

# The Influence of the Number of Somatic Cells and the Number of Bacterial Colonies on the Hygienic Quality of Raw Cow's Milk in Some Individual Producers

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## Abstract

This work aimed to initially determine the hygienic condition based on the increase in the number of somatic cells (SCC/ml) and the number of bacterial colonies (CFU/ml) and in raw cow's milk, as the important elements in determining the hygienic quality of milk, and the description of some factors that lead to the increase in the number of somatic cells and the growth of bacterial colonies in raw cow's milk. In this research, a total of 45 raw milk samples were examined, which were taken in fresh stalls after milking from individual milk producers in several locations in Tetovo in the period January-March 2019. After the analyzes carried out in the laboratory, it was found that the results obtained in relation to the total number of microorganisms ( $\leq 100,000$  CFU/ml) show that no single sample meets the criteria in accordance with the national regulation and the criteria of the European Union. As for the total number of somatic cells ( $\leq 400,000$  SCC/ml), it was found that 76.74% of the examined samples meet the criteria in accordance with the National Regulation and the criteria of the European Union. The results show insufficient hygiene during the rearing of dairy cows, incorrect handling of milk after milking, and all this is the result of insufficient knowledge of farmers on the basic hygienic procedures of milk production.

**Keywords:** milk quality, number of somatic cells, number of bacteria, hygienic quality

## 1. Introduction

The number of somatic cells and the number of bacterial colonies have long been used as parameters according to which the hygienic correctness of raw cow's milk is evaluated.

Quantity, composition and hygienic safety play an important role in milk production. Milk is a very valuable food in the human diet, therefore the quality of raw milk is a basic prerequisite for successful processing. In addition to the chemical composition, the quality of milk is determined by indicators of hygienic correctness, and these are the total number of microorganisms and the number of somatic cells [8]. In the milk of cows, under physiological conditions,

different types of cells are constantly found: neutrophil granulocytes (polymorphonuclear granulocytes), lymphocytes, eosinophils, macrophages and epithelial cells [13]. This cellular content is known as "mammary somatic cells - SCC". The number of somatic cells (Somatic Cell Counts-SCC) is an indicator of the hygienic quality of milk and is a general indicator of udder health [14]. In the milk of healthy udder quarters, SCC is less than 200.000 cells/ml, and epithelial cells and leukocytes (polymorphonuclear neutrophils, lymphocytes, macrophages and other cells) are

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primarily represented. Milk from a quarter of a diseased under may contain up to 5.000.000 somatic cells/ml.

Factors that affect the number of somatic cells in milk are: genetic and environmental factors. We can influence the set of genetic factors through selection. The heritability for SCC is very low, so reducing the number of somatic cells by selection is slow and difficult to achieve. It is considered that there is a possibility of improving immunity to mastitis by indirect selection of the morphological characteristics of the of the udder and teats. Environmental factors have the most important influence on the change in the number of somatic cells, many of which can be influenced by the farmer himself. The most important are: udder infection status, age of the cow, stage of lactation, order of lactation, breed, the breeding regime, geographical area and season, herd size, stress factors, excessive physical activity, milking and education of the milker. Milk from cows with mastitis is often mixed with milk from healthy dairy cows and sent to dairies, especially if the somatic cell count in the pooled milk as well as the milk of individual producers is not regularly monitored [8].

Somatic cell count can be an indicator of subclinical mastitis and as such can be used in the breeding program for healthy cows. The number of somatic cells in the milk of healthy cows varies from  $160\text{--}450 \times 10^3/\text{ml}$ , and the limit value of the number of cells in 1 ml of milk of healthy cows is  $500 \times 10^3/\text{ml}$  (Schalm, 1971). At the beginning of lactation, the number of somatic cells can go up to  $2.500.000/\text{ml}$  [6]. Of the leukocytes in the milk of healthy udder quarters, polymorphonuclear granulocytes ( $23. \pm 9.8\%$ ), followed by macrophages ( $10.1 \pm 7.4\%$ ) and lymphocytes ( $23.9 \pm 17.4\%$ ) of which the percentage of helper T lymphocytes is  $5 \pm 4.2\%$ , while that of cytotoxic T lymphocytes is  $11.7 \pm 6.8$  (Chaffer 2000). Pillai et al., (2001) in their research declare that in the milk of healthy cows, the dominant cell type is macrophages, while neutrophils are the dominant cell type during infection. In milk samples taken immediately before milking, the percentage of polymorphonuclear granulocytes is similar to that in the total milk sample (40-50%), while in the sample taken after milking the

percentage decreases to only 8% [12]. The number of cells in milk varies, and much depends on the stage of lactation. As lactation progresses, the total number of neutrophils increases and close to dry can reach up to 40% (Concha, 1986). With the cessation of milking, the tissue of the mammary gland undergoes strong physiological changes. At the onset of drying, especially in the first seven days, the number of cells increases to reach about  $2\text{--}5 \times 10^6/\text{ml}$ , then decreases and remains at  $1\text{--}3 \times 10^6/\text{ml}$  [10]. During the process of udder involution the number of somatic cells in milk increases to 1.000.000 cells/ml, probably as a result of cessation of milking, so that before birth the number of cells decreases to normal values [11]. The initial increase in the number of somatic cells during the dry period is probably a consequence of the cessation of milking, as well as the resorption of milk components. The total number of cells remains at the highest level during the longest drying period. During drying, the most abundant mammary cell types are macrophages, while colostrum shows an increase in polymorphonuclear leukocytes (PMNL), as in all mammary gland infections.

## 2. Material and Methods

The research was carried out on a total of 45 raw milk samples, which were taken in fresh stalls after milking by individual milk producers in several locations in Tetovo in the period January-March 2019. To determine the number of somatic cells (SCC/ml), 45 samples of raw cow's milk were analyzed, while a total of 45 samples were analyzed to determine the total number of bacteria (CFU/ml).

The samples intended for the examination of the number of somatic cells (SCC/ml) and the determination of the total number of bacteria (CFU/ml) in milk were taken as follows: milk samples were taken under aseptic conditions before regular milking. The udder is first washed with soap and lukewarm water, especially the teats and wiped with a clean cloth. Disinfection the tips of the teats of the was performed with cotton wool soaked in 70% ethanol. First, the farthest quarters were disinfected, then the closest

quarters. Three streams of milk were expressed from each udder quarter prior to sampling. After disinfection, the milk is made in special sterile plastic cups with a quantity of 60 ml. After sampling, fresh raw milk samples were placed in a hand cooler (temperature 4°C) and transported directly to an authorized laboratory for bacteriological examination. Raw milk samples were stored at 4°C until laboratory examination began.

The samples intended for the determination of somatic cells and the number of bacterial colonies were analyzed in the laboratory, which is accredited in accordance with the ISO 17025/2005 standard by the Accreditation Institute of the Republic of Macedonia (IARM) with certificate number LT - 033. The samples were analyzed according to internationally recognized ISO standards, namely:

1. The number of somatic cells was performed according to the standard MKS ISO 13366-2:2010. Somatic cells in raw milk were determined using an electronic cell counter. A laboratory uses an instrumental method for counting somatic cells in milk with the Somacount SS 150 device.
2. The total number of microorganisms in raw milk will be determined according to the MKS EN ISO 21187:2011 standard. This is quantitative determination of the bacteriological quality of raw milk with an

instrumental method for counting the total number of bacteria in milk with the Bactocount IBC apparatus.

### 3. Results and Discussion

The research results are compiled in tabular form and processed according to statistical methods applied in the field of scientific research.

From the statistical processing of the obtained results presented in **Table 1.** for determining the number of somatic cells in the examined samples, the obtained results show that the minimum number of somatic cells was  $1 \times 10^3 \text{ SCC/ml}$ , while the maximum number of somatic cells in the samples was  $2.009 \times 10^3 \text{ SCC/ml}$ . The arithmetic mean value was  $\bar{x} = 320.37 \times 10^3 \text{ SCC/ml}$  somatic cells, where the standard deviation is  $\text{SD} = 446.20 \times 10^3 \text{ SCC/ml}$ , while the coefficient of variation was  $\text{CV} = 139.28\%$ .

During the examination for the determination of the total number of microorganisms, it can be observed that the minimum and maximum values fluctuate from  $249 \times 10^3 \text{ CFU/ml}$  to  $7.541 \times 10^3 \text{ CFU/ml}$ , respectively, with an average value of  $4.489.07 \times 10^3 \text{ CFU/ml}$ . The standard deviation from the mean value for the total number of microorganisms in milk is  $\text{DS} = 2,366.35 \times 10^3 \text{ CFU/ml}$  in milk, with a coefficient of variation of  $53.71\%$ .

**Table 1.** Tabular presentation of the total number of samples analyzed by farmers with unsatisfactory results from the first control

N=45	min	max	$\bar{x}$	SD	CV
SCC/ml x $10^3$	1	2.009	320,37	446,20	139,28%
CFU/ml x $10^3$	249	7.541	4.489,07	2.366,35	53,71%

According to the results presented in **Table 2.** from the examination of the number of somatic cells ( $N=45$ ), it can be observed that 33 samples (76.74%) meet the criteria according to the *Regulation for special requirements for safety and hygiene and the way and procedure of performing official controls for milk and*

*milk products* (Official Gazette of the Republic of Macedonia 26/12, 145/14, 59/16, 197/16 and 16/2019) and European Union criteria (Council Directive 92/46 EEC). While the remaining 12 samples (26.66%) deviate from the requirements defined in the Regulation and the number of somatic cells is  $\leq 400,000 \text{ SCC/ml}$ .

**Table 2.** Tabular presentation of the obtained results of the total number of somatic cells by category

SCC/ml x 10 <sup>3</sup>	Number of samples	Percentage (%)
< 400	33	73.33 %
from 401 to 1000	6	13.33 %
>1001	6	13.33 %

In **Table 3.** are presented the total number of analyzed samples (N=45) for the total number of microorganisms. At the same time, we can note that not a single sample meets the criteria in accordance with the *Regulation on special requirements for safety and hygiene and the manner and procedure for carrying out*

*official controls on milk and milk products* (Official Gazette of the Republic of Macedonia 26/12, 145/14, 59/16, 197/16 and 16/2019 and the criteria of the European Union (Council Directive 92/46 EEC), regarding the total number of microorganisms ( $\leq 100,000$  CFU/ml).

**Table 3.** Tabular presentation of the obtained results of the total number of microorganisms according to categories

CFU/ml x 10 <sup>3</sup>	Number of samples N=45)	Percentage (%)
$\leq 1.000$	5	11.11 %
1.001-5.000	21	46.66 %
$\geq 5.001$	19	42.22 %

In **Table 1.**, the minimum values of the total number of microorganisms are  $\min = 249 \times 10^3$  CFU/ml, while the maximum number of somatic cells in the samples was  $\max = 7,541 \times 10^3$  CFU/ml.

This number of microorganisms present not only makes the milk unsuitable for processing into dairy products, but can also pose a risk to the safety of milk and milk products.

The number of microorganisms in milk is directly influenced by the initial contamination with microorganisms from the environment (bedding, feces, urine) which in significant numbers can populate the surfaces of the milking equipment and the surfaces with which the milk comes into contact. [1], [15] indicates that the bedding may have been contaminated with a large number of microorganisms as a result of the presence of urine and feces and the same may have been the cause of the infection of dairy cows. The teats of cows lying on straw bedding contain higher levels of streptococci than those of cows lying on sawdust and shavings. Because of this, Causin (1982) [4] in his research determined that reducing the level of contamination of milk from these sources significantly

reduces the level of psychrophilic microorganisms. Thus, from the moment of milk storage in the farm's milk refrigerator, transportation and storage in the tank for bulk milk as well as from the beginning of processing, the number of microorganisms present in the milk is kept at the lowest level.

In Table 2, from a total of 45 samples examined, 33 (76.74%) meet the criteria, while only 12 samples (26.66%) deviate from the quality requirements of raw milk, which are the result of inadequate control of breast health and the appearance of subclinical mastitis in dairy farms.

The number of somatic cells in milk today represents an internationally recognized indicator of breast health, so we determine it in the breast and in milk to check the health of the mammary gland, to prevent the occurrence of mastitis and to improve the quality of milk. Their number in the milk of healthy cows varies from 50.000 cells/ml to 200.000 cells/ml [9]. The somatic cell count from a normal, uninfected udder is usually below 200.000 cells/ml, but may be below 100,000/ml during the cow's first lactation. A greater number of somatic cells than the above is abnormal and is indicative of

breast inflammation [7]. The increase in the number of somatic cells in milk is mainly influenced by genetic and environmental factors. The most important environmental factors are the infection status of the udder, the age of the lactating animal, the stage of lactation, the sequence of lactation, breed, farming method, geographical area and season, herd size, stress factors, excessive physical activity and milking method [3]. The increase in the number of somatic cells is a consequence of breast inflammation and is reflected by changes in milk secretion and the chemical composition, physical, bacteriological and technological properties of milk [2]. Given these findings from Antunac et al., (1997) we can say that in our examinations only a small proportion of cows had problems with teat infections, in accordance with the high percentage of cows that had milk with a number of satisfactory of somatic cells (76.74%).

#### 4. Conclusions

Based on the results obtained from the examined samples of raw milk, we can conclude the following:

1. From a total of 45 samples examined, in terms of the number of somatic cells, 33 samples, ie 76.74% of the samples meet the criteria according to the Regulation for special requirements. for safety and hygiene and the way and procedure of carrying out official controls of milk and milk products (Official Gazette of RM 26/12, 145/14, 59/16, 197/16 and 16/2019 and European Union criteria (Council Directive 92 /46 EEC) Samples that do not meet the criteria  $\leq 400,000$  SCC/ml are 10 or expressed in percentage 23.25% of the total number of samples examined
2. From a total of 45 samples examined, from the point of view of the total number of microorganisms, no sample examined meets the criteria from the Regulation on special requirements for safety and hygiene and the manner and procedure of conducting official controls. for milk and milk products (Official Gazette no. 26/12, 145/14, 59/16, 197/16 and 16/2019) and European Union criteria (Council Directive 92/46 EEC), which is  $\leq 100,000$  CFU / ml of raw milk.

#### 6. References

1. **Akam, F.D., Dodd, F.H. & Quick, A.J.** (1989) *Milking, Milk Production Hygiene and Udder Health*, Report No. 78, Food and Agriculture Organization of the United Nation, Rome, pp. 56–95.
2. **Antunac, N., Lukač-Havranek, J., Samaržija, D.** (1997): Somatic cells and their influence on the quality and processing of milk. *Dairy* 47 (3), 183-193.
3. **Čačić, Z., Kalit, S., Antunac, N., Čačić, M.** (2003) Somatic cells and factors affecting their number in milk. *Dairy* 53, 23-36.
4. **Causin, M.** (1982): Presence and activity of psihrotrophic microorganisms in milk and dairy products: a review. *Journal of Food Protection* 45, 172-207.
5. **Concha** (1986): Cell types and their immunological functions in bovine mammmary tissues and secretion- a review of the literature. *Nord. Vet. Med.* 38.
6. **Frerking H.**, (1961): Yur Feststellung von Enterstörungen und Enteren-t zündungen in Vorzugsmilhbetriebe ben unter Verwendung geeigneter Laboratoriumsverfahren. *Vet.med. Diss.* Hanover
7. **Harmon, R.J.** (1994.): Physiology of mastitis and factors affecting somatic cell count. *Journal of Dairy Science*, 77: 2103-2112.
8. **Kalit, S., havranek, J.** (1998.): Current status of somatic cell count (SCC) inthe milk from individual farm in Croatia. *Milchwissenschaft*, 53 (4), 183-184.
9. **Kalit, S., Lukač-Havranek, J.** (1999) Incidence od subclinical mastitis on the farms with various numbers of cows. *Mljekarstvo* 49, 9-14.
10. **Mc Donald JS, Anderson AJ** (1981): Total and diferential somatic cell counts in secretion from noninfected bovine mammary glands: the peripartal period. *Am. j. Vet. Res.* 42.1366-8.
11. **Nickerson S.C.** (1985): Immune mechanisms of the bovine udder an overview. *J. Am. Vet. Med. Assoc.* Jul.1. 187 (1), 41-45.

12. **O'Brien B, Mehra R, Connolly JF, Harrington D**, 1999, Seasonal variation in the composition of Irish manufacturing and retail milks 1. Chemical composition and renneting properties. *Irish J Agricult Food Res* 38, 53-64
13. **Pillai S.R., Kunze E., Sordillo L.M., Jayarao B.M.**, (2001): Application of differential inflammatory cell count as a tool to monitor udder health. *J.Dairy Sci*, 84,1313-1420.
14. **Rupić, V.** (1988): Maternity. U: Veterinarian in the house. The second book, Logos, Split, 153-246.
15. **Schalm O.W.**, (1971): Bovine mastitis. Philadelphia, USA.
16. **Slaghuis, B.A., Te Giffel, M.C., Beumer, R.R. and André, G.** 1997. Effect of pasturing on the incidence of *Bacillus cereus* spores in raw milk. *International Dairy Journal* 7: 201-205.