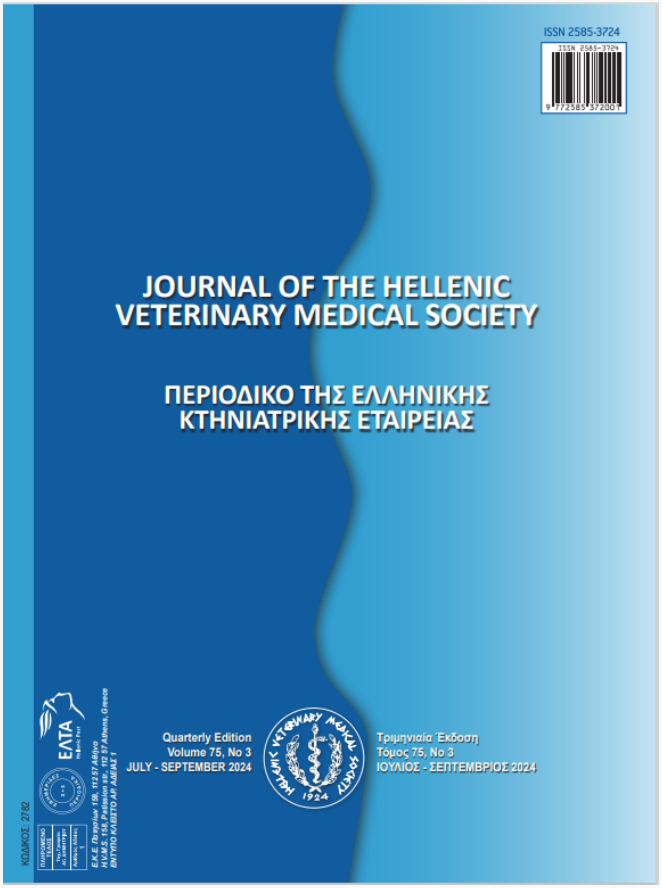
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 3 (2024)



Comparison of methods for measurement of somatic cell count in goat milk

NM Zazharska

doi: 10.12681/jhvms.35582

To cite this article:

Zazharska, N. (2024). Comparison of methods for measurement of somatic cell count in goat milk: somatic cells. *Journal of the Hellenic Veterinary Medical Society*, *75*(3), 7791–7800. https://doi.org/10.12681/jhvms.35582

J HELLENIC VET MED SOC 2024, 75 (3): 7791-7800
ΠΕΚΕ 2024, 75 (3): 7791-7800

Research article
Ερευνητικό άρθρο

Comparison of methods for measurement of somatic cell count in goat milk

N.M. Zazharska*

Dnipro State Agrarian and Economic University, Faculty of Veterinary Medicine, Yefremov Str., 25, Dnipro, 49000, Ukraine

ABSTRACT: The aim of the study was to compare the efficiency of determining the number of somatic cell count in goat milk by different methods. Somatic cells in individual samples of milk from 28 goats were studied using analyzers "Somatos", "SomaCount Flow Cytometer" and by the counting method in milk films stained according to Romanovsky - Giemsa, May-Grünwald and with pyronin Y. For the counting of somatic cells in goat milk by Prescott and Breed method, it is recommended to stain milk films by the May-Grünwald method, because the cytoplasm and nuclei of somatic cells are clearly stained, and the cost of dyes is much less than the pyroninY method. When counting cells in milk films stained by any method, no samples with the SCC up to 100 x 10^3 cells/ml were found. Direct counting of somatic cells in milk films stained by any method reveals a greater number of cells than with the help of devices. It was found that the fat content in goat milk with SCC >3000 x 10^3 cells/ml is significantly higher ($P < 0.05$) compared to milk with SCC < 1000 x 10^3 cells/ml. The quality of goat's milk smears for counting of somatic cells quantity stained by the May-Grünwald method corresponds to the recommended method with pyronin Y, and the cost of dyes is 28.4 times lower. The distribution of somatic cell ranges is similar between different methods of staining smears, which once again proves the accuracy of the method of direct cell counting in milk films, although it is more labor-intensive than hardware methods.

***Keywords*:** somatic cell count; goat milk; milk films

Corresponding Author:
Zazharska N.M., Dnipro State Agrarian and Economic University, Faculty of Veterinary Medicine, Yefremov Str., 25, Dnipro, 49000, Ukraine
E-mail address: zazharskayan@gmail.com

Date of initial submission: 06-10-2023
Date of acceptance: 13-11-2023

INTRODUCTION

Sociological and economic factors significantly affect the food industry and, as a result, new products with characteristics according to marketing requirements have appeared. Goat's milk has significant advantages over cow's milk, it is a valuable dairy product and an integral part of a healthy diet, it is used as a nutritional source for babies and children, as well as a functional food (Silanikove et al., 2010; Vasylieva, 2021).

The ability to identify goats with subclinical mastitis caused by *S. aureus*, is important in udder health monitoring programs. In Norway, the somatic cell count (SCC) is recommended as a screening tool and bacteriological examination is a confirmatory test to detect goats with subclinical mastitis. The researchers evaluated the accuracy of pathogen detection, deoxyribonucleic acid diagnostics, and counting of somatic cells. Bacterial culture and polymerase chain reaction had high specificity and relatively high sensitivity (Smistad et al., 2022). Scientists from Slovakia evaluated the presence of pathogens in goat milk and their relationship with the SCC and milk composition. Bacterial pathogens were detected in only 13% of samples. As a diagnostic tool, SCC is probably less useful in goats than in cows (Tvarožková et al., 2023).

The effectiveness of SCC alone in goat milk to detect subclinical mastitis as a single test is now being questioned. Determination of a total number of bacteria is proposed as an auxiliary test. Some authors are engaged in the discovery of new biomarkers that show a relationship between high SCC values and bacterial contamination in goat milk samples. Lactate dehydrogenase is a potential biomarker for the detection of high somatic cell counts and total bacterial counts in individual or bulk tank milk samples of goats (Gómez-Gascón et al., 2022). Lithuanian scientists found some statistically significant relationships between the SCC and amino acids and fatty acids. Therefore, improving milk quality by reducing the number of somatic cells may benefit farmers by improving the fatty acid and amino acid composition of goat milk and may serve as a biomarker for dairy goats (Šlyžius et al., 2023). Somatic cell counts showed a direct effect on protease activity, with the highest value in goat milk (compared to cow and buffalo milk). The correlation coefficients of protease activity with the stage of lactation and SCC were positive, which indicates a direct influence of somatic cells and lactation on it (Gautam et al., 2023).

Scientists from Greece analyzed the growth retardation of microorganisms to detect antibiotic residues, the isolation of *Staphylococcus aureus* and *Escherichia coli* strains as microbiological indicators of sensitivity to antimicrobial drugs, the counting of somatic cells and coagulase-negative staphylococci (Rozos et al., 2022). High SCC in goat milk (reflecting the presence of mastitis) and gastrointestinal parasitic infections (mainly Teladorsagia infection) have a more significant effect on milk fat and protein content compared to factors unrelated to infection (Lianou et al., 2022). Erduran (2023) found that stages of lactation and daily milk yield significantly affected SCC, pH, total solids and freezing point of goat milk.

Scientists from Brazil claim that the addition of organic selenium to the diet of dairy goats for 60 days reduced the number of somatic cells and, as a result, improved milk quality (De Vasconcelos et al., 2023). Scientists at our university in Ukraine study the effect of various substances on pathogens (Zazharskyi et al., 2019; Gotsulya et al., 2020; Sklyarov et al., 2020; Zazharskyi et al., 2020; Zazharskyi et al., 2021; Borovuk & Zazharska, 2022).

The aim of the study was to compare different methods of determining the number of somatic cells in goat milk.

MATERIALS AND METHODS

Individual samples of milk from 28 goats of Alpine and German White breed of farm in the Dnipropetrovsk region (Ukraine) were studied. The system of keeping animals is stall-walking. The goats had free access to feed and water. The goats were of 3rd-5th lactation, milk yield 1-3 liters. Milking took place twice a day with a milking machine. An average sample of 60 ml of well-mixed milk was taken from the morning milk yield of one goat. The milk was filtered and cooled to a temperature of 4 ± 2ºC in a cooler bag with a refrigerant.

Determination of SCC by Hardware Mehtods

Determination of SCC was carried out on the device "SomaCount Flow Cytometer" (Bentley, USA) (flow cytometry method) in the laboratory of DMS ("Dairy Management System") of the Dnipropetrovsk regional public organization "Agricultural Consultancy Service". Flow cytometry is a research method in which cells are labeled with a special fluorescent dye and then exposed to laser radiation. The intensity of

fluorescence induced by laser radiation is proportional to the content of DNA in cell molecules. The deviation does not exceed ±0.5%.

Another hardware method used to determine SCC was the viscometric analyzer "Somatos" (Kostil, Russia). The researches were carried out at Parasitology and Vet Expertise Department of Dnipro State Agrarian and Economic University. The principle of operation of the device is based on the change in viscosity - the time of outflow through the capillary of the milk sample, which is mixed with the drug "Mastoprim" (Reagent, Ukraine), depending on the SCC. This reagent destroys somatic cells, DNA molecules move into the intercellular space, while changing the viscosity, resulting in the formation of a gelatinous clot. The relative error of conventional viscosity measurement is no more than 5%.

Counting of somatic cells in milk smears

Smears were also made from all samples of goat milk, stained with pyronin Y and methyl green, according to Romanovsky - Giemsa, according to May-Grünwald. Somatic cells were counted according to the Prescott and Breed method under a microscope (100 objective) (Kavitha et al., 2011).

Goat milk smears were made using an improved technique. To make smears of goat's milk, a stencil with cut-out squares of 1 cm^2 was made on millimeter paper, placed under a glass on a dark background. A mixed sample of milk (0.005 ml) was applied to areas on a slide with a micropipette.

The milk was distributed with a needle in a uniform thin layer over the entire area of the square - in this way milk smears were made.

Milk films were labeled, air-dried, immersed in a beaker with Carnoy's fixative (15 ml chloroform, 5 ml glacial acetic acid, 30 ml 100% ethanol) for 5 minutes. Then the smears were transferred to 50% ethanol (kept for 1 min.), in 30% ethanol (also kept for 1 min). Then smears were dipped in distilled water for 1 minute. After air drying, the smears were stained.

a) staining of milk smears with pyronin Y and methyl green.

Dye preparation: pyronin Y (250 mg) and methyl green (140 mg) were added to a flask with demineralized water (49 ml) and mixed thoroughly. The solution was filtered through a suitable filter.

After fixing the smears of milk, they were stained according to the following scheme: pyronin Y dye solution with methyl green - for 6 minutes; quickly washed with n-butyl alcohol and then with xylene. The finished smears were dried in the air.

b) staining of goat milk smears according to May-Grunwald.

Staining was carried out with a ready solution of May-Grünwald paint. 1-2 ml May-Grunwald paint with water 1:1 was applied to the fixed smear and left for 2-3 minutes. Then the smear was washed with water and restained according to Romanovsky - Giemsa for 10-15 min. (1-2 drops of Romanovsky - Giemsa paint per 1 ml of distilled water). Next, the milk film was washed with water and dried in air.

c) staining of milk smears according to Romanovsky - Giemsa.

A working solution of Romanovsky-Giemsa paint was prepared - 1-2 drops of Romanovsky-Giemsa paint per 1 ml of distilled water. Smears were painted for 20-25 min at 37 °C. After staining, the smears were washed with water and air-dried. From own experience, it is not necessary to create a wet chamber in the thermostat for smears of goat's milk - in this case, the smear is washed off the slide.

Stained cells were counted using MICROmed XS-3320 and Olympus CX21FS1 binocular microscopes at 100 objective magnification (the Prescott and Breed method). In each milk film, 100 fields of view were viewed.

The calculated number of cells was multiplied by a coefficient taking into account the values of the objective and the eyepiece and their number in 1 ml of milk was determined. To derive the coefficient, the diameter of the field of view of the microscope was determined with the help of an object-meter and the area of one field of view was calculated using the formula πR^2. Then quantity of fields of view of the microscope which placed on an area of 1 cm^2 was calculated. The obtained result was converted to volume.

Determination of physicochemical parameters of goat milk was carried out using an ultrasonic analyzer Ekomilk Milkana Kam 98-2a (Bulteh, Bulgary). Limits of permissible absolute error when measuring the mass fraction of fat - ± 0.1%, protein - ± 0.15%, freezing temperature - ± 0.01ºC.

Statistical Analysis

Data were analyzed using Microsoft Excel and Statisica 12 (Stat Soft) computer software. The data in the tables are presented as x ± SE (x ± standard error). Differences between values in the groups were determined using the Tukey test, where the differences were considered significant at $P < 0.05$ (subject to the Bonferroni Amendment).

RESULTS

During the process of making smears, Carnois fixative must be used. If fixed in an alcohol solution, most of the milk films "slip" off the slide.

The arbitration method for SCC determining is counting the somatic cells of milk by the Prescott and Breed method (direct microscopic method of ISO 13366-1: 200X/IDF 148-1:2008), according to which it is recommended to stain smears of goat milk with pyronin Y and methyl green. However, dyes for research are expensive, so the recommended method was compared with more affordable ones. In this study, May-Grünwald method of staining blood smears was used to stain goat milk films.

The best results for testing milk films were obtained using the May-Grünwald method compared to other methods of staining smears. Somatic cells in this method appear with clearly defined cytoplasm and nuclei (Fig. 1).

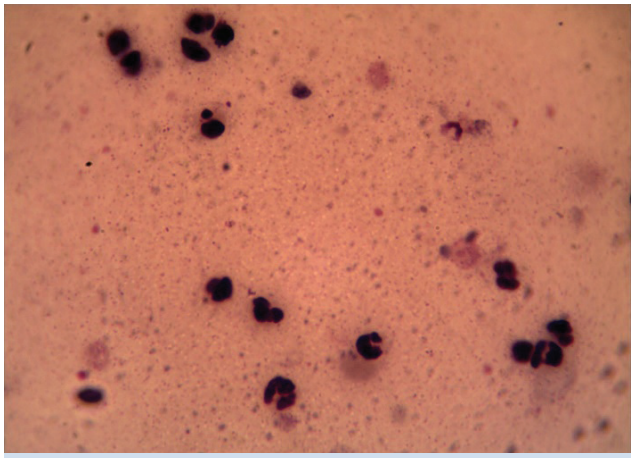


Fig. 1. Somatic cells in goat's milk smears: May-Grünwald stain; bar - 10 μm

In the case of smear staining with pyronin Y and methyl green (Fig. 2) somatic cells are well stained, but due to the high cost of materials, this method is almost not used in Ukraine. In addition, the May-Grünwald method takes less time to stain goat milk films than the pyronin Y method.

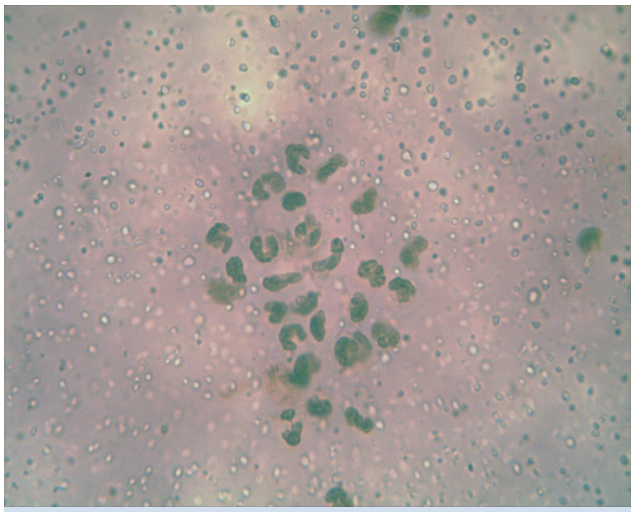


Fig. 2. Somatic cells in goat's milk smears: pyronin Y stain; bar - 10 μm

The cost of reagents for examining milk smears by the May-Grünwald method is 28.4 times lower compared to staining smears with pyronin Y.

In addition to somatic cells, goat milk also contains extracellular membrane material, nuclear particles, and cell fragments (cytoplasmic fragments) originating from the distal alveolar secretory cells of the mammary gland. The number of cytoplasmic particles in goat milk is very high compared to other species due to the apocrine type of milk secretion. The method of staining smears according to Romanovsky - Giemsa is not completely acceptable for goat's milk, because "shadows" of cells, so-called cytoplasmic particles, particles of paint are often detected (Fig. 3).

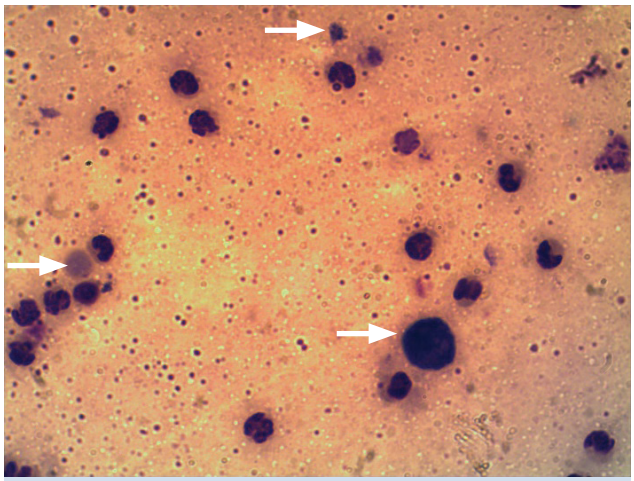


Fig. 3. Disadvantages in milk films: Romanovsky - Giemsa stain; bar - 10 μm; *a -"shadow" of the cell, b - cytoplasmic particle, c - piece of paint*

During the counting somatic cells in goat's milk films stained according to Romanovsky - Giemsa, higher in-

dicators were obtained than according to other methods. Moreover, the process of staining smears according to Romanovsky - Giemsa includes the stage of exposure in a humid chamber at a temperature of 37 °C. This caused most of the smears to "slip" off the slide.

Therefore, to count the number of somatic cells, staining smears of goat's milk using the May-Grünwald method is recommended. Somatic cells stain well, and the cost of dyes is 28.4 times lower than the pyronin Y method.

To facilitate processing, the results of the goat milk study were divided into 5 ranges according to the SCC (Table 1). The smallest number of somatic cells at the last level (> 3 million/ml) was noted during counting with the "Somatos" device. The method of counting somatic cells by flow cytometry with the help of "SomaCount Flow Cytometer" is considered more accurate compared to the viscometric method.

When counting cells in milk films stained by any method, no samples with the somatic cell count up to 100 x 10^3 cells/ml were found. Direct counting of somatic cells in milk films stained by any method revealed a greater number of cells than with the help of devices.

The distribution of somatic cell ranges was similar between different methods of smear staining, which once again proves the accuracy of the method of direct cell counting in milk films, although it is a more time-consuming method than hardware methods.

Among the various smear staining methods, the largest SCC in the range > 3 million/ml was noted when using pyronin Y and methyl green - almost 9 million cells in a milliliter of milk and according to Romanovsky-Giemsa - almost 10 million/ml. Such a large indicator opposes the indicator obtained with the help of "Somatos" - 5.5 million/ml. This once again proves that the viscometric device "Somatos" is only an indirect method of SCC determination, and in goat's milk this difference between the methods is more significant (Table 1).

According to the results of research on the "Somatos" and "SomaCount Flow Cytometer" devices, 32.1 and 7.1% of milk samples, respectively, with the SCC up to 100 000/ml were found (Fig. 4).

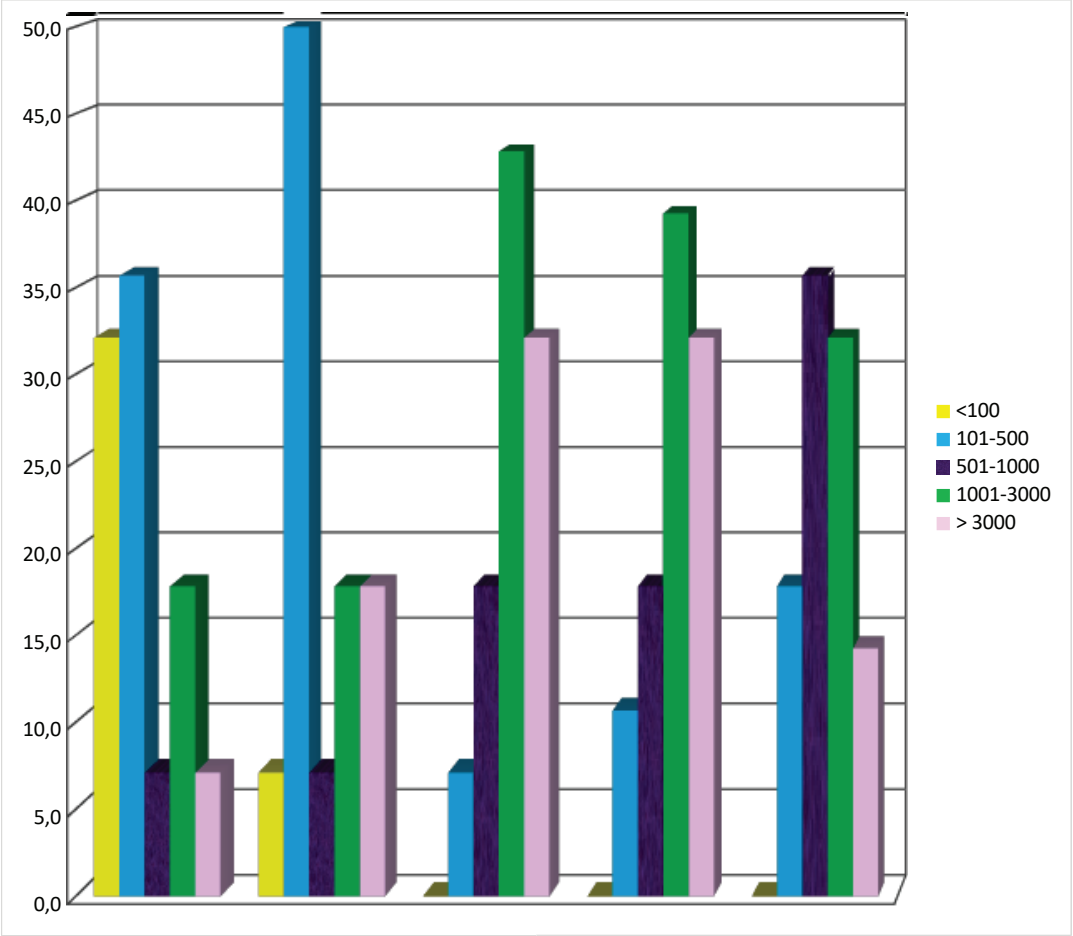


Fig. 4. Distribution of milk samples according to different methods of counting somatic cells, (n=28)

Table 1. Determination of SCC in goat milk by various methods (x ± SE, x 10^3 cells/ml)

Number of somatic cells in the milk	SCC by various methods				
	on devices		count in smears of milk, stained by		
	«Somatos»	«SomaCount Flow Cytometer»	Romanovsky - Giemsa method	pyronin Y	May-Grünwald method
<100	72 ± 6	66 ± 20	-	-	-
101-500	254 ± 38	302 ± 35	425 ± 12	392 ± 47	354 ± 136
501-1000	766 ± 141	741 ± 71	851 ± 62	811 ± 357	734 ± 57
1001-3000	1768±342	1518 ± 205	2293 ± 163	1790 ± 205	1706 ± 164
> 3000	5500±501	8865±3374	9869 ± 3730	8903 ±3520	7677 ± 3326

As already mentioned above, when examining milk films stained by any of the methods, the range of SCC is up to 100 thousand/ml not found.

According to the results obtained on the "Somatos" and "SomaCount Flow Cytometer" devices, the largest part of milk samples belonged to the level - 101-500 x 10^3 cells/ml - 35.7 and 50% respectively. The largest part of milk samples with regard to staining methods - according to Romanovsky - Giemsa (42.9%) and according to May-Grünwald (39.3%) belonged to the range of 1001-3000 x 10^3 cells/ml, while smears stained with pyronin Y and methyl green (35.7 %) - up to the range of 501-1000 x 10^3 cells/ml.

This proves that direct counting of somatic cells in smears of goat's milk stained by any method reveals a greater number of cells than with the help of devices. Figure 4 shows that the distribution of somatic cell ranges is similar between different smear staining methods, which once again proves the accuracy of the method of direct somatic cell counting in milk smears, although these are more time-consuming methods than hardware methods.

With an increase of SCC, fat indicators gradually increased (Table 2). It was found that the fat content in goat milk with SCC >3000 x 10^3 cells/ml is significantly higher ($P < 0.05$) compared to milk with SCC < 1000 x 10^3 cells/ml. Perhaps this is explained by the smaller volume of milk in goats with a large SCC.

With the increase in the level of somatic cells, the content of protein and lactose increased, but in the last group (more than 3 million/ml.) there was a sharp decrease in indicators. The opposite trend was observed in relation to the freezing temperature of milk.

DISCUSSION

Some scientists note that in the milk of most species of animals, somatic cells in the uninfected gland are represented by leukocytes, consisting of lymphocytes, polymorphonuclear neutrophils and macrophages, which serve as important components of the mammary gland's defense against potential pathogens (Vasil' et al., 2017, Hisira et al. 2023). Milk also contains desquamated epithelial cells. In the uninfected gland of cows, somatic cells consist of epithelial cells by 40%, and in goats by 27% (Silanikove et al., 2010; Boutinaud et al., 2004)

There are several ways to count the number of somatic cells: from conventional methods to integrated modern SCC methods based on biosensors (Sun et al.,

Table 2. Milk indicators depending on the level of somatic cells (x ± SE)

Indicator	Milk samples depending on the level of somatic cells			
	101-500, n = 5	501-1000, n = 10	1001-3000, n = 9	>3000, n = 4
SCC (the pyronin Y method), x 10^3 cells/ml	392 ± 47	811 ± 357	1790 ± 205	8903 ±3520
Fat, %	2,62 ± 0,37^a	2,74 ± 0,32^a	3,05 ± 0,37^{ab}	4,37 ± 0,64^b
Protein, %	3,13 ± 0,12	3,16 ± 0,04	3,33 ± 0,07	2,82 ± 0,52
Lactose, %	4,72 ± 0,17	4,77 ± 0,06	5,01 ± 0,10	4,18 ± 0,81
Freezing point, ^{o}C	-0,558 ± 0,021	-0,565 ± 0,007	-0,592 ± 0,011	-0,515 ± 0,078
Conductivity, mS/cm	5,338 ± 0,179	5,286 ± 0,092	5,378 ± 0,161	5,740 ± 0,358

Note: different letters indicate selections that significantly ($P < 0.05$) within the line differ from each other according to the results of the Tukey test, with Bonferroni correction; if there are no letters above the numbers in the line, then no significant difference between any selections is registered.

2023). Soquila (2023) also compared direct and indirect methods for determining the SCC in goat milk: direct microscopic somatic cell counting and the California mastitis test. Milk films stained by Modified Methylene Blue stain. Both methods had a correlation coefficient (r) of 0.986 in detecting subclinical mastitis in goats. The apocrine type of milk secretion in goats results in a high SCC, which generally reverses the generally accepted relationship between SCC and the presence of the bacterial causative agent of mastitis in goats. The SCC in goat's milk is generally much higher than in cow's milk, even in a healthy udder. Stage of lactation, estrus, milking method, season, breed and number of lactations can affect SCC. California mastitis test has the advantage of being an animal test that is easy to perform, but bacterial testing is required for confirmation (Zigo et al 2022).

According to Yangilar (2013), the average protein content in the milk of British Saanen goats is 2.6%, Nubian in England - 3.6%, Alpine and Saanen in France - 3.2%, fat content 3.5%, 4.9% and 3.6%, respectively. According to the own results, the protein content in goat's milk is observed at the level 2.82-3.13%.

Lianou et al. (2022) in a study of 119 goat herds in Greece found that the average fat/protein content of bulk tank goat milk was 4.77 ± 0.44 %/3.23 ± 0.30 %, respectively. Month of lactation of goats and SCC in milk appeared to be significant factors in multivariate analysis for simultaneous high fat and protein content.

According to the own data, an increase in the fat content was also observed in parallel with the increase in the level of somatic cells in milk: the fat indicators were from 2.62% (with the lowest number of somatic cells) to 4.37% (with the SCC >3 million/ml), $P < 0.05$. It was also found that when the protein content decreased, the freezing temperature increased. This tendency was also observed in other studies described in previous own publications (Fotina et al., 2018; Zazharska et al., 2021).

Research by Hungarian scientists also revealed a relationship between SCC and protein content and the absence of a relationship between SCC and lactose and freezing point. They claim that with an increase in SCC, the protein, pH, and Na significantly increase, but the lactose content and freezing point decrease (Pajor et al., 2013). According to Bagnicka et al. (2016) in goat milk, no correlation between the SCC and the content of protein, fat, and lactose is observed. Israeli scientists claim that bacterial infections and stages of lactation are the two most important factors that affect the quality of goat milk. A positive relationship between casein, lactose and suitability for cheesemaking, and a negative relationship between the last indicator and SCC are associated with bacterial infection and with milk of late lactation (Silanikove et al., 2014).

SCC is the most informative for the diagnosis of udder health in cows, may not be a reliable parameter for determining subclinical mastitis in goats. This indicator changes a lot (for example, during the lactation period of goats) and often a value of more than 1 million/ml is determined without the appearance of subclinical mastitis. The number of somatic cells can increase only due to physiological factors (for example, breed, stage of lactation, estrus), hygienic parameters and milking equipment. The gold standard for the detection of mastitis is the determination of bacteriological status, but the analysis is time-consuming and expensive and is not often used. It is proposed to use as an alternative parameter of subclinical mastitis in goats, such as SCC, the California mastitis test, electrical conductivity, milk composition (fat, protein, lactose), N-acetyl-β-D-glucosaminidase (NAGase), lactoferrin, β- glucuronidase and lactate dehydrogenase (Stuhr et al., 2013.)

According to the own research, with an increase in SCC, the indicators of electrical conductivity increase almost gradually. An increase in the electrical conductivity of milk is usually associated with the occurrence of subclinical mastitis in cows, that is, with an increase in SCC.

The increase of SCC in the bulk tank milk of goats is a problem in the production of dairy products from goat milk. In Norway, SCC in goat milk has been recorded at a level of more than 1 million / ml in recent decades, and in healthy goats it should be below 500 thousand / ml (Solverod, 2013). According to Looper (2013) and Zigo et al. (2014, 2019) there are two ways to reduce SCC in milk. The first method is culling the cows, a process that can quickly reduce SCC in the bulk tank milk. The second method is management of mastitis, which includes examination of milk samples from each cow every month, prevention through feeding, sanitary measures during milking of cows, dryness, first lactation. Addition of selenium and vitamin E into the diet improves the condition of the tissue in the mammary gland.

The normal level of somatic cells in the uninfected udder of goats (~ 300 thousand / ml) and sheep (~ 200 thousand / ml) is significantly higher than in dairy cows (~ 70 thousand / ml), therefore, this indicator in the infected udder of goats and sheep, as a rule, is much higher than that of cows. This means that milk grading schemes based on SCC in goats and sheep must be specifically adapted to these two species, and cannot simply be replicated from those established in cows. Many noninfectious factors can also cause significant differences in SCC in goat milk. One of the problems unique to goats is a marked increase of this indicator in milk from a healthy udder near the end of lactation (Raynal-Ljutovac et al., 2005; Fotina et al., 2018; Zazharska et al., 2021).

Based on the prevalence of bacterial infections and a number of studies both at the herd level and at the level of mammary gland infection in individual animals, Leitner et al. (2008) proposed a classification scheme for goat and sheep milk. According to this proposal, milk with SCC > 3.5 million/ml should not be accepted for milk processing plants. Therefore, to sort the quality of goat milk for production purposes, other criteria than SCC are needed (Raynal-Ljutovac et al., 2007).

CONCLUSIONS

To count the number of somatic cells staining smears of goat milk using the May-Grunwald method is recommended. Somatic cells stain well, and the cost of dyes is 28.4 times lower than the pyronin Y method.

Direct counting of somatic cells in milk films stained by any method reveals a greater number of cells than with the help of devices. The distribution of somatic cell ranges is similar between different methods of staining smears, which proves the accuracy of the method of direct cell counting in milk films, although it is more labor-intensive than hardware methods.

With an increase of somatic cell count, the indicators of fat increased. It was also found that when the protein content decreased in goat milk, the freezing point increased.

ACKNOWLEDGMENT

Thanks to the laboratory of DMS (“Dairy Management System”) of the Dnipropetrovsk regional public organization “Agricultural Consultancy Service” for help in researching goat milk samples.

CONFLICT OF INTEREST

The author does not declare any conflict of interest.

REFERENCES

Bagnicka E, Łukaszewicz M, Ådnøy T (2016) Genetic parameters of somatic cell score and lactose content in goat's milk. J Anim Feed Sci 25(3):210-215. https://doi.org/10.22358/jafs/65552/2016

Borovuk I, Zazharska N (2022) Evaluation of broiler meat in experimental listeriosis. J Adv Vet Anim Res 9(1):155-165. https://doi.org/10.5455/javar.2022.i580

Boutinaud M, Guinard-Flament J, HélèneJammes (2004) The number and activity of mammary epithelial cells, determining factors for milk production. Reprod Nutr Dev 44(5):499-508. https://doi.org/10.1051/rnd:2004054

de Vasconcelos ÂM, Martins TP, de Souza V, Bonfim JM, Pompeu RCFF, Façanha DAE et al (2023) Effect of a 60-day organic selenium-supplemented diet on the decrease of somatic cell counts in goat milk. Trop Anim Health Prod 55(2). https://doi.org/10.1007/s11250-023-03526-2

Erduran H (2023) Effect of parturition time and photoperiod on milk production, quality, and somatic cell count traits of pure and crossbred goats in a different production system. Trop Anim Health Prod 55(3). https://doi.org/10.1007/s11250-023-03560-0

Fotina T, Fotina H, Ladyka V, Ladyka L, Zazharska N (2018) Monitoring research of somatic cells count in goat milk in the eastern region of Ukraine. J Hellenic Vet Med Soc 69(3):1101-1108. https://doi.org/10.12681/jhvms.18882

Gautam PB, Sharma R, Atbhaiya Y, Gandhi K, Mann B (2023) Activities of indigenous proteases in cow, buffalo and goat milk of Indian subcontinent and their correlation with somatic cell count. Int Dairy J 139:105567. https://doi.org/10.1016/j.idairyj.2022.105567

Gómez-Gascón L, Galán-Relaño A, Cardoso-Toset F, Barrero-Domínguez B, Astorga RJ, Luque I, et al (2022) Lactate dehydrogenase: Detecting high bacterial and somatic cells counts in goats from whole milk samples. Small Rumin Res 208:106632. https://doi.org/10.1016/j.smallrumres.2022.106632

Gotsulya AS, Zazharskyi VV, Davidenko PO, Zazharska NM, Kulishenko OM, Panasenko OI, et al (2020) Features of experimental modeling of tuberculosis in guinea pig with the participation of N'-(2-(5-((thephylline-7'-yl)methyl)-4-R-1,2,4-triazole-3-ylthio)acethyl)isonicotinohydrazide. Ukr J Ecol 10(4):191-194. doi: 10.15421/2020_187

Hisira V, Zigo F, Kadaši M, Klein R, Farkašová Z, Vargová M, Mudroň P (2023) Comparative analysis of methods for somatic cell counting in cow's milk and relationship between somatic cell count and occurrence of intramammary bacteria. *Vet. Sci. 10:*468. https://doi.org/10.3390/vetsci10070468

Kavitha G, Isloor S, Rathnamma D, Rajeshwari Y, Nagaraja C, Sadish S (2011) Induction of mastitis in rabbit mammary glands with bovine mastitis bacterial strains. Int J Pharma Bio Sci 2(3):266-276.

Leitner G, Silanikove N, Merin U (2008) Estimate of milk and curd yield loss of sheep and goats with intrammamary infection and its relation to somatic cell count. Small Rumin Res 74(1-3):221-225. https://doi.org/10.1016/j.smallrumres.2007.02.009

Lianou DT, Michael CK, Gougoulis DA, Cripps PJ, Vasileiou NGC, Solomakos N, et al (2022) High milk somatic cell counts and increased teladorsagia burdens overshadow non-infection-related factors as predictors of fat and protein content of bulk-tank raw milk in sheep and goat farms. Foods 11(3):443. https://doi.org/10.3390/foods11030443

Looper M (2013) Reducing Somatic Cell Count in Dairy Cattle. Agr Nat Resour https://www.uaex.edu/publications/PDF/FSA-4002.pdf

Pajor F, Sramek A, Toth G, Poti P (2013) Effect of somatic cell count on some chemical, physical and bacterial properties of milk in a Hungarian Alpine goat farm. Goat Milk Quality Regional IGA Conference, Norway. Abstracts 2013:38.

Raynal-Ljutovac K, Gaborit P, Lauret A (2005) The relationship between quality criteria of goat milk, its technological properties and the quality of the final products. Small Rumin Res 60(1-2):167-177. https://doi.org/10.1016/j.smallrumres.2005.06.010

Raynal-Ljutovac K, Pirisi A, de Crémoux R, Gonzalo C (2007) Somatic cells of goat and sheep milk: Analytical, sanitary, productive and technological aspects. Small Rumin Res 68(1-2):126-144. https://doi.org/10.1016/j.smallrumres.2006.09.012

Rozos G, Skoufos I, Fotou K, Alexopoulos A, Tsinas A, Bezirtzoglou E et al (2022) Safety issues regarding the detection of antibiotics residues, microbial indicators and somatic cell counts in ewes' and goats' milk reared in two different farming systems. Appl Sci 12(3):1009. https://doi.org/10.3390/app12031009

Silanikove N, Merin U, Leitner G (2014) On effects of subclinical mastitis and stage of lactation on milk quality in goats. Small Rumin Res 122(1-3):76-82. https://doi.org/10.1016/j.smallrumres.2014.07.018

Silanikove N, Leitner G, Merin U, Prosser C (2010) Recent advances in exploiting goat's milk: Quality, safety and production aspects. Small Rumin Res 89:110-124. https://doi.org/10.1016/j.smallrumres.2009.12.033

Sklyarov P, Fedorenko S, Naumenko S (2020) Oxidant/Antioxidant Balance in Cows and Sheep in Antenatal Pathology. Ukr J Ecol 10(5):26-28. https://doi.org/10.15421/2020_201

Šlyžius E, Anskienė L, Palubinskas G, Juozaitienė V, Šlyžienė B, Juodžentytė R et al (2023) Associations between somatic cell count and milk fatty acid and amino acid profile in alpine and saanen goat breeds. Animals 13(6):965. https://doi.org/10.3390/ani13060965

Smistad M, Vatne MK, Sølverød L, Dean KR (2022) Sensitivity and specificity of bacterial culture, qPCR, and somatic cell count for detection of goats with Staphylococcus aureus intramammary infection using Bayesian latent class models. Prev Vet Med 209:105793. https://doi.org/10.1016/j.prevetmed.2022.105793

Soquila SS (2023). Direct and indirect measurement of somatic cell count in detecting subclinical mastitis in goats. TSRJ 18(6):1085-1090. https://doi.org/10.17605/OSF.IO/9Z82Q

Stuhr T, Aulrich K, Barth K, Knappstein K, Larsen T (2013) Influence of udder infection status on milk enzyme activities and somatic cell count throughout early lactation in goats. Small Rumin Res 111(1-3):139-146. https://doi.org/10.1016/j.smallrumres.2012.09.004

Sun X, Zhao R, Wang N, Zhang J, Xiao B, Huang F et al (2023) Milk somatic cell count: From conventional microscope method to new biosensor-based method. Trends Food Sci Technol 135:102-114. https://doi.org/10.1016/j.tifs.2023.03.020

Solverod L (2013) Udder health in Norwegian goat dairy herds. Goat Milk Quality Regional IGA Conference, Norway. Abstracts 2013:49.

Tvarožková K, Tančin V, Uhrinčať M, Oravcová M, Hleba L, Gancárová B et al (2023) Pathogens in milk of goats and their relationship with somatic cell count. J Dairy Res. 2023:1-5. https://doi.org/10.1017/s0022029923000237

Vasil' M, Farkasova Z, Elecko J, Illek J, Zigo F (2017) Comparison of biofilm formation by Staphylococcus aureus and Staphylococcus epidermidis strains isolated from sheep milk using three diagnostic methods. Pol J Vet Sci 20(4):795-801. doi: 10.1515/pjvs-2017-0100. PMID: 29611650.

Vasylieva N (2021) Food security in times of covid-19: price aspects in Ukraine and neighboring EU countries. MNJE 17(3):21-30. https://doi.org/10.14254/1800-5845/2021.17-3.2

Yangilar, F., 2013. As a potentially functional food: Goats' milk and products. J. Food Nutr. Res., 1(4): 68-81.

Zazharska N, Fotina T, Yatsenko I, Tarasenko L, Biben I, Zazharskyi V et al (2021) Comparative analysis of the criteria for goat milk assessment in Ukraine and France. Ukr J Ecol 11(2):144-148. http://doi.org/10.15421/2021_91

Zazharskyi V, Bigdan O, Parchenko V, Parchenko M, Fotina T, Davydenko P et al (2021) Antimicrobial Activity of Some Furans Containing 1,2,4- Triazoles. Arch Pharm Pract 12(2):60-65. https://doi.org/10.51847/rbjb3waubb

Zazharskyi V, Parchenko M, Fotina T, Davydenko P, Kulishenko O, Zazharskaya N et al (2019) Synthesis, structure, physicochemical properties and antibacterial activity of 1,2,4-triazoles-3-thiols and furan derivatives. Vopr Khimii i Khimicheskoi Tekhnologii 6:74-82. https://doi.org/10.32434/0321-4095-2019-127-6-74-82

Zazharskyi V, Parchenko M, Parchenko V, Davydenko P, Kulishenko O, Zazharska N (2020) Physicochemical properties of new S-derivatives of 5-(5-bromofuran-2-yl)-4-methyl-1,2,4-triazol-3-thiols. Vopr Khimii i Khimicheskoi Tekhnologii 6:50-58. https://doi.org/10.32434/0321-4095-2020-133-6-50-58

Zigo F, Elečko J, Farkašová Z, Zigová M, Vasiľ M, Ondrašovičová S, Lenka K (2019) Preventive methods in reduction of mastitis pathogens in dairy cows. J Microbiol Biotechnol Food Sci 9(1):121-126. https://doi.org/10.15414/jmbfs.2019.9.1.121-126

Zigo F, Farkasóvá Z, Eleko J, Lapin M, Chripková M, Czerski A (2014) Effect of parenteral administration of Selenium and vitamin E on health status of mammary gland and on selected antioxidant indexes in blood of dairy cows. Pol J Vet Sci 17(2):217-223. https://doi.org/10.2478/pjvs-2014-0031

Zigo F, Farkašová Z, Výrostková J, Regecová I, Ondrašovičová S, Vargová M, Sasáková N, Pecka-Kielb E, Bursová Š, Kiss DS (2022) Dairy Cows' Udder Pathogens and Occurrence of Virulence Factors in Staphylococci. Animals 12(4):470. https://doi.org/10.3390/ani12040470