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**Ερευνητικός έλεγχος για τη μέτρηση των σωματικών κυττάρων στο γάλα κασίκας στην ανατολική περιοχή της Ουκρανίας**

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## ■ Monitoring research of somatic cells count in goat milk in the eastern region of Ukraine

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**ABSTRACT.** The aim of research was to analyze the contents of somatic cells (SC) in goat milk in the East of Ukraine, level of SC translocation in the process of milking, speed of SC evacuation in their secret, to conduct monitoring and ranging on the level of SC goat milk of herds in the Eastern region during 2015 seasonally. Somatic cell count was studied on a large number of animals in different zones of the East of Ukraine (1800 milk samples). Somatic cell count in goat milk of the Eastern region of Ukraine appears up to  $800 \times 10^3$  cells/ml at 65-71% of studied milk samples by the method of laser-running cytometry with the account of only those cells having DNA in the nucleus. While analyze of SC subpopulations in goat milk it is shown that at increase to 2 million cells/ml and more the concentration of neutrophils and lymphocytes does not change and the number of macrophages and eosinophils ( $p \leq 0,05$ ) rises from 1,5 to 4 times accordingly. It is marked that SC contents in goat milk in the morning and evening yield of milk is different. In evening yield of milk, the somatic cell count is up to 30 % higher than in the morning one. A clear pattern of distribution of somatic cells in milk portions during milking was not detected. Average sample of yield is required to determine the somatic cell count in the milk of goats. Six of the eight goats over six months of lactation were given milk with a fairly constant somatic cell count. Low and fairly constant somatic cells content ( $15$  to  $63 \times 10^3$  cells / ml) is observed in the milk of primiparous goats.

**Keywords:** goat milk, somatic cell count, cell subpopulations, lactation, yield

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## INTRODUCTION

Nowadays a big question in the science and dairy industry remains the establishment of referent levels of somatic cells (SC) in goat milk, and estimation of top concentration limit which can lie in pricing and establishing of milk quality grade. It is known that somatic cell count in milk is the indicator of udder health and subclinical mastitis. Determination of somatic cells is a very important factor in the estimation of milk quality which is used besides other parameters such as chemical composition of milk (concentration of fat and protein) for determination of milk cost (Dankow et al., 2003; Shapovalov et al., 2015). In addition, somatic cell count can be an excellent index in the monitoring programs of mastitis. The increase of somatic cells is connected with the reduction of milk yield and changes in its composition.

An inflammatory process in the mammary gland of goats as a result of pathogenic microorganisms influence, toxins or tissue damage leads to the changes of milk secretion, its amount, composition and properties, increase of albumen contents in a whey, decline of milk components secretion (casein, fat, lactose), reduction of thermal stability of milk, increase of time for coagulation of milk, and its safety (Katic et al., 1994; Heeschen, 1995, 2000, Bernacka, 2006). On the basis of the above-mentioned it is possible to make a conclusion that the level increase of somatic cells in goat milk influences on its appropriateness for processing (Auldust and Hubble, 1998).

Goat milk on the average has a higher level of somatic cells (can be several millions in 1 ml) comparing with cow milk (Dankow et al., 2003). When correlative dependence between the level of SC and health of udder is clearly defined for cows the question of mastitis diagnostics by the level of SC in goat milk is debatable. So, in the United States (USA) the possible index of somatic cell count for cow milk is  $750 \times 10^3$  cells/ml, for goats and sheep - 1 million/ml. In the European Union (EU), (Directive 92/46 EEC, 1992) - the possible level of SC in cow milk is set at  $400 \times 10^3$  cells/ml, but also legislatively, the level of SC is not defined for goat and sheep milk. At present the procedure of milk quality control is regulated by international standards (By regulation of Commission (EU) № 1662/2006 - is regulation with alteration made in Regulation (EU) № 853 /2004

about hygienical rules for the food products of animal origin) in which a possible level limit of SC in goat milk also is not set. Reference levels of SC are shown only for a raw goat milk at a temperature of  $30\text{ }^{\circ}\text{C}$ ,  $\leq 1500 \times 10^3$  cells/ml, if it is intended for production of thermally processed products and  $\leq 500 \times 10^3$  cells/ml if it is intended for making products without thermal processing. Determination of SC level is suggested to express as the average geometrical meaning received during two months. However, the last research of the Norwegian scientists at IGA Conference "Quality of Goat Milk" (2013) in Tromse showed that concentration of SC in milk of healthy goats must be at the level of  $500 \times 10^3$  cells/ml (Solverod, 2013). The level of SC in milk set by the method of direct count in some herds of goats in Norway was within the limits of  $549 \times 10^3$  cells/ml, and at the end of lactation  $415 \times 10^3$  cells/ml, on other farms a maximal level of SC was:  $943 \times 10^3$  cells/ml in 2009,  $839 \times 10^3$  cells/ml in 2010,  $842 \times 10^3$  cells/ml in 2011,  $824 \times 10^3$  cells/ml in 2012 (method of direct count) (Solverod, 2013). Somatic cell count in goat milk of herds in Brazil in 2010 (state San Paulo) depending on the stage of lactation was: 159, 508 and  $277 \times 10^3$  cells/ml (Madureira et al., 2010, Gomes et al., 2006).

Divergences in received values are connected first with methods of SC level determination and usage of direct microscopic methods of count or methods with painting DNA SC and estimation of their amount by the method of laser running cytometry from one side and also with the features of goat milk secretion. As it is generally known in the process of secretion for goats and sheep cytoplasmic, apocrine particles are evolved in milk, these structures have similar diameter and morphology of leucocytes (of the basic SC population) though they contain more albumen and RNA but not DNA (Andrade et al., 2001, Dulin et al., 1982). At microscopic count, these particles are mistakenly considered as SC of goat milk. Perhaps contradictory data are also explained by the fact that the row of works was done in the period when the method of laser-running cytometry at painting DNA with etidium bromide and with the following multiple detection was not used yet. It is also necessary to mark that technical requirements outlined in the project of standard of the Eurasian council of metrology standardization and certification, Minsk 2013 "MILK

is WHOLE DRINKABLE GOAT” are the following - contents of SC in goat milk must be no more than  $750 \times 10^3$  cells/ml and for the product intended for a preschool and school feed no more than  $500 \times 10^3$  cells/ml. In Ukraine, long-term research results of T. N. Ryzhkova concerning the study of goat milk quality (2000 – 2014) became the base of the national standard of Ukraine “Goat milk raw material” elaborated and put into operation since 01.01.2010. This standard shows that for the first grade it is 500, for the second 600, for the third  $800 \times 10^3$  cells/ml.

The aim of research was: to analyze the contents of SC in goat milk in the East of Ukraine (Sumy, Kharkov, Donetsk regions), to determine the level of SC translocation in the process of milking, to offer the optimal methods of sampling, to conduct monitoring and ranging on the level of SC goat milk of herds in the Eastern region seasonally.

## MATERIALS AND METHODS

Experimental part of work included studies of lactating goats in the period of 2015. For experiment, clinically healthy goats were selected in accordance with the generally accepted methods of zootechnic research. The level of SC was studied on the greater number of animals (527 goats) in different zones of the East of Ukraine. 26 herds were involved in monitoring research. Herds of Saanen and local breed goats were from 8 to 30 animals. The goats were of 1 – 5 lactations, hand milking twice daily. In summer goats graze, while in the cold season they stay indoors. The goats have free access to feed and water. The data collection and sampling of milk was conducted in a few stages: the first two

stages: since 13 till 20 of April and since 12 till 12 of June 2015 in the Sumy region, others during a year since 14 till 17 of every following month: February, April, July, October in the Sumy region: Sumy, Lebedyn, Krasnopillja, Nedryhailiv districts, Kharkov region, Donetsk region: Starobeshevsk district, Dnepropetrovsk region. Samples of milk from each experimental goat on a farm were filtered and cooled to the temperature of  $(6 \pm 2)$  °C. Determination of SC (1800 milk samples) was conducted in the Trial center of stock-raising institute of National agrarian academy of science of Ukraine, which is accredited according to requirements of SSDU ISO/IEC 17025: 2006 (ISO/IEC 17025 : 2005, certificate of accreditation № 2T621 in the National accreditation agency of Ukraine). In the laboratory, the samples of milk were heated to  $40 \pm 0,9$ , homogenized and conducted measuring on Somacount 150 (USA) using principle of laser running cytometry of fluoro - optic electronic method in accordance with ISO 13366-2: 2007 (IDF 148-2: 2006). For contents determination of certain SC subpopulations in goat milk (eosinophils, neutrophils, monocytes, lymphocytes) was used the running cytometer with the method of immunophenotyping with specific monoclonal antibodies of CD 45 marked by fluorescent dye.

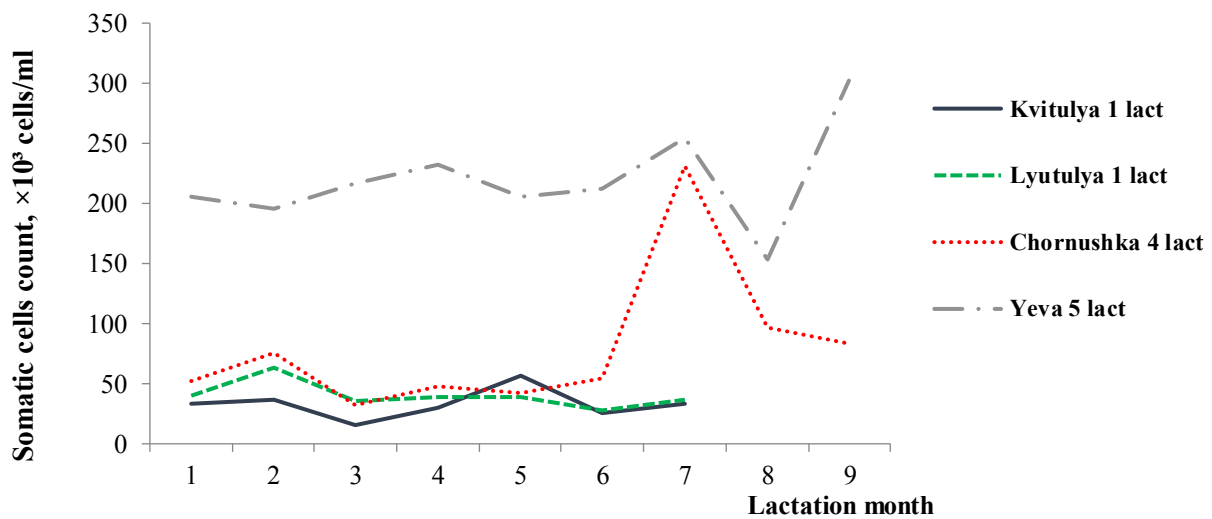
## RESULTS

While ranging the results of goat milk quality monitoring in the Eastern region of Ukraine in 2015, almost the same distribution of somatic cells in milk of goats by seasons is marked (table. 1).

Somatic cell count in the goat milk was up to  $800 \times 10^3$  cells/ml at 65-71% of all studied animals by

**Table 1:** Ranging of somatic cell count in goat milk by seasons in 2015 in the Eastern region of Ukraine

Level of ranging of somatic cell count	Spring		Summer		Autumn		Winter	
	n	% from sampling	n	% from sampling	n	% from sampling	n	% from sampling
A: $< 100 \times 10^3$	75	14	66	17	88	20	77	17
B: $100 \times 10^3 - 400 \times 10^3$	121	23	60	16	86	19	75	17
C: $400 \times 10^3 - 600 \times 10^3$	89	17	58	15	87	20	74	17
D: $600 \times 10^3 - 800 \times 10^3$	77	15	64	17	54	12	69	16
E: $800 \times 10^3 - 10^6$	76	14	62	16	71	16	67	15
I: $\geq 2 \times 10^6$	89	17	75	19	60	13	80	18



**Fig 1:** Somatic cell count in goat milk depending of lactation month

seasons, that is considerably lower than in researches of other authors. SC contents more than 2 million/ml was from 13 to 19% during a year, thus a minimum percent was in autumn time. This research can become pre-condition in the analysis of goat milk rating in the Eastern region of Ukraine.

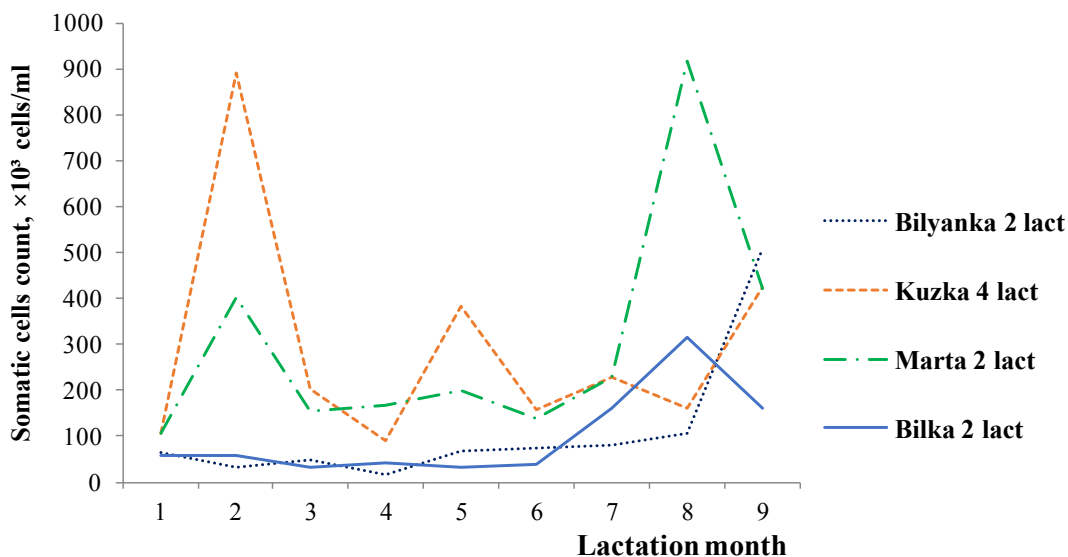
At contents determination of certain SC subpopulations in goat milk: epithelial cells and eosinophil's, neutrophils, macrophages, lymphocytes, it was shown that level of epithelial "shelling cells" was in the range of 2,1-3,7% and did not have reliable differ-

ences in milk with the different level of summary SC contents. The count of other cells was taken later on for 100% and their mass stake was determined with the use of running cytometry method (table 2).

It is shown that lymphocytes level in goat milk remains permanent enough and does not have reliable distinctions even in case of SC increase in goat milk more than 1 million/ml. However, considerable level increase of eosinophil's (to 4 times) and macrophages (1, 5 times) has been marked. The level of neutrophils at general increase of SC goes down not for certain,

**Table 2:** Ranging of SC contents, contents of SC subpopulations in goat milk in 2015 conducted in the result of milk quality monitoring in the Eastern region of Ukraine

Level of ranging	n	Mass portion of sampling, %			
		Neutrophils	Macrophages	Lymphocytes	Eosinophils
A: $< 100 \times 10^3$	30	87,80	8,1	2,10	2,0
		$\pm 4,8$	$\pm 3,1$	$\pm 0,2$	$\pm 0,7$
B: $100 \times 10^3 - 400 \times 10^3$	12	84,50	11,1	2,00	2,4
		$\pm 6,2$	$\pm 5,6$	$\pm 0,1$	$\pm 0,5$
C: $400 \times 10^3 - 600 \times 10^3$	14	82,90	11,8	2,40	2,9
		$\pm 5,3$	$\pm 3,4$	$\pm 0,4$	$\pm 0,1$
D: $600 \times 10^3 - 800 \times 10^3$	14	81,10	12,0	2,40	4,5
		$\pm 2,5$	$\pm 2,1$	$\pm 0,7$	$\pm 0,1$
E: $800 \times 10^3 - 10^6$	15	77,40	14,2	2,40	6,0
		$\pm 9,7$	$\pm 3,1$	$\pm 0,7$	$\pm 0,1$
I: $\geq 2 \times 10^6$	17	73,40	15,8	2,70	8,1
		$\pm 5,4$	$\pm 2,7$	$\pm 0,5$	$\pm 0,2$



**Fig 2:** Somatic cell count in milk from other goats depending of lactation month

perhaps due to the increase of other subpopulations of lymphocytes.

It has been discovered that the range of SC con-

tents in goat milk was from 11 thousand to 2 million when using method of laser-running cytometer (painting DNA of every SC) (table. 3). A difference

**Table 3:** Somatic cell count in goat milk in the morning and evening yield of milk at farm in Sumy region

Names of experiment animals	Spring (March) 2015				Summer (June) 2015			
	14.04 evening, ×10 <sup>3</sup> cells/ml	15.04 morning, ×10 <sup>3</sup> cells/ml	Δ evening / morning, ×10 <sup>3</sup> cells/ml	Δ evening / morning, %	13.06 evening, ×10 <sup>3</sup> cells/ml	14.06 morning, ×10 <sup>3</sup> cells/ml	Δ evening / morning, ×10 <sup>3</sup> cells/ml	Δ evening / morning, %
Belka	442	198	244	55,2	703	282	421	59,9
Kvitka	1824	1053	771	42,3	716	297	419	58,5
Rosa	255	141	114	44,7	721	731	-10	-1,4
Chernushka	2070	1458	612	29,6	1416	1488	-72	-5,1
Dina	571	268	303	53,1	504	444	60	11,9
Anfisa	619	465	154	24,9	494	614	-120	-24,3
Ljuta	63	37	26	41,3	567	633	-66	-11,6
Aza	53	34	19	35,8	598	621	-23	-3,8
Marta	190	64	126	66,3	71	90	-19	-26,8
Berezka	170	71	99	58,2	62	80	-18	-29,0
Roza	175	116	59	33,7	52	47	5	9,6
Marta	177	115	62	35,0	45	27	18	40,0
Belka	526	471	55	10,5	839	744	95	11,3
Kvitka	362	376	-14	-3,9	1131	536	595	52,6
Romashka	11	12	-1	-9,1	1441	2177	-736	-51,1
Mean value	500,5	325,3	175,3	34,5	624,0	587,4	36,6	6,0

between the morning and evening yield of milk in individual samples was from 1 to 770 thousand. A middle difference of SC contents in goat milk between the morning and evening yield of milk was from 35 to 150 thousand cells.

Somatic cell count during lactation of eight goats is presented in two charts (fig.1-2). Lactation curves of animals milks which are characterized by a more or less constant and low somatic cell count are placed at Figure 1. Kvitulya, Lyutulya – primiparous goats, their first lactation lasted only 7 months. But milk differed of very low and fairly constant somatic cell content (from 15 to  $63 \times 10^3$  cells/ml). Figures 1, 2 shows that six of the eight goats were given milk with a fairly constant level of somatic cells over six months of lactation. On the contrary, milks of goat Kuzka (fourth lactation) and Marta (second lactation) are characterized of frequent changes of somatic cell count (fig. 2).

Somatic cell count at the last month of lactation in three goats increase, in the other three goats, on the contrary, – decrease compared to eighth month.

An important achievement is that goat milk of

the first month of lactation (5-8 days after kid birth) contains a very low somatic cell count – from 33 to  $107 \times 10^3$  cells/ml. The exception is goat Eva milk which contains  $206 \times 10^3$  cells/ml. But milk from this animal is researched for several years, and her level of somatic cells is always  $250-300 \times 10^3$  cells/ml in the main months of lactation, which is her physiological norm.

## DISCUSSION

According to Ukraine requirements it is permitted to  $500 \times 10^3$  cells / ml for the highest quality goat milk. In Europe, the best goat milk is considered with somatic cells count  $\leq 1$  million / ml. Somatic cell count in the goat milk was up to 1 million cells/ml at 81-87% of all studied animals according to our results. We have to review Ukrainian requirements for somatic cells count of goat milk.

It is defined that in spring period of experiment on the herd of goats at farm in Sumy region general contents of SC in the evening milk was at the level of  $500 \times 10^3$  cells/ml and in the morning milk -  $325 \times 10^3$  cells/ml i.e. 34 % more (table. 3).

**Table 4:** Somatic cell count,  $\times 10^3$  cells/ml in goat milk in different milking portions

	Milk portions			Ratio accumulation of SC in %		
	1*	2**	3***	$\Delta$ 2/1	$\Delta$ 2/3	$\Delta$ 3/1
Sabina	27	27	449	0,00	93,99	93,99
Ljuta	77	85	225	9,41	62,22	65,78
Regina	106	98	103	-8,16	4,85	-2,91
Marta	205	300	335	31,67	10,45	38,81
Dina	450	550	620	18,18	11,29	27,42
Kvitka	700	1252	1658	44,09	24,49	57,78
Kuzka	37	40	47	7,50	14,89	21,28
Marta	1033	642	1388	-60,90	53,75	25,58
Chornushka	39	43	45	9,30	4,44	13,33
Bilka	43	41	43	-4,88	4,65	0,00
Kvitulya	90	111	74	18,92	-50,00	-21,62
Lyutulya	45	47	46	4,26	-2,17	2,17
Bilyanka	47	50	50	6,00	0,00	6,00
Eva evening 19.12.13	346	391	600	11,51	34,83	42,33
Eva morning 20.12.13	334	374	448	10,70	83,48	25,45
Eva evening 28.01.14	431	448	351	3,79	-27,64	-22,79
Eva morning 29.01.14	650	700	675	7,14	-3,70	3,70

Notice: \* cisternal, \*\* transit - alveolar, \*\*\* alveolar

Analogical researches were got by F. Cedden et al. in 2008 in Turkey, however in a summer period such regularity was practically at the level of tendency (on the average 6% higher) in count to 1000/ml, but at count to the total evening and morning yield of milk SC contamination was higher in the evening milk.

Such difference may be explained by influence of different factors: the factor of photoperiodism, greater motion activity in the interval of light day and correspondingly the speed of metabolism, the interval of time from milking to milking - the bigger interval the more yield of milk and value of milk components concentrations. Thus, it is shown that this index is very variable even for one animal when milking in different time of the day. Others study led for this same conclusion (Zazharska N. et al., 2015). 170 milk samples from eight goats were studied during more than year and a half. In each animal there was an increase of somatic cell count as the evening and morning. The dependence of the number of somatic cells occasionally yield time was not found. The index can change twice and more during the day. The somatic cell count in goats increases with age, late lactation and influenced by other factors, even without infectious agents (Maurer J. et al., 2013). Noted the big somatic cell count ( $> 2000$  thousand / ml) at low bacterial contamination ( $19,6 \times 10^3$  CFU / mL) of goat milk (Zazharska N., 2016).

On the next stage, the level of SC at milk excretion was studied (table. 4). It is known that at milking the first streams differentiate on chemical composition: less high-fat than in the last streams. It is explained by complicated mechanisms and concerted reflex reactions of myoepitely and smooth musculature of udder, walls and sphincter of nipple. It is found that somatic cells count in different portions of milk within a milking change in healthy goats. It is shown that amount of SC in 65% samples in cisternal milk is considerably lower than in alveolar one (from 2 to 94%). In four samples, largest number of somatic cells is defined in the middle of milking and in two cases – observed in cisternal milk. Dependence SC content in the alveolar milk from primary level in cisternal one is also not defined. Perhaps stimulation of milk giving simultaneously causes the intensive excretion of SC by alveolar epithelium that leads to their rapid and considerable

increase in the following portions of milk yield. It is also set by numerous studies that alveolar portion is relatively steady while cisternal part of milk yield varies considerably. Their ratio changes depending on individual distinctions of animals and stages of lactation, age of goats, interval and stereotype of milking, capacity of udder, cisternal pressure and other. Moreover, even in milk samples of one animal somatic cells are distributed differently. For example, at the eighth month of lactation of goat Eva the largest somatic cells count is noticed in the end of milking, and next month before dry period – in the middle of milking. That also points to the great volatility of this parameter. So, sample of goat milk cannot be taken at the beginning, middle or end of milking for determination of somatic cell count. It is necessary to select an average sample.

## CONCLUSIONS

Somatic cell count in goat milk of the Eastern region of Ukraine appears up to  $800 \times 10^3$  cells/ml at 65-71% of studied milk samples by the method of laser-running cytometry with the account of only those cells having DNA in the nucleus.

At ranging of SC contents, contents of SC subpopulations in goat milk it is shown that at increase to 2 million cells/ml and more the concentration of neutrophils and lymphocytes does not change for certain and the number of macrophages and eosinophils certainly ( $p \leq 0,05$ ) rises from 1,5 to 4 times accordingly.

It is marked that SC contents in goat milk in the morning and evening yield of milk is different. In evening yield of milk, the level of SC is up to 30 % higher than in the morning one. It causes the necessity of milk sampling during two adjoining days proportionally to daily yield of milk for determination of SC concentration in milk.

A clear pattern of distribution of somatic cells in milk portions during milking was not detected. Average sample of yield is required to determine the somatic cell count in the milk of goats.

Six of the eight goats over six months of lactation were given milk with a fairly constant level of somatic cells. In the first month of lactation milk of seven goats contains a very low somatic cells count from 33 to  $107 \times 10^3$  cells / ml. Low and fairly con-



stant somatic cells content ( $15$  to  $63 \times 10^3$  cells / ml) is observed in the milk of goats-firstborn, though their first lactation lasted only 7 months.

## CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest. ■

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