Flóra Mária PETRÓCZKI[1,3], Béla BÉRI[2], Ferenc PELES[1]

DOI: https://doi.org/10.52091/EVIK-2021/2-3-ENG
Submitted: July 2020 – Accepted: November 2020

The effect of lactation number and lactation stage on milk yield, on the composition and on the microbiological properties of raw cow milk in a Hungarian dairy farm

Keywords: lactation number, lactation stage, cow's milk, milk quantity, milk composition, microbiology

1. SUMMARY

Changes in the composition and hygienic properties of milk affect producer price, so it is essential for the responsible dairy farmer to collect information on changes in these parameters due to various factors. In their study, the authors seek to answer the question whether there is a fluctuation in the daily milk yield of cows and in the composition (fat and protein content) and microbiological properties (somatic cell count, total plate count, coliform and *S. aureus* count) of raw cow's milk in primiparous and multiparous cows or at different stages of their lactation. Based on the data of a Hungarian large-scale dairy farm, it was found that there was no difference in the fat and protein content of the milk, but the daily milk yield was higher in the case of multiparous cows and, compared to the milk of primiparous cows, their milk had a higher somatic cell count and larger amounts of coliform bacteria. The daily milk yield decreased in the successive stages of lactation, but the fat and protein content of the milk increased, which is presumably due to the concentrating effect of the decreasing milk yield. No significant change was observed in the colony count of microorganisms at the different stages of lactation.

[1] University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science
[2] University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Environmental Protection, not independent Faculty of Animal Husbandry
[3] University of Debrecen, Doctoral School of Animal Science

Flóra Mária PETRÓCZKI	petroczki.flora@agr.unideb.hu	https://orcid.org/0000-0001-9878-4656
Béla BÉRI	beri@agr.unideb.hu	https://orcid.org/0000-0001-9952-0089
Ferenc PELES	fpeles@yahoo.com	https://orcid.org/0000-0002-9226-3777

2. Introduction

Cow's milk has a high nutritional value; it contains fats, proteins, carbohydrates, vitamins and minerals, among other things **[1]**. Examination of the composition of milk is a routine practice on dairy farms for monitoring the hygiene, nutritional and health aspects of dairy cows **[2]**. The composition of milk can be influenced by a number of factors, such as the number of lactations, the stage of lactation, the season and the feeding technology **[3, 4, 5]**. Thus, the composition of milk may vary during lactation, and as a result of the interactions of different environmental factors, there may be differences between the different dairy farms **[6]**. According to Dürr et al., the creation of databases in order to determine the causes and consequences of differences in milk yield and milk composition is of paramount importance. A database should also include records related to these parameters, as well as to lactation-related events and to the individual cows **[7]**.

Due to its nutritional value, high water activity and neutral pH, milk serves as an excellent medium for various microorganisms, which may include pathogenic organisms such as *Campylobacter jejuni*, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, etc. **[8, 9]**. During the primary infection of milk, sick animals themselves are the sources of infection. In case of dairy animals suffering from a systemic disease accompanied by the spread of pathogens, the pathogens may be excreted in the milk. During secondary contamination, the contamination of milk is of environmental origin. Due to improper milking hygiene, milk can be contaminated by the faeces of the animals or the equipment used in milking (milking machines, milk lines, milk storage tanks), among other things **[10]**. As the milk enters the teat cistern and then the teat canal, it can become infected with various microorganisms, of environmental origin, so the bacterial infection of the first milk jets is remarkably high. At the beginning of milking, it is therefore advisable to separate the first jets of milk from milk that is milked subsequently, and to ensure that they are destroyed **[11]**. To reduce the number of bacteria in milk, heat treatment is most commonly used. The initial microbiological state of the milk is not only important from a food safety point of view, but can also affect the quality of the dairy products made from it **[12]**.

Coliform bacteria can cause mastitis in dairy animals. Mastitis caused by coliform bacteria can reduce milk production in dairy animals, causing economic losses to dairy farms **[13]**. The presence of these bacteria in the environment is common, so their presence in food may indicate environmental contamination **[14, 15].**

Raw milk can be contaminated with *S. aureus* from a number of sources, such as the environment, milkers' hands, milking equipment, and so on **[16]**. The economic damage due to mastitis caused by *S. aureus* comes from a decrease in the quantity and quality of the milk produced, an increase in the number of somatic cells measured in it, a reduction in the purchase price of lower quality milk, which also leads to a decrease in the turnover of dairy farmers **[17]**. Prevention is an effective means of controlling *S. aureus* infection. It can be prevented by adhering to appropriate housing and milking technologies, more frequent fly extermination, pre- and post-disinfection of the teats, the use of disposable udder wipers and regular laboratory testing **[18]**.

External and internal factors can affect the composition of milk, as well as the microbiological state of raw milk. The latter is mostly determined by the hygienic condition of the surfaces that come into direct contact with the milk **[19]**. Research by Peles et al. found that different husbandry and milking methods at dairy farms with different cow numbers had influencing effects on the microbiological quality of milk **[20]**. Tessema sought to find a correlation between breed, the age of the animals, the number of lactations and the stage of lactation, as well as the likelihood of *S. aureus* occurrence.

In his study, he found a significant difference in the prevalence of *S. aureus* in milk for the two cattle species studied. *S. aureus* was found in higher proportion of crossbred cows and in older individuals **[21]**. Examining the milk of different varieties, Bytyqi et al. found no difference in the colony counts obtained **[22]**.

Although fewer Hungarian studies have been conducted on the subject, several international publication have examined whether the number of lactations and the stage of lactation have an influence on the daily milk volume of the animals, the composition of the milk and its microbiological parameters. According to Tessema, there is a significant difference in the prevalence of *S. aureus*, depending on how many lactations the animals have been through. In his study, he found that *S. aureus* was more common in the milk of cows that had calved more than twice and produced a positive California Mastitis Test **[21]**. Tenhagen et al. also found that the incidence of *S. aureus* increases with the age of the animals **[23]**. This may be related to the fact that the milking machine may damage the teats during milking, allowing microorganisms to enter the udder from the environment **[24]**. Another possible reason is that the health status of dairy animals may deteriorate with advancing age, which may have an adverse effect on the somatic cell count of milk **[25]**.

The objective of our study was to determine at a Hungarian large-scale dairy farm whether there is a difference in the daily milk yield of primiparous and multiparous cows, as well as cows at different stages of lactation, and also in the composition (fat and protein content) and microbiological parameters (somatic cell count, total plate count, coliform and *S. aureus* count) of primiparous and multiparous cows, as well as cows at different stages of lactation.

3. Materials and methods

3.1. Place and time of sampling

A dairy farm in Hajdú-Bihar county (Hungary) was the site of our studies. 440–450 Holstein-Friesian cows are milked on the farm. The farm uses deep litter livestock-keeping and monodiet feeding. Milking takes place in a milking parlor, and no post-disinfection is carried out after milking.

The data on the daily amount of milk, fat and protein content and somatic cell count used for the calculations are derived from the milking results, i.e., from the examination results of the milk samples collected monthly by the Állattenyésztési Teljesítményvizsgáló Kft. All milking results of 38 individuals between May 2015 and January 2020 were used in the calculations. During the calculations, data on the first lactation of the cows (n=387), data on the 2^{nd} to 5^{th} lactation of the cows (n=446), as well as data on the early (under 100 days; n=275), middle (100 to 200 days; n=249) and late (over 200 days; n=309) stages of the lactation of the cows were summarized for the abovementioned time period.

Microbiological tests were performed between May 2018 and October 2019. A total of 62 milk samples were taken from 38 randomly selected, clinically healthy individuals for the microbiological tests. According to which lactation cycle the animals were in and in which stage of lactation during the sampling, the samples taken from the individual cows were classified as follows: 26 samples were taken from 15 primiparous cows and 36 samples were taken from 23 multiparous (2 to 5 calvings) Holstein-Friesian cows. Of the total 62 samples taken, 23 were taken in the early stage of lactation, 21 samples in the middle stage of lactation and 18 samples were taken from cows in the late stage of lactation.

Following pre-disinfection of the teats, wiping them dry using paper towels and milking of the first milk jets, samples were taken from all four udder quarters into sealable sterile plastic sampling vessels with a capacity of 50 ml. The vessels were transported in coolers to the microbiology laboratory of the Institute of Food Science of the University of Debrecen within two hours of sampling. Samples were processed within 24 hours of sampling.

3.2. Microbiological tests

Preparation of the milk samples and the subsequent microbiological tests were performed according to the procedure described by Petróczki et al. **[26]**. Sample preparation was carried out according to standard MSZ EN ISO 6887-1:2017 **[27]**, the samples were stored at 4 °C until the beginning of the analysis, and they were homogenized by shaking before processing. To prepare the dilution series, peptone water was used which was prepared by dissolving 8.5 g of sodium chloride (VWR International Kft., Hungary) and 1.0 g of peptone (Merck Kft., Hungary) in 1,000 ml of distilled water.

After weighing the appropriate amounts (9 ml each) into test tubes, the diluent was sterilized in a pressure cooker at 120 °C for 30 minutes, then it was cooled and the decimal dilution series was prepared.

The total plate count was determined according to standard MSZ EN ISO 4833-1:2014 **[28]**, which prescribes the use of a tryptone-glucose-yeast agar (Plate Count Agar, PCA) culture medium (Biolab Zrt., Hungary) supplemented with milk powder. After performing the prescribed plate casting method, the plates were incubated at 30 °C for 72 hours.

The amount of coliform bacteria was determined by the plate casting method according to standard ISO 4832:2006 **[29]**, using sterile crystal violet-bile-lactose agar (Violet Red Bile Lactose, VRBL, Biolab Zrt., Hungary). The incubation period was 24 hours at 30 °C.

The determination of *S. aureus* was performed according to standard MSZ EN ISO 6888-1:2008 **[30]** by the spreading method, for which Baird-Parker agar (BPA, Biolab Zrt., Hungary) supplemented with egg yolk-tellurite emulsion (LAB-KA Kft., Hungary). The incubation period was 48 hours at 37 °C. *S. aureus* was isolated from other *Staphylococcus* species using a latex agglutination test (Prolex Staph Xtra Kit, Ferol Kft., Hungary).

3.3. Statistical analysis

For the analysis of our experimental results, for the calculation of descriptive statistics, for the logarithmic transformation of the amount of microorganisms and for performing t-tests and analysis of variance, the SPSS v.22.0 **[31]** software was used.

In the case of lactation number, variables were compared using unpaired t-tests and non-parametric Mann-Whitney tests, while in the case of lactation stage, the comparison was performed by one factor analysis of variance and non-parametric Kruskal-Wallis tests. Since the total plate count, the coliform count and the somatic cell count were not found to be normally distributed variables in several cases, a logarithmic transformation was used for these parameters. During statistical analyses, a value of $P<0.05$ was considered to indicate a significant difference.

4. Results

4.1. Effect of lactation number on milk yield, raw milk composition and its microbiological parameters

Mean and standard deviation values of the daily milk yield of primiparous and multiparous cows, as well as those of the fat and protein content, somatic cell count, total plate count, coliform and *S. aureus* count of the milk produced by them are shown in ***Table 1***. Cows selected for the study were classified into groups based on the number of lactations (and calvings) into groups of primiparous and multiparous individuals. The average milk yield was 25.67 kg/day for primiparous cows, while in the case of multiparous cows it was 31.04 kg/day. The difference is significant ($P<0.05$), which means that our experiments confirmed the findings of Bondan et al. and Yang et al., that the daily amount of milk produced by multiparous cows is higher than that of primiparous cows **[5, 32]**. In the case of crossbred Holstein-Friesian cows, Gurmessa and Melaku studied, inter alia, the effect of calving number on milk yield, but found no difference between the daily milk yield of primiparous and multiparous cows **[33]**. Pratap and his research group also found no difference in the average daily milk yield of primiparous and multiparous cows (6.43±1.39 and 5.89±2.37 l/day, respectively) **[34]**.

The average fat content of milk during the first lactation of cows selected during our research was 3.74±0.40%, while the average fat content of milk samples taken during the second or later lactation was 3.75±0.36%. The difference was not found to be significant ($P>0.05$). Similarly to our own results, no difference was found between the average fat content of the milk of primiparous and multiparous crossbred Holstein-Friesian cows by Gurmessa and Melaku, or Pratap et al. **[33**, **34]**. On the other hand, it was found by Bondan and his group that the number of lactations did have an effect on the fat content of milk in Holstein-Friesian cows. While the fat content was 3.47±0.67% in the case of cows lactating for the first time, it was 3.43±0.68% and 3.41±0.67% for cows lactating for the 2nd or 3rd time and for cows that had calved at least 4 times, respectively **[5]**. In their study in Sudan, Shuiep and his working group examined changes in the fat content of milk with the number of lactations in local and crossbred cows. In the case of local cows, cows in their fourth lactation period had a lower milk fat content (4.82±0.55%) than cows with fewer calvings (1:5.16±0.32; 2:5.22±0.34; 3:5.14±0.34). No difference was observed in the case of crossbred cows **[6]**. In contrast, Yang et al. found in their research that Holstein-Friesian cows lactating for the first time had a lower milk fat content (3.88%) **[32]**. Based on the varied results of our study and other literature references, we came to the conclusion that the fat content of milk may be influenced by other factors besides the lactation number.

During the first lactation of the Holstein-Friesian cows selected by us, the average protein content of the milk was 3.24±0.19%, while the average protein content of milk samples taken during the second or later lactation was 3.31±0.16%, with the difference being not significant ($P>0.05$). This is in line with the findings of Gurmessa and Melaku, as well as those of Pratap et al., as these authors did not find any difference between the average protein content of the milk of primiparous and multiparous cows **[33**, **34]**. In contrast, the research group of Bondan found that the lactation number affected the protein content of milk in Holstein-Friesian cows. While the protein content was 3.24±0.37% in the case of cows lactating for the first time, it was 3.23±0.38% and 3.19±0.37% in the case of cows lactating for the 2nd or 3rd time and for cows that had calved at least 4 times, respectively **[5]**. Changes in the protein content of milk with the lactation number was also studied by the research group of Shuiep. In the case of local cows, the protein content of milk was lower in the case of cows in their fourth lactation period (3.67±0.19%), than in the case of cows with fewer calvings (1:4.01±0.11; 2:3.82±0.12; 3:3.84±0.12). No difference was observed in crossbred cows **[6]**.

According to our results, the average somatic cell count [242.2×10^3 (5.12±0.42 lg) cell/ml] in the milk of primiparous cows is less ($P<0.05$) then in the milk of multiparous cows [356.3×10^3 (5.39±0.39 lg) cell/ml]. In none of the cases did the averages exceed the limit value [$M=400.0\times10^3$ (5.60 lg) cfu/ml] laid down in Regulation (EC) No 853/2004 **[35]**. The results obtained at the Hungarian dairy farm are in line with relevant literature data. According to Mikó et al., as the lactation number increases, the somatic cell count of milk may also increase **[25]**. This finding was also found to be true by Yang and his group **[32]**. Sheldrake et al. observed a significant relationship between the calving number and the somatic cell count. They found that with the increase in calving number, there was a smaller change in the udder quarters of healthy animals, however, in the case of udder quarters infected with *S. aureus*, the somatic cell count increased significantly **[36]**. The research team of Bondan found that as the lactation number of the Holstein cows studied increased, so did the somatic cell count in milk. While the average somatic cell count in primiparous cows was 4.83±1.73 lg cell/ml, it was 5.31±1.72 és 5.84±1.62 lg cell/ml in cows that had calved 2 or 3 times and cows that calved 4 or more times, respectively **[5]**.

In the milk samples taken from Holstein-Friesian cows that only calved once, i.e., cows in their first lactation period, the average total plate count was 5.1×10^3 (3.36±0.58 lg) cfu/ml, while it was 4.6×10^3 (3.30±0.59 lg) cfu/ml in the milk samples taken from multiparous cows, however, the difference was not significant ($P>0.05$).

However, the average coliform count [1.1×10^3 (1.35±1.20 lg) cfu/ml] in the milk of multiparous cows was more ($P<0.05$) than the average coliform count [1.1×10^1 (0.65±0.61) lg) cfu/ml] measured in the milk of primiparous cows. Tenhagen et al. also included clinically healthy cows in their study, which found that although coliform bacteria were found in higher quantities in the milk of multiparous cows, the difference was not significant **[23]**.

S. aureus was present in only one of the 26 milk samples taken from primiparous cows, in an amount of 5.0×10^1 (1.70 lg) cfu/ml, while it could be detected in eight of the 36 milk samples taken from multiparous cows. The mean *S. aureus* count in these samples was 1.5×10^2 (1.92±0.56 lg) cfu/ml. The values do not exceed the limit value [$M=5.0\times10^2$ (2.70 lg) cfu/ml] specified by EüM decree 4/1998 (XI. 11.) **[37]**. In the case of the individuals in whose milk *S. aureus* could be detected during the microbiological tests, the average somatic cell count was between 44.3×10^3 (4.65 lg) cell/ml and 357.2×10^3 (5.55 lg) cell/ml on the basis of milking data. In his study, Tessema also found that the prevalence of *S. aureus* was higher in the case of multiparous cows (i.e., who had calved more than twice) (who produced a positive California Mastitis Test) **[21]**. According to Tenhagen et al., the incidence of *S. aureus* increases with the age and lactation stage of the animals **[23]**.

Table 1. Milk yield of primiparous and multiparous cows and compositional and microbiological properties of milk collected from them

		Primiparous ($\bar{x}\pm\sigma$)	**Multiparous ($\bar{x}\pm\sigma$)**
Production parameter	Milk yield (kg/day)	25.67 ± 4.42^a	31.04 ± 5.08^b
Composition	Fat content (%)	3.74 ± 0.40^a	3.75 ± 0.36^a
	Protein content (%)	3.24 ± 0.19^a	3.31 ± 0.16^a
Microbiological parameter	Somatic cell count (lg cell/ml)	5.12 ± 0.42^a	5.39 ± 0.39^b
	Total plate count (lg cfu/ml)	3.36 ± 0.58^a	3.30 ± 0.59^a
	Coliform count (lg cfu/ml)	0.65 ± 0.61^a	1.35 ± 1.20^b
	S. aureus count (lg cfu/ml)	1.70	1.92±0.56

$^{a,\,b}$ *The values marked with different letters in the same rows of the table differ significantly ($P<0.05$)*

4.2. Effect of lactation stage on milk yield, raw milk composition and its microbiological parameters

Mean and standard deviation values of the daily milk yield of cows in the early, middle and late stages of lactation, as well as those of the fat and protein content, somatic cell count, total plate count, coliform and *S. aureus* count of the milk produced by them are shown in ***Table 2***. The cows selected for the study were classified into groups of early, middle and late lactation stage individuals, based in their stage of lactation. When examining the changes in the daily milk yield of the individuals with the stage of lactation, the finding in the literature that the daily milk yield decreases towards the end of lactation was confirmed. While the average daily milk yield of cows in the early stage of their lactation was 32.10±4.73 kg/day, that of cows in the middle stage of their lactation was 29.08±5.09 kg/day, while that of cows in the late stage of lactation was 23.36±3.63 kg/day. The difference was significant ($P<0.05$). Bondan and his group came to a similar conclusion in their study. The average milk yield between days 6 and 60 of the lactation of the Holstein-Friesian cows studied by them was 29.4±8.72 l/cow/day; for cows between days 61 and 120 of their lactation, it was 29.2±8.66 l/cow/day; for cows between lactation days 121 and 220, the milk yield was 26.2±8.01 l/cow/day, and at the end of the lactation (>220 days), it was 22.0±7.49 l/cow/day **[5]**. Gurmessa and Melaku, as well as Pratap et al. also found that the daily milk yield of the animals was higher at the beginning of lactation (6.81±1.45 l/day) than at the end of lactation (5.48±0.05 l/day). In their studies, the daily milk yield of crossbred Holstein-Friesian cows in the middle stage of their lactation was the highest (7.17±0.05 liter) **[33**, **34]**. According to Auldist et al., the effect of lactation stage on milk yield (e.g., a decrease) may be due to a change in the number and activity of the secretory cells within the mammary gland because of physiological reasons **[2]**.

The fat content of the milk of the individuals studied shows a change as we progress towards the end of lactation. The average fat content of cows in the early and middle stages of lactation was 3.65±0.43% and 3.59±0.41%, respectively, which were lower (P<0.05) than the fat content of the milk of cows in the late stage of lactation (3.99±0.47%). The increase in milk fat concentration at the end of lactation may be associated with a decrease in milk yield as lactation progresses, as a decrease in milk yield may have a concentrating effect on milk composition **[2]**. The fat content of milk also varies at the three stages of lactation according to the publication of Gurmessa and Melaku. For cows in the early or late stages of lactation, the average fat content of the milk (4.46±1.44% and 4.46±1.44%, respectively) was significantly higher than that of cows in the middle stage of lactation (3.70±0.89%) **[33]**. The publication of Bondan et al. states that the fat content of milk at the end of lactation (>200 days) is higher (3.55±0.67%) than at earlier stages of lactation. However, they also found that the measured average fat content (3.30±0.66%) was lowest between days 61 and 120 of the lactation of the cows. Between days 6 and 60, and between days 121 and 220, average fat contents of 3.40±0.65% and 3.40±0.66% were measured, respectively **[5]**. Shuiep et al. studied changes in the fat content of milk of local and crossbred cows in Sudan with lactation stage. In the case of the local breed, there was no difference in the fat content between the early (5.31±0.51%), middle (4.67±1.56%) and late (5.28±0.75%) stages of lactation. However, in the case of crossbred cows, the fat content was higher at the end of lactation (4.45±1.43%) than at the beginning of the lactation (3.41±1.09%) or in the middle stage of lactation (3.33±1.05%) **[6]**.

Similar to the fat content, a change in protein content can also be observed as we progress towards the end of lactation. The average protein content measured at the beginning of lactation was 3.08±0.15%, it was 3.20±0.19% in the middle of lactation and 3.56±0.20% at the end of lactation, the difference being significant (P<0.05). Bondan et al. came to a similar conclusion: a higher protein content (3.41±0.36%) was measured at the end of lactation (>200 days) than at earlier stages of lactation. It was also found that the measured average protein content (3.03±0.31%) was the lowest between days 61 and 120 of the lactation of the cows. The measured average protein content between days 6 and 60 of the lactation and between days 121 and 220 were 3.05±0.36% and 3.18±0.32%, respectively **[5]**. Gurmessa and Melaku, as well as the research group of Pratap did not find any difference in protein content between cows in the early (3.55±1.43%), middle (3.17±0.15%) and late (3.33±0.16%) stages of their lactation **[33**, **34]**. In the case of local and crossbred cows in Sudan, changes in milk protein content with the lactation stage were investigated by Shuiep et al. In the case of the local breed, the protein content of the milk of the animals was higher at the beginning (3.87±0.52%) and in the middle (3.91±0.18%) of lactation than at the end of lactation (3.67±0.17%). In the case of crossbred cows, there was no difference in protein content at the beginning (3.67±0.17%), middle (3.69±0.16%) and end (3.63±0.22%) of lactation **[6]**.

The average somatic cell count in the early stage of lactation of the cows selected for our research was 195.1×10^3 (5.07±0.43 lg) cell/ml, it was 370.6×10^3 (5.28±0.50 lg) cell/ml in the middle of lactation, and it was 336.4×10^3 (5.33±0.41 lg) cell/ml in the late stage of lactation. The somatic cell count was higher (P<0.05) in the milk of individuals in the late stage of lactation than in the case of cows at the beginning of lactation. As lactation progresses, somatic cell counts also show an increasing trend according to Bondan et al. While in the milk of Holstein-Friesian cows between days 6 and 60 of their lactation the average somatic cell count was 4.79±1.90 lg cell/ml, between days 61 and 120 it was 4.89±1.90 lg cell/ml, between days 121 and 220 it was 5.21±1.75 lg cell/ml, and in the case of lactations lasting more than 220 days, this parameter was 5.53±1.53 lg cell/ml in the milk of the cows studied **[5]**.

Total plate counts were also determined during the different stages of lactation of Holstein-Friesian cows. At the beginning of lactation, the average total plate count was 6.8×10^3 (3.42±0.67 lg) cfu/ml, in the middle of lactation it was 4.4×10^3 (3.39±0.46 lg) cfu/ml, while at the end of lactation it was 2.7×10^3 (3.13±0.56 lg) cfu/ml. No significant difference was found between the total plate count values obtained (P>0.05).

Regarding the number of coliform bacteria, the highest count [1.3×10^3 (1.30±1.23 lg) cfu/ml] was measured in samples taken at the beginning of the lactation of the cows, while the lowest average coliform count [2.2×10^1 (0.76±0.79 lg) cfu/ml] was detected in samples taken in the middle stage of lactation. The average coliform count in the samples taken at the end of lactation was 1.50×10^2 (0.90±0.94 lg) cfu/ml. There was no significant difference between the results (P>0.05).

S. aureus was present in a total of 9 samples (14.52%) out of 62 individual milk samples, with an average count of 1.4×10^2 (1.89±0.53 lg) cfu/ml. In the six samples (9.68%) taken from cows in the early stage of lactation, the average *S. aureus* count was 1.2×10^2 (1.81±0.56 lg) cfu/ml, for the single animal in the middle of lactation (1.61%) the *S. aureus* count was 8.2×10^1 (1.91 lg) cfu/ml, while for the two cows at the end of lactation (3.23%) it was 2.4×10^2 (2.15±0.70 lg) cfu/ml.

Table 2. Milk yield of cows in their early, mid and late lactation stages and compositional and microbiological properties of milk from them

		Early lactation ($\bar{x}\pm\sigma$)	Mid lactation ($\bar{x}\pm\sigma$)	Late lactation ($\bar{x}\pm\sigma$)
Production parameter	Milk yield (kg/day)	32.10 ± 4.73^a	29.08 ± 5.09^b	23.36 ± 3.63^c
Composition	Fat content (%)	3.65 ± 0.43^a	3.59 ± 0.41^a	3.99 ± 0.47^b
	Protein content (%)	3.08 ± 0.15^a	3.20 ± 0.19^b	3.56 ± 0.20^c
Microbiological parameter	Somatic cell count (lg cell/ml)	5.07 ± 0.43^a	$5.28\pm0.50^{a,b}$	5.33 ± 0.41^b
	Total plate count (lg cfu/ml)	3.42 ± 0.67^a	3.39 ± 0.46^a	3.13 ± 0.56^a
	Coliform count (lg cfu/ml)	1.30 ± 1.23^a	0.76 ± 0.79^a	0.90 ± 0.94^a
	S. aureus count (lg cfu/ml)	1.81±0.56	1.91	2.15±0.70

$^{a, b, c}$ *The values marked with different letters in the same rows of the table differ significantly (P<0.05)*

5. Conclusions

In the course of our research work in a Hungarian dairy farm, it was proven that, under large-scale husbandry conditions, for primiparous cows the average daily milk yield is significantly lower (P<0.05) than in the case of multiparous cows. This is probably due to the fact that cows in their first lactation need more amino acids and fat to develop their bodies compared to animals that have already undergone several lactation periods **[38]**.

Regarding the fat and protein content of the milk, there was no significant difference between primiparous and multiparous cows. Since one can find in the literature findings that both support and contradict our results, we assume that the fat and protein contents of milk are also influenced by other factors (e.g., breed, season, etc.), but mapping out of these factors was not the objective of our study. For example, Shuiep et al. reported in their paper different results for two different cattle breeds when examining the changes in the fat and protein contents of milk with the lactation number **[6]**.

Based on our measurement results, we found that the somatic cell count, as well as the coliform count and the *S. aureus* count were significantly higher (P<0.05) in the milk samples taken from multiparous cows compared to the milk samples taken from primiparous cows. The reason why microorganisms can be measured in larger amounts in milk samples taken from multiparous cows is probably that the teats may have been damaged during the lactations (due to, for example, the milking machine), which may have increased the chances of microorganisms entering the udder **[24]**. Another reason may be that as the age progressed or the number of lactations increased, the condition of the dairy animals may have deteriorated, which may have had an adverse effect, for example, on the somatic cell count of the milk **[25]**.

It was also proven that, in the stages of lactation, a decreasing trend in daily milk yield can be observed, but the fat and protein contents show an increase. This is presumably due to the concentrating effect of the decreasing milk yield.

There was no difference in milk samples taken at different stages of lactation in terms of total plate count and coliform count, however, in the late stage of lactation, the somatic cell count showed an increase.

6. Acknowledgment

This publication was supported by the project EFOP-3.6.3-VEKOP-16-2017-00008. The project was supported by the European Union and co-financed by the European Social Fund.

We are grateful to the management and staff of the dairy farm for their helpful assistance.

7. Literature

[1] Hill B., Smythe B., Lindsay D., Shepherd J (2012): Microbiology of raw milk in New Zealand. International Journal of Food Microbiology 157 2 pp. 305-308. https://doi.org/10.1016/j.ijfoodmicro.2012.03.031

[2] Auldist M. J., Walsh B. J., Thomson N. A. (1998): Seasonal and lactational influences on bovine milk composition in New Zealand. Journal of Dairy Research 65 (3) pp. 401-411. https://doi.org/10.1017/S0022029998002970

[3] Heck J. M. L., van Valenberg H. J. F., Dijkstra J., van Hooijdonk A. C. M. (2009): Seasonal variation in the Dutch bovine raw milk composition. Journal of Dairy Science 92 (10) pp. 4745-4755. https://doi.org/10.3168/jds.2009-2146

[4] Lambertz C., Sanker C., Gauly M. (2014): Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. Journal of Dairy Science 97 (1) 3 pp. 19-329.https://doi.org/10.3168/jds.2013-7217

[5] Bondan C., Folchini J. A., Noro M., Quadros D. L., Machado K. M., González F. H. D. (2018): Milk composition of Holstein cows: a retrospective study. Ciência Rural 48 (12) pp. 1-8. https://doi.org/10.1590/0103-8478cr20180123

[6] Shuiep E. S., Eltaher H. A., El Zubeir I. E. M. (2016): Effect of Stage of Lactation and order of Parity on Milk Composition and Daily Milk Yield among Local and Crossbred Cows in South Darfur State, Sudan. SUST Journal of Agricultural and Veterinary Sciences (SJAVS) 17 (2) pp. 86-99.

[7] Dürr J. W., Ribas N. P., Costa C. N., Horst J. A., Bondan C. (2011): Milk recording as an indispensable procedure to assure milk quality. Revista Brasileira Zootecnia 40 pp. 76-81.

[8] Quigley L., O'sullivan O., Beresford T. P., Ross R. P., Fitzgerald G. F., Cotter P. D. (2011): Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. International Journal of Food Microbiology 150 (2-3) pp. 81-94. https://doi.org/10.1016/j.ijfoodmicro.2011.08.001

[9] Claeys W. I., Cardoen S., Daube G., De Block J., Dewettinck K., Dierick K., De Zutter L., Huyghebaert A., Imberechts H., Thiange P., Vandenplas Y., Herman L. (2013): Raw or heated cow milk composition: Review of risks and benefits. Food Control 31 (1) pp. 251-262. https://doi.org/10.1016/j.foodcont.2012.09.035

[10] Laczay P., Lehel J., Lányi K., László N. (2016): A nyers tejben potenciálisan jelen levő kórokozók közegészségügyi jelentősége. Magyar Állatorvosok Lapja 138 pp. 231-242.

[11] Laczay P. (2008): Élelmiszer-higiénia - Élelmiszerlánc-biztonság. Mezőgazda Kiadó, Budapest.

[12] Cilliers F. P., Gouws P. A., Koutchma T., Engelbrecht Y., Adriaanse C., Swart P. (2014): A microbiological, biochemical, and sensory characterisation of bovine milk treated by heat and ultraviolet (UV) light for manufacturing Cheddar cheese. Innovative Food Science & Emerging Technologies 23 pp. 94-106. https://doi.org/10.1016/j.ifset.2014.03.005

[13] Mbuk E. U., Kwaga J. K. P., Bale J. O. O., Boro L. A., Umoh J. U. (2016): Coliform organisms associated with milk of cows with mastitis and their sensitivity to commonly available antibiotics in Kaduna State, Nigeria. Journal of Veterinary Medicine and Animal Health 8 (12) pp. 228-236. https://doi.org/10.5897/JVMAH2016.0522

[14] Altalhi A. D., Hassan S. A. (2009): Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers. Food Control 20 (10) pp. 913-917. https://doi.org/10.1016/j.foodcont.2009.01.005

[15] Mhone T. A., Matope G., Saidi P. T. (2011): Aerobic bacterial, coliform, *Escherichia coli* and *Staphylococcus aureus* counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. International Journal of Food Microbiology 151 (2) pp. 223-228. https://doi.org/10.1016/j.ijfoodmicro.2011.08.028

[16] Markus G. (2001): A tejelő tehenek tőgygyulladása III. MezőHír. 9

[17] Ózsvári L., Fux A., Illés B. CS., Bíró O. (2003): A *Staphylococcus aureus* tőgygyulladás által okozott gazdasági veszteségek számszerűsítése egy nagyüzemi holstein-fríz tehenészetben. Magyar Állatorvosok Lapja 125 pp. 579-584.

[18] Rosengren Å., Fabricius A., Guss B., Sylvén S., Lindqvist R (2010): Occurrence of foodborne pathogens and characterization of *Staphylococcus aureus* in cheese produced on farm-dairies. International Journal of Food Microbiology 144 (2) pp. 263-269. https://doi.org/10.1016/j.ijfoodmicro.2010.10.004

[19] Anderson D., Dulmage D., McDougall M., Séguin G. (2003): General guidelines for effective dairy equipment cleaning. https://www.milk.org/Corporate/pdf/Farmers-UdderEquipmentCleaning.pdf (Aquired: 21. 02. 2020.)

[20] Peles F., Máthéné Sz. Zs., Béri B., Szabó A. (2008): A tartástechnológia hatása a nyers tej mikrobiológiai állapotára. Agrártudományi Közlemények 31 pp. 67-75. https://doi.org/10.34101/actaagrar/31/3009

[21] Tessema F. (2016): Prevalence and Drug Resistance Patterns of *Staphylococcus Aureus* in Lactating Dairy Cow's Milk in Wolayta Sodo, Ethiopia. EC Veterinary Science 2 (5) pp. 226-230.

[22] Bytyqi H., Vehapi I., Rexhepi S., Thaqi M., Sallahi D., Mehmeti I. (2013): Impact of Bacterial and Somatic Cells Content on Quality Fresh Milk in Small-Scale Dairy Farms in Kosovo. Food and Nutrition Sciences 4 (10) pp. 1014-1020. https://doi.org/10.4236/fns.2013.410132

[23] Tenhagen B. A., Köster G., Wallmann J., Heuwieser W. (2006): Prevalence of Mastitis Pathogens and Their Resistance Against Antimicrobial Agents in Dairy Cows in Brandenburg, Germany. Journal of Dairy Science 89 (7) pp. 2542-2551. https://doi.org/10.3168/jds.S0022-0302(06)72330-X

[24] Hamann J., Mein G. A., Wetzel S. (1993): Teat tissue reactions to milking: effects of vacuum level. Journal of Dairy Science 76 pp. 1040-1046. https://doi.org/10.3168/jds.S0022-0302(93)77432-9

[25] Mikó E., Baranyi A., Gráff M. (2015): Analysis of somatic cells in cow's milk. Lucrări Ştiinţifice 17 (1) pp. 290-293.

[26] Petróczki F. M., Tonamo T. A., Béri B., Peles F. (2019): The effect of breed and stage of lactation on the microbiological status of raw milk. Acta Agraria Debreceniensis 1 pp. 37-45. https://doi.org/10.34101/actaagrar/1/2367

[27] Magyar Szabványügyi Testület (MSzT) (2017): Élelmiszerek és takarmányok mikrobiológiája. A vizsgálati minták, az alapszuszpenzió és a decimális hígítások elkészítése mikrobiológiai vizsgálathoz. 1. rész: Az alapszuszpenzió és a decimális hígítások elkészítésének általános szabályai. Magyar szabvány MSZ EN ISO 6887-1:2017. Magyar Szabványügyi Testület, Budapest.

[28] Magyar Szabványügyi Testület (MSzT) (2014): Az élelmiszerlánc mikrobiológiája. Horizontális módszer a mikroorganizmusok számlálására. 1. rész: Telepszámlálás 30 °C-on lemezöntés módszerrel. Magyar szabvány MSZ EN ISO 4833-1:2014. Magyar Szabványügyi Testület, Budapest.

[29] International Organization for Standardization (ISO) (2006): Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique. ISO 4832:2006

[30] Magyar Szabványügyi Testület (MSzT) (2008): Élelmiszerek és takarmányok mikrobiológiája. Horizontális módszer a koagulázpozitív sztafilokokkuszok (*Staphylococcus aureus* és más fajok) számának meghatározása. 1. rész: Baird-Parker-agar táptalajos eljárás. Magyar szabvány MSZ EN ISO 6888-1:2008. Magyar Szabványügyi Testület, Budapest.

[31] SPSS (2013): SPSS 22.0 for Windows. SPSS Inc., Chicago, IL, USA. Copyright © SPSS Inc., 1989-2013.

[32] Yang L., Yang Q., Yi M., Pang Z. H., Xiong B. H. (2013): Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in northern China. Journal of Dairy Science 96 (11) pp. 6863-6869. https://doi.org/10.3168/jds.2013-6846

[33] Gurmessa J., Melaku A. (2012): Effect of Lactation Stage, Pregnancy, Parity and Age on Yield and Major Components of Raw Milk in Bred Cross Holstein Friesian Cows. World Journal of Dairy & Food Sciences 7 (2) pp. 146-149.

[34] Pratap A., Verma D. K., Kumar P., & Singh A. (2014): Effect of Pregnancy, Lactation Stage, Parity and Age on Yield and Components of Raw Milk in Holstein Friesian Cows in organized Dairy form in Allahabad. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 7 (2) pp. 112-115. https://doi.org/10.9790/2380-0721112115

[35] 853/2004/EK: Az Európai Parlament és a Tanács 853/2004/EK rendelete az állati eredetű élelmiszerek különleges higiéniai szabályainak megállapításáról

[36] Sheldrake R. F., Hoare R. J. T., McGregor G. D. (1983): Lactation Stage, Parity, and Infection Affecting Somatic Cells, Electrical Conductivity, and Serum Albumin in Milk. Journal of Dairy Science 66 pp. 542-547. https://doi.org/10.3168/jds.S0022-0302(83)81823-2

[37] 4/1998. (XI. 11.) EüM rendelet az élelmiszerekben előforduló mikrobiológiai szennyeződések megengedhető mértékéről

[38] Oltner R., Emanuelson M., Wiktorsson H. (1985): Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livestock Production Science 12 (1) pp. 47-57. https://doi.org/10.1016/0301-6226(85)90039-9