have been identified in rumen microbial communities: *Methanococcales, Methanobacteriales, Methanomicrobiales, Methanosarcinales and Thermoplasmatales* (Janssen and Kirs, 2008; Poulsen *et al*., 2013). Three major genera and 3 minor genera of methanogens belonging to the Archaea domain have been identified, although it is likely that more exist (Wright *et al*., 2006; Janssen and Kirs, 2008; Liu and Whitman, 2008). Only 8 methanogen species have been cultured (Kong *et al*., 2013). Methanogens are found in the hindgut as well as the rumen, although the population structure, ecology, and microbial metabolism differ between the 2 compartments (Knapp *et al*., 2014). Methanogenic microorganisms remove H_2 produced during fermentation of organic matter in the rumen and hind gut (Cottle *et al*., 2011). Enteric fermentation is thermodynamically favourable only when a hydrogen sink is present and the major hydrogenutilising microorganisms in the rumen are hydrogenotrophic methanogens. Hydrogenotrophic species belonging to the genus *Methanobrevibacter* are frequently the most active and abundant methanogens in the rumen of cattle and sheep (Wright *et al*., 2008).

A primary factor for enteric methane production is dietary carbohydrate, which influences the rate of fermentation, rate of rumen passage, and animal intake (Johnson and Johnson, 1995). The digestibility of ingested plant biomass, which is determined by the ratio of insoluble cell wall fibre to soluble carbohydrates, directs enteric fermentation to the preferential production of certain end products (Migwi *et al*., 2013). Highly fibrous, poorly digestible plant biomass leads to the production of higher proportions of methanogenic substrates and reduces rumen passage rates, resulting in higher rates of methane production (Ellis *et al*., 2009). Organisms involved in cellulose, hemicellulose, cellobiose, xylan, lipid and protein metabolism are important for animal. Most of these organisms are closely associated with particulate plant biomass and other microflora to facilitate syntrophic interactions such as plant biomass degradation and interspecies electron transfer (Edwards *et al.,* 2008; Leng, 2014; Finn *et al*., 2015).

The final products of enteric fermentation include acetate, formate, methanol, carbon monoxide, carbon dioxide and hydrogen gas, all of which are substrates for methanogenesis (Johnson and Johnson, 1995; Moss *et al.*, 2000; Merino *et al*., 2011). It was found that 89 % gases are excreted through the breath and only 11 % through the anus (Madsen *et al*., 2010).

Manure methane production

Animal manure is a valuable source of nutrients and renewable energy in the agriculture. On the other hand, livestock manure management is extremely challenging and resultant gaseous emissions may contribute to global warming. Manure from livestock operations is most often stored in solid or liquid form before being applied to agricultural land.

Methane is produced from freshly deposited manure due to bacterial processes, and from storage lagoons and settling basins due to anaerobic degradation (Hensen *et al*., 2006; Chagunda *et al.*, 2009; Borhan *et al.*, 2011a). Many of the emission pathways are controlled by microorganisms, and thus, by the optimum temperature for each specific microorganism involved (Chianese *et al.*, 2009). Klevenhusen *et al*., (2011) and Bell *et al*., (2011a) support the hypothesis that slurry methanogenesis strongly depends on storage temperature and duration, with the diet type being less important. The variation in CH_4 emission from slurry stored at cold temperature for 15 weeks was of low

importance. At a low storage temperature CH_4 production is almost negligible (Klevenhusen *et al*., 2011). CH_4 emission and oxidation rates are moisture dependent. The natural crust must stay dry in order to allow for optimal aerobic conditions inside the crust. A crust that is subjected to rainfall gets wet and anaerobic. As a result, the rate of CH_4 oxidation will strongly be reduced.

Methane production from manure (faecal material) depended on the type of waste, temperature, and duration of storage, and the manner in which the manure is handled. Emissions during composting of dung depend on factors such as aeration rate, water content, thermal insulation, weather conditions, and dung composition. During anaerobic fermentation, organic wastes are biologically degraded in the absence of oxygen to CH_4, CO_2, N_2, and H_2S. The content of organic matter labile fractions is negligible in cattle faeces but the content of the anaerobically degradable fraction is utilizable. It depends not only on feed quality and quantity but also on all factors of enteric fermentation and processes determining the digestion of ruminants (Kolář *et al*., 2010). Methanogenic fermentation of organic materials occurs under strictly anaerobic and low redox potential conditions where sulphate and nitrate concentrations are low. Methanogens produce methane by breaking down organic matter in the absence of oxygen (anaerobically), releasing CO_2 and CH_4. Methane production during composting is linked to the lack of oxygen in the decomposing biomass (Saggar *et al*., 2004). Wulf *et al*. (2001) showed that anaerobic digestion of the slurry reduced CH_4 emissions after field application, because the easily degradable organic compounds were already converted to CO_2 and CH_4 during digestion in the biogas plant.

The main factor determining the extent of CH_4 production is the amount of degradable organic matter in the effluent. This fraction is commonly expressed in terms of biochemical or chemical oxygen demand. The higher the biochemical or chemical oxygen value, the more CH_4 is produced (Saggar *et al*., 2004). The potential amount of CH_4 formation from animal faeces will depend on the amount of faecal matter excreted, the physical form of the deposit (shape, size), excretal form (solid, slurry), climatic and soil conditions, and the length of time these deposits remain intact before being decomposed. Chadwick *et al*. (2000) measured CH_4 emissions from grassland following application of pig manure, beef manure, pig slurry, dairy-cow slurry, and dilute dairy-cow effluent during different times of the year. Methane emissions were greater from dairy-cow slurry than from pig slurry, but pig manure produced much greater amounts of CH_4 (47.8 $mg.kg^{-1}$) than did beef manure (2.7 $mg.kg^{-1}$) (Saggar *et al*., 2004).

Methane production in ruminants

Methane emissions in animal husbandry originate from fermentative digestion in animals, natural anaerobic ecosystems, storage of manures, and field application. Within livestock, ruminants (cattle, sheep, and goats) are the primary source of emissions. Other livestock (swine, horses, and poultry) are of lesser importance for nearly all countries. Among the ruminants, cattle population contributes most towards enteric CH_4 production (Johnson and Johnson, 1995; Zijderveld van *et al.*, 2011; Sejian and Naqvi, 2012). Emissions from enteric fermentation exceed those from storage of slurry and manure and are regarded a key source in greenhouse gas emission reporting. However, the assessment of emissions from stored manures is difficult due to lack of experimental data (Dämmgen *et al*., 2012).

The amount of CH_4 produced by ruminants is affected by various factors including animal type and size, growth rate, level of production, and energy consumption digestibility and quantity of feeds, intake of dry matter, total carbohydrates, digestible carbohydrates, and environmental temperature. Both animal and dietary factors play an important role in predicting CH_4 production (Johnson and Johnson, 1995; Yan *et al*., 2000; Monteny *et al*., 2006; Chianese *et al*., 2009; Shibata and Terada, 2010).

Enteric fermentation emissions for ruminants are estimated by multiplying the emission factor for each species. The emission factors are an estimate of the amount of CH_4 produced (kg) per animal, and are based on animal and feed characteristics data, average energy requirement of the animal, the average feed intake to satisfy the energy requirements, and the quality of the feed consumed. The country level emission from enteric fermentation is computed as a product of the ruminant population under each category and its emission coefficient (Chhabra *et al.* 2009; Sejian and Naqvi, 2012).

Environmental temperature

Environmental temperature also influences CH_4 production and the production rate. Since the digestibility of feed tends to increase with the lower feed intake and slower rates of passage under high temperatures, it may be considered that energy loss as CH_4 decreases. However, in a high temperatures environment, the contents of the cell wall, acid detergent fiber and lignin tend to increase, causing lower digestibility of feed and higher energy loss, and resulting in an increase in CH_4 production per unit of product through the decrease in the efficiency of animal production. These phenomena occur in tropical regions but will also occur more and more frequently in temperate regions as global warming progresses (Shibata and Terada,

2010). Eckard (2011) and Cottle *et al.* (2011) found that mature beef cows emit approximately 350 g CH_4 daily in the tropics and 240 g daily in temperate zones; dairy cows emit approximately 430 $g.d^{-1}$ at peak lactation down to 250 $g.d^{-1}$ as milk yield declines. Kurihara *et al.* (1999) reported that the amount of CH_4 production in dry cows was decreasing as the environmental temperature was increasing because of decreased feed intake. However, CH_4 production per DMI increases under high temperatures. Kurihara *et al*. (1995, cited by Shibata and Terada, 2010) established a significant regression equation between DMI and CH_4 production at 18 °C and 30 – 32 °C, respectively, and concluded that CH_4 production per DMI increased at high temperatures and was about 10 % higher at temperatures above 26 °C than at 18 °C in cows at the maintenance level of feeding. The same authors also found that the effects of environmental temperature were different depending on the type of feed given: CH_4 production per DMI in lactating cows increased with temperature in high-roughage feeding while there were no significant differences among temperatures in high-concentrate feeding (Shibata and Terada, 2010). Temperature and manure storage time are the most important factors influencing CH_4 emissions because substrate and microbial growth are generally not limited (Monteny *et al.*, 2001; Chianese *et al*., 2009).

Feeding

The type and amount of feed consumed are the primary drivers affecting emissions (Sejian and Naqvi, 2012). Daily CH_4 emissions were higher in grass-based systems than in intensive systems (Arias *et al.*, 2015). Gerber *et al*. (2013b) wrote that higher emission intensities are in low productivity systems. It can be explained by low feed digestibility (leading to higher enteric and manure emissions), poorer animal husbandry and lower slaughter weights (slow growth rates leading to more emissions per kg of meat produced) and higher age at slaughter (longer life leading to more emissions). Generally, the CH_4 emission intensity of milk production is the lowest in industrialized regions of the world, compared with regional averages. Better animal feeding and nutrition reduce CH_4 and manure emissions.

But sometimes there are contradictory results. According to Pedreira *et al.* (2009), intensive managed pasture systems, with fertilized pasture and concentrate use, do generate more CH_4; methane emission by heifers grazing fertilized pasture was greater than that of heifers on unfertilized pasture.

Emissions from enteric fermentation and manure are also influenced by the composition of ruminants diets (Beauchemin *et al.*, 2008; Sasu-Boakye *et al.*, 2014). A large proportion of the variation in enteric CH_4 emissions from animals can be explained by diet composition and feed intake (Bell *et al*., 2012; Bell *et al*., 2014a). Ricci *et al.* (2014) observed significant differences between diets in finishing steers, emissions were greater for the low concentrate ration than the high concentrate ration. Jiao *et al.* (2014) demonstrated that offering concentrates to grazing dairy cows increased milk production per cow and decreased CH_4 emissions per unit of milk produced. Methane emissions of grazing animals are strongly related to feed intake, which is likely to vary with seasonal pasture conditions. When the beef cattle were grazed on pasture, they produced significantly (3.5 times) higher CH_4 than the same cattle fed a highly digestible, high-grain diet. These measurements clearly document higher CH_4 production for cattle receiving low quality, high-fiber diets than for cattle fed high-grain diets (Harper *et al.*, 1999).

Lovett *et al*. (2005) found that CH_4 production. $kg\ MY^{-1}$ was unaffected by concentrate supplementation, but CH_4 production.$kg\ FCM^{-1}$ decreased with increasing concentrate feed level. Young and Ferris (2011, cited by Jiao *et al*., 2014) observed that daily CH_4 emissions were unaffected by concentrate feeding, however, CH_4 emissions per $kg\ DMI^{-1}$ and per $kg\ ECM^{-1}$ decreased with increasing concentrate level.

The CH_4 production during feed ration 30 % hay and 70 % concentrate was significantly lower than that in 70 % hay and 30 % concentrate (Shibata *et al*., 1992). It is also known that fat supplements reduce CH_4 production (Beauchemin *et al*., 2009; Ramin and Huhtanen, 2013; Moate *et al*., 2014). Fraser *et al*. (2015) indicated that forage type had a greater impact than breed type on CH_4 emissions from growing weaned lambs.

Internal and genetic factors

Variation in enteric CH_4 emission has been reported between animals, between breeds, and across time, providing potential for improvement through genetic selection (Haas de *et al.*, 2011). It was concluded that CH_4 emissions vary considerably between dairy cows housed under commercial conditions, but ranking of cows for CH_4 emissions is consistent across time. Variation is related to LBW, MY, parity, and stage of lactation, in accordance with changes in metabolizable energy requirements (Garnsworthy *et al*., 2012b). There was no indication of individual cows with persistently low or high CH_4 yield.$kg\ DMI^{-1}$ and CH_4 yield.$kg\ MY^{-1}$ (Münger and Kreuzer, 2008). Pinares-Patiño *et al*. (2008) tested low bloat vs. high bloat cows. The mean CH_4 emissions were not different from each other.

CH_4 production is significantly different among animal species and breeds. Heifers produced about 7 times and 9 times as much as sheep and goats, respectively (Pedreira *et al*., 2009). Lactating cows produced more methane than dry cows and heifers.

Holstein cows produced less CH_4 per unit of dry matter intake than the crossbred (Pedreira *et al*., 2009). Holstein and Simmental cows had a similar CH_4 emission rate for dry period and entire lactation, while that of the Jersey cows was lower (Münger and Kreuzer, 2008). CH_4 values were significantly higher for the crossbred steers with 67 % of Angus (Limousine 33 %) compared with 67 % of Limousine (33 % Angus) (Ricci *et al*., 2015). Higher DM intake and a longer lactation period were positively correlated with lower lifetime CH_4 emissions.kg ECM^{-1} (Bell *et al*., 2011a).

Emission from excrements

Manure has often been identified as a significant source of CH_4 production. It carries an appropriate population of microorganisms, and has a readily available supply of substrate carbon (Saggar *et al.*, 2004). Methane emission rates vary depending on the type of dung. Measurements made by Jarvis *et al*. (1995) on dung patches from dairy cows, heifers, calves, and steers fed various diets at different times of the grazing season, showed a good deal of variability in emission rates amongst dung types. The total CH_4 emissions during a 10-day measurement period ranged between 300 and 2040 $mg.m^{-2}$ of dung pat. Williams (1993) also noted that CH_4 emission rates with dung from similar types of animals varied markedly, and suggested this might reflect the variation in the number of dung microorganisms that are responsible for CH_4 production. Williams (1993) measured methane emissions from fresh cattle faecal deposits and found the emissions were low but highly variable, and the dung deposits quickly dried out in the hot, dry climate. Rahman *et al*. (2013) reported CH_4 emission rates from the pen surface of a beef feedlot 38 $g.d^{-1}$.

Methane emissions from animal excreta are influenced by how they are stored (Saggar *et al*., 2004). The same authors concluded that CH_4 emission from dung would be greatly reduced if the cattle were allowed to spend most of their time in pastures during the grazing season. The highest emission measured from the pat in the field was only 11 % of the emission that would have resulted from solid manures, or 4 % of that from slurry. Methane emission factors from cattle manure produced under diverse climates (cool, temperate, and warm), systems (intensive, semi-intensive, and extensive) and cattle production functions (dairy, non-dairy, and dual purpose) have recently been studied (González-Avalos and Ruiz-Suarez, 2001). Results suggest that the dominant factor in CH_4 emissions is the feed ration, followed by fermentation temperature and the excreta moisture content.

Methane is also generated when manure is stored in anaerobic and warm conditions (Cassandro *et al.*, 2013). Most of the CH_4 emission from manure is produced under anaerobic conditions during storage with very little following land application. Manure produces less CH_4 when handled as a solid (e.g., in stacks or pits) or when deposited on pasture or rangelands. Therefore, opportunities to reduce CH_4 emission are centred on preventing anaerobic conditions during storage or capturing and transforming the CH_4 that is produced, if anaerobic conditions are present (Montes *et al*., 2013). Data summarized by Chianese *et al.* (2009) indicate average CH_4 emissions from covered slurry, uncovered slurry, and stacked manure to be 6.5, 5.4, and 2.3 $kg.m^{-2}.yr^{-1}$ although rates vary with temperature and time in storage. CH_4 emissions from manure storage averaged 4.5 $kg.m^{-3}.yr^{-1}$ being about half that from stacked manure.

It was observed that the faecal matter of animals grazing in the morning emitted much more methane than that of steers grazing in the afternoon. The difference in the emissions was in qualitative agreement with the pronounced loss of organic matter from the morning samples (Priano *et al.*, 2014).

Composting is the natural biological breakdown of dung into more stable organic substances and is an alternative to conventional management of agricultural wastes. Composting reduces volume and mass and the composted product can be trucked further distances, stored, and spread on land with little or no odour, fly breeding potential, pathogens, or weed seeds. There are four general types of composting methods on farms: passive, windrows, aerated piles, and in-vessel composting. These results suggest that composting could contribute to about one-third of CH_4 emission from livestock agriculture (Saggar *et al*., 2004). Amon *et al.* (2001) found much higher CH_4 emissions during storage and after spreading of manure from the anaerobically stacked manure than from the composted manure. Soil type had no effect on these emissions, and interaction with soil appeared to be relatively minor. It is apparent that emissions from stored animal excreta are much higher than from the dung voided in the field.

List of abbreviations

AC = accumulation chamber
AL = ad libitum
ASDM = air sampled during milking
CM = concentrate mixture
CS = corn silage
d = day
DIM = days in milk
DMI = dry mater intake
ECM = energy corrected milk
FC = flux chamber
FCM = 4 % fat corrected milk
FMFT = flux method from feed trough
FS = fattening steers
FTIR = Fourier transform infrared spectroscopy
GA = gas analyzer
GC = gas chromatography
GF = green feed system (head position sensors)
GLAS = emissions measuring from ground-level area sources
GS = grass silage
H = hay
HA = haylage
HCD = high concentrate diet
HE = heifers
IPCC Tier 2 = guidelines for national greenhouse gas inventories, method Tier 2
LBW = live body weight
LBWG = gain of live body weight
LMD = laser methane detector
LU = live unit (500 kg of LBW)
M = month
MBIGA = mass balance method from 24 h gas sampling
MF = milk fat
MHA = methane hydrocarbon analyzer
MMT = micrometeorological mass technique
MP = milk protein
MR = milk replacer
MS = manure system
MULTI = multiparous
MY = milk yield
OMA = open-path methane analyser
OPL = open-path laser
PCM = protein–corrected milk
PRIMI = primiparous
RC = respiration chamber
S = silage
SF_6 = sulphur hexafluoride tracer technique
SMAMS = snifer method in automatic milking station
SMFT = snifer method from feed trough
TDL = tuneable diode laser absorption spectrometer
yr = year

Table 1: Methane production and emission factors of dairy cattle

Calf, LBW 41 kg - 125 kg, LBWG 0.67 kg.d^{-1}; IPCC Tier 2, 9.4 kg.yr^{-1} (Dämmgen *et al.*, 2013)

23 - 50 Holstein, 1 yr; pasture, grass; FTIR, 342 g.d^{-1} (Griffith e*t al*., 2008)

12 Holstein heifers, 8 M, LBW 230 kg; rotationally grazed (flowers, clover, ryegrass); GF, 164 g.d^{-1}, 18.8 g.kg DMI^{-1} (Hammond *et al*., 2015)

12 Holstein heifers, 8 M, LBW 230 kg; rotationally grazed (flowers, clover, ryegrass); SF_6, 186 g.d^{-1}, 21.5 g.kg DMI^{-1} (Hammond *et al*., 2015)

4 Holstein HE, 14 M, LBW 317 kg; CS, GS; GF, 198 g.d^{-1}, 26.6 g.kg DMI^{-1} (Hammond *et al*., 2015)

4 Holstein HE, 14 M, LBW 317 kg, GS; RC, GA, 215 g.d^{-1}, 28.3 g.kg DMI^{-1} (Hammond *et al.*, 2015)

4 Holstein HE, 14 M, LBW 339 kg; ryegrass HA, clover, trefoil and flowers; GF, 208 g.d^{-1}, 27.8 g.kg DMI^{-1} (Hammond *et al.*, 2015)

4 Holstein HE, 14 M, LBW 339 kg; ryegrass HA, clover, trefoil and flowers; RC, GA, 209 g.d^{-1}, 27.7 g.kg DMI^{-1} (Hammond *et al*., 2015)

HE, grass, clover (grazed), RC (750 cm^2), GC, 1 kg dung, exposed 30 min., 1143 mg CH_4.m^{-2} (Jarvis *et al*., 1995)

147 Holstein HE, feedlot; TMR, H; SF_6, 631 L.d^{-1} (Kaharabata *et al*., 2000)

6 Holstein FS, LBW 334 kg; TMR, 41.4 % CS, 23.4 % grass H, 35.2 % CM; MBIGA, 103 g.d^{-1}, 0.31 g.kg LBW^{-1}, 13.6 g.kg DMI^{-1} (Newbold *et al*., 2014)

10 Holstein FS, LBW 215 kg; grazing morning, oat; RC, GC, 92.24 mg.kg fecal matter^{-1}, 576.5 mg.kg DM^{-1}, 0.067 kg.yr^{-1} (Priano *et al.*, 2014)

10 Holstein FS, LBW 215 kg; grazing afternoon, oat; RC, GC, 16.13 mg.kg fecal matter^{-1}, 89.6 mg.kg DM^{-1}, kg.yr^{-1} (Priano *et al*., 2014)

Holstein FS; alfalfa H, rice straw; RC, GA, 259.32 L.d^{-1}, 33.85 L.kg DMI^{-1} (Shibata *et al*., 1993)

6 Holstein HE, LBW 401 kg, H 66.7 %, 33.3 % MC; RC, GA, 230.9 L.d^{-1}, 28.4 L.kg DMI^{-1} (Shibata *et al*., 1992)

9 Holstein FS, LBW 150.5 kg; TMR, HCD; RC, MHA, 1.99 g.h^{-1} (Stackhouse *et al*., 2011)

9 Holstein FS, LBW 336.4; TMR, HCD; RC, MHA, 3.16 g.h^{-1} (Stackhouse *et al*., 2011)

9 Holstein FS, LBW 529.5 kg; TMR, HCD;RC, MHA, 4.15 g.h^{-1} (Stackhouse *et al*., 2011)

4 Holstein HE, 18 M, LBWG 0.7 kg.d^{-1}; CS, alfalfa H; SF_6,168 g.d^{-1} (Westberg *et al*., 2001)

Table 2: Methane production and emission factors of dairy cows

12 Holstein, LBW 600 kg, 38.9 kg ECM, 48, 125, 164, and 212 DIM; CS, clover S, CM; RC, GA, 669 L.day^{-1}, 30.6 L.kg DMI^{-1}, 24.2 L.kg ECM milk^{-1} (Alstrup *et al.*, 2015)

12 Holstein, LBW 600 kg, 38.9 kg ECM. 48, 125, 164, and 212 DIM; CS, clover S, rapeseed, CM; RC, GA, 588 L.day^{-1}, 29.8 L.kg DMI^{-1}, 17.7 L.kg ECM milk^{-1} (Alstrup *et al*., 2015)

12 Holstein, LBW 600 kg, 38.9 kg ECM, 48, 125, 164, and 212 DIM; CS, clover S, CM, vegetable fat; RC, GA, 622 L.day^{-1}, 28.5 L.kg DMI^{-1}, 17.4 L.kg ECM milk^{-1} (Alstrup *et al*., 2015)

12 Holstein, LBW 600 kg, 38.9 kg ECM, 48, 125, 164, and 212 DIM; CS, clover S, CM, calcium soaps of palm, hydrogenated palm; RC, GA, 564 L.day^{-1}, 25.6 L.kg DMI^{-1}, 14.9 L.kg ECM milk^{-1} (Alstrup *et al*., 2015)

12 Holstein, LBW 600 kg, tie-stall, slurry MS or straw MS; mobile RC, FTIR, GC, 194.4 g.d^{-1}, 194.4 g.d^{-1} (Amon *et al.*, 2001)

36 Holstein, LBW 664 kg, MY 33.3 kg.d^{-1}; TMR, 36.0 GS, 21.0 CS, 17.8 WS; ASDM, 0.24 mg.L^{-1} (Bell *et al*., 2014b)

36 Holstein, LBW 661 kg, MY 31.5 kg.d^{-1}; TMR, 36.1 CS, 19.3 GS, 18.4 WS; ASDM, 0.24 mg.L^{-1} (Bell *et al*., 2014b)

36 Holstein, LBW 662 kg, MY 29.7 kg.d^{-1}; TMR, 22.6 GS, 25.3 CS, 21.5 WS; ASDM, 0.25 mg.L^{-1} (Bell *et al*., 2014b)

Holstein, LBW 598 kg, MY 6970 L.lactation^{-1}, MF 273 kg.lactation^{-1}, MP 228 kg.lactation^{-1}; model, enteric 340 g.d^{-1}, manure 32 g.d^{-1} (Bell *et al*., 2013)

Jersey, LBW 444 kg, MY 5030 L.lactation^{-1}, MF 243 kg.lactation^{-1}, MP 188 kg.lactation^{-1}; model, enteric 281 g.d^{-1}, manure 26 g.d^{-1} (Bell *et al*., 2013)

Holstein, LBW 632 kg, lactation milk 8965 kg, milk fat 358 kg; model, enteric 395 g.d^{-1}, manure 114 g.d^{-1}, enteric 144 kg.yr^{-1}, manure 42 kg.yr^{-1} (Bell *et al*., 2015)

700 Holstein, FTIR, January, March, June, September, combined emissions (pens and storage pond) 0.34, 0.55, 0.21, and 0.20 kg.d^{-1}, combined emissions 120 kg.yr^{-1} (Bjorneberg *et al*., 2009)

3500 Holstein, free-stall, TMR (wheat H, WS, alfalfa H, CS, CM; FC, GC, 836 g. d^{-1} (Borhan *et al*., 2011a)

500 Holstein, free-stall (barn, manure lane and bedding area, loafing pen, lagoon, settling basin, silage pile, walkway); TMR (wheat H, WS, alfalfa H, CS, CM; FC, GLAS, summer, 1.04, 0.66, 21.5, 85.0, 166.0, 0.26, 0.3 g.d^{-1}, total 274 g.d^{-1} (Borhan *et al*., 2011b)

500 Holstein, free-stall (barn, manure lane and bedding area, loafing pen, lagoon, settling basin, silage pile, walkway), TMR (wheat H, WS, alfalfa H, CS, CM; FC, GLAS, winter, 0.58, 0.27, 5.1, 40.9, 4.7, 0.05, 0.25 g.d^{-1}, total 52 g.d^{-1} (Borhan *et al*., 2011b)

4 Holstein, LBW 592 kg, MY 34.3 kg, 143 DIM; 54 % CS, 46 % GS, forage to MC 50:50, supplements rapeseed meal, rapeseed cake, cracked rapeseed and rapeseed oil; RC, GA, 569 L.d^{-1}, 20.4 L.kg ECM^{-1}, 29.6 L.kg DMI^{-1}, 531 L.d^{-1}, 19.0 L.kg ECM^{-1}, 29.9 L.kg DMI^{-1}, 478 L.d^{-1}, 16.9 L.kg ECM^{-1}, 25.8 L.kg DMI^{-1}, 462 L.d^{-1}, 16.7 L.kg ECM^{-1}, 26.4 L.kg DMI^{-1} (Brask *et al.*, 2013)

11 Holstein, MY 17.46 kg, 180 DIM, grass, CS, H, CM; SF_6, 429 g.day^{-1}, 21.9 g.kg milk^{-1} (Dehareng *et al*., 2012)

8 Holstein, LBW 528 kg, 45.5 % cracked corn grain, 44.6 % alfalfa H; SF_6 vs. RC, GA, 22.3 g.kg DMI^{-1}, 431 g.d^{-1} vs. 21.9 g.kg DMI^{-1}. 455 g.d^{-1} (Deighton *et al*., 2014)

4 Holstein, LBW 542 kg, MY 16.9 kg; TMR ad libitum vs. reduced to 2/3 (70 % silage, 4 % hay, 26 % CM); RC, GA, 420 L.d^{-1}, 328 L.d^{-1} (Derno *et al.*, 2009)

100 Holstein, MY 27.0 kg, TMR, GS, CS, CM; RC, GA, 381 g.day^{-1}, 21.5 g.kg DMI^{-1} (Dijkstra *et al.*, 2011)

8 Holstein, LBW 536 kg, MY 24.9 kg, 195 DIM; grazing, grass vs. legume, SF6, 372 g.d^{-1}, 521 L.d^{-1}, 20.6 g.kg FCM^{-1}, 22.7 g.kg DMI^{-1} vs. 364 g.d^{-1}, 510 L.d^{-1}, 18.6 g.kg FCM^{-1}, 21.6 g.kg DMI^{-1} (Dini *et al.*, 2012))

82 Holstein, LBW 454 to 786 kg, MY 11 to 61 L, DIM 20 to 430, parity 1 to 4; AL TMR; CM at milking, ASDM, GA, 369 g.d^{-1} (Garnsworthy *et al.*, 2012a)

12 Holstein, MY 20 to 40 L; AL TMR, GS, CS, alfalfa H; CM at milking, RC, GA, 395 g.d^{-1} (Garnsworthy *et al*., 2012a)

215 Holstein, LBW 602 kg, MY 33 kg, DIM 161, parity 3; TMR AL, CM at milking; ASDM, 2.07 g.min^{-1}, 379 g.d^{-1} (Garnsworthy *et al*., 2012b)

18 Holstein, LBW 660 kg, MY 31.7 kg; TMR, CM 27.5 % vs. 21.7 % digestible carbohydrates; ASDM, 447 g.day^{-1} vs. 438 g.day^{-1} (Haque *et al*., 2014b)

12 pregnant Holstein, LBW 646 kg, MY 38.4 kg, GS:CS 70 : 30 vs. 30 : 70; SF6, 409 g.day^{-1}, 19.5 g.kg DMI^{-1}, 15.5 g.kg milk yield^{-1}, 316 g.kg milk fat^{-1}, 104 g.kg milk solids^{-1} vs. 397 g.day^{-1}, 17.8 g.kg DMI^{-1}, 14.7 g.kg milk yield^{-1}, 349 g.kg milk fat^{-1}, 99 g.kg milk solids^{-1} (Hart *et al*., 2015)

16 Holstein, DIM 302.4, parity 2.8; group SL, TMR, GS 600 g.kg DMI^{-1}, CM 400 g.kg DMI^{-1},starch fermentation slowly, inclusion level

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low; RC, GA, 597 L.d^{-1} (Hatew *et al*., 2015)

16 Holstein, DIM 302.4, parity 2.8; group SH, TMR, starch fermentation slowly, inclusion level high, GS 600 g.kg DMI^{-1}, CM 400 g.kg DMI^{-1}; RC, GA, 545 L.d^{-1} (Hatew *et al*., 2015)

16 Holstein, DIM 302.4, parity 2.8; group RL, starch fermentation rapidly, inclusion level low, GS 600 g.kg DMI^{-1}, CM 400 g.kg DMI^{-1}; RC, GA,581 L.d^{-1} (Hatew *et al*., 2015)

16 Holstein, DIM 302.4, parity 2.8; group RH, starch fermentation rapidly, inclusion level high, GS 600 g.kg DMI^{-1}, CM 400 g.kg DMI^{-1}; RC, GA,557 L.d^{-1} (Hatew *et al.*, 2015)

7 Dairy farms, no straw bedding, total (animals and manure), mobile TDL, 700 g.d^{-1} (Hensen *et al*., 2006)

3 Dairy farms with strawbedding, total (animals and manure), mobile TDL, 1400 g.d^{-1} (Hensen *et al*., 2006)

7 Dairy farms, slurry manure storage, winter, 1200 m^3, mobile TDL, 11 g.m^{-3}.d^{-1} (Hensen *et al*., 2006)

32 Swedish Red, LBW 664 kg, MY 30.2 kg, DIM 134; TMR (60 % forages, 40 % CM), CM from feed trough units; FMFT, 453 g.d^{-1}, SMFT 1405 ppm (Huhtanen *et al*., 2015)

107 Holstein, LBW 675 kg, MY 29.5 kg, LBWG 0.55 kg, TMR (60 % forages, 40 % concentrates), CM from feed trough AMS; FMFTAMS 447 g.d^{-1}, SMAMS 758 ppm (Huhtanen *et al*., 2015)

Dairy cow, grass, clover (grazed), CM; RC (750 cm^2), GC, 1 kg dung exposed 30 min., 1702 mg.m^{-2} (Jarvis *et al.*, 1995)

Dairy cow, S, CM; RC (750 cm^2), GC, 1 kg dung exposed 30 min., 716 mg.m^{-2} (Jarvis *et al*., 1995)

Dairy cow, fertiliser grass, CM; RC (750 cm^2), GC, 1 kg dung exposed 30 min., 2040 mg.m^{-2} (Jarvis *et al*., 1995)

40 Holstein (12 PRIMI, 28 MULTI), grazing ryegrass, CM (2.0, 4.0, 6.0, and 8.0 kg.d^{-1}); SF6, 287, 273, 272, and 277 g.d^{-1}, 20.0, 19.3, 17.7, and 18.1 g.kg DMI^{-1}, 15.4, 12.9, 11.2, 10.8 g.kg milk^{-1} (Jiao *et al*., 2014)

36 Holstein, LBW 600 kg, MY 32.3 kg; diet 2.3 % fat; SF_6, 16.2 g.h^{-1}, 543 L.d^{-1}, 16.8 L.kg milk^{-1} (Johnson *et al.*, 2002)

36 Holstein, LBW 600 kg, MY 39.3 kg; diet 4.0 % fat; SF_6, 16.4 g.h^{-1}, 550 L.d^{-1}, 14 L.kg milk^{-1} (Johnson *et al*., 2002)

36 Holstein, LBW 600 kg, MY 39.1 kg; diet 5.6 % fat; SF_6, 19.0 g.h^{-1}, 637 L.d^{-1}, 16.3 L.kg milk^{-1} (Johnson *et al.*, 2002)

90 Holstein, LBW 600 kg; TMR and 1.5 kg H (timothy, alfalfa); SF_6, 542 L.cow^{-1}.d^{-1}, 19 L.kg of milk^{-1} (Kaharabata *et al*., 2000)

118 Holstein, tie-stall, LBW 602 kg, MY 28.5 kg; TMR, CM; MBIGA, 587 L.d^{-1}, after subtracting manure contribution 552 L.d^{-1}, 19.4 L.kg of milk^{-1} (Kinsman *et al.*, 1995)

67 lactating cows, LBW 583 kg, MY 17 kg; RC, 420 L.d^{-1}, 24.7 L.kg milk^{-1} (Kirchgessner *et al.*, 1991, cited by Boadi *et al.*, 2004)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0; TMR corn diet (corn, ryegrass, barley, mixture of forage and CM 0.45 : 0.55; RC, GA, 303 g.d^{-1}, 22.8 g.kg DMI^{-1}, 22.1 g.kg milk^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0; TMR corn diet (corn, ryegrass, barley, mixture of forage and CM 0.45 : 0.55; slurry stored 7 weeks at 14 °C vs. 27 °C; RC, GA, 0.4 g.d^{-1} vs. 9.8 g.d^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0; TMR corn diet (corn, ryegrass, barley, mixture of forage and concentrate 0.45 : 0.55, slurry stored 15 weeks at 14 °C vs. 27 °C, RC, GA, 6.1 g.d^{-1} vs. 131.3 g.cow^{-1}.d^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0, TMR barley diet (barley, corn, ryegrass, mixture of forage and CM 0.45 : 0.55); RC, GA, 364 g.d^{-1}, 24.0 g.kg DMI^{-1}, 23.6 g.kg milk^{-1} (Klevenhusen *et al.*, 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0, TMR barley diet (barley, corn, ryegrass, mixture of forage and CM 0.45 : 0.55); slurry stored 7 weeks at 14 °C vs. 27 °C, RC, GA, 0.6 g.d^{-1} vs. 7.5 g.d^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), BW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0, TMR barley diet (barley, maize, ryegrass), mixture of forage and concentrate (0.45 : 0.55), slurry stored 15 weeks at 14 °C vs. 27 °C, RC, GA, 5.6 g.d^{-1} vs. 108.1 g.d^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), BW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0; hay-only diet (low starch); RC, GA, 338 g.d^{-1}, 25.1 g.kg DMI^{-1}, 23.6 g.kg milk^{-1} (Klevenhusen *et al*., 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3.0; hay-only diet (low starch); slurry stored for 7 weeks of storage at 14 °C vs. 27 °C; RC, GA, 1.5 g.d^{-1} vs. 15.8 g.d^{-1} (Klevenhusen *et al.*, 2011)

18 cows (11 Holstein, 7 Brown Swiss), LBW 649 kg, MY 16.9 kg, 215 DIM, parity 3, hay-only diet (low starch), slurry stored for 15 weeks at 14 °C vs. 27 °C; RC, GA, 11.2 g.d^{-1} vs. 74.8 g.d^{-1} (Klevenhusen *et al*., 2011)

10800 Holstein, 20 open-lot pens (60 ha), wastewater storage pond (10 ha), compost yard (10 ha), LBW 635 kg; TMR; MBIGA, 490 g.d^{-1},

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103 $g.m^{-2}.d^{-1}$, 13.5 $g.m^{-2}.d^{-1}$, combined emissions (lots, wastewater pond and compost) 1.39 $kg.d^{-1}$ (Leytem *et al.*, 2010)

24 Holstein, LBW 582 kg, MY 24.5 kg, 231 DIM; ryegrass, meadow, CM 1 kg vs. 6 kg; SF_6, 346 $g.d^{-1}$ vs. 399 $g.d^{-1}$, 19.60 g.kg DMI^{-1} vs. 17.83 g.kg DMI^{-1}, 19.26 g.kg FCM^{-1} vs. 16.02 g.kg FCM^{-1} (Lovett *et al*., 2005)

4 Holstein cows, LBW 705 kg, 113 DIM, MY 29.3 kg; TMR (60 : 40 forage : CM); SF6, 326.6 $g.d^{-1}$, 15.8 g.kg DMI^{-1}, 11.7 g.kg $milk^{-1}$, 13.2 g.kg FCM^{-1} (Meale *et al*., 2014)

10 Holstein, LBW 593 kg, milk per lactation 6502 kg, dry period, entire lactation; grass, H AL; RC, GA, 196 $g.d^{-1}$, 394 $g.d^{-1}$ (Münger and Kreuzer, 2006)

10 Jersey, LBW 354 kg, milk per lactation 4097 kg, dry period, entire lactation; grass, H AL; RC, GA, 149 $g.d^{-1}$, 309 $g.d^{-1}$ (Münger and Kreuzer, 2006)

10 Simmental, LBW 636 kg, milk per lactation 5578 kg, dry period, entire lactation; grass, H AL; RC, GA, 222 $g.d^{-1}$, 392 $g.d^{-1}$ (Münger and Kreuzer, 2006)

20 (4 Norwegian, 4 Norwegian × Holstein, 12 Holstein), 4 PRIMI, 16 MULTI, MY 22.9 kg, 56 DIM; GS, CM 45 % DM basis; SF_6, 469 $g.d^{-1}$, RC, GA 422 $g.d^{-1}$, 24.3 g.kg DMI^{-1}, 19.9 g.kg $milk^{-1}$ (Muñoz *et al*., 2012)

24 Holstein, LBW 494 kg, 70 DIM, parity 3.4; grazing ryegrass, 1 kg CM vs. 5 kg CM (reduce herbage intake by 1.8 kg $DM.d^{-1}$ compared to cows receiving 1 kg CM); SF_6, 323 $g.d^{-1}$, 357 $g.d^{-1}$ (Muñoz *et al*., 2015)

24 Holstein, 70 DIM, LBW 494 kg, parity 3.4; grazing ryegrass, 1 kg CM or 5 kg CM (reduce herbage intake by 4.4 kg DM/d, compared to cows receiving 1 kg CM); SF_6, 349 $g.d^{-1}$, 390 $g.d^{-1}$ (Muñoz *et al.*, 2015)

164 – 195 Holstein, LBW 600 kg, MY 31 – 33 kg; GS, CS, CM; MBIGA, 9.0 – 13 $g.LU^{-1}.h^{-1}$ (Ngwabie *et al*., 2009)

141 lactating Holstein vs. 75 dry Holstein; model, 363 $g.d^{-1}$ vs. 241 $g.d^{-1}$ (Ngwabie *et al*., 2014)

141 lactating Holstein, 75 dry, model; enteric 312 $g.d^{-1}$, indoor manure 73 $g.d^{-1}$ (Ngwabie *et al*., 2014)

9 Friesian x Jersey, LBW 407 kg, 3 yr, 167 DIM; grazing, ryegrass and white clover; SF_6, 327 $g.d^{-1}$ (Pinares-Patiño *et al.*, 2007)

9 Friesian x Jersey, LBW 455 kg, 3 years, non-lactating, non-pregnant; fresh pasture forage; SF_6, 301 $g.d^{-1}$, 26.4 g.kg DMI^{-1} (Pinares-Patiño *et al*., 2007)

12 Friesian x Jersey, LBW 402, 3 yr; pasture ryegrass, white clover, 2 periods; SF_6, 144.5 $g.d^{-1}$, 147.9 $g.d^{-1}$, 346 mg.kg LBW^{-1}, 345 mg.kg LW^{-1} (Pinares-Patińo *et al*., 2008)

88 – 109 Holstein, LBW 600 kg, MY 29 kg; TMR, CS 30 %; alfalfa HA 26 %; H 9 %, CM 35 %; MBIGA, 622 $L.d^{-1}$, 21.4 L.kg $milk^{-1}$ (Sauer *et al*., 1998)

6 Holstein, LBW 603 kg, MY 37.1 kg, 3.6 yr, 62 DIM; TMR, CS, alfalfa H, corn, CM; RC, GA, 557 $L.d^{-1}$, 15 L.kg $milk^{-1}$ (Sechen *et al.*,1989)

Holstein, pregnant, dry; CS, alfalfa H, H, CM; RC, GA, 268.43 $L.d^{-1}$, 33.84 L.kg DMI^{-1} (Shibata *et al*., 1993)

Holstein lactating; CS, alfalfa H, H, CM; RC, GA, 464.04 $L.d^{-1}$, 27.17 L.kg DMI^{-1} (Shibata *et al*., 1993)

9 dry Holstein, Free-stall, LBW 770 kg; TMR, alfalfa, oat H, CM; MBIGA, cow and manure 12.35 $g.h^{-1}$ (Sun *et al*., 2008)

9 lactating Holstein, Free-stall, LBW 565 kg, MY 31 kg; TMR, Corn, alfalfa, oat H, cottonseed meal, CM; MBIGA, cow and manure 18.23 $g.h^{-1}$ (Sun *et al.*, 2008)

720 Holstein, LBW 602 kg; MBIGA, 305 $g.d^{-1}$ (Zhu *et al*., 2011)

4 Holstein, LBW 673 kg, MY 22 kg; alfalfa based diet; SF_6, 446 $g.d^{-1}$ (Westberg *et al*., 2001)

4 Holstein, LBW 673 kg, MY 22 kg; corn based diet; SF_6, 405 $g.d^{-1}$ (Westberg *et al*., 2001)

CONCLUSION

Agriculture is a major contributor to GGE, in particular of methane. The actual rate of CH_4 emission is highly dependent on the management strategies implemented on a farm. Consequently, improvements in management practices and changes in demand for livestock products will affect future CH_4 emissions.

Knowledge of experimental studies that quantify CH_4 production from agriculture is important in order to better establish typical emission ranges for farms and the effect of management factors on these emissions.

Further research will address these limitations through direct measurement of livestock methane emissions from a range of forages and through the integration of selected forage inputs. New approaches will be required in genetics and nutrition to provide perspective on the contribution of CH_4 emission from ruminants to global GHG emissions. Specifically, data are needed on CH_4 emissions from manure storage and housing facilities.

Table 3: Methane production and emission factors of beef cattle

Simbrah HE (5/8 Brahman, 3/8 Simmental), 1 yr; grazing, bermudagrass, bahiagrass, and ryegrass, winter bahiagrass H, CM;SF_6, 89 – 180 $g.d^{-1}$ (DeRamus *et al.*, 2003)

Simbrah cows (5/8 Brahman, 3/8 Simmental), 3 to 7 yr, grazing, bermudagrass, bahiagrass, and ryegrass, winter bahiagrass H, CM, SF_6, 165 – 294 $g.d^{-1}$ (DeRamus *et al*., 2003)

4 Murray Gray x Charolais x Angus HE, 19 M, pregnant 3 M, LBW 435.5 kg;grazing, Yorkshire fog, Phalaris, Dead grass vs. feedlot, oats, alfalfa; MMT, 260 $g.d^{-1}$vs. 66 $g.d^{-1}$ (Harper *et al.*, 1999)

Calf, fertilized (N) grass (grazed); RC (750 cm^2), GC, 1 kg dung exposed 30 min., 1655 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

Heifer, grass-clover (grazed), RC (750 cm^2), GC, 1 kg dung exposed 30 min., 1143 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

Heifer, low-N grass (grazed), RC (750 cm^2), GC, 1 kg dung exposed 30 min., 423 mg $CH_4.m^{-2}$ (Jarvis *et al.*, 1995)

Steer, grass-clover (grazed), RC (750 cm^2), GC, 1 kg dung exposed 30 min., 406 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

Steer, low (N) grass (grazed), RC (750 cm^2), GC, 1 kg dung exposed 30 min., 503 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

Steer, unfertilized (N) grass (grazed), RC (750 cm^2), GC, 1 kg dung exposed 30 min., 300 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

Suckler cow, rough grazing, RC (750 cm^2), GC, 1 kg dung exposed 30 min., 922 mg $CH_4.m^{-2}$ (Jarvis *et al*.,1995)

13 Brahman steers (*Bos indicus*), LBW 227 kg; 22 diets, 5 tropical grass, 5 legumes; RC, GA, from 42.0 to 159.0 $g.day^{-1}$ or from 17.5 to 22.4 $g.kg\ DMI^{-1}$ (Kennedy and Charmley, 2012)

HE, enteric fermentation, 61 $kg.yr^{-1}$ (Lima *et al*., 2010; citated by Mazzetto *et al.*, 2015b)

Cow, enteric fermentation, 63 $kg.yr^{-1}$ (Lima *et al*., 2010; citated by Mazzetto *et al*., 2015b)

Bull, enteric fermentation, 55 $kg.yr^{-1}$ (Lima *et al.*, 2010; citated by Mazzetto *et al.*, 2015b)

Calf, enteric fermentation, 42 $kg.yr^{-1}$ (Lima *et al.*, 2010; citated by Mazzetto *et al*., 2015b)

Steer, enteric fermentation, 42 $kg.yr^{-1}$ (Lima *et al*., 2010; citated by Mazzetto *et al.*, 2015b)

Beef cattle, 13,800, feedlot, LBW 265 - 620 kg vs. 16,500, feedlot, LBW 280 - 700 kg; high grain diets; OPL, model, 146 $g.d^{-1}$ vs. 166 $g.d^{-1}$ (Loh *et al*., 2008)

Beef cattle, faeces, 0.08 $kg.yr^{-1}$ (Mazzetto *et al.*, 2014)

13,800 beef cattle, feedlot, Australia, LBW 350 - 600 kg vs. 22,500 beef cattle, feedlot, Canada, LBW 265 - 620 kg; high grain diet; OPL, model, 166 $g.d^{-1}$ vs. 214 $g.d^{-1}$ (McGinn *et al*., 2008)

30 Brahman cattle (*Bos indicus*), LBW 425 kg, grazed, Rhodes grass, Sabi grass, and Verano Stylo; OPL, 240 – 250 $g.d^{-1}$ (McGinn *et al*., 2015)

6 Angus steers, 1 yr; pastures, tall fescue, white clover; SF_6, 95 to 200 $g.d^{-1}$ (Pavao-Zuckerman *et al*., 1999)

4 Angus cows, 3 yr; pastures, tall fescue, white clover; SF_6, 150 – 240 $g.d^{-1}$ (Pavao-Zuckerman *et al*., 1999)

192 cattle, feedlot; corn, distillers grains, CS, H; air samples, GC,2.66 ppm, overall emissions 1.32 $g\ m^{-2}\ d^{-1}$ (Rahman *et al.*, 2013)

8 Belmont Red steers, LBW 436; Rhodes grass H, CM; RC, GA, 174.1$g.d^{-1}$, 20.0 $g.kg\ DMI^{-1}$, 0.36 $g.kg\ LBW^{-1}$ (Ramírez-Restrepo *et al*., 2014)

72 Angus and Limousin crossbred, steers, LBW 673 kg, 16 M, low concentrate diet (48:52 forage to concentrate ratio (40 % grass silage, 35 % barley silage, 15 % barley grain, and 10 % maize distillers dark grains) vs. high concentrate diet (8:92 forage to concentrate ratio (12 % straw, 68 % barley grain, and 20 % maize distillers dark grains); RC, GA, 205 $g.d^{-1}$ vs. 145 $g.d^{-1}$ (Ricci *et al*., 2015)

9 Black Angus crossed steers, LBW 340 kg, high concentrate diet; RC, MHA, 2.85 $g.h^{-1}$ (Stackhouse *et al*., 2011)

9 Black Angus crossed steers, LBW 544 kg, high concentrate diet; RC, MHA, 4.18 $g.h^{-1}$ (Stackhouse *et al*., 2011)

9 Brahman (*B. indicus*) and 9 Belmont Red (*Bos taurus* x *African Sanga*) steers, LBW 222 kg; grazed, pasture Rhodes grass, OPL, 136.1$g.d^{-1}$, 29.7 $g.kg\ DMI^{-1}$, 0.57 ± 0.067 $g.kg\ LW^{-1}$ (Tomkins *et al.*, 2011)

9 Brahman (*B. indicus*) and 9 Belmont Red *(Bos taurus* x *African Sanga*) steers, LBW 222 kg; freshly cut Rhodes grass; OPL, 114 $g.d^{-1}$, 30.1 g $kg\ DMI^{-1}$, 0.49 $g.kg\ LW^{-1}$ (Tomkins *et al*., 2011)

12bulls, LBW 498 kg, 9 M; pasture good (spring), poor (fall), winter feed diet;SF6, 231 $g.d^{-1}$, 188 $g.d^{-1}$,228 $g.d^{-1}$ (Westberg *et al*., 2001)

4 suckling calves, LBW 206 kg, 4 M; pasture;SF_6, 53 $g.d^{-1}$ (Westberg *et al.*, 2001)

16 cows, LBW 585 kg, 4 yr; pasture, good (spring), poor (fall), winter feed diet, early lactating diet;SF_6, 231 $g.d^{-1}$, 188 $g.d^{-1}$, 211 $g.d^{-1}$, 201 $g.d^{-1}$ (Westberg *et al*., 2001)

12 HE, LBW 225 – 275 kg, 18 M; grower diet, good pasture, poor pasture; SF_6, 135 $g.d^{-1}$, 179 $g.d^{-1}$, 223 $g.d^{-1}$ (Westberg *et al*., 2001)

8 beef, feedlot, LBW 544 kg, LBWG 0.9 kg vs. 0.5 kg, 12-17 M; high-grain finishing diet vs. stocker diet; SF_6, 193 $g.d^{-1}$ vs. 175 $g.d^{-1}$ (Westberg *et al.*, 2001)

Table 4: Methane production and emission factors of goats and sheep

4 Japanese goats, 2 years, LBW 26 kg; timothy H, alfalfa H, corn, MC; RC, GA, 31 mL.g DMI^{-1} (Bhatta *et al.*, 2008)
Sheep, Scottish grey face; grazing, ryegrass, 10.8 ha; OMA, 20.5 $g.d^{-1}$, 7.4 kg yr^{-1} (Dengel *et al.*, 2011)
16 weaned lambs, Welsh Mountain vs. Welsh Mule × Texel, fresh cut ryegrass, RC, GA, 15 $g.d^{-1}$ vs. 17 $g.d^{-1}$, 16.1 g.kg DMI^{-1} vs. 16.7 g.kg DMI^{-1}, 5.4 $kg.yr^{-1}$ vs. 6.3 $kg.yr^{-1}$ (Fraser *et al*., 2015)
16 weaned lambs, Welsh Mountain vs. Welsh Mule × Texel, fresh cut permanent pasture, RC, GA, 12 $g.d^{-1}$vs. 14 $g.d^{-1}$, 16.7 g.kg DMI^{-1} vs. 18.8 g.kg DMI^{-1}, 4.3 $kg.yr^{-1}$ vs. 5.1 $kg.yr^{-1}$ (Fraser *et al.*, 2015)
9 lambs, 90 d, LBW 20.9 kg; grass H; GA, 19.9 $g.d^{-1}$, 116.3 g.kg $LBWG^{-1}$, 31.1 g.kg DMI^{-1} (Haque *et al*., 2014a).
9 lambs, 90 d, LBW 21.8 kg, 2.5 $L.d^{-1}$; 50:50 MR, dairy cream; GA, 3.2 $g.d^{-1}$, 11.5 g.kg $LBWG^{-1}$, 4.3 g.kg DMI^{-1} (Haque *et al*., 2014a).
9 lambs, 150 d, LBW 33.7 kg; grass H; GA, 19.1 $g.d^{-1}$, 113.9 g.kg $LBWG^{-1}$, 34.3 g.kg DMI^{-1} (Haque *et al*., 2014a).
9 lambs, 150 d, LBW 34.7 kg, 2.5 $L.d^{-1}$; 50:50 MR, dairy cream; GA, 2.4 $g.d^{-1}$, 9.1 g.kg $LBWG^{-1}$, 1.1 g.kg DMI^{-1} (Haque *et al*., 2014a).
4 wether sheep, 1.5 yr, LBW 51.0 kg; white clover; RC, GA, 25.7 $g.d^{-1}$, 22.5 kg.DMI^{-1} (Hammond *et al*., 2014)
4 wether sheep, 1.5 yr, LBW 51.0 kg, ryegrass; RC, GA, 24.5 $g.d^{-1}$, 22.0 kg.DMI^{-1} (Hammond *et al*., 2014)
30 wether sheep, 5x6, LBW 51.4 kg; ryegrass, 0.50, 0.76, 1.02, 1.26, 1.51 kg $DM.d^{-1}$; RC, GA, 13.1 $g.d^{-1}$, 27.0 g.kg DMI^{-1}; 19.5 $g.d^{-1}$, 27.0 g.kg DMI^{-1}; 23.2 $g.d^{-1}$, 25.2 g.kg DMI^{-1}; 27.1 $g.d^{-1}$, 25.3 g.kg DMI^{-1}; 31.9 $g.d^{-1}$, 23.9 g.kg DMI^{-1} (Hammond *et al*., 2014)
Sheep, H, CM; RC (750 cm^2), GC, 1 kg dung exposed 30 min., 598 mg $CH_4.m^{-2}$ (Jarvis *et al*., 1995)
4 Korean native black goats, LBW 23.5 kg; 50:50 forage, CM; RC, GA, 11.6 $g.d^{-1}$, 24.7 g.kg DMI^{-1} (Li *et al*., 2010)
41 sheep, metaanalysis, LBW 47.6 kg; 19.0 $g.d^{-1}$, 20.3 g.kg DMI^{-1} (Patra, 2014)
20 Romney sheep, 14 M, LBW 45 kg; grazing, ryegrass, white clover; SF_6, 28.9 - 35.5 $g.d^{-1}$ (Pinares-Patiño *et al.*, 2003)
24 Scottish Mule ewes, 29 DIM, 5.5 yr, LBW 68 kg; alfalfa AL vs. restricted alfalfa (0.8 of AL); RC, LMD, 109.7 $g{\cdot}pair^{-1{\cdot}d^{-1}}$, 83.2 $g{\cdot}pair^{-1{\cdot}d^{-1}}$ (Ricci *et al*., 2015)
160 ewes, 50:50 alfalfa H, oaten H; MBIGA, 22.2 $g.d^{-1}$ (Robinson *et al*., 2014)
10 wethers sheep, Corriedale, LBW 71 kg; 66.7:33.3 H, CM; RC, GA, 34.3 $L.d^{-1}$, 25.9 L.kg DMI^{-1} (Shibata *et al.*, 1992)
11 wether goats, Japanese native, LBW 39 kg; 66.7:33.3 H, CM; RC, GA, 25.2 $L.d^{-1}$, 27.1 L.kg DMI^{-1} (Shibata *et al*., 1992)
Sheep, goats; H, CM; RC, GA, 28.55 $L.d^{-1}$, 26.70 L.kg DMI^{-1} (Shibata *et al*., 1993)

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KNOWLEDGE OF MILK TRAITS IN SLOVAK DAIRY SHEEP: A REVIEW

M. ORAVCOVÁ

NAFC - Research Institute for Animal Production Nitra, Slovak Republic

ABSTRACT

The objective of this review is to summarize the knowledge of milk traits, lactation curves and genetic evaluation of dairy sheep in Slovakia. Thus, an emphasis was given on milk yield (daily and milking period yield), fat content and protein content. The special attention was drawn to modelling of lactation curves for these traits using the Ali and Schaeffer regression model. The following breeds: Tsigai, Improved Valachian i.e. breeds of local provenience providing low milk yields, and Lacaune (a specialized dairy breed providing high milk yield), were involved in the analyses. Various sources of information: test day records with daily milk yield, milking period yield, fat content and protein content incorporated in single-trait models and multi-trait models were reviewed. Accordingly, the experience with estimations of genetic parameters and proportions of variance components for milk traits was covered. Approaches based on alternative strategies treating milk yield (fat and protein content) in individual months of lactation either as the same trait or as a different trait were documented (Tsigai chosen as a model breed). The review attempts to summarize the recent experience with description of milk traits (lactation curves and genetic evaluation) in dairy sheep in Slovakia.

Key words: Tsigai; Improved Valachian; Lacaune; dairy; lactation curve; genetic evaluation

INTRODUCTION

Sheep industry is an important branch of livestock production in Slovakia with about 400 thousands heads in total, out of which 270 thousands are ewes. Milk and cheese production predominate; about 168 thousand ewes are milked. In 2013, marketed milk production was 11000 tons (Gálik, 2014). The most numerous dairy sheep in Slovakia are breeds of local provenance: Tsigai and Improved Valachian. The less numerous are imported dairy breeds: Lacaune and East Friesian. At present, a size of synthetic population of Slovak dairy sheep with genetic portion of Lacaune and East Friesian is increasing. The proportion of ewes in milk performance recording is up to 10 % of dairy ewes. An average milk yield per ewe in recorded flocks is about 110 kg in Tsigai and Improved Valachian, and about 210 kg in Lacaune (see Results of Milk Performance Testing of Sheep and Goats, 2012, 2013, 2014). The first study dealing with analyses of milk yield and milk composition in sheep kept in the territory of Slovakia was done in the beginning of the 20th century (Laxa, 1908). In recent times, studies have been aimed at investigating lactation curves and genetic evaluation of dairy sheep based on either milking period or daily milk yield (Margetín and Milerski, 2001; Oravcová *et al.*, 2005, 2006a,b, 2007; Oravcová and Peškovičová, 2008). However, no comprehensive review summarizing up-to-date knowledge of milk traits in dairy sheep in Slovakia has been published yet. The objective of this review is to summarize the knowledge of milk traits, lactation curves and genetic evaluation of dairy sheep in Slovakia.

Correspondence: E-mail: oravcova@vuzv.sk
Marta Oravcová, NAFC - Research Institute for Animal Production Nitra,
Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 378 Fax: +421 37 6546 361

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Milk traits

The most important milk traits in Slovak sheep are considered milk yield, fat and protein content. The first study dealing with investigations on these traits in ewes kept on the territory of Slovakia originated in the beginning of the 20th century (Laxa, 1908). Further studies were conducted since 1950s (Šulc, 1957; Janotík, 1958; Semjan, 1972; Špánik and Mikuš, 1988). Recently, either milk traits of ewes in individual flocks (Čapistrák *et al*., 1995, 2002, 2005) or milk traits of ewes in milk performance recording (Margetín *et al*., 1998a, 1998b, 2005; Oravcová *et al*., 2006, 2007) have been analyzed. Milk performance testing of Slovak sheep using the AC method as defined by ICAR (2014) has been routinely recorded under the guidance of the Breeding Services of the Slovak Republic since 1995.

Local Tsigai and Improved Valachian ewes produce lower milk yields than most breeds in Europe. Daily milk yield (Oravcová *et al*., 2015) of ewes in milk performance recording was 0.640 kg (Tsigai) and 0.667 kg (Improved Valachian) during the period 2006-2010, reflecting the effort to increase milk yield from 0.583 kg (Tsigai) and 0.562 kg (Improved Valachian) during the period 1995-1999. Daily milk yield of Lacaune ewes was 1.053 kg (Oravcová *et al*., 2006). This corresponds with the fact that Lacaune ewes in Slovakia are of lower milk yields (Oravcová, 2007) than Lacaune ewes in France (Barillet *et al*., 2001; Berger, 2004). Opposite to daily milk yield, fat and protein content decreased between the periods 2006-2010 and 1995-1999: 8.22 vs. 7.59 % (Tsigai) and 7.92 vs. 7.51 % (Improved Valachian). Fat and protein content in Lacaune ewes were lower in comparison to Tsigai and Improved Valachian ewes i. e. 6.97 and 5.62 % (Oravcová *et al*., 2007). In the beginning of milk performance testing (years 1995 and 1996), Margetin *et al*. (1998a, 1998b) reported the following milk yields: 0.55 L (Improved Valachian) and 0.53 L (Tsigai), fat contents: 8.15 % (Improved Valachian) and 8.46 % (Tsigai), protein contents: 5.73 % (Improved Valachian) and 6.74 % (Tsigai). Comparisons with earlier study of Špánik and Mikuš (1988) showed that fat and protein contents changed minimally during last thirty years. For local sheep breeds, these authors reported fat content about 7.8 % and protein content about 6.05 %.

Among factors affecting the variability of milk traits in Slovak dairy sheep the most important were: flock-test day effect, parity, number of lambs born, days in milk, and also, direct additive genetic and permanent environmental effect of ewe. The statistical models applied to study the influence of factors affecting milk traits in Slovak dairy sheep were able to explain 49 % to 59 % of the total variability (Oravcová *et al*., 2006a, 2007).

Lactation curves

Breed-specific lactation curves of daily milk yield were modelled using the Ali and Schaeffer (Ali and Schaeffer, 1987) regression (Oravcová *et al*., 2002, 2006a, 2006b, 2015) and Wood (Wood, 1967) model (Krupová *et al*., 2009). In modelling breed-specific lactation curves for fat and protein content the Ali and Schaeffer model was employed (Oravcová *et al*., 2007, Oravcová, 2015). Lactation curves were modelled as submodels incorporated in general linear model (SAS, 2002-2003) and mixed model methodology (variance component estimation). Formerly, regression coefficients for days in milk were estimated for the first, second and third (and later) parity separately (Oravcová *et al*, 2006 and 2007) i.e. different shapes of lactation curves resulted for each milk trait (milk yield, fat and protein content) in each investigated parity. In the recent analysis (Oravcová *et al*., 2015), regression coefficients of days in milk were estimated for each breed regardless of parity: shifting between parities was estimated on the basis of different intercepts. Due to limited number of test-day measurements in the first month after parturition (lambs are weaned about 55 days on average), lactation curves were estimated since day 30 (Oravcová *et al*., 2015). The shape of lactation curves for milk yield, fat and protein content in Slovak sheep was in accordance with the shape of lactation curves reported in literature. Lactation curves of Slovak Lacaune breed corresponded to lactation curves of dairy sheep (lower persistency, higher changes in milk traits between earlier and later days in milk), whereas lactation curves of Tsigai and Improved Valachian breeds corresponded to lactation curves of multipurpose breeds (higher persistency, smaller changes in milk traits between earlier and later days in milk). Milk yield decreased along with increasing days in milk, and fat and protein content increased along with increasing days in milk, regardless of difficulties with modelling the beginning and ending phases of lactation curves (Oravcová *et al*., 2015). When lactation curves were estimated for individual parity separately, some atypical shapes were revealed (Oravcová *et al*., 2006, 2007). When lactation curves were estimated (only for milk yield) with Wood model by Krupová *et al.* (2009), these were found to be of typical shape for both Tsigai and Improved Valachian. These curves slightly differed from lactation curves estimated by Oravcová *et al*. (2006), mainly in the beginning and end of lactation (less test-day measurements available and related underestimation or overestimation of milk yield).

Genetic evaluation

Single-trait and multi-trait animal models were employed in genetic evaluation of dairy sheep in Slovakia.

Genetic evaluation of milk traits can either be based on individual test day records or cumulative milking period records. Single-trait models based on cumulative milking period records were used in the beginning of effort aimed at adopting genetic evaluation in Slovak dairy sheep (Margetín and Milerski, 2001). Predicting accurate breeding values, however, needs all effects affecting the traits to be accounted for, to be known. Genetic evaluation based on individual test day records has a number of advantages. One main advantage, apart from operational ease lies in a better possibility to account for sources of variation affecting each test day (Swalve, 1998). Estimates of variance (covariance) components and predicted breeding values were calculated by means of univariate and multivariate animal models (test-day models) taking into account similar effects as statistical models analyzing most important factors affecting variability of milk traits in Slovak dairy sheep. Variance (covariance) components were estimated using REML (Restricted Maximum Likelihood) method as applied in VCE 5 (Kovač *et al*., 2002) and VCE 6 (Groeneveld *et al*., 2010) softwares. Breeding values were predicted using PEST software (Groeneveld *et al*., 1993). All these methodologies are incorporated in the routine genetic evaluation of Slovak sheep which is done by the Breeding Services of the Slovak Republic on a yearly frequency.

Estimated coefficients of heritability for daily milk yield ranged from 0.10 (Improved Valachian) to 0.19 (Tsigai), for fat content ranged from 0.06 (Improved Valachian) to 0.12 (Tsigai) and for protein content ranged from 0.07 (Improved Valachian) to 0.25 (Lacaune). These were found on the lower values reported for dairy sheep in literature (Oravcová *et al*., 2005, Oravcová, 2007, Oravcová and Peškovičová, 2008). Breeding values expressed as averages across birth years of animals involved in the analyses and environmental changes expressed as averages of flock-test day solutions over years and months of milk performance testing were used to analyze genetic and environmental trends in investigated populations. These are useful when revealing patterns how genetic and environmental effects influence variability of milk traits (mainly milk yield) depending on time (Oravcová and Peškovičová, 2008). With breeding values, research on their reliability in males has been done (Oravcová *et al*., 2005) recently. It showed that reliability increased with the number of daughters tested per male. Males and/or their sons with the higher breeding values as well as higher reliabilities should be preferred in selection. Thus, analyzed milk traits can be improved genetically.

The strategy of treating test day measurements in individual months of lactation as a different trait has been also investigated in Slovak dairy sheep recently (Oravcová 2014, 2015). These analyses, undertaken on milk performance data of Tsigai breed, showed that milk yield, fat and protein content are mostly correlated in the middle of lactation (0.95 to 0.98 for milk yield, 0.94 to 0.99 for fat content and 0.95 to 0.99 for protein content). When effects involved in animal models were defined similarly as in repeatability models, where milk yield (fat and protein content) was treated as the same trait, heritability estimates differed minimally for each milk trait (see Oravcová *et al*., 2005, Oravcová and Peškovičová, 2008 vs. Oravcová, 2014, 2015 for comparisons).

CONCLUSION

The study attempts to summarize the knowledge of milk traits, lactation curves and genetic evaluation of dairy sheep in Slovakia. The information provided here, however, may not be considered as complete. For instance, recent research aimed at milkability and economic importance of milk traits in Slovak dairy sheep was not included in the present study.

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