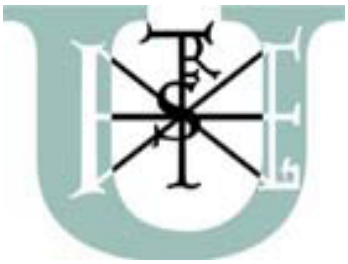

SZENT ISTVÁN UNIVERSITY

FACTORS AFFECTING THE MATERNAL TRAITS OF THE EWES

PhD Thesis

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The PhD school

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1. Introduction, object of the research

Milk and milk product are consumed by humans from the beginning of history. Importance of that kind of food even increased as our knowledge about nutrition improved. Sheep milk has better nutritional values, than cow milk, and based on its unique flavour popular worldwide. Sheep milk is also having a beneficial physiological value due to its macro- and microelement content. Sheep milk products can be sold almost without limits in the world market, as there's no quota applied on its export.
Profitability of the branch can be improved based on sheep milk production, there're a lot of possibilities still in Hungarian sheep milk production.
I'm analysing the maternal traits of ewes in my study, based on the principles bellow.

2. Aims of the study

The aims of my research were the following ones.

1. Evaluating the maternal traits (reproduction rate, number of lambing) of sheep belonging to different genotypes (Hungarian Merino, German Mutton Merino, German Blackhead Mutton, Lacaune).
2. Analysing the temperament of dairy sheep: the effect of temperament on milk quality and production traits in a Lacaune population.
3. Milk quality studies: the effects of the somatic cell count on the chemical and physical traits of sheep milk.
4. Studying udder morphology: analysing udder morphology of dairy sheep, correlations between udder morphology and the hygiene quality (somatic cell count, defining bacteria species harmful to udder health).

3. Material and methods

As combines various points of views, more experimental designs were necessary for the 1st aim. So for the 1st point I've conducted three studies, while in case of the 2nd, 3rd and 4th points draw my conclusions based on one experimental design for each aim.

3.1. Material and methods connected to Aim 1

The first experiment connected to my first aim was designed to study what is the effect of the age, lactation number, parity, weaning weight and life weight of dams to its milk production.

a.) Analysis of some factors affecting the milk production of Lacaune ewes

The study was conducted in Mórichida, Győr-Moson-Sopron County, Kisalföld region, Hungary. The farm is specialised to breeding and milking Lacaune sheep, the approximately 375 ewes are under permanent production control of the Hungarian Sheep Breeders Association (Magyar Juhtenyésztők Szövetsége). Ewes taking part in the study (n=110) were chosen randomly among those having a closed lactation, their milking started at the same time in March and lasted for 228 day in average. Age of animals was between 1 and 6 years, and they were in their 1st to 5th lactation. Ewes were milked twice a day by a milking machine in Hungaro Lact milking parlour with 2x24 parallel milking stands. Milking was between 5-6 a.m., and 5-6 p.m., so 12 hours between them. Sheep were grazed in lawn, which needed

renovation (most common species: perennial ryegrass/*Lolium perenne*, smooth meadow-grass/*Poa pratensis*, red fescue/*Festuca rubra*, common bird's-foot trefoil/*Lotus corniculatus*) from April until November (190 days) with pastoral grazing. Ewes were fed 400 g fodder (60% corn, 40% oats) daily, meadow and alfalfa hay and mineral lick next to grazing. Dams were lambed once a year, during spring.

I've analysed the effect of the age of dams, lactation number, parity, and live weight at weaning, at one year of age and as matured among the factors influencing milk production. Ewes were grouped into three (bellow 20 kg, 20-25 kg and over 25 kg) at weaning and four at one-year-old (30-39 kg, 40-49 kg, 50-59 kg, 60-69 kg) based on live weight, I've studied the lactation milk yield and the length of lactation according to this.

Statistical analysis was conducted by SPSS 23.0 (Shapiro-Wilk test, Levene test, GLM, Tukey post hoc test) according to the following equation:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + E_m + e_{ijklm}$$

Y_{ijklm} = trait studied; μ =mean, A_i = effect of age (fix effect: 4 groups), B_j = effect of number of lactation (fix effect: 5 groups), C_k= effect of parity (fix effect: 3 groups), D_l= effect of weaning weight (fix effect: 3 groups), E_m= effect of life weight at one year (fix effect: 4 groups), e_{ijklm} = error.

In the second experiment according to Aim 1 I was analysing the life production and the effect of year.

b.) The effect of the year to the life production of ewes

I've studied the life production of dams and the effect of year in Törtel, Pest County, Hungary at a breeding station. There're approximately 1000 dams in the farm, belonging to Hungarian Merino, German Black-headed Mutton and German Mutton Merino genotypes. Animals for the study were chosen randomly from all genotypes, Hungarian Merino (n=40), German Mutton Merino (n=40) and German Black-headed Mutton (n=40). I've analysed the life production of 10-10 ewes from each year. Ewes in the study were born in 2004, 2005, 2006 and 2007. Ewes were involved into breeding at about 15 months of age, all with artificial insemination. Ewes in oestrus were chosen by test rams (Hungarian Raczka and Hungarian Czigaja). After the first lambing all breeds were lambed in every 8 months, oestrus synchronizing was applied on German Black-headed Muttons before insemination when not in the main season. Feeding of the breeding animals varied among years, according to the nutritional value of the feed, but must be stressed, that they were always in optimal breeding condition.

I've used SPSS 20.0 for the statistical analysis, analysed variance and used t probes.

In the third experiment for the Aim 1 I've studied the possibilities of crossbreeding.

c.) The effect of crossing a Hungarian Merino population with Landschaf Merino on the reproduction traits of ewes

This experiment took place in Kardoskút (Körös-Maros National Park, Békés County, Hungary) in a commercial sheep farm. Hungarian Merino and Hungarian Merino x Landschaf Merino F_1 ewes were inseminated in two breeding seasons. The average age of dams was 4 years. Hungarian Merino (n=50-50) and Hungarian Merino x Landschaf Merino F_1 (n=50-50) ewes were inseminated by Hungarian merino rams is the autumn and in the spring breeding season, the mating system was harem mating in all four cases.

Ewes were additionally fed by 10 dkg fodder through whole year, while during rearing lambs got 70 dkg fodder (corn, oats and barely in equal quantity). After lambing dams with their lambs were housed separately in hosts, where they've spent 2-3 days. Lams were fed alfalfa hay *ad libitum*, and mixture of cracked corn and oats till weaning (70 days in average). Sheep were grazing with traditional, pastoral method. The age of rams was 5-7 years, while their live weight was between 88-110 kg when used for stud.

I've studied the fertility percentage of the dams, the number of lambs per lambing, the ratio of twin lambing, the number of lambs dead till weaning, and the lamb number at weaning. I've used SPSS 21.0 for the statistical analysis (average, standard deviation, Chi^2 probe).

3.2. Material and methods connected to Aim 2

This study took part in a Hungarian dairy sheep farm (Mórichida: 47º 29'10,5 „N 17ő 25'18,4" E. 42). Lacaune dams which already lambed earlier (were in their 2nd to 5th lactation) and were free from the clinical signs of mastitis and feet disease took part in the analysis. I've recorded the length of suckling, the lactation milk yield and measured the fat, protein, and lactose content of the milk, so as the pH and its electrical conductivity and the somatic cell count.

Experiment and milking started in the middle of March. Animals in the same stage of lactation were housed in barns. Ewes were milked twice a day, in 2x24 Hungaro Lact milking parlour. Grazing was through the whole experiment, which started in the middle of April. Next to it, animals were fed by a concentrate (400 g/day; EI: 7,1 MJ/kg DM; rough protein 180 g/kg DM), containing vitamins (A, D3, E). Animals in the experiment could choose the licking salt whenever wanted.

I've evaluated the temperament of the dams on the 48th, 74th and 103rd days of suckling, during the morning and evening milking. A 5 score system was used for evaluating temperament, during the preparation of the udder and the whole process of milking (BUDZYNSKA et al., 2005), with direct scoring:

1.) Very nervous, constant and forceful stepping and kicking;
2.) Constant and forceful stepping, but no kicking;
3.) Sometimes pronounced leg movements;
4.) Quiet animal, few leg movement;
5.) Very quiet, no leg movement.

Milk samples from the ewes were collected three times after scoring the temperament. I've used LactoScope™ (Delta Instruments Ltd., Netherlands) machine for analysing the milk composition (fat, protein and lactose). Extech EC500 instrument was used for measuring the

pH and the electric conductivity of the sheep milk. Somatic cell count was determined in Livestock Performance Testing Ltd (Gödöllő, Hungary) by Bentley FCM machine.

Statistical analysis

I've used SPSS 23.0 statistical package for the analysis. I've combined the data of the collected milk samples and the temperament scores for the statistical evaluation. I merged the groups score 2 (n=3) and score 3 (n=5), due to their small sample size. I've studied the effect of temperament (fix effect, 3 groups) to the chemical and physical traits of the milk, and to somatic cell count and production (dependent variables). Before the actual analysis I've performed Shapiro-Wilk test for investigating whether the sample shows a normal distribution. Repeated ANOVA was used for the chemical and physical parameters of the milk, correlations between groups were computed by Tukey post-hoc test ($P<0.05$). I've used one-way ANOVA, with Tukey post-hoc test ($P <0.05$) for determining the significant differences in case of the length of lactation and milk production.

3.3. Material and methods connected to Aim 3

In this study I've analysed the fat, protein and lactose content of sheep milk, and it's pH and electric conductivity in correlation with somatic cell count. The study took part in a sheep breeding station in Győr-Moson-Sopron County, Hungary, in 2013. Lacaune breed is kept in this farm, approximately 300 ewes. 42 Lacaune ewes, in their different lactation were chosen randomly for the study. Milking of the ewes started at the end of March, and in general lasted for 189 days. Average milk yield per ewe was 184 kg. Milking animals got fodder (400 g/day) as feed supplementation as well. Traditional pastoral grazing was applied in the farm. Sheep were milked twice a day, by milking machine in a Hungaro Lact milking parlour with 2x24 stands. I've collected milk samples from mixture milk of the fully milked udder three times, in the second (48^{th} day), third (74^{th} day) and fourth month (103^{rd} day) of lactation, at the same time, when the Hungarian Sheep and Goat Breeders Association collected the monthly samples.

I've determined the milk yield of dams based on the measured monthly samples. We1ve collected 2x50 ml milk sample from each ewe in tubes, first was utilised for determining the somatic cell count, while the second for measuring the milk protein, milk fat and lactose content, the pH and the electric conductivity. For determining the content LactoScope™ machine (Delta Instruments Ltd., Netherlands) was used, while pH and electric conductivity was measured with Extech EC500 machine. We've used a machine applying fluorescent optoelectric technique (Bentley FCM) for determining the somatic cell count (ÁT Kft., Gödöllő, Hungary).

I've determined three categories based on mean somatic cell count values in in my study:

- < 200 thousand/ml (n=19);
- 200 thousand /ml – 1000 thousand /ml (n=17)
- 1000 thousand /ml < (n=6).

SPSS 22.0 software was used for the statistical analysis (checking normality and homogeneity, ANOVA, Tukey post-hoc test, Pearson correlation). I've applied Kolmogorov-Smirnov test for checking normality. Based on it my data showed a normal distribution – except somatic cell count – so I've applied parametric tests. I've checked the homogeneity of

data with Levene test before analysing variance. As I've measured the content and the physical parameters of the milk on every ewe three times, I've used analysis of variance for repeated measures (GLM). In case of milk yield of the ewes I've used a one factor ANOVA. Somatic cell count category was the fixed effect in both cases. I've applied Tukey post-hoc test, as there was a difference in the number of individuals among groups. I've taken the logarithm (log base ten) of the somatic cell count, then applied Pearson correlation for finding the connection of the traits studied.

3.4. Material and methods connected to Aim 4

This study took part in two Lacaune breeding stations, both in Győr-Moson-Sopron County, in 2009, 2011 and 2012. Further I refer to those as farm 1 and farm 2. I've analysed the following udder morphology traits: udder size, udder shape, udder placement, udder symmetry, length and diameter of teats, teat placement and shape. Udder size, shape, width, placement and teat placement were scored on a 1 to 5 scale (1 meaning bad, 5 meaning excellent), while length and diameter of the teat was measured in mm precisely.
I've evaluated the udder traits according the linear scoring system described by De la Funte at al. (1996), modified in a way, that the original uses a 1 to 9 score system, while I've applied a 1 to 5 one, when 5 means excellent, 4 good, 3 average, 2 weak and 1 bad qualification.
I've evaluated the udder of 51 ewes in farm 1, and 216 in farm 2 in 2009. In 2011 the number of dams milked increased, to 70 in farm1, and to 283 in farm 2. I've 86 ewes in farm 1, and 282 in farm 2 in 2012. SPSS 21.0 software was used for the statistics.

4. Results

4.1. Maternal traits in Lacaune and other sheep breeds, based on life production, number of lambs and the effect of crossing on reproduction

Results of the study in the breeding station in Mórichida:

Lacaune ewes (n=110) chosen randomly took part in this experiment. The age of animals were between 2-6 years, while their lactation number between 1 and 5. I've classified the animals in three groups (20> kg, 20-25 kg and 25 kg<) based on weaning weight, and in four groups, based on live weight at one year old (30-39 kg, 40-49 kg, 50-59 kg, 60-69 kg) and analysed the length of milking and the lactation milk yield according to this.

The age of ewes, the number of lactation and heir live weight at one year old had an effect on the lactation milk yield according to my results. Lacaune ewes, which were between 60-69 kg at one year old, produced significantly (P <0.05) more milk than animals that weighted less. Weaning weight and parity didn't have an effect on milk production of the ewes.

Results of the study in the breeding station in Törtel:

Reproduction, reproductive traits are the base of sheep breeding being economically productive (MARSELEK – ABAYNÉ HAMAR, 2008). Ewes born is 2004, 2005, 2006 and

2007, belonging to Hungarian Merino (n=40), German Merino Mutton (n=40) and German Black-headed Mutton (n=40) took part in this experiment. Ewes were involved in breeding at 15 moths of age, with artificial insemination.

Dams were lambing in every 8 month after the first lambing. Keeping system was the same in case of all three breeds.

Reproduction traits of the German Merino Mutton increased in parallel with the number of lambing, until the 10th lambing according to my results. Birth year didn't have an effect on reproduction traits in case of appropriate keeping system and feeding.

The effect of crossing in case of Hungarian Merino x Landschaf Merino on the reproduction traits of ewes:

This study took part in a commercial sheep farm in Kardoskút, Hungary. Hungarian Merino (n=100), and Hungarian Merino x Landschaf Merino F_1 (n=100) crossed ewes were in the study. Ewes were grouped in two in case of both genotypes and all inseminated with Hungarian merino rams.

Average age of the ewes was 4 years. Reproduction traits of the Hungarian Merino x Landschaf Merino F_1 dams were more advantageous ($P < 0,05$) according to my results, than of purebred Hungarian Merino ewes. **Improving reproductive traits are necessary for the productive sheep husbandry in Hungary, and one of the possibilities of it is the utilization of crossbred dams (which are more prone to twin lambing) in commercial farms.**

4.2. Correlation of temperament and somatic cell count and their effect on the chemical and physical parameters of the mil and on milk yield in Lacaune sheep breed

42 Lacaune ewes took part in this study. Temperament of the ewes was measured on a 5-point scale (1 meaning very nervous, while 5 meaning calm) during milking.

Results of applied ethology are getting more and more important in animal husbandry. Animal – human – environment- technology interactions are in the focus of that field of study for being able to set the optimal live circumstances for the animals through describing their demands (GYÖRKÖS et al, 1995; GERE and CSÁNYI, 2001).

Length of lactation, milk yield, milk composition (fat, protein and lactose), pH, electric conductivity and somatic cell count was analysed in this part of my study. According to my measures the lactose content of the milk was significantly lower, while electric conductivity higher in the nervous group, compared to calmer ones. **Next to it, significant differences were found between temperament categories in the somatic cell count of the milk: calmer dams show lower somatic cell number (5.78 log/cm^3), than the more nervous ones (5.67 log/cm^3; $P < 0.05$). Calmer ewes had a significantly longer lactation** (score 4: 211 days; score 5: 199.8 day), and higher milk yield (score 4: 201.7 kg; score 5: 194.2 kg), compared to the nervous animals (128.9 days and 129.0 kg; $P < 0.05$).

4.3. Correlation of somatic cell count with lactation milk yield and of some chemical and physical parameters of the milk in Lacaune ewes

I've analysed the milk production, the composition of the milk and the changes in some physical parameters of the milk in correlation with the somatic cell count in Lacaune ewes (n=42). I've measured three times during the study the fat, protein and lactose content of the milk, and the pH, the electric conductivity and the somatic cell count, and also evaluated the days milking lasted and the lactation milk yield. I've divided the animals into three groups based on somatic cell count: Group 1: <200 thousand/ml, Group 2: 200 thousand – 1000 thousand /ml, Group 3: > 1000 thousand /ml.

Somatic cell number had a significant effect on the lactose content and electric conductivity of the sheep milk. I've found positive correlation ($P < 0.01$) between the somatic cell count and protein content (r=0,35), and pH value (r=0.45) and electric conductivity (r=0.37). There's a negative correlation between somatic cell count and lactose content (r=-0.58), and lactation milk yield (r=-0.38).

Based on my results it can be concluded that somatic cell count – especially in the category over one million cells – has a significant effect on sheep milk's yield, composition, and its physical and hygienic traits. Somatic cell count is a good indicator of udder health as even in case of a subclinical mastitis the yield of the produced milk is decreasing.

4.4. Analysing udder conformation traits in Hungarian Lacaune populations

I've analysed udder morphology traits of milking ewes in three years. Conformation traits of the udder and the teats have a medium to high heritability, so selection can be successful even in one or two generations. In my experiment udder conformation treats improved already in three years.

Using Lacaune breed more frequently can be advised based on my results, also as a crossing partner. Selection based on udder conformation improves the morphological traits of the udder and the teats already in a short term.

5. New scientific results

1.) Age of ewes and next to it, the number of lactation and live weigh at one year old has an effect on lactation milk yield in case of Lacaune ewes. Lacaune dams between 60-69 kg live weight at one year old produced significantly ($P < 0.05$) more milk, than lighter individuals. Ewes produced the most milk during their second lactation ($P < 0.05$). Weaning weight and parity didn't have an effect on milk production.

2.) Reproduction rate significantly decreased starting from the 6th lambing Hungarian Merino and German Black-headed Mutton, while in German Merino Mutton increased until the 9-10th lambing. It is advised to keep for replacement the ewes born from dams kept in breeding for a long time, with advantageous reproduction traits.

3.) Weaning lamb number per dam in Hungarian Merino can be significantly increased with targeted crossing with Landschaf Merino. Reproduction traits of Hungarian Merino x Landschaf merino F_1 dams were significantly better ($P < 0.05$), than of the Hungarian Merino ewes.

4.) Temperament of ewes has a significant effect on milk production traits, length of lactation, lactation milk yield, milk composition (fat, protein, lactose), pH, electric conductivity of the milk and on somatic cell count in Lacaune sheep breed. Lactose content of the milk is significantly lower, while electric conductivity is higher in nervous ewes than in calmer animals. Somatic cell count of calmer dams is lower (5.78 log/cm^3), than of teh more nervous ones (5.67 log/cm^3; $P < 0.05$), also lactation of the calmer ewes is significantly longer (score 4: 211 days; score 45: 198.8 days) and their milk yield is higher (score 4: 201.7 kg, score 5: 199.2 kg), than of the more nervous ewes (128.9 days and 129.0 kg, $P < 0.05$).

5.) Somatic cell count – especially in the category over one million cells – is in correlation with the yield, composition of the produced sheep milk, and with its physical and hygienic traits. There's a positive correlation ($P < 0.01$) between somatic cell count and protein content ($r = 0.35$), pH value ($r = 0.45$) and electric conductivity ($r = 0.37$). Correlation is negative between somatic cell count and lactose content ($r = -0.58$), and lactation milk yield ($r = -0.38$).

6.) Pronounced improvement can be achieved in short term - even in 2-3 generations – with the udder conformation judgement (concerning udder placement, udder width and length, udder depth and the length and diameter of teats) applied in Lacaune breed, which is also visible in the decreased accidence of mastitis.

6. Discussion and recommendations

6.1. I've analysed the maternal traits in case of different sheep genotypes (Hungarian Merino, German Merino Mutton, German Black-headed Mutton and crossed Hungarian Merino x Landschaf Merino)

German Merino Mutton ewes had better reproduction indicators as Hungarian Merino ones, because twin lambing was more frequent is case of that breed. I've concluded, that German

Merino Mutton dams are exceeding the results of Hungarian Merino dams in case of reproduction traits and life production.

Reproduction traits of dams can be improved with targeted crossing, in case of Hungarian Merino x Landschaf Merino F_1 ewes lamb number per a dam can be significantly increased.

Improving reproduction is the base of economically effective sheep husbandry, so my results are advised to be applied. In case of appropriate crosses fertility can be enhanced in commercial sheep farms.

Reproduction traits of German Merino Muttons improved in parallel with the consecutive lambing, so it is advised to keep for breeding the female lambs born from ewes kept in breeding after their 8^{th} lambing. Fertility traits improved until the 6^{th} lambing, then decreased in case of Hungarian Merino breed. Offspring of dams with long and high life production are advised to be kept for breeding animal replacement.

6.2. I've studied how age, lactation number, parity, so as weaning and one year old live weight affects milk production in Lacaune ewes

It could be concluded that age of ewes, lactation number and live weight at one year all affect the quantity of milk produced during lactation. Lacaune ewes between 60-69 kg at one year produced significantly ($P < 0.05$) more milk, than lighter individuals.

Lactation number had a significant effect on milk yield, however didn't affect the length of lactation based on my results. The highest milk yield was measured in dams in their 2^{nd} lactation ($P < 0.05$).

6.3. I've analysed the effect of the temperament of lactating sheep on the milk quality and milk yield

Length of lactation, milk yield, milk composition (fat, protein, lactose), pH, electric conductivity and somatic cell count was analysed in this study. There was no significant difference between temperament categories in case of milk fat, protein content and pH. That is in contradiction with a previous reported result (MURRAY et al., 2009), which found higher protein content in calm ewes than in nervous ones.

Temperament had a significant effect on lactose content ($P = 0.022$) and on electric conductivity ($P = 0.004$). The more nervous group (score 2 and 3) had lower lactose content and higher electric conductivity, than the calmer group. Calmer dams showed lower somatic cell number

Furthermore, calmer dams produced more milk, next to lower somatic cell count, than more nervous ones.

6.4. Milk production of Lacaune dams, and the effect of somatic cell count on the chemical and physical parameters of sheep milk

Disadvantageous udder health significantly affects the quantity and quality of milk produced, based on my results. Increased somatic cell count had an effect on days of lactation and on lactation production. Decreased milking period and milk yield was recognized in categories with higher somatic cell number (over 200 000).

Lactose content significantly decreased in parallel with increasing somatic cell count. Electric conductivity values increased as somatic cell count also increased.

Number of days in milking were 27% lower, while quantity of produced milk 38% less in the group of somatic cell count over one million.

Constant control of udder health, and fast intervention is case of negative changes is necessary based on my results.

6.5. Analysis of udder conformation traits in Hungarian Lacaune populations

Udder morphology traits are not taken in consideration pronounced among selection criteria in Hungarian dairy sheep farms nowadays, although their effect on milk production is known (KUKOVICS et al., 1993; 1999).

Udder of the dams have to be huge, and of glandular substance. Teats have to be sound, cylinder shape, their length 20 mm, and diameter at their base 15 mm. Two developed teats have to be on the udder. Teats in horizontal direction to the ground give the best possibility for milking.

Investigating udder and teat conformation is an important part of selection against mastitis. Size of teats is also important in rearing the lambs. I've studied the udder conformation traits in two Lacaune breeding stations. Selection for udder morphology resulted is fast advance in both farms.

Publications by the author in topics connected to the study (grouped as described by the PhD school)

Publications:

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