Influence of different milking methods on milk quality based on somatic cell count and basic composition

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Abstract
Introduction and Objective. Correlations between the number of milk somatic cells (SCC), the number of microorganisms, and the content of basic components of milk were studied on five farms (F1–F5) with cows of the same breed, but with different milking systems.

Materials and Method. From each farm, 50 Holstein Friesian milk samples were collected once a month (250 samples/month; n=3,000) during March 2022 – February 2023. Samples from farms F1 and F5 were tested for fat, protein, lactose, no fat dry matter content (FTIR spectroscopy), for the SCC (Fossomatic 7), and for the differential cells (Vetscan DC-Q).

Results. The highest fat content was confirmed on farm F5 (3.85 ± 1.70%) and F4 (3.82 ± 0.21%) with automatic milking system (AMS). However, from the point of view of protein content, these farms showed slightly lower values (<0.05). F1 did not meet the minimum required amount for fat content (2.84 ± 0.81%) set by the legislation of the Slovakia. The comparison shows that there is not much difference in cell size between healthy cells and mastitis cells. The average size of healthy cells was approximately 8.77 ± 0.49 μm. In the monitored period, the average values determined were at the level of 292,000/mL (5.46 ± 0.72 log10 SCC) in cow milk samples, while for the rest of the year, the values remained at 256,000/mL (5.40 ± 0.80 log10 SCC). F1 was categorized as a positive farm with a high TLC (total milk leucocyte count) concentration (5.58 log10 cells/mL), and a predominant NEU fraction (61%). Farms F2, F4, and F5 were classified as negative farms (TLC was 4.70 ± 0.26 log10 cells/ml).

Conclusions. According to the results, the size of SCCs in healthy milk does not differ from SCCs found in mastitis milk. From the results, it can be concluded that the transition to the latest generation of robotic milking method can positively affect milk production and its quality.

Key words
somatic cells, size, milk components, method of milking, seasonality, quality, mastitis

INTRODUCTION

Milk contains several types and different numbers of cells which are a very important criterion of udder health, and an indicator of the technological, hygienic and health quality of milk [1]. Cells enter the milk either from the udder itself or from the bloodstream of the dairy cow.

Somatic cells include mainly milk-secreting epithelial cells that have been shed from the lining of the gland, and white blood cells (leukocytes – monocytes, granulocytes, and lymphocytes) that have entered the mammary gland in response to injury or infection [2].

Somatic cell count (SCC) in milk is highly correlated with udder health, and somatic cells are generally the standard in its diagnosis [3, 4].

Some authors [5] state that mastitis is an inflammatory disease of the mammary gland, which is characterized by physical, chemical and microbial changes, as well as an increase in the number of somatic cells and other changes in the composition of milk. Mastitis is among the most economically serious livestock diseases. Due to its high prevalence and negative impact on the economy, mastitis is a major problem in the dairy industry [6]. This problem is widespread and causes a decrease in the quantity as well as the quality of milk produced. It is estimated that cows affected by subclinical mastitis produce 25–42% less milk than healthy cows [7, 8, 9].

The SCC increases as leukocytes – particularly polymorphonuclear leukocytes (PMNs) are recruited from the blood into the mammary gland to phagocytize invading organisms. This immune response is crucial for preventing further infection by pathogens. Because the magnitude of the SCC is closely linked to the extent of inflammation in the mammary gland, cows with the lowest averages of SCC during lactation are deemed to have the highest resistance to intramammary infection (IMI) and mastitis [10].
In 2017, a new tool – the differential somatic cell count (DSCC) – was described for determining the proportion of PMNs and lymphocytes in somatic cells during monthly DHI testing (Dairy Herd Information). Given that lymphocytes typically constitute a small proportion of milk cells, fluctuations in DSCC primarily reflect changes in PMN levels [11].

In cows affected by mastitis, almost all the basic components of milk are changed. Therefore, data on the composition of milk are often used for general estimation of animal health and udder condition, as well as an indicator for detecting subclinical mastitis, feeding errors, causes of metabolic disorders. The composition of milk is influenced by other factors, such as breed, number of lactations, stage of lactation, technology of housing, season, feeding, environmental factors [12].

However, few studies have evaluated the association between breed and IMI, or mastitis associated pathogens. Previous studies found that some breed variation may exist, for example, Staphylococcus haemolyticus is more common in quarter milk samples from Swedish Holsteins compared with Swedish Reds [13], and Staphylococcus devriesei was more common on the teat ends of red and white Holstein Friesien cows compared with black and white Holstein Friesien cows [14]. Conversely, another study identified no impact of breed when evaluating pathogens associated with clinical mastitis among Swedish dairy cows [15, 16].

Another influence on milk quality is the method of milking (manual, machine). In general, automatic milking system (AMS) has been adopted as a realistic alternative for milking in the ‘traditional’ milking parlour. Systems have gradually been improved and, maybe even more importantly, farmers have become more familiar with their potential and limitations, both technically and in herd management. The number of farms milking with AMS has increased worldwide [17]. The technical design and the functionality of an AMS could influence the behaviour and milking characteristics of dairy cows, both directly and indirectly.

All developed countries use the milk SCC as a marker to monitor the prevalence of mastitis in dairy herds. Among all the different screening tests for milk quality, it is the number of somatic cells in milk that is the most effective method for detecting the subclinical form of mastitis [18]. Regulation of the European Parliament and Council No. 853/2004 of 29 April 2004, established special hygiene regulations for food of animal origin, i.e. the criterion for the number of somatic cells in raw cow’s milk in a pool sample is a maximum of 400,000 cells/mL [19]. The number of somatic cells is a mandatory indicator in Europe. In the USA, the legal maximum SCC count for Grade A farm bulk milk is 750,000 cells/mL; this limit is high compared to many international standards. Much of Europe, Australia and New Zealand, has a limit of 400,000 cells/mL, and Canada a limit of 500,000 cells/mL of raw milk. Milk SCC is a diagnostic figure for subclinical mastitis [20].

Slovakia is a landlocked central European country with a climate that can be described as a typical European continental [21].

Cow’s milk production is an important and traditional sector of agricultural primary production in Slovakia. The current article presents results that provide a framework picture of the hygienic quality of raw cow’s milk on five monitored Slovak farms for a period of one year (March 2022 – February 2023). The aim of the study, therefore, was to investigate the quality of milk and to compare the influence of the number of somatic cells on the content of milk components on selected farms. The location, effect of the season and method of milking on the mentioned parameters were taken into account. Somatic cells were analyzed in terms of morphological characteristics – size and number of cells, i.e. total number of milk leukocytes, such as Lymphocytes, Macrophages, Neutrophils.

**MATERIALS AND METHOD**

Individual samples of raw cow’s milk were obtained from five farms (F1 – F5) from different regions of Slovakia (Fig. 1). Dairy cows are stabled on all farms with deep bedding, the food regime was the same on all farms.

Sampling was based on the real conditions of breeding practice in the Slovak Republic. Individual milk samples (n = 3,000) were collected from Holstein Friesien cattle. Fifty samples of milk of the Holstein Friesien cattle were taken from each farm once a month (250 samples/month) for a period of one year (March 2022 – February 2023). Dairy cows were not treated with antibiotics for mastitis or any other disease. Dairy cows had first to fifth lactation (2.67 ± 1.52) and the 12-month average DIM on farms was 270–300 days.

Farm F1 (500 dairy cows) is located in the southeast of the Slovak Republic, has a lowland-hilly character (200 m a.s.l.). The territory belongs to the northern temperate zone with average annual temperatures of around 10°C. Southwest of the city of Košice (the metropolis of eastern Slovakia), F2 is located (400 dairy cows) which also belongs to the Košice region. F3 (300 dairy cows) is located in the southeastern part of the Republic (170 m a.s.l.) and borders the Republic of Hungary. All three farms use Boumatic parallel milking equipment. F4 and F5 (350 dairy cows) were among the first unified farming cooperatives in Slovakia, located in the Banskobystricky Region at the foot of the Polana mountain range at an altitude of 400 m a.s.l. The farms use a robotic method of milking (F4 – Lely Astronaut A4; F5 – Lely Astronaut A4).

**Figure 1. Geographic location of sampling from five farms (F1 – F5)**

At each farm, feeding was carried out using complete mixtures of feed rations, the so-called TMR (total mixed ration). One of the biggest advantages of TMR is the stable composition of the ration.

The milk samples were transported to the laboratory in a refrigerator (4°C) and processed for analysis within 12 hours. Samples from farms F1 – F5 were delivered to the Examinala testing laboratory (Zilina, Slovak Republic), which is the central laboratory for testing raw milk in Slovakia.
Determining the basic components of milk. In samples from all farms fat, protein, but not fat solids (SNF) content, were tested by FTIR spectroscopy using MilkoScan FT 6000 (Foss Analytical A/S, Hillerød, Denmark), according to the equipment manual.

Somatic Cells Analysis. SCC in samples from all farms was analyzed with the use of a Fossomatic 7 flow cytometer (FOSS, Denmark) according to ISO 13366-2 [22]. All examinations are accredited by SL Examinala by the Slovak National Accreditation Service according to ISO/IEC 17025.

Differential cells were also counted using a Vetscan DC-Q milk analyzer (AAD Advanced Animal Diagnostics, NC, USA) that provides the concentration (cells/mL) of total milk leukocyte count (TLC), defined as SCC without epithelial cells. The results of SCC were transformed to logarithmic form. Differential cells were used to calculate the differential somatic cell count (DSCC), defined as the sum of neutrophils (NEU) and lymphocytes (LYM) as a percentage of total SCC. According to many authors [23, 24], this index (DSCC) increases during intramammary infections. The Vetscan DC-Q milk analyzer uses fluorescence imaging, as described by Godden [25], and interprets the results through secret? algorithms that identify intramammary infections.

Microbial analyses. Preparation of milk samples, initial suspension, and decimal dilutions were performed according to ISO 6887-5 (2011) [26].

All procedures were performed according to Lobacz et al. [27]. The milk samples were decimally diluted in peptone physiological salt solution, and an inoculum of 100 μL was used. Colonies on plate count agar (Oxoid Ltd., Basingstoke, UK) were counted after 72 h incubation at 30°C.

Subsequently, the Pure Milk Test (PM test) was used – for farm diagnosis of mastitis (LabMediaServis, CZ). The PM test, which is used for farm diagnosis of mastitis, is a quick and very effective solution for correct diagnosis and, thanks to this, targeted treatment of the causative agents of the mammary glands in dairy cows. The diagnostic set included a three-sector petri dish with special chromogenic agars. The milk sample was applied using an inoculation stick to the surface of all three agar Petri dishes, and incubated for 22–26 hours at 37.5°C. Subsequently, the type of causative agent of mastitis was determined using the Atlas of Agents [28]. The results were expressed in several colony forming units (CFU/mL).

Statistical analysis. In the statistical evaluation, the differences were compared in milk quality between the location, influence of the season, and the method of milking. The results were evaluated using MS Excel (Microsoft, Redmond, Washington, USA). Basic statistical characteristics, such as mean, and standard deviation, were calculated for numerical data.

Statistical analysis was performed by one-way analysis of variance. ANOVA and Tukey’s test for multiple comparisons of means with a confidence interval set at 95% were performed using GraphPad Prism statistical software 8.3.0.538 (GraphPad Software, San Diego, CA, USA).

Table 1. Means and their standard deviation (SD) of the basic indicators of cow’s milk, depending on the month of milking

<table>
<thead>
<tr>
<th>Season</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Avg. per year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
<td>Jun</td>
<td>Jul</td>
</tr>
<tr>
<td>FAT (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>2.90</td>
<td>2.90</td>
<td>3.10</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>F2</td>
<td>3.20</td>
<td>3.34</td>
<td>3.43</td>
<td>3.41</td>
<td>3.32</td>
</tr>
<tr>
<td>F3</td>
<td>3.30</td>
<td>3.22</td>
<td>3.30</td>
<td>3.39</td>
<td>3.25</td>
</tr>
<tr>
<td>F4</td>
<td>4.03</td>
<td>3.90</td>
<td>3.80</td>
<td>3.67</td>
<td>3.95</td>
</tr>
<tr>
<td>F5</td>
<td>4.03</td>
<td>3.98</td>
<td>3.80</td>
<td>3.85</td>
<td>3.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRO (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>3.60</td>
<td>3.48</td>
<td>3.62</td>
<td>3.59</td>
<td>3.44</td>
</tr>
<tr>
<td>F2</td>
<td>3.28</td>
<td>3.36</td>
<td>3.33</td>
<td>3.31</td>
<td>3.56</td>
</tr>
<tr>
<td>F5</td>
<td>3.35</td>
<td>3.37</td>
<td>3.30</td>
<td>3.18</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNF (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PRO – protein; SNF – solids not fat
a, b, c – within a row, means without a common superscript differ (P<0.05)
The month of milking significantly affected all quality traits, as above. The content of fat, and not fat solids, increased with the time of lactation (P<0.05).

The annual average showed statistically significant (P<0.05) positive correlation between the content of fat, protein, and solids not fat content (Figure 2).

The highest and almost identical fat content was confirmed on farm F5 (3.85 ± 1.70%) and F4 (3.82 ± 0.21%) which used the Lely Astronaut robot method of milking. However, from the point of view of protein content, these farms showed slightly lower values (<0.05).

F1 did not meet the minimum required amount for fat content (2.84 ± 0.81%) set by the legislation of the Slovak Republic.

Within the seasons, the values for fat were highest in spring (3.48 ± 0.44%), proteins (3.38 ± 0.14%) and SNF (9.34 ± 0.36) only in the summer months.

In this study, statistical significance was demonstrated between SCC and their size (P<0.005), as well as between SCC and fat content in all farms (P<0.005). The relationship between cell size and seasons (Fig. 3) was not statistically proven (P>0.05). Changes in the number of somatic cells within the seasons are presented in Figure 3.

The comparison shows that there is little difference in cell size between cells from healthy and infected mammary glands. The average size of healthy cows ranged from approximately 8.77 ± 0.49 μm.

The smallest size of SCC (6 μm) was shown by the sample from F5 in the spring, the highest size of SCC (12.13 μm) was recorded on F2, also in spring (Tab. 2). This means that the size of somatic cells is not decisive for health.

The correlation between SCC and cell size on monitored farms is shown in Figure 4.

Using an analyser, it can be assessed that in the negative sample there were mainly epithelial cells of the milk ducts, and a small amount of leukocytes. Table 3 shows the results of analyses using the Vetscan DC-Q milk analyzer.

The F1 farm was categorized as a positive farm with a high TLC concentration (5.58 log10 cells/mL; 406.65 ± 53.80 × 10⁵ cells/mL) and a predominant NEU fraction (61%). The results from F3 reached borderline values (386.17 ± 21.36 × 10⁵ cells/mL) with a TLC of 4.9 log10 cells/mL and a higher NEU percentage of 63.10%. Farms F2, F4, and F5 were classified as negative farms. TLC, on average, was 4.70 ± 0.26 log10 cells/mL, while the percentage of NEU (52.0 ± 14.15%) was lower than the percentage of positive cows. In addition, the number of MAC in the milk of F2, F4, and F5 cows was higher (19%) than that of F1 milk (8.70%).

The aggregated microbiological quality of raw cow’s milk samples from five farms was presented in Figure 5. The results are satisfactory, although on F1 (90.16 ± 8.14 × 10⁻⁵ CFU/mL; 4.94 ± 0.03 log CFU/mL) bordering on the upper limit for maximum limits (100,000 CFU/mL), which can be justified mainly by the current state of hygiene in the process of obtaining milk. The results from the F1 and F2 farms show the occurrence of mastitis in dairy cows.

F4 was the opposite, where the results of the arithmetic mean of the tested samples were 26.33 ± 18.19 × 10⁻⁵ CFU/mL (4.33 ± 0.25 log CFU/mL) because very strict hygienic criteria for milking and housing dairy cows are observed on the farm.

The results show the lowest number of total bacteria count (TBC) on farms with AMS (4.45 ± 0.19 log CFU/mL) (Fig. 6). In May – July, values were stable on farms, the highest increase was during the transition from winter to spring. Whereas in general the highest incidence of diseases were recorded in August and September, in the current study there was an increase in autumn.

The study demonstrates a statistical significance between SCC and TBC within the monitored period on the monitored farms (P<0.005).
Table 2. Relationship between SCC and cell size on five farms with different milking methods during the year

<table>
<thead>
<tr>
<th>Farm</th>
<th>Value</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAX</td>
<td>6.53</td>
<td>10.98</td>
<td>6.70</td>
<td>12.13</td>
<td>6.73</td>
<td>10.72</td>
</tr>
</tbody>
</table>

Means SD = 5.60 ± 0.86, 5.40 ± 0.74, 5.58 ± 0.80, 5.27 ± 0.65, 5.26 ± 0.61

pValue = <0.05, <0.05, <0.05, <0.05, <0.05

SCC – somatic cell count (× 10³ cells/mL); Size – average cell size (μm); Size – average cell size (μm)
AVG – average (× 10³ cells/mL)

Mean ± SD – means and their standard deviations of log10 SCC in cow milk samples on different months of milking

Table 3. Results of the total milk leukocyte count and their differential cell count (Lymphocytes, Macrophages, Neutrophils) in milk on various farms in Slovakia

<table>
<thead>
<tr>
<th>Value</th>
<th>TLC [log10 cells/mL]</th>
<th>Lymphocytes [%]</th>
<th>Macrophages [%]</th>
<th>Neutrophils [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVG</td>
<td>4.70 ± 0.26</td>
<td>26 ± 14.90</td>
<td>35.70 ± 15.59</td>
<td>52.00 ± 14.15</td>
</tr>
<tr>
<td>Min</td>
<td>4.60</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Max</td>
<td>5.58</td>
<td>68.20</td>
<td>63.90</td>
<td>90.50</td>
</tr>
</tbody>
</table>

TLC – total milk leukocyte count; AVG – average (log10 cells/mL)

DISCUSSION

The importance of the influence of the season on the quality of milk has been discussed and confirmed by various authors [12] because geographical and climatic conditions cannot be influenced. The time of year is often associated with different eating regimes, which allow for sufficient intake, good digestion for metabolism; on the other hand, they can affect the composition of milk. Some authors report that changes in milk composition are more related to feeding rather than genetic factors; hence, for better correlations among variables (composition) the food intake is more important than the content of nutritive matters in a diet. Nevertheless, the season of the year considerably affects the food intake [29].

SCC is influenced by many factors, such as cattle breed, level of milk production, stage and order of lactation, season, month of measurement, individual and environmental factors, teat and udder morphology, as well as management practices [30, 31, 32, 33].

The results obtained in the current study confirm [34] that from the turn of April – May to the turn of September – October, a slight increase in the number of somatic cells are be observed. When dairy cows are housed on deep bedding, the increase is also noticeable during the winter months. This fact has also been confirmed by other authors [35], who in their study for 2015, demonstrate an increase in the number of somatic cells between May – August to a value approaching 280,000 cells/mL, while for the rest of the year the values remained at 230,000 cells/mL. In the monitored period from May – August in the current study, the average values determined were at the level of 292,000/mL (5.46 ± 0.72 log10 SCC) in cow milk samples, while for the rest of the year the values remained at 256,000/mL (5.40 ± 0.80 log10 SCC). Other authors [36] state that somatic cells reach their highest values in August and September. However, the main factor affecting PSB in milk is infection of the mammary gland [37].

An important factor in describing the efficiency of an AMS is the cow-individual SCC, is that it is not influenced mainly by the milking technique [38], but that the changeover of the milking system could have an effect on the SCC of a cow. In one study [39], the authors found that the highest SCC occurred in the first six months after the introduction of an AMS, after which the SCC normalized and stabilized. Another study [40] compared two AMS from different manufacturers regarding milk yield, milking frequency, milking interval, teat-cup attachment success rate, and length of milking procedure. They found differences in teat-cup attachment success, duration of several milking phases, and milking frequency regarding different AMS.

Therefore, one of the objectives of the current study was to discover whether a different milking method or the latest generation AMS has advantages in terms of efficiency and animal health, compared to the previous model of the same manufacturer.

One research [41] revealed that high SCS levels do not necessarily negatively affect milk production as long as DSCC is also high [42]. This finding suggests that cows with increased SCS and DSCC may be in the early stages of infection when the infection is well controlled by abundant PMNs. Conversely, when SCC is high while DSCC is also high [42]. This finding suggests that cows with increased SCS and DSCC may be in the early stages of infection when the infection is well controlled by abundant PMNs. Conversely, when SCS is high while DSCC is low, indicates chronic infection and cow productivity is significantly impaired.

The total bacteria count (TBC) is considered an indicator of the microbiological purity of milk and thus of the environment [43]. Concentrations of colony-forming units can be expressed using logarithmic notation, where the value shown is the base 10 logarithm of the concentration.

There are many management factors that play the most important role in the development of infectious diseases like mastitis in dairy animals, among them unsanitary conditions are more important in increasing the chances of intramammary infection (IMI) and leading to high SCC [44].

The study also evaluated the prevalence of microbial organisms and the association with the number of milk...
CONCLUSIONS

Based on the results obtained, it can be concluded that the hygienic quality of the raw cow’s milk produced on the selected Slovak farms is at a relatively very good level, with the majority of samples (85%) meeting all legislative limits.

The results confirmed a higher occurrence of microorganisms in milk in the summer season on two of the farms, which were attributed to climatic conditions, unsuitable litter, insufficient animal hygiene, as well as inappropriate feed rations from a qualitative and quantitative point of view. The insufficient nutrition resulted in an increase in the number of somatic cells in milk.

The results of this study confirmed that the production and quality of milk are significantly influenced by the already mentioned external and internal factors, and also confirmed that the size of somatic cells in healthy udder does not differ from the somatic cells found in an infected udder. Furthermore, it was shown that the size of the somatic cells was not decisive in the occurrence of mastitis, as the differences in cell sizes were small.

From the results, it can be concluded that the transition to the latest generation robotic method of milking can positively affect milk production and its quality. The results of this study indicate a higher level of milk quality influenced by milking time, milk flow and milking interval. Even if the milking time is reduced, the milk yield, milk flow and milking interval will increase in the robot. Significant differences were found in milk quality between the two systems.

For the management of the health status of dairy cows and the further processing of milk, further investigation certainly makes sense, but it requires a deeper investigation in relation to the technological quality of raw milk, as well as from the point of view of the possible presence of microorganisms involved in the development of inflammation of the udders of cows.

Acknowledgements


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