Hungarian University of Agriculture and Life Sciences

MILK PRODUCTION AND UDDER HEATH STUDIES IN HUNGARIAN DAIRY CATTLE AND SHEEP POPULATIONS

DOCTORAL DISSERTATION THESIS

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1. INTRODUCTION

The milk production in 2022 exceeded 930 million tons in the World (FAO, 2024), out of which cattle milk had been 753,3 million tons, while sheep milk 10 million tons (FAO, 2024). There are 191 thousand dairy cows registered now in Hungary (KSH, 2023), their number is a little bit decreasing through the recent years. However, quantity of the milk produced slightly increased in previous years, in 1949 thousand litres, while in 2022 already 2040 thousand litres (FAO, 2024). Milk production is foremost influenced by udder health. Mastitis in dairy cows still counts as a major problem in dairy farms, as being the one of the most frequent diseases, involving high costs, through decreased income from milk market, culling of animals, curing them (e.g. with antibiotics), and by the cost of extermination of the milk (Halasa et al., 2007; Kovács et al., 2015). Udder health is mainly described by the somatic cell count of milk.

Mastitis in mainly caused by some pathogenic bacteria, which multiplies in udder quarters, causing tissue damages. As a conclusion, maintaining and controlling hygienical background of animal populations is crucial for milk and milk product safety. It's well-known, that processing ability of the detreated quality milk (udder pathogenic bacteria present, increased somatic cell count) is much lower (Ryhanen et al., 2005).

Demand for quality sheep dairy products is continuously increasing, both all over Europe and both worldwide, causing increased interest in high milk yield dairy sheep breeds (Li et al., 2022). Lacaune, a French breed is one of those, which used purebred and as a crossing partner also all over the world for increasing the milk yield of local sheep populations (Barillet et al., 2001). Price of sheep milk is twice - three times higher, than of cow milk in many countries, therefore makes farms more profitable (Legarra et al., 2007). Next to these, sheep milk contains important bioactive materials for maintaining human health (Fenyvessy and Csanádi, 1999; Flis and Molik, 2021). Sheep milk production in Hungary is 1.5 million litres per year (2022), so marginal compared to the world production, (2022: 10.138 thousand t; FAO, 2024). The low quality of sheep milk production nowadays must be the main reason, that makes the whole dairy production branch in sheep itself uneconomical. The main prerequisite of sheep dairy branch is producing sheep milk in sufficient quality and quantity. Low production quality in Hungary itself can make sheep milk and dairy production uneconomical, therefore studying factors influencing milk production is crucial is sheep husbandry. Main aim must be increasing milk quality and ingredient content, next to maintaining a level quantity.

Based on those written about cattle industry, udder heath of dairy animals has a huge effect on milk quality, so quality milk production is based on understanding the factors effecting udder health. Acclimatization to milking technology is one of those factors, therefore I've studied the effects of udder morphology and milking order in dairy sheep, while milking order, the elements of milking technology and housing technology in dairy cows on milk yield and milk quality.

1.2. Aims of the study

The main aims of my study were the following.

- analysing factors affecting milking order in Lacaune ewes, next to determining the correlations of milking order with milk yield and milk quality traits;
- analysing factors influencing the udder morphology of first lactation Lacaune ewes, next to the correspondences of udder morphology with milk yield and milk somatic cell count;
- analysing the effect of bedding powder on milk somatic cell count of Holstein-Friesian dairy cattle kept in large-scale farm without bedding;
- analysing the accommodation of first lactation Holstein-Friesian dairy cows to automated milking systems, and its correlations with milk yield and milk quality, both in thermal neutral and thermal stress (winter and summer) periods;
- analysing milking order of dairy cows (Hungarian Simmental and Holstein-Friesian) both in small-scale and large-scale farms, and the effect of milking order to milk yield and milk quality.

3. MATERIALS AND METHODS

3.1. Effect of milking order to milk yield and milk quality of ewes

3.1.1. Effect of milking order on milk yield of ewes

I've recorded the milking order of 143 dairy ewes in Mórichida, Hungary. Lambs were weaned on the 50^{th} day in average. Individual milk yield (total and standard) of the ewes was counted based on the monthly milking data of performance test by the Hungarian Sheep and Goat Breeders Association (Sheep Performance Test Codex of Magyar Juh- és Kecsketenyésztők Egyesülete, MJKSZ, 2013). Ewes were milked twice a day, at 5.00 a.m. and 17.00 p.m., by Hungarolact milking system, with 2 × 24 parallel stands. Animals spent 10 minutes in average in the milking system, while consuming mixed feed. The housing system was half-intense, for 190 days (between April-November) animals were kept outside, grazing and got 400 g mixed feed (NEl: 7.1 MJ/kg dry material (DM); rude protein: 180 g/kg DM, containing A-, D3-, E-vitamin), and alfalfa hay *ad libitum* (NEl: 5.1 MJ/kg DM; rude protein: 189 g/kg DM). Main plant species on the pasture were *Lolium perenne*, *Poa pratensis*, Festuca *rubra* and *Lotus corniculatus.* Licking salt containing selenium was permanently available for animals.

I've recorded the order of ewes entering to milking parlour four times, on the 48^{th}, 74^{th}, 103^{rd} and 132^{nd} days of lactation, during the evening milking, and day before the milk quality assessments. Ewes were milked in six rounds; they've entered the milking parlour by their own will. Next to recording the milking order I've also scored the temperament of the ewes while they'd stayed in the milking stand - so during udder preparation and milking – and obtained a milk sample from every animal. Milk composition (fat and protein content) has been determined by LactoScope™ (Delta Instruments Ltd., Drachten, Netherlands) instrument. Matured live weight of the ewes (referred as live weight further) has been measured at 28 months of age, according to the performance test regulation of the Hungarian Sheep and Goat Breeders' Association (MJKSZ, 2013). Mean live weight of ewes was 62 kg. I've grouped the ewes into two categories based on their live weight: low weight (<62 kg, abbreviated as KTS / LW) and hight weight (≥62 kg, abbreviated as NTS / HW) groups. I've used a 5-score scale, according to Budzynska et al., (2005) for the temperament assessment during milking. After the initial experiment I'd chosen 22 ewes (out of the 143) which always grouped in the first round (abbreviated as FG) for milking, and 18 animals, which always entered the milking parlour in the last round (abbreviated as LG). I've compared the two groups according to all factors recorded (age, parity, litter size, live weight, temperament) and milk performance traits (daily milk yield, lactation milk yield, standard milk yield, length of lactation), milk quality traits (fat and protein percentage, daily fat and protein yield).

I've used SPSS 25.0 software package (IBM Corporation, Armonk, NY, USA) for the statistical evaluation. Distribution of the data was checked by Kolmogorov-Smirnov test, all recorded parameters show normal distribution. In case of temperament animals scored 1 and 2 (1+2), were united in one group, due to their low number (n = 3 and n = 5). I've analysed the effect of live weight (<62 kg and ≥62 kg), age (two, three, four, five and six years old), parity (first, second, third, fourth and fifth pregnancies), litter size (single, twin and triplet), and temperament (score 1+2, 3, 4 and 5) to milking order and milk production traits (length of lactation, total milk yield, standard milk yield, daily milk yield) in this study. I've applied general linear model (GLM) method according to the following formula:

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

when: Y_{ijklm} – trait studied; μ – mean, A_i – effect of live weight (fixed effect: 2 groups), B_j – effect of age (fixed effect: 5 groups), C_k –effect of parity (fixed effect: 5 groups), D_l – effect of litter size (fixed effect: 3 groups), E_m – effect of temperament (fixed effect: 4 groups) and e_{ijklm} – error. Between group differences were analysed by Tukey test.

Significance of the differences in milk production factors recorded according to the milking parlour entering order (first or last group) and milk quality traits (fat and protein content) were analysed by F and t-tests. For analysing differences in the temperament of the two groups I've used Mann-Whitney U test. Consistency of the observations about the entering order of the ewes was analysed by Kendall W test.

3.1.2. Effect of milking order to the milk quality of ewes

The study took place within the environment described in the previous, 3.1.1. chapter. I've collected milk samples in 10-10 ml jars from both udder halves, tits were soaked in sterilising solution, then dried with paper towels before sampling. During the milk quality assessment individual mixture milk samples were collected in 50 ml tubes (two 50 ml tubes per every animal). I've collected the samples three times, at the beginning of the lactation (9th of June), in the middle of it (7t^{h} of July), and at the end of lactation (1st of September), all during the evening milking. I've recorded the milking order of the ewes and scored their temperament three times, according to the method described earlier (based on Budzynska et al., 2005). 18 ewes grouped to the first entering class, while 22 ewes to the last entering class, so I've analysed the data of 41 ewes altogether.
LactoScope™ equipment (Delta Instruments Ltd., Drachten, Netherlands) was used for analysing the composition (dry material, milk protein, milk fat, milk sugar content) of the 50 ml milk samples. I've determined somatic cell count by LactoScan SCC equipment (Milkotronic Ltd., Nova Zagora, Bulgaria). The 10 ml milk samples were used for checking for mastitis causing bacteria by surface splitting method (ÁT Ltd, Gödöllő). I've used HI98163 pH-measuring equipment (Hanna Instruments, Carrolton, TX USA) of determining the pH of the milk samples, and Cond 340i conductometer with WTW Tetracon 325 measuring head

(WTW, Weilheim, Germany) for determining electrical conductivity. Methil-esters of fatty acids were determined by gas chromatography (GC 2010, Shimadzu, Kyoto, Japan), with flame ionizing detector (FID) and column (Zebron ZB-WAX, 30 m × 0.25 mm × 0.25 µm)(Campus Kaposvár, MATE). Given fatty acids were calculated based by their peak areas compared to the sum area of all fatty acids.

I've used SPSS 29.0for statistical analysis. Distribution of data was determined by Kolmogorov-Smirnov test, they showed normal distribution. I've applied F and t tests for determining the differences between the early entering and late entering groups in milk production, milk composition and microbiological traits. I've chosen Mann-Whitney U test for comparing the temperament differences of the two groups.

3.2 Effect of udder morphology on milk yield and milk quality of ewes

Udder morphology traits of 64 first parity Lacaune ewes were evaluated in 2023, in Mórichida, Győr-Moson-Sopron County, Hungary. Farm parameters were as described in chapter 3.1.1. I've collected milk samples four times, parallel to control milking dates 1-4. Animals in this study were first mated in two different ages: either at 9 or at 18 months old. Length of average rearing period was 55 days. I've grouped the ewes in two classes based on it: those whose rearing period was shorter than the average (with mean 45 days) and those with longer rearing period (mean 68 days). Based on weaned lambs set three groups of the ewes: first, whose lamb died before weaning 9 animals belonged to it, second consisted of those ewes, which weaned one lamb (n=47), while third group meant those ewes, which weaned two lambs (n=8).

1've collected milk samples together with test milkings. In those milk checks the individual milk yield of ewes was determined by Tru-Test Sheep Meter (Tru Test Ltd, Auckland, New Zeeland) volume measuring equipment. First milk check was due to between 30 days after the start of lactation. Next test milking were 28 days after the first, until the end of lactation, when ewes were set dry. Conformation judgement of the udder morphology was performed by me, during the first test milking, on a 9-score scale. The following traits made the base of scoring: depth of the udder, frontal udder joint, shape of the udder (de la Fuente et al., 1996), udder suspension (Casu et al., 2006), joint of the udder base (Eurosheep, 2018), symmetry of the two udder halves (Margatho et al., 2020), placing and length of the teats (de la Fuente et al., 1996).

I've determined the ingredients of the milk (dry material, milk protein, milk fat, milk sugar) with LactoScope™ equipment (Delta Instruments Ltd., Drachten, Netherlands). For somatic cell count I've used LactoScan SCC equipment (Milkotronic Ltd., Nova Zagora, Bulgaria).

I've used SPSS 29.0 software package for the statistical analysis (normality and homogeneity checks, mean, standard deviation, F and t-test, Mann-Whitney test, Levene test, analysis of variance, Tukey post hoc test, Kruskal-Wallis test). I've checked the distribution of my data by Kolmogorov-Smirnov test, they proved to show normal distribution. Multivariate analysis of variance was used for studying the factors influencing the teat morphology of ewes (time of first mating, length of rearing period, number of lambs weaned).

$$Y_{ijk} = \mu + A_i + B_j + C_k + e_{ijk}$$

when: Y_{ijk} = trait studied; μ =mean, Ai = effect of mating age (fixed effects: two groups: 1. and 2. year), B_j = effect of rearing period (fixed effect: two groups: early and late weaning), C_k = effect of the number of lambs weaned (fixed effects: three groups: deceased, singleton and twin), e_{ijk} = error. I've also studied the correlation of different factors, but as those doesn't turn out to be significant, later I describe only the main factors themselves.

Multivariate regression analysis, the so called backward eliminating model was used for determining the correlations of teat traits somatic cell count of the milk, milk yield, lactation length. The best models based on R^2, error, F and P values were chosen for further analysis.

3.3. Effect of bedding powder on cow milk quality

I've conducted these experiments between February and May in 2018 in a large-scale dairy cattle farm, situated in South-West Hungary. 83 Holstein-Friesian cows belonged to the experimental group, where stable powder was applied in the barn, while 79 animals to the control group, whose barn I didn't use bedding powder. I've disinfected the udders before getting the milk samples with sterile rubber gloves. I started to apply the stall powder in 21st of February (day 0). Lactation length of experimental and control groups was the same (212 days in average). No litter bedding at all was used in the control group. First four weeks of applying the drying agent were considered as acclimatization period, then stared to gather milk samples in the consecutive week. I've collected the milk samples for somatic cell counts on 21st February, 22nd and 28th of March, on 4th, 11th , 19th and 26th of April and on 3rd of May (so on the 212nd, 241st, 247th, 254th, 261st, 269th, 276th and 283rd days of lactation). Bedding powder was applied manually on every second day of the experiment, with 100g/m^2 density. Bentley FCM equipment was used to determine somatic cell count, and Bentley FTS FTIR for determining the fat, protein and fat content of the milk samples (ÁT Kft, Gödöllő, Hungary).

I've used SPSS 25.0 software package for the statistical analysis. I've checked the distribution of data with Kolmogorov-Smirnov test. As somatic cell count data didn't show normal distribution. I've taken the logarithm of those values for further analysis. I've applied F and t tests between the experimental and control groups in every sample collection data, as well as at the start and final date of the study.

3.4. Studies about accommodation to automated milking systems

This study took place at a Holstein-Friesian dairy cattle farm where 6 robotic milking machines are applied (GEA DairyRobot R9500). Milking system is controlled; animals can visit the milking robots three-four times a day. Cows are transported to this barn right after calving, the first milking is also done there. I've studied the milking traits of 17 cows, which had calved in summer (between 15t^h June – 31^st July) and 17, which calved in the winter (December - January). Heifer were first mated at 16 months of age is average in that farm. The experiment lasted for four weeks, and the following parameters were recorded: milking time (minutes), milk yield per milking (kg), electric conductivity (mS/cm), time interval between two milking (hour), number of milking per day, number and ratio of unsuccessful milking. The automated milking system also provided information about the type of unsuccessful milking. I've recorded those: number and ratio (%) of unjustified milking attempts, number and ration (%) of manual leat cup placements, number and ratio (%) of decreased milk yield compared to expected. Somatic cell counts from milk samples taken at control milking periods were determined by Bentley FCM equipment, while milk fat and milk protein contents with Bentley FTS FTIR (ÁT Kft, Gödöllő, Hungary).

I've used SPSS 29.0 software package for the statistical analysis. All data showed normal distribution, based on Kolmogorov-Smirnov test. I've applied the following tests to compare the milking data, physical and chemical composition of milk between the summer and the winter groups: F and t tests. In case of unsuccessful milking attempts Chi^2 probe was applied.

3.5. Effect of milking order on milk yield and milk quality of cows in small-scale and large-scale farms

3.5.1. Effect of milking order on milk yield and milk quality of cows in a small-scale farm

I've studied 7 Hungarian Simmental and 6 Holstein-Friesian milking cows in this experiment. Cows could visit the two-stand milking parlour by their own will. I've recorded their order of visits in the milking parlour for a week, both during morning and evening milkings. My aim was to find correspondences between the average morning and evening milkings, separately on each seven days, then altogether as well. Then I've analysed the milk production traits of the first 6 and last 6 animals, based on average milking orders during the week. Afterwards also concluded a long-term study about milking orders, 30 days after that one-week period, then again after 30-30 days, altogether four times. Data about the milk yields, milk fat, milk protein content and somatic cell count was provided from the official test milkings by the Animal Breeding Production Control Company (Állattenyésztési Teljesítményvizsgáló Kft, Gödöllő, Hungary). I've analysed the data a month before my experiment and in three consecutive months, so three datasets.

I've used SPSS 29.0 software package for the statistical analysis. All data showed normal distribution, based on Kolmogorov-Smirnov test. I've applied F and t tests for comparing the milking parameters and physical and chemical properties of the milk between the two groups, the first and last cows in the milking order. I've used Spearman rank correlation for analysing the milking order of given days, months.

3.6.2. Effect of milking order on milk yield and milk quality of cows in a large-scale farm

There were 63 Holstein-Friesian cows got in this experimental group. I've recorded milking order for 7 days, in the milking parlour, both during the morning and the evening milking. Cows could attend the milking parlour freely. I've recorded the order of their attendance as milking order. I was analysing correlations between the morning and evening orders individually on the seven days, then based on their average morning and evening rank as well. I've studied the milk production traits of the first 20 and last 20 cows based on milking order. The milking system had a built-in Afilab measuring equipment, which recorded the milk yield, the somatic cell count, the milk fat, milk protein, milk sugar, and the length of milking.

I've used SPSS 29.0 version for the statistics. I've applied Kolmogorov-Smirnov test for checking normality, and data proved to show normal distribution. F, then t tests were used for comparing the first and last milking order groups according, milking and milk production data, physical and chemical traits. In case of comparing the milking orders in morning and evening daily, as well as in average I've applied Spearman rank correlation.

4. RESULTS AND DISCUSSION

4.1. Effect of milking order to milk yield and milk quality of ewes

4.1.1. Connection between milking order and milk production

Neither live weight, age or litter size of ewes affect significantly milk yield (total, standard and daily milk yields neither). On the contrary, number of lactations and temperament of ewes had a significant effect on production traits. Ewes in their third lactation had a higher lactation milk yield (209 kg), standard milk yield (129 kg) and daily milk yield (0.91 kg), than ewes in their first (170 kg, 102 kg and 0.76 kg) or in their fifth (185 kg, 100 kg and 0.80 kg) lactation. However, calmer ewes had higher milk quantities (209 kg, 125 kg and 0.92 kg), than more nervous ones (182 kg, 118 kg and 0.81 kg). None of the factors studied effected the fat- or protein content of milk.

Age, parity and litter size of the ewes didn't have a significant effect on milking order. However, live weight and temperament significantly affected the order of entering to the milking parlour. Heavier sheep entered the parlour earlier, than those whose live weight was lower. Based on live weight 65 animals were under the average of the experimental group (<62 kg, KTS/ LW) and 78 animals above it (≥62 kg, NTS / HW). Mean live weight in low weight group was 56 kg, while in high weight group 68 kg. There was a difference in average entrance order between the two groups: LW 3.62, while in HW 3.27 was the mean rank value, so heavier ewes entered earlier. I could conclude, that with increasing temperament scores the entering rank into the milking parlour decreased. More nervous animals (scored 1-2-3) got into the parlour later than their calmer (scored 5) counterparts. In my study 43.4% of the Lacaune ewes (n = 62) could score 5, 39.2% (n = 56) scored 4, 11.9% (n = 17) scored 3, while 5.6% (n = 8) scored 1, or 2.

Based on my observations – four times under these experimental conditions – I've also analysed the long-term stability of milking order of ewes. Long-term consistency was low ($W < 0.030$; $P < 0.05$). between the milking parlour entrance based on Kendall W-test.

There were 22 ewes who entered the parlour in the first group (FG), and 18 ewes always remained in the last group (LG) according to the four recorded observations. I've found significant differences in live weight and temperament scores between the FG and LG groups. Milking order didn't affect the length of lactation: I've found almost equal values (approximately 229 days) in FG and LG groups. On the contrary, entering order had a significant effect on milk yield. Ewes in FG gave 203 kg milk, while those in LG 168 kg ($P < 0.05$). Difference between the two groups is remarkable, 35 kg. However, there were no differences in milk fat and protein content between FG (7.84 and 5. 81%) and LG (7.52 and 5.74%). Nonetheless, daily fat and protein yield was higher in FG (69.8 and 51.8 g), than in LG (55.7 and 42.5 g; $P < 0.001$)(Table 1).

Table 1

Changes body weight, age, lactation number, litter size, temperament and milk production of ewes according to milking order (first group, n=22; last group, n=18)

Items	First group (n = 22)		Last group (n = 18)		P
	mean	SD	mean	SD	
Body weight, kg	1	9.65	57.13	7.57	<0.05
Age, year	4.35	1.73	4.56	1.51	N.S.
Parity, pc	2.94	0.97	3.11	1.90	N.S.
Litter size, pc	1.88	0.49	1.78	0.44	N.S.
Temperament, score	4.59	0.80	3.56	1.01	<0.001
Length of lactation, day	228.52	6.77	229.58	5.44	N.S.
Mil production, kg	203.10	36.28	168.34	38.95	<0.05
Standard milk production, kg	132.1	35.28	102.0	37.73	<0.05
Daily milk yield, kg	0.89	0.15	0.74	0.17	<0.05
Milk fat, %	7.84	0.89	7.52	0.74	N.S.
Milk fat yield, g	69.79	7.93	55.68	1.59	<0.001
Milk protein, %	5.81	0.18	5.74	0.14	N.S.
Milk protein yield, g	51.75	1.59	42.49	1.05	<0.001

N.S.= not significant difference

4.1.2. Connections between milking order and milk quality

Based on the three observations, there were 18 eses who entered the milking parlour in the first round (first group, FG), while 23 entered in the last round (last group, LG). Milking order had a significant effect on the live weight and on the temperament of the eses. I've found higher live weight (61.1 kg) and more favourable temperament scores (4.0 score) in the first group, than in the last group (56.8 kg and 2.96 scores). On the contrary, milking order doesn't affect the age and the parity of the eses. Age and lactation number in FG were 3.86 years and 2.28 bits in FG, and 4.32 years and 3.54 bits in the LG.

Milking order didn't affect dry material, fat or protein content, neither pH value. Ewes producing more milk went to the milking parlour in the first round (FG: 200 kg), while those producing less in the last round LG: 147 kg). Similar tendency is visible in case on standard lactation milk yield and daily milk yield (FG: 120.9 kg and 1.2 kg/day, LG: 101.7 kg and 1.0 kg/day). Consequently, significant differences were found in fat and protein yield between the two groups. Electric conductivity of the milk was significantly lower in the first group (3.66 mS/cm), than is the last one (4.06 mS/cm; $P < 0.001$). Next to it, somatic cell count was also enhanced in the last milking group (556.26 thousand cells/ml), while milk samples from the first-round ewes contained only 200.51 *thousand* cells/ml (P < 0.01).

Prevalence of udder pathogenic bacteria remarkably differed between the two groups. I've found more favourable values in the FG, when it was only 4.6%, while in LG 16.7% (P < 0.05). Coagulase-negative *Staphylococcus* (CNS) (80%) and *S. aureus* (20%) had been the most frequent bacteria in the infected samples belonging to FG. On the contrary, in LG *E. coli* (43%) was the most frequent, followed by CNS (30%) and *S. uberis* (17%). Total frequency of large effect udder pathogenic bacteria in first group was 20%, compared to the positive samples. That is significantly lower, than in the last group (69.6%; P<0.001)(Table 2).

Table 2

Average milk production, chemical and physical properties of milk and somatic cell count of Lacaune ewes according to milking order (based on milking order: first or last group)

Items	**First group (n = 18)**		**Last group (n = 23)**		**P**
	mean	**mean**	**Átlag**	**SD**	
Electrical conductivity, mS/cm	3.66	0.36	4.06	0.52	<0.001
Somatic cell counts, log cell/ml, (thousands cell/ml)	4.93 (200.51)	0.43	5.18 (556.26)	0.65	<0.01
Negative samples, %	95.4		83.3		<0.05
Positive samples, %	4.6		16.7		<0.05
Total major udder pathogens, %	20.0		69.6		<0.001

N.S.= not significant difference

Entering order of the ewes has a slight effect on the fatty acid profile of the milk. Concentration of capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), saturated fatty acids, C-16 shorter fatty acids and *de novo* fatty acids was significantly higher, while concentration of oleic acid (c11 C18:1), arachidonic acid (20:4,n-6), monounsaturated fatty acids, C16 longer fatty acid was significantly lower in FG, than is LG.

4.2 Effect of udder morphology on milk yield and milk quality of ewes

4.2.1. Analysis of factors influencing udder morphology

48 ewes in my study population were involved into breeding at 9 months of age, 16 animals at 18 months of age. Breeding age didn't have a significant

effect on most udder conformation traits, only udder depth differed between the two groups. Mean udder depth of ewes mated first at 9 months of age was 4.51 scores, while those mated later had deeper udders, 6.36 scores are average ($P<0.01$). It can be explained by younger ewes having less mature udders than those involved to breeding later.

There was a significant difference in udder depth and udder suspension scores between short (average weaning time 45 days) and long (average weaning at 68 days) rearing periods. Those ewes which had shorter rearing scored 4.36 for udder depth and 4.85 for udder suspension. Ewes with longer rearing period scored higher for udder depth, 5,57 in average, so their udder was deeper compared to the other group. Score for udder suspension is lower, 3.04 in the group of ewes with longer rearing, meaning the suspension ligament in their udder is not as tight, less visible.

Number of weaned lambs had a significant effect on udder conformation of the ewes. Significant differences were found between the groups in udder depth, udder suspension, udder symmetry and teat length. Group with deceased lambs got 4.13 scores in average for udder depth, while group with weaned twins 6.47 scores. In udder suspension there was also a significant difference between the first group, scored 6.29, and the third, scored only 3.17. In udder symmetry the difference was significant between group one – means score 5.71 - and group two, with one weaned lamb, whose mean score was 5.96. In teat length the difference again was significant before the first and third groups, first scored 3.5, while third 5.43 in average. Animals with weaned twin lambs got higher scores for udder depth – so their udder hanged deeper down - than those whose lamb died. Twin ewes also got less favourable scores for udder suspension, compared to ewes with deceased offspring. Teats were significantly longer in second group, compared to first one. I've also found significant differences between groups with different number of weaned offspring in udder symmetry.

4.2.2 Effect of udder morphology on milk production and milk quality

Udder conformation traits can be usefully applied in selection for improving udder health, milk quality and ability for robotic milking.

In correlation analysis of milk production traits, the statistical package offered five regression models. Factors in all five models significantly affected the milk yield of the sheep. Based on R^2 values, accuracy of the estimation (error) and strength of the effect I've chosen the most suitable models for further analysis. Based on the fourth and fifth model, udder depth, frontal udder joint, udder shape had significant effects on milk production of the ewes. Paralell with more favourable udder conformations the quantity of produced milk also increased. Higher frontal udder joint and udder shape scores led to higher milk yield. I could conclude from the multivariate regression analysis, that all scored udder conformation traits influenced milk yield. Udder depth, frontal udder joint, udder shape and udder symmetry had the greatest impact on milk production.

Regression analysis also made up more models when studied the effect of udder conformation traits on somatic cell count. Factors in in all five models had a significant effect on somatic cell count. Based on R^2 values, accuracy (error) of the estimation and strength of the effect fourth and fifth models suited the best. According to those models, udder suspension, joint of the udder base and udder symmetry had the highest effect on somatic cell count. Somatic cell count is more favourable with more tight suspension ligament, with bigger udder base - when connected to the body of the animal – and with as symmetric udder halves as possible. As a conclusion, based on multivariate regression analysis between somatic cell count and udder conformation traits, udder suspension, udder base connection and udder symmetry had the greatest effect on milk production.

4.3. Effect of bedding powder on cow milk quality

I've analysed the milk samples of the dairy cows in this research eight times. First occasion had been the day before starting the experiment (control), on this control, test milking day cows were in the middle of their lactation, on the 212nd day. Somatic cell count values increased in both the experimental, both the control groups as the lactation of the animals were going on, but to different extent. This is in concordance with the results published by several authors before (Laevens et al., 1997; Gonçalves et al., 2018). Somatic cell count in the experimental group only raised slightly, there was no significant difference at the end compared to the starting value (212nd day: 5.01 log cell/ml, 283rd day: 5.23 log cell/ml; $P > 0.05$). On the contrary, difference in the control group proved to be significant (212nd day: 5.04 log cell/ml, 283rd day: 5.57 log cell/ml; $P < 0.05$). Analysing somatic cell counts through the experiment it is visible, that the difference between the experimental and the control group developed for the 4th week. Starting from the 4th week I could show a significant difference between the somatic cell count values of the two groups. Based on my results, we have to be aware in practice, that the beneficial effect of the stable powder, applied according to the manufacturer's instructions (quantity and time) isn't visible from the start in somatic cell count. Improvement in the udder health status of the cows is expected after 7-8 weeks. Further, it can be advised to use bedding powder for a longer time, continuously in powder maintaining a beneficial barn climate.

4.4. Studies about accommodation to automated milking systems

I've studied the milking parameters of Holstein-Friesian cows after their first calving - either in the summer, either in the winter - sampled in the first, second, third and fourth week of their lactation, producing in an automated milking system. I've found no significant differences in time interval between milkings and daily number of milkings between the two groups, so those calved in the summer, and those calved in the winter. On the contrary, season had a great effect on milking time, on milk yield per milking, on electric conductivity and on frequency of milking errors. Milking time was shorter in the summer, and in parallel, quantity of milk also decreased. Electric conductivity is an important trait in milk quality, as it is well-known, that there's a positive correlation between conductivity and somatic cell count. Milk electric conductivity values were higher in the summer period than during the winter. Moreover, frequency of unsuccessful milkings significantly increased during summer (19%), compared to winter (7%)(Table 3).

Table 3

The average values of milking traits of first-calving Holstein-Friesian cows milked in an automatic milking system during the first four weeks of their lactation in the summer and winter periods (mean±SD)

Items	**Winter (n=17)**	**Summer (n=17)**	**P**
Milking time, min	5.81±2.35	4.13±1.88	<0.001
Milk yield per milking time, kg	12.28±3.22	11.46±4.64	<0.001
Electrical conductivity, mS/cm	5.01±0.37	5.30±0.50	<0.01
Time between two milkings, hour	10.15±3.23	9.62±4.61	N.S.
Number of daily milking, pc	2.36±0.71	2.47±1.19	N.S.
Ratio of failed milking, %	7	19	<0.05

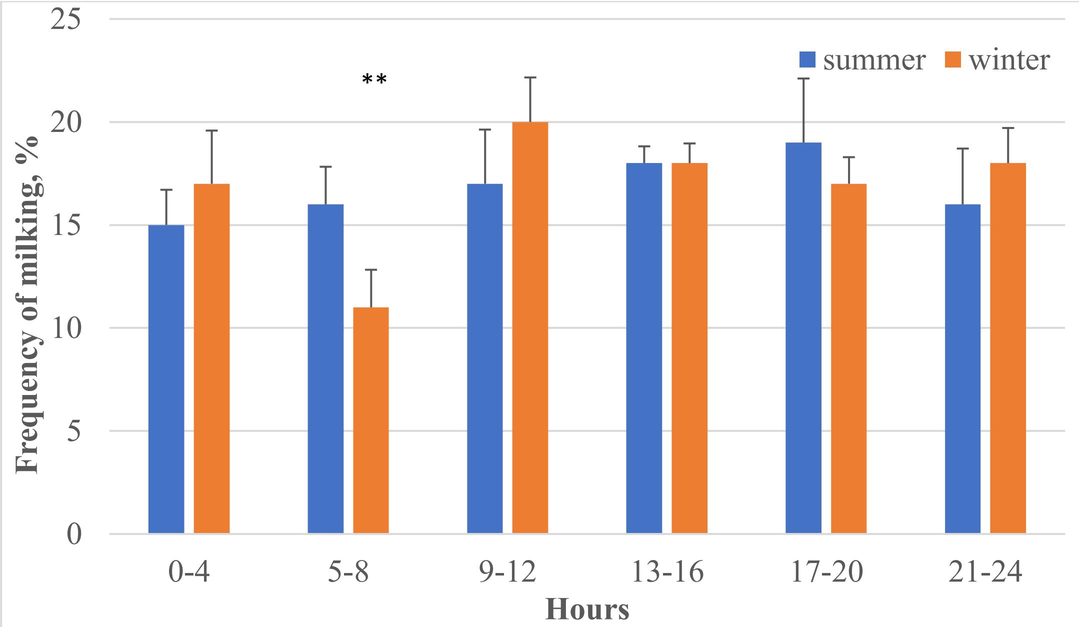
N.S.= not significant difference

Frequency of unsuccessful milkings from various reasons differed between the two dates (first day and first week) studied. Ratio of unsuccessful milkings both on the first day (19%) and on the first week (22%) in the summer had been twice as much than in the winter period (% and 11%). The most numerous and frequent reason of unsuccessful robotic milking was the need of manual admission of the lead cups, which requires plenty manpower. The frequency of such intervention at the beginning of milking significantly increased during the summer period (11%), while in the winter its ratio was evanescent (0.6%%; P<0.001). No significant differences were found between the two periods in the frequency of other reasons of unsuccessful milking during the first week of the experiment.

I've found significant difference between the two groups in the time of the day when animals visited the milking parlour. There was a significant difference in the frequency of milking attempts during dawn period (P<0.01). Due to the hot weather, during summer cows appeared more frequently early morning in the milking machine, than during winter (Figure 1).

Figure 1

Milking frequency during the summer (n=17) and winter (n=17) periods during the four weeks



4.5. Effect of milking order on milk yield and milk quality of cows in small-scale and large-scale farms

4.5.1. Studies about milking order in small-scale and in large-scale farm

In the small-scale farm correlation of milking order of given cows was strong within a day, between morning and evening milkings (r=0.49-0.99; P < 0.05). I could also show a strong positive correlation for the whole experimental period, so between the average morning and evening ranks of the seven days (r= 0.97; P < 0.001). On given days the correlation was a bit weaker, that must have been influenced by animals being in oestrus. Milking rank in cows considered to be relatively stable, based on the daily date of the one-week experimental period. Milking ranks recorded for four months also show strong correlations between the moths. Therefore, I could conclude, that in a small-scale farm milking order is a stable trait.

In the large-scale farm correlation between the morning and evening milkings, within a day was moderate. Correlation calculated from the average values of morning and evening ranks during seven days was strong (r= 0.78 P < 0.001). However, strength of the correlations' coefficients were lower, than in the small-scale farm. But based on the daily ranks of a week period milking orders of cows also considered to be almost constant. Correlations were between r=0.6-

0.7, which means strong. As a conclusion, milking order is a stable trait in large-scale farms as well.

4.5.2. Effect of milking order to the milk production of cows in small-scale and in large-scale farms

My results show, that milking order didn't affect milk yield neither in small-scale, nor in large-scale farm, however had a significant effect on the somatic cell count of the milk. In the small-scale farm the somatic cell count of the cows belonging to the first group (n=6) was lower (5.02 log cell/ml), than of those visiting the milking parlour in the last group (n=6; 5.69 log cell/ml; $P < 0.05$).

I've analysed the milk production data of the first 20 and last 20 cows in the milking order in the large-scale farm. As mentioned, milking order wasn't connected to milk yield there either, nevertheless, it had a significant effect on milking time and milking speed. Cows belonging to the first group had shorter milking time and faster milking speed than their counterparts in the last group. Again, milking rank didn't affect the composition of the milk, however, was significant to somatic cell count. Similarly, cows grouped first had lower somatic cell count (4.50 log cell/ml), than those grouped last (5.05 log cell/ml; $P < 0.05$) (Table 4).

Table 4

Milk production and milking speed values according to the milking order of cows in a large-scale dairy farm (mean±SD)

Items	**Daily milk yield, kg**	**Length of milking time, sec**	**Milking speed, kg/min**	**Somatic cell counts, cell log/ml**
First group (n=20)				
Mean	30.93	379.75	2.49	4.50
SD	7.30	72.19	0.60	0.61
Last group (n=20)				
Mean	31.23	450.40	2.12	5.05
SD	8.29	114.68	0.51	0.83
P	N.S.	<0.05	<0.05	<0.05

N.S.= not significant difference

5. CONCLUSIONS

5.1. Effect of milking order to milk yield and milk quality of ewes

I've determined based on my four-months experiment, that milking order in the large population size (over 200 animals) Lacaune farm was variable, therefore it is advised to record milking order not only once, but continuously. According to my results live weight and temperament of the ewes highly influenced the order of their entrance into the milking parlour. Calmer and heavier ewes entered earlier, than more nervous and lighter animals. Moreover, electric conductivity (3.66 mS/cm), somatic cell count (4.93 log cell/ml), and frequency of pathogen bacteria (5 %), was significantly lower in ewes on the first batch in the milking parlour, compared to those remaining to the last group (4.06 mS/cm, 5.18 4.93 log cell/ml, 17%; $P<0.05$). My results show the usefulness of milking order on dairy purpose selection, therefore advice registering it more than once.

5.2. Effect of udder morphology on milk yield and milk quality of ewes

5.2.1. Study of factors affecting udder morphology traits

First breeding time (9 or 18 months) didn't influence significantly udder traits. However, length of rearing period (shorter or longer than 55 days) moderately affected udder conformation in first parity Lacaune ewes. Animals with shorter rearing period, so having their lambs suckling for a shorter time had more advantageous udder conformation. Number of lambs weaned had a significant effect on udder depth, udder suspension, udder symmetry and on the length of teats: ewes with twins had the least favourable udder conformation.

5.2.2 Effect of udder morphology to the milk production and milk quality of ewes

Udder conformation traits scored by me significantly affected the length of lactation, the milk yield and the somatic cell count in the milk. Especially advantageous udder depth and frontal udder joint lifted the length of lactation and milk yield significantly, while udder suspension, join of the udder base and udder symmetry reduced the somatic cell count in first-lactation Lacaune ewes.

5.3. Effect of bedding powder on cow milk quality

Udder health of dairy cows can be successfully improved by applying stable powder, as my results show. Average somatic cell count was significantly lower in the experimental group, where bedding powder had been used, compared to the control (124 thousand vs. 234 thousand cell/ml). I've also determined that it is advised to apply bedding power for at least 7-8 weeks to achieve this advance. Several authors reported previously that this quantity of difference on somatic cell count is already associated with milk yield, no matter that both values reach the acceptable limit otherwise.

5.4. Studies about accommodation to automated milking systems

I've found significant differences between summer and winter seasons in milking traits of first lactation Holstein-Friesian cows, producing in automated milking system. Milking time and milk yield was lower in the summer, while electric conductivity of the milk was higher at the same time. The need for manual milking lead cup admission was increased - among milking errors - during summer (from 0.6% to 11%; P<0,01). Cows visited the robotic milking system in higher frequency (with 45%) in the early dawn hours during heat stress periods in the summer, which might lead to spending less time in rest, leading to negative effects in milk production.

5.5. Effect of milking order on milk yield and milk quality of cows in small-scale and large-scale farms

5.5.1. Studies about milking order of dairy cows in a small-scale and in a large -scale farm

Morning and evening milking order of cows on given days strongly correlated in a small-scale farm (r=0.49-0.99), while moderately in a large-scale farm (r=0.35-0.61). Mean morning and evening milking orders during the one-week experiment were very strong in the small-scale farm (r=0.97) and strong in the large-scale farm (r=0.78). My results support the previous scientific opinion, that milking order in dairy cows is almost constant.

5.5.2. Effect of milking order to milk production in dairy cows, in a small-scale and in a large-scale farm

I've concluded, that milking order didn't influence milk yield in neither of the farm sizes, cows with larger production didn't enter earlier the milking parlour. Milking group didn't affect the ingredients of milk either. However, order had a significant effect on somatic cell count, furthermore in large-scale farm on the length of milking time and on milking speed as well. Cows grouped first in milking order had shorter milking time and higher milking speed and lower somatic cell counts in the large-scale farm, than animals belonging to the last milking batch.

6. NEW SCIENTIFIC RESULTS

1. I've verified, that milking order of ewes is significantly influenced by their live weight and temperament. Ewes with calmer temperament and higher live weight have entered the milking parlour earlier, than their more nervous and smaller counterparts. I've also determined, that in a large population dairy Lacaune farm, milking order of ewes recorded on 48th, 74th, 103rd and 132nd days of lactation cannot be considered as stable.

2. I've identified at the first time, that electric conductivity of the milk (3.66 mS/cm), somatic cell count (4.93 log cell/ml), and ratio of udder pathogenic bacteria (5 %), was lower in those ewes which entered the milking parlour first, compared to those entered in the last round, at the end of milking (4.06 mS/cm, 5.18 4.93 log cell/ml, 17%; $P < 0.05$).

3. I've proved, that udder traits of the first parity Lacaune ewes are determined by the length of rearing period and the number of lambs. Longer rearing period (over 55 days) and higher number of lambs (twin pregnancies) had an unfavourable effect on udder quality traits of the ewes. On the contrary, time of first mating (9 vs. 18 months) didn't have a significant effect on the udder traits of the ewes.

4. I've determined at the first time, that start of the milking period significantly influences the accommodation to automated milking systems is first parity Holstein-Friesian cows. Milk yield per milking in the first month and persistency was lower in first parity cows starting their lactation in summer months, at the same time, electric conductivity of their milk and ratio of unsuccessful milking - due to various reasons - were increased, compared to first parity cows starting their lactation in winter.

5. I've proved, that first parity Holstein-Friesian cows starting their lactation in summer visited automated milking system mostly in early morning hours, while frequency of visiting the milking parlour also decreased compared to those starting their lactation in winter, due to heath stress. The milking frequency of the dawn period increased by 45% in summer, compared to winter ($P<0.01$).

6. I’ve proved the effect of milking order to milk somatic cell count in Holstein-Friesian cows. Somatic cell count of cows entering the milking parlour earlier was more advantageous (small-scale farm: 5.02 log cell/ml vs. 5.69 log cell/ml; $P<0.05$; large-scale farm: 4.50 log cell/ml vs. 5.05 log cell/ml; $P<0.05$). Moreover, milking speed in a large-scale farm in the beginning of milking was significantly higher in cows arriving earlier, than those arriving later ($P<0.05$).

Scientific publications of the author according to the topic so far

Scientific publications:
Peer-reviewed publications with impact factor

Libis-Márta, K., Póti, P., Egerszegi, I., Bodnár, Á., Pajor F. (2021): Effect of selected factors (body weight, age, parity, litter size and temperament) on the entrance order into the milking parlour of Lacaune ewes, and its relationship with milk production. Journal of Animal and Feed Sciences, 30, 2, 111–118.

Peer-reviewed publications

Márta K., Molnár Á., Gulyás L., Bodnár Á., Póti P., Pajor F. (2024): Első ellésű lacaune anyajuhok tőgytulajdonságainak összefüggése a tejtermelésükkel. Animal Welfare, Etológia és Tartástechnológia. 20, 1, 3–15

Márta K., Molnár Á., Gulyás L., Bodnár Á., Póti P., Pajor F. (2024): Egyes tényezők hatása első ellésű lacaune anyajuhok tőgytulajdonságaira. Állattenyésztés és Takarmányozás. 73, 2, 120–129.

Libis-Márta K., Bócsi B., Szalai Sz., Varga, FB., Pajor F., Kovács L., Bodnár Á. (2022): A hőtűrés javítására irányuló egyes szarvasmarha keresztezések és jelentőségük a világ szarvasmarhatartásában. Animal Welfare, Etológia és Tartástechnológia. 18, (1), 35–50.

Libis-Márta, K., Pajor, F., Póti, P. (2021). Az alompor felhasználásának hatása a tejelő tehenek tőgyegészségére. Animal Welfare, Etológia és Tartástechnológia, 17, (2), 125–131.

Publications in scientific conference issues:

Libis-Márta K., Póti P., Egerszegi I., Bodnár Á., Pajor F. (2023): A fejési sorrend hatása a lacaune anyajuhok tejtermelésére és tejminőségére. 39. Óvári Tudományos Nap Konferencia. 2023. november 16. 53.

Libis-Márta K., Zemplényi L., Fényes F., Bodnár Á., Egerszegi I., Póti P., Pajor F. (2022): Tőgyegészségügyi vizsgálatok egy hazai lacaune tenyészetben. VIII. Gödöllői Állattenyésztési Tudományos Nap Nemzetközi Konferencia. 2022. november 25.

Libis-Márta K., Pajor F., Póti P. (2021): Az alompor felhasználhatósága a tejelő tehenek tartástechnológiájában: tőgyegészségügyi vizsgálatok. XXXVIII. Óvári Tudományos Nap. 2021. november 11. 33.

References:

1. Barillet, F., Marie, C., Jacquin, M., Lagriffoul, G., Astruc, J.M. (2001): The French Lacaune dairy sheep breed: use in France and abroad in the last 40 years. Livestock Production Science, France, 71, 17–29.
2. FAO (2024): Food and Agriculture Organisation of the United Nation. https://www.fao.org/faostat/en/#home (utolsó letöltés: 2024. 03. 06.)
3. Fenyvessy J., Csanádi J., (1999): A kiskérődzők (juh, kecske) tejalkotórészeinek táplálkozási megítélése). Tejgazdaság 59, 23–26.
4. Flis, Z., Molik, E. (2021): Importance of Bioactive Substances in Sheep's Milk in Human Health. International Journal of Molecular Sciences, 22, 4364.
5. Halasa, T., Huijps, K., Østerås, O., Hogeveen H. (2007): Economic effects of bovine mastitis and mastitis management: A review. Veterinary Quartely, 29, 18–31.
6. Kovács P., Tibold J., Ózsvári L. (2015): A Staphylococcus aureus tőgygyulladás elleni védekezés egy nagyüzemi holstein-fríz állományban és a fertőzés gazdasági hatásai. Magyar Állatorvosok Lapja, 137, 707–718.
7. Legarra, A., Ramon, M., Ugarte, E., and Perez-Guzman, M. D. (2007): Economic weights of fertility, prolificacy, milk yield and longevity in dairy sheep, Animal, 1, 193–203,
8. Li, R., Ma, Y., Jiang, L. (2022): Review: research progress of dairy sheep milk genes. Agriculture, 12(2), 169.
9. MJKSZ (2013): Juh Teljesítményvizsgálati Kódex (9. kiadás): https://portal.nebih.gov.hu/documents/10182/43858/JUH_TELJESITMENYVIZSGALATI_KODEX_2013.pdf/db820d53-01f6-43d6-bf91-140a8a73d72f (utolsó letöltés: 2024. 04. 05.)
10. Ryhanen E.L., Tallavaara K., Griinari J.M., Jaakkola S., Mantere-Alhonen S., Shingfield K.J. (2005): Production of conjugated linoleic acid enriched milk and dairy products from cows receiving grass silage supplemented with a cereal-based concentrate containing rapeseed oil. International Dairy Journal, 15, 207–217.