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# SEASONAL VARIATIONS IN SOMATIC CELL COUNT AND THEIR IMPACT ON THE PHYSICOCHEMICAL COMPOSITION OF RAW COW MILK

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**Abstract**

Understanding milk quality is essential for ensuring dairy products meet required standards. Somatic cell count (SCC) serves as a commonly used measure of milk quality within the dairy sector. While it offers valuable insights into the udder health of dairy cows, its reliability is influenced by various non-infectious factors, notably seasonal variations. This study aimed to investigate the impact of seasonal variations on SCC in raw cow milk, and subsequently, how these variations may lead to changes in its physicochemical composition. Over a year, 4,287 samples were collected from three dairies in Bitola, Macedonia. Standard accredited methodologies were employed, adhering to ISO 21187:2011 for total bacteria count (TBC), ISO 13366-2:2010 for SCC determination, ISO 9622:201 for analyzing fat, protein, and lactose content, and ISO 5764:2010 for determining freezing point. Statistical analysis using SPSS 15.0 was conducted on the acquired data. Significant differences were observed in the lactose content ($p<0.05$), but not in the milk fat content ($p>0.05$), while for the other parameters (proteins, freezing point, and solids-non-fat) seasonal variations were observed with the increase in SCC in summer and autumn. TBC in summer is the highest (1,992 x 103 CFU/ml) while in winter is the lowest (1,168 x 103 CFU/ml), which is not the case for SCC where the highest values are also in summer (537 x 103 SCC/ml), but the lowest values were determined in spring (449 x 103 SCC/ml). These findings underscore the importance of considering seasonal variations when evaluating milk quality and suggest potential implications for dairy industry practices and regulations.

*Keywords:* somatic cell count, physicochemical composition, milk quality, seasonal variation

## 1. Introduction

Milk is a biological fluid secreted by the mammary glands of female mammals, and has a very complex composition in which there are important elements that are needed for the human body. It also contains all the nutritious and protective ingredients such as: proteins, fats, carbohydrates, minerals and vitamins and is characterized by easy digestibility. Since milk is synthesized in the mammary gland of the animal, the care of its quality begins precisely with the health and well-being of the animal. Improper nutrition and storage, hereditary and acquired diseases or various infections can significantly affect the quality of milk and milk products. Milk is created from specific ingredients that pass from the blood to the mammary gland, where many complex biochemical processes of biosynthesis take place. Thus, in very complex processes of biosynthesis, milk fat, milk sugar (lactose) and typical milk proteins (casein, α-lactalbumin and β-lactoglobulin) are produced. The other ingredients, such as mineral ingredients, enzymes, vitamins, albumins and immunoglobulins, pass directly from the blood to the mammary gland and become part of the composition of the milk (Tratnik, 1996).
The composition of milk can vary from several factors, which can be categorized as genetic, physiological, environmental, and pathological (Forsbäck, 2010), such as: the health of the animals, the order (number) and stage of lactation, the diet, the season, the method of milking (hand/automatic), the time and the number of milkings and finally the individual itself (age,

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body weight, movement of the animal, etc.). Most of the constituents of milk undergo changes as a result of the onset of mastitis (Walstra et al., 1999). Mastitis is one of the most widespread and economically significant diseases in dairy cattle. In doing so, there is reduced milk production and deteriorating milk quality, deterioration of animal welfare, and increased costs for antibiotics and labor (Halasaet al., 2007; Seegers et al., 2003, Bradley, 2002). It is an inflammation of the mammary gland caused by microorganisms, mostly pathogenic bacteria, but it can also be mycoplasmas and fungi that penetrate the udder through the teat canal or injuries and wounds on the udder skin (Forsbäck, 2010). In addition to microorganisms, immediate causes of mastitis are mechanical injuries as well as thermal injuries (hot water, steam, very low ambient temperature), and chemical injuries (acids, bases that damage the skin) (Havranek & Rupić, 2003). The reaction of the cow to the bacterial infection results in an inflammatory response characterized by redness, swelling, heat, pain, and loss of function (Forsbäck, 2010). The main function of inflammation is to destroy the infectious agent and heal the udder. The influx of somatic cells into milk is a primary function of inflammation (Sandholm et al., 1995; Larson & Anderson, 1985).
The milk of healthy animals contains from 50 to 200,000 SCC per ml, which is 60% of the total number of cells in the milk. In the milk of the mastitic udder, the number of these cells is 90-95%. Somatic cells are a combination of lymphocytes, neutrophils (polymorphonuclear leukocytes), and epithelial cells in a correlation of 1:1.5:14 (Antunac et al., 1997; Harmon & Reneau, 1993). Cows with SCC of less than 200,000 cells/ml are considered to be producing healthy milk, but cows with more than 300,000 cells/ml may have subclinical mastitis (Brolund, 1985; Smith, 1996). During infection, the SCC increases and changes – this correlation increases. When considering the result, the age and stage of lactation, the way of feeding the cows, the way of keeping them, the geographical area and the time of the year should be considered as important factors that affect the SCC after calving or before drying and then it is not a matter of inflammation of the udder. The measurement of the SCC is taken as a control of the health status of the udder and is also a measure of the severity of mastitis.
The milk in the Republic of Macedonia is classified and valuated into three groups, according to the total number of bacteria and total SCC in three categories (Table 1):

**Table 1:** Classification of milk according to the TBC and SCC

| Classes | TBC (CFU/mL) | SCC (SCC/mL) |
|---|---|---|
| Extra class | ≤ 100.000 | ≤ 400.000 |
| I class | 100.001-700.000 | 400.001-500.000 |
| II class | 700.001-1.500.000 | 500.001-600.000 |

When the somatic cell count (SCC) exceeds 400,000/mL, it triggers changes in milk, causing decreased secretion and modifications in its chemical composition, along with changes in its physical, bacteriological, and technological characteristics (Antunac et al., 1997).

## 2. Methodology

The study was carried out in three dairies located in the Pelagonia region of Bitola. Raw milk, obtained separately from each milk producer and designated for processing in these dairies, was utilized as the main testing material. The samples analyzed were collected as a component of the official dairy control procedure aimed at monitoring the raw milk quality. The milk sampling from the milk producers was conducted by the dairies in compliance with Regulation (Official Gazette no. 151, 2011) and transported to the laboratory using portable refrigerators maintained at temperatures of up to 7°C. The milk samples designated for microbiological testing were preserved using Broad Spectrum Microtabs. Conversely, for the physicochemical analyses, the raw milk was transported to the laboratory fresh and chilled, without the addition of any preservatives. All analyses were performed in an accredited laboratory, following the standard ISO/IEC 17025: Testing and calibration laboratories. Accredited methods were utilized for each parameter by established protocols. The SCC in milk was determined using Somacount FCM (Bentley Instruments, Hungary), according to the standard MKC EN ISO 13366-2:2010: Milk - Counting of somatic cells - Part 2: Instructions for use with the fluoro-opto-electronic counter ISO 13366-2:2006. The milk samples, which had been preserved beforehand, were warmed to a temperature of 40°C using a water bath before analysis. The results were reported as one thousand somatic cells per milliliter. The total number of microorganisms was analysed by epifluorescence flow cytometry according to the standard MKS EN ISO 21187:2010 Milk-Quantitative determination of bacteriological quality – guidelines for establishing and improving the conversion between the results of the routine and the basic method. The measurement was carried out on Bactocount IBC equipment (Bentley Instruments, Hungary). The chemical analysis of raw milk was performed following the standard MKC ISO 9622 IDF 141C:2013 Milk - determination of fat, lactose, protein, and solids-non-fat (SNF) content. The analysis was carried out using the LACTOSCOPE C4+ instrument manufactured by Advanced Instruments. This automated device utilizes infrared spectrometry for accurate analysis of milk components and has a measurement range within the limits of: Fat: 0-55%, Proteins: 0-15%, Lactose: 0-20%, SNF: 0-60%. The assessment of the freezing point, aimed at identifying added water in the milk, was conducted following the standard MKC EN ISO 5764:2010 Determination of the freezing point - Cryoscopic method with the thermistor Cryoscope 4250 Advanced instruments. The results were analyzed employing standard statistical techniques, incorporating descriptive statistical parameters such as mean value and standard deviation. To ascertain the statistical significance among the various categories of examined milk at a significance level of 5% ($p<0.05$), Student's t-test was employed. The data are shown in tabular form. The results were processed using Microsoft Office Excel and SPSS 20 statistical software.

## 3. Results and discussion

According to the obtained results, shown in Table 2, it can be noted that the physicochemical parameters in milk show seasonal variations between the studied seasons. The percentage of milk fat is the highest in the winter period (3.93%), and the lowest is in the summer season (3.75%). In addition, it can be noted that fat is a very variable ingredient in milk, precisely because of this, statistically significant differences were observed between the studied seasons ($p<0.05$), except the spring and autumn seasons ($p>0.05$). The situation with proteins is similar to that of fats. In our tests, we determined that the highest percentage of protein is in the winter season (3.39%), and the lowest in the summer season (3.21%). Proteins show significant changes during the examined period, except the autumn and winter seasons where the statistical significance is $p>0.05$. This is complemented by the research of Bernabucci, (2015), who noted that of the total proteins, the biggest changes were in casein (especially in γ-casein), which was the lowest during the summer and the highest during the winter period. In addition, the content of IgG and serum albumins are higher during the summer period, compared to the winter and spring periods.

Seasonal variations of lactose range from 4.25% to 4.42% in autumn and spring seasons, respectively. The solids non-fat during the winter period have the highest percentage of 8.68%,

while in the summer period, the percentage is the lowest 8.36%. The freezing point ranges from -525.82 to -535.17 m° C in the winter and summer seasons, respectively. Significant changes in freezing point values were determined between the seasons spring and summer, summer and winter, and autumn and winter ($p<0.05$).
The TBC in summer is the highest (1,992 x 103 CFU/ml) while in winter it is the lowest (1,168 x 103 CFU/ml), which is not the case for somatic cells where the highest values are also in summer (537 x 103 CFU/ml), but the lowest values were determined in spring (449 x 103CFU/ml).
The differences in the values of the total number of bacteria between the groups of examined samples are significant, except for the samples from the summer and autumn groups ($p>0.05$), while the differences in the values of the SCC between the examined groups are significant between the samples from the spring and autumn groups. summer, spring and autumn, spring and winter, and autumn and winter ($p<0.05$).

**Table 2:** Values for the physicochemical and microbiological parameters by season

| Examined parameters x̄ ± SD | | | | | | | |
|---|---|---|---|---|---|---|---|
| Season | Fat (%) | Protein (%) | Lactose (%) | Solids non-fat (%) | Freezing point (m°C) | TBC (CFU/ml x $10^3$) | SCC (CFU/ml x $10^3$) |
| Spring **(N=1076)** | 3,86 ± 0,68$^a$ | 3,29 ± 0,31$^a$ | 4,42 ± 0,22$^a$ | 8,62 ± 0,38$^a$ | -529,86 ± 64,24$^a$ | 1.479± 1.827,67$^a$ | 449 ± 567,98$^a$ |
| Summer **(N=1102**) | 3,75 ± 0,63$^b$ | 3,21 ± 0,37$^b$ | 4,31 ± 0,25$^b$ | 8,36 ± 0,49$^b$ | -535,17 ± 52,81$^{bc}$ | 1.992± 2.095,39$^b$ | 537 ± 661,87$^{bc}$ |
| Autumn **(N=1083)** | 3,90 ± 0,71$^a$ | 3,38 ± 0,30$^c$ | 4,25 ± 0,25$^c$ | 8,39 ± 0,43$^b$ | -531,99 ±25,89$^{ca}$ | 1.945± 2.038,69$^b$ | 500 ± 599,86$^c$ |
| Winter **(N=1026)** | 3,93 ± 0,51$^c$ | 3,39 ± 0,28$^c$ | 4,31 ± 0,23$^b$ | 8,68 ± 0,31$^c$ | -525,82 ± 38,11$^{da}$ | 1.168± 1.636,12$^c$ | 465 ± 577,62$^b$ |

**Differences of values with different superscripts in the same column are statistically significant at the level: a:b, a:c, a:d, b:c, b:d, c:d; p<0.05*

In our studies, we found that the SCC varies throughout the year depending on the season. Namely, according to the results we obtained (table 2), the SCC in the collected milk is the highest during the summer and autumn periods, 537 x $10^3$ CCC/ml and 500 x $10^3$ CCC//ml, respectively, while during the spring and in the winter we have a decrease (449 x $10^3$ CCC/ml and 465 x $10^3$ CCC/ml, respectively). Such differences are statistically significant at the $p<0.05$ level. It is primarily due to the increased incidence of mammary gland disease in the investigated farms during summer and autumn compared to the other two seasons. Similar results were obtained by Moosavi et al. (2014). The same conclusions were reached by (Al Hussien & Dang, 2018), which indicate that microclimatic conditions significantly affect the number of SCC in milk. Any change in microclimatic conditions, stress conditions, the way of keeping, the way of feeding the cows, the geographical area and the time of year are significant factors that affect the SCC. This is because extreme temperatures not only impose stress on animals but also affect food intake. High humidity in some seasons such as hot-humid and the lack of micronutrients due to the poor quality of fodder can also cause the creation of suitable conditions for the reproduction of pathogenic bacteria that lead to a decrease in immunity in dairy animals. In their studies, they determined that changes occur in the casein content, which decreases during the summer and winter periods, while serum albumin increases in the summer, compared to the winter and spring periods (Al Hussien & Dang, 2018).

In addition, a large number of factors can affect the increased SCC in milk, among them milk yield, animal health, management of dairy animals as well as environmental factors. A correlation exists between the SCC and milk yield. According to Mukherjee & Dang (2011), high-milk cows are more exposed to stress and as a result, there is a decrease in immunity, which leads to an increase in the SCC in the milk. Cinar et al., (2015) state that high SCC does not negatively affect milk yield, but affects the composition and quality of raw milk. The stage of lactation significantly affects the SCC in milk. It is observed that their number is the highest immediately after parturition, then it decreases and has the lowest value from 25 to 45 days of lactation, then the value gradually increases during the rest of the lactation period (Kennedy et al., 1982). In addition, primiparous cows secrete less milk and have a lower SCC compared to multiparous cows (Goncalves et al., 2018).

## 4. Conclusions

The SCC in milk is an indicator of its hygienic correctness, and on the other hand, the increased number of somatic cells has an impact on the physicochemical parameters in raw milk. The season as a factor influences the changes that occur in raw milk, but other factors that can influence these changes should not be excluded, such as: the stage of lactation, milk yield, the number of lactations, mastitis, and similar. At the same time, the SCC in the collected milk is the highest during the summer and autumn periods, 537 x 103CCC/ml and 500 x 103 CCC/ml, respectively. Although fat is one of the most variable components in milk and is within the range of 3.93% in the winter period, and the lowest values are in the summer period of 3.75%, no significant differences were observed between the examined milk samples and milk fat content. In our tests, we determined that the highest percentage of proteins is in the winter season (3.39%), and the lowest percentage of proteins is in the summer season (3.21%). The changes in the protein content show significant changes during the examined period, except the autumn and winter seasons where the statistical significance is $p>0.05$. In the defined categories according to the SCC, there were changes in the proteins in the summer and autumn seasons ( $p<0.05$). Seasonal variations of lactose range from 4.25% to 4.42% in autumn and spring seasons, respectively. During the spring, summer, and autumn seasons, a statistically significant difference was observed at the $p<0.05$ level, i.e. lactose levels decreased with increasing SCC. The only exception is the winter season, where no significant differences were observed ($p>0.05$). During the studied period, the freezing point ranged from -525.82 to -535.17 m°C, showing significant variations in the studied seasons. The solids non-fat during the winter period have the highest percentage of 8.68%, while in the summer period, the percentage is the lowest 8.36%. From the obtained results, it can be concluded that the SCC affects the changes in the physicochemical composition of milk, where significant differences were observed in the lactose content ($p<0.05$), but not in the milk fat content ($p> 0.05$), while for the other parameters (proteins, freezing point and solids non-fat) seasonal variations were observed with the increase in the SCC in summer and autumn. The TBC significantly increases with the increase of SCC ($p<0.05$), which points to the fact that attention should be paid to the hygienic conditions in which dairy animals are kept.

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