Chapter 1. Assessment of safety and quality of goat's milk

N. M. Zazharska
Dnipro State Agrarian and Economic University

The criteria for safety and quality assessment of goat's milk[1]

According to the European regulation 853/2004 only microbiological criteria have been put forward for goat milk: bacterial contamination at a temperature of 30 °C is ≤ 1500×10^3 CFU/cm^3; in milk intended for the production of products without heat treatment the content of microorganisms – ≤ 500×10^3 CFU/cm^3. It is substantiated that the National Standards of Ukraine (DSTU) "Goat's milk. Raw. Specifications: DSTU7006: 2009" does not meet the international requirements for goat milk (Zazharska, 2018). According to the requirements of existing normative document DSTU7006:2009 the number of mesophilic aerobic and extra-anaerobic microorganisms of milk of the second grade (the extreme limit) corresponds to the best milk in accordance with the European regulations. According to Ukraine requirements it is permitted somatic cell count to 500×10^3 cells/cm^3, bacterial contamination ≤ 100×10^3 colony forming units (CFU)/cm^3 for the highest quality goat milk. In most European countries and the USA, the best goat milk is considered with somatic cell count ≤ 1 million/cm^3, bacterial contamination ≤ 500×10^3 CFU/cm^3. Consequently, the requirements for the indexes of bacterial contamination and somatic cell count in the Ukrainian normative document are very strict.

Indicators of density and acidity of goat milk are not regulated in EU countries, but regulated in Ukrainian standard.

[1] Zazharska N. M. (2019). Assessment of safety and quality of goat's milk. In: Current problems of agrarian industry in Ukraine. Accent Graphics Communications & Publishing, Vancouver, Canada. – P. 3–33. Doi:10.15421/511902

The aim was to scientifically substantiate the requirements of the Ukrainian standard for goat milk, especially for indicators of acidity, density, somatic cell count and bacterial contamination.

836 samples of goat milk were taken from farms of Dnipropetrovs'k and Transcarpathian oblasts during 2013–2017 years. Physico-chemical parameters (acidity, density, indicators of fat and protein) of goat's milk were determined using the ultrasound analyzer "Ekomilk". The somatic cell count was determined by viscosimetric method and flow cytometry.

According to our research the indicators of the acidity in goat milk vary from 14 °T to 27 °T, of the density – from 25.6 °A to 35.4 °A. The smallest somatic cell count in the goat milk was observed in the autumn 265 ± 41 × 10^3 cells/cm^3, the highest – in the winter – 451 ± ± 46 × 10^3 cells/cm^3 ($p < 0.05$) (viscosimetric method).

The smallest somatic cell count throughout the life of the animal was observed in the milk of goats of the first lactation – 712 ± 174 × 10^3 cells/cm^3. In goats from the second to the fourth lactation, somatic cell count was recorded at the level of 880–1092 × 103 cells/cm^3 (flow cytometry method).

Indicators of fat and protein in goat milk are drastically reduced in the summer, therefore the requirements of the Ukrainian standard of 3.3 and 3.0% respectively are not substantiated.

According to the obtained data it was determined that the somatic cell count in goat milk exceeds the index from DSTU7006: 2009 "Goat's milk. Raw. Specifications: DSTU7006: 2009", twice or more. It was found that the existing standard does not meet the international requirements for goat milk based on the main indicators (bacterial contamination, somatic cells, acidity and density). The ineffectiveness of the criteria of density and acidity for goat milk assessment was substantiated because of their insignificance. It was proposed to exclude the indexes of density and acidity of goat's milk from DSTU, to decrease the requirements for the fat and protein content, and to align the requirements for the somatic cell count and bacterial contamination in accordance with European standards.

Parameters of subclinical mastitis in goats

The universal definition of a somatic cell count threshold to distinguish between healthy and sick udder halves in goats does not exist yet.

The aim of this work was to establish the possible diagnostic parameters of subclinical mastitis in goats. 27 samples milk of goats were researched.

The main parameters of milk were analyzed by means of ultrasonic analyzer of milk of "Ekomilk type MILKANA KAM 98–2a" (Bulgary), the somatic cell count was determined at viscometric analyzer "Somatos-M" (Russia), milk films were stained with pironin Y and May-Grünwald methods. Chloride ion content in milk was determined by titrimetric method. Chlorine-sugar number was counted, settling test and mastidin test were also conducted.

The data were analysed in Statisica 6.0 (StatSoft Inc., USA). The data in the tables are presented as x ± SE (x ± standard error). The differences between the values in groups were determined using Tukey test, where the differences were considered significant at $P < 0.05$ (with taking into account the Bonferroni correction).

The samples were divided into 3 groups after determination of biochemical parameters, according to the chloride content in the milk: group I – < 250 mg%; group II – 250–300 mg%; group III – > >300 mg% (tab. 1). The indexes of chloride content were significantly different between three groups of samples ($P < 0.001$).

The index of fat content increased by 0.4% in goat's milk with a chloride concentration > 300 mg%, protein – by 0.24%, lactose – by 0.28%, milk solids – by 0.66%, pH – by 2.8%, the freezing point decreased by 7.2% in comparison to I group of milk samples (with a chloride content < 250 mg%), but it was not statistical difference.

In the group of samples with a chloride content > 300 mg%, the somatic cell count increased by 3.2–5.7 times compared to the group with chloride concentration < 250 mg%, depending on the method of study ($P < 0.05$ and $P < 0.001$ accordingly). The parameters of somatic cell count of the second group (with a chloride content

250–300 mg%), were 2.1–3.8 times higher compared with the first group ($P < 0.05$ and $P < 0.01$ respectively).

The chlorine-sugar figure in samples of milk of healthy goats (with a chloride content < 250 mg%) is average 5 (from 4.1 to 5.9). The chlorine-sugar figure is 7.2 (from 6.5 to 7.9) in milk samples of III group (with a chloride content > 300 mg%).

Table 1. – Parameters of goat milk depending on the content of chlorides (x ± SE)

Parameter	Milk sample groups according to the content of chlorides, mg%		
	I, n = 8	II, n =13	III, n = 6
Chloride content, mg%	223.7 ± 4,7[a]	270.4 ± 3,8[b]	344.4 ± 14,8[c]
somatic cell count, × 10^3 cells/ml: "Somatos"	439 ± 159[a]	1672 ± 292[b]	2500 ± 316[b]
May-Grünwald	634 ± 169[a]	1569 ± 323[b]	2149 ± 560[b]
pironin Y	703 ± 213[a]	1484 ± 276[b]	2273 ± 539[b]
Fat,%	4.35 ± 0,74	4.28 ± 0,47	4.75 ± 0,62
Dry non-fat milk solids,%	8.20 ± 0.27	8.13 ± 0.10	8.86 ± 0.34
Density, °A	27.2 ± 1.4	27.0 ± 0.4	29.6 ± 1.7
Protein,%	3.06 ± 0.09	3.02 ± 0.04	3.30 ± 0.12
Freezing point, °C	–0.540 ± ± 0.015	–0.533 ± ± 0.006	–0.579 ± ± 0.023
Lactose,%	4.52 ± 0.16	4.48 ± 0.05	4.80 ± 0.24
Conductivity, mS/cm	4.73 ± 0.26[a]	5.35 ± 0.13[b]	6.21 ± 0.46[b]
pH	6.71 ± 0.04	6.67 ± 0.04	6.90 ± 0.16
Chlorine-sugar figure	5.00 ± 0.23[a]	6.05 ± 0.14[b]	7.21 ± 0.27[c]
Bacterial contamination, CFU/cm^3	1.1 ± 0.24 × ×10^5	9.0 ± 4.9 × ×10^5	1.6 ± 0.5 × ×10^6
Mastitis pathogens	–	–	+
Mastidin test	–	+	+
Settling test	–	–	+

Note: different letters within the line correspond to the selections which had significant differences between one another according to the results of Tukey's test ($P < 0.05$) with Bonferroni correction

The chlorine-sugar figure in the milk samples of the I group was significantly less than in the II and III groups by 20.9% and 44% respectively ($P < 0.001$). This parameter in the goat milk of the III group is more than in the II group by 19% ($P < 0.01$).

The electrical conductivity in the samples of the I group was less than in the II by 13.1% and than in the III – by 31.3%, ($P < 0.05$). H. Schüppel & M. Schwope determined the average electrical conductivity in goat milk 6.6 ± 0.5 mS/cm (Schüppel & Schwope, 1999). Unfortunately, these indicators do not coincide with the results of our research – the electrical conductivity of milk in healthy goats was within the limits 4.73 ± 0.26 mS/cm. However, the absolute threshold for the differentiation of infected and uninfected mammary glands of goats has not yet been found (Barth, 2009). A significant correlation between the electrical conductivity and the somatic cell count that is known in dairy cows does not seem to exist in dairy goats (Park, 1991).

Positive settling test was observed in the samples of III group (with a chloride content > 300 mg%). Positive mastidin test with goat's milk was observed in the samples of II and of III group.

Bacterial contamination increased with the growing of the chloride content in goat's milk, but statistical difference was not been detected. *Streptococcus agalactiae* was isolated in 2 of 6 milk samples of the III group. It was established that combination of such indexes as the somatic cell count > 2 million/ml, the chloride ion content > 300 mg%, chlorine-sugar figure 7 and above, a positive settling test and mastidin test, can serve as a criterion for detecting subclinical mastitis in goats (Zazharska et al., 2017 b).

The study of goat milk on subclinical mastitis and comparison of different methods of determining the somatic cell count was carry out. The herd of goats, German white, Anglo-Nubian and Alpine and local breeds, was studied on the subclinical mastitis twice: in the fall 83 dairy goats were examined, in the spring – 144 animals. The first portions of milk were collected, after which they were examined by mastidin test and Californian mastitis test. Samples of milk were taken to the Laboratory of Food Hygiene at the Department of Parasitology, Veterinary and Sanitary Expertise at the Dnipro State

Agrarian and Economic University. The settling test was conducted there. The determination of somatic cell count in milk was carried out by viscosimetric method. We also made milk films and stained them by the May-Grünwald method (Fig. 1).

After that the somatic cell count using microscope was calculated. As a result of bacteriological research of milk on pathogens mastitis *Staphylococcus aureus* was isolated in autumn. For six months the number of goats with subclinical mastitis decreased from 12 to 8%, by improving the control of the udder health of the animals on the farm. The mastidin test was better than the California mastitis test with goat milk, due to the formation of a tighter clot. From the milk samples which were positive or questionable on mastidin test, 29% samples were found consistent with the requirements of DSTU7006: 2009 "Goat's milk. Raw. Specifications" for second grade of goat's milk by viscosimetric method and settling test.

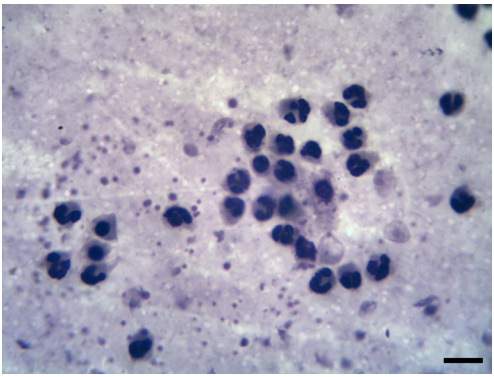

Figure 1. Somatic cells in smears of milk of goat with subclinical mastitis: May-Grünwald stain; bar – 10 μm

But by the arbitration method (direct microscopy) the somatic cell count did not meet the requirements of DSTU7006:2009 in all samples. The exact somatic cell count in goat's milk should be determined only by direct microscopic or fluoroptoelectronic counting. In milk samples, which revealed the largest somatic cell count in milk films (> 20 million/ml), only $2818 \pm 956 \times 10^3$ cells/cm^3 was determined by viscometric method, which proves the accuracy of the arbitration

method. To obtain milk with a low somatic cell count using the viscometric method (< 600 thousand/cm^3) for high-quality cheese production a continuous renovation of the herd was recommended because the lowest number of somatic cells in milk observed in goats of the first lactation (primiparous goats) (Zazharska & Rosenko, 2018).

Comparison of methods efficiency for determination of somatic cell count in goat milk

It was compared different methods of determination of somatic cell count in goat milk (Zazharska & Zharko, 2016). Somatic cell count of individual milk samples from 28 goats were analyzed by means of analyzer "Somatos" (viscosimetric method), "SomaCount-FlowCytometer" (flow cytometry) and the by counting of cells in milk films stained with pironin Y, at May-Grünwald and Romanovsky – Giemsa methods.

There were not found the samples with somatic cells count to 100 × 10^3 cells/ml while counting cells in milk films stained by any method. According to the research by means of "SomaCountFlowCytometer" and "Somatos" most of milk smears be-longed to the level of – 101–500 × 10^3 cells/ml – 35.7 and 50% re-spectively. The biggest part of milk smears – stained by Roma-novsky – Giemsa method – 42.9% and by May-Grünwald method – 39.3% related to the range of 1001–3000 × 10^3 cells/ml, whereas films, stained with pironin Y and methyl green – 35.7% – to a range of 501–1000 × 10^3 cells/ml.

The greater indexes of somatic cell count in direct counting of cells in goat's milk smears, stained by any method was determined than using devices. It was proved the accuracy of direct counting method because the distribution ranges of somatic cells was similar between different methods of film staining.

The cytoplasm and nuclei of somatic cells are well stained in goat milk films stained by May-Grünwald method (Fig. 2). So, this method is proposed for counting of somatic cells by Prescott and Breed method in goat's milk. It is proved that the quality of goat's milk films stained by the May-Grünwald method for counting of somatic cells corresponds

to the recommended method with pyro-nin Y (Fig. 3), and the cost of dyes is lower by 28.4 times.

The Romanovsky – Giemsa method is not quite acceptable for goat's milk films, because we have received higher rates of somatic cell count than other methods of staining because of cytoplasmic particles. Also "shadows" of cells, particles of a paint appear often in milk smears (Fig. 4). So, for the veterinary medicine practice, it is proposed: method of the May-Grünwald for staining of goat's milk films for counting somatic cells by Prescott and Breed method.

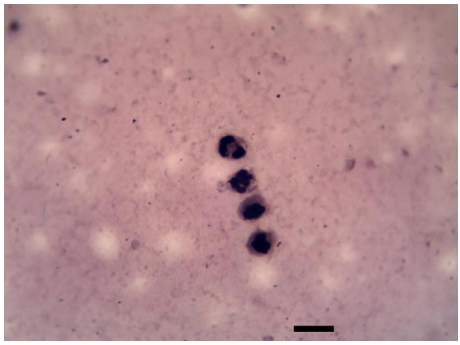

Figure 2. Somatic cells in goat's milk smears: May-Grünwald stain; bar – 10 μm

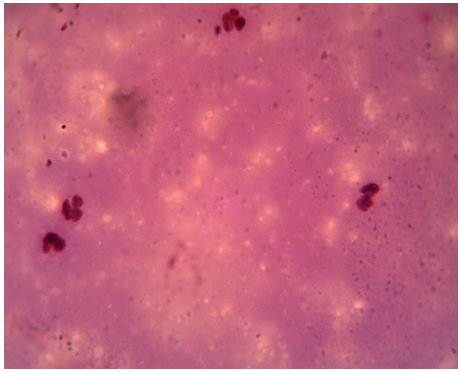

Figure 3. Somatic cells in goat's milk smears (control): pyronin Y stain; bar – 10 μm

The content of fat in goat's milk was gradually raised while increasing of somatic cell count, but significant difference was not

found. Also a positive correlation between the somatic cell count and protein and lactose was found, but in the milk with somatic cell count of more than 3 million/ml there was a sharp decrease. The opposite trend was observed concerning the freezing point of milk. The freezing temperature increased while reducing of protein in goat milk.

The study of Hungarian scientists also showed a positive correlation ($P < 0.01$) between the somatic cell count and the protein content ($r = 0.67$; $P < 0.001$) and a negative correlation between the somatic cell count and the lactose content ($r = –0.41$, $P < 0.001$) and the freezing point ($r = –0.33$) in goat's milk (Pajor et al., 2013).

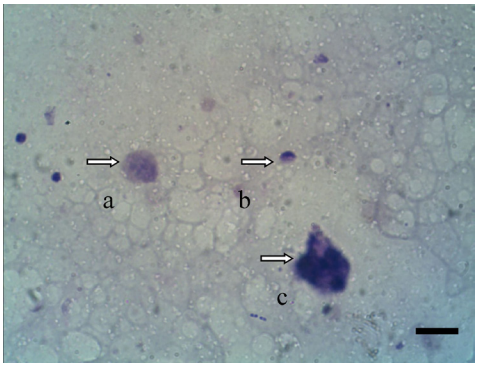

Figure 4. Disadvantages in goat's milk smears: Romanovsky – Giemza stain; bar – 10 µm; *a – cytoplasmic particle, b – "shadow" of the cell, c – piece of paint*

There was no correlation between the somatic cell count and the content of protein, fat and lactose in goat milk according to the results of other scientists (Bagnicka et al., 2016). Contrary, polish scientists point out that there is an effect of the somatic cell count on lactose content in goat milk (Czopowicz et al., 2013).

Monitoring studies of goat's and cow's milk in France and Ukraine

The safety and quality of raw milk in Ukraine remained the biggest problem in retooling of dairy enterprises by newest processing lines, the introduction of modern quality control systems. In France

every farmer is interested in improving of product quality because it affects the price of milk. Monitoring of goat milk indexes was conducted in Ukraine, compared with similar ones in the milk laboratory of LILCO (Laboratoire Interprofessionnel Laitiere du Centre Ouest – Interprofessional milk laboratory of center and west), Surgères, France.

LILCO – one of 16 laboratories for control of milk quality in France. LILCO serves more than 5 thousands farmers who get milk from cows and goats. Laboratory analyses the milk from the herd of each farmer three times a month. Based on the results of milk analysis laboratory forms the price, that the dairy company has to pay to the farmer. In Ukraine, unfortunately, dairy plants do not accept goat milk for processing, although it is a valuable raw material than cow milk (Zazharska, 2015).

Laboratory LILCO determines fat, protein, somatic cells count, freezing point, lipolysis, microbial contamination, inhibitors, butyric bacteria in the milk of cows and goats. All physical and chemical indexes of milk were determined by devices FossomaticTM FC and MilkoScanTM FT+. To 16 thousand samples of milk per day are analyzed in the laboratory. Many methods of verification of the accuracy of analyzes, including repeatability and reproducibility, tracage, reference methods take place to control the operation of very expensive and modern apparatuses. Reference methods for fat (acid Gerber method), protein (with amide black), freezing point (by cryoscope) perform daily to control the accuracy of machines MilkoScan. There is also an internal control sample and "gamma" (10 samples with known parameters) from the reference-laboratory Ceca Lait. Microbial contamination of milk is determined by epifluorescence microscopy (FOSS Integrated Milk Testung BactoScanFC), reference method – passaging through the nutrient medium (Zazharska, 2016b).

Laboratory LILCO was accredited to ISO 17025 by Committee of Accreditation COFRAC (Comité français d'accréditation). Assurance of quality of testing and research methods of analyses was provided by CNIEL (Centre national interprofessionnel de l'economie

laitière – National interprofessional center of dairying). Laboratory data of 2013, 2014 years were statistically processed during the internship.

Laboratory analyzed per year more than 3.5 million of milk samples for determination of fat, protein, somatic cells count. Number of samples of goat milk was about 4 times less than of cow milk, the majority of samples were from individual animals.

The objective of the study was to compare the parameters of cow's and goat's milk on the basis of analysis in French laboratory during two years. Fat content in cow milk in France was higher than in goats. In cow milk indicators of fat were almost constant throughout the year, from 4.0% in summer to 4.3% in winter (fig. 5).

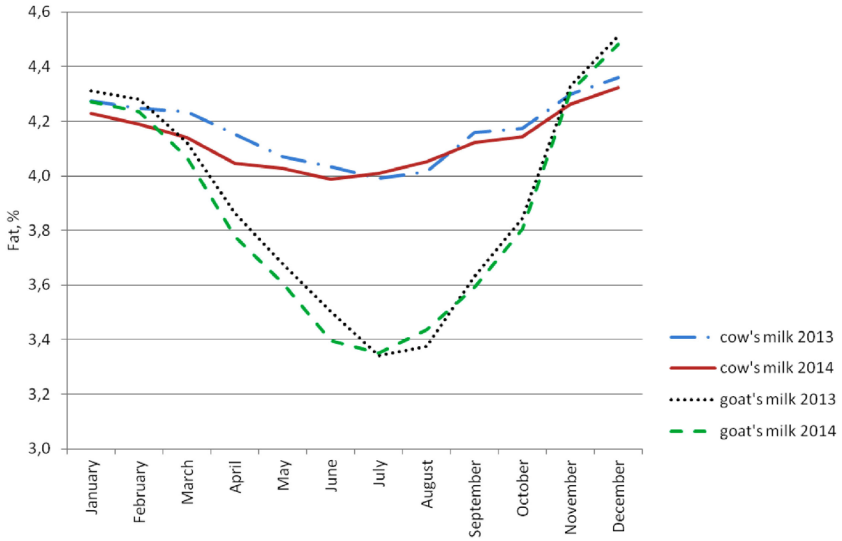

Figure 5. Fat content in milk of cows and goats for two years (%, n ~ 292 thousand samples per month, according to the data of LILCO)

Fluctuations of this indicator in goat milk were more significant – from 3.3% in summer to 4.5% in winter. A similar tendency was observed in regard to protein (Fig. 6).

In cow milk protein content was almost at the same level (3.3%) throughout the year, decreasing slightly to 3.2% in June and July. The protein content in goat milk fluctuated greatly: from 3.7% in January the figure gradually decreased to 3.1% in June and July, and then increased to 3.9% in December.

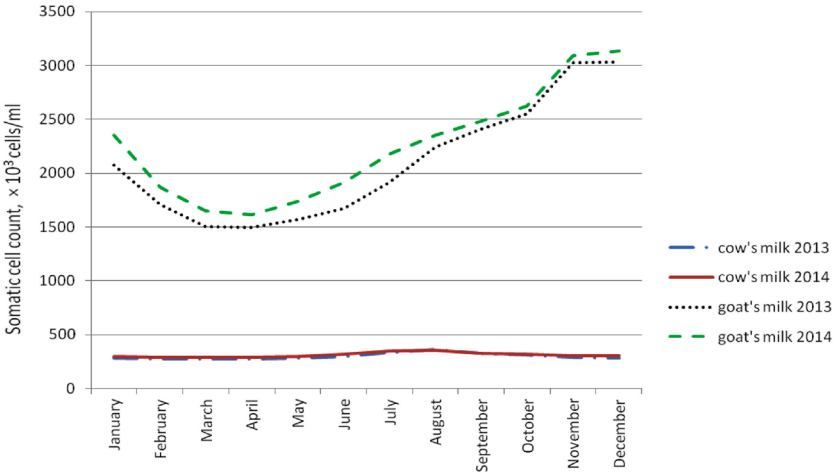

Figure 6. Protein content in milk of cows and goats for two years (%, n ~ 292 thousand samples per month, according to the data of LILCO)

So, according to the processing of statistical data obtained in the LILCO in cow milk indicators of fat and protein were almost constant throughout the year, in goat milk – are gradually decreased in the summer. Somatic cell count in cow milk during the year was in average 307 ± 5 × 10^3 cells/ml; in goat milk in March – the lowest level of about 1577 ± 77 × 10^3 cells/ml, and in December was 2 times more (flow cytometry method) – (Fig. 7).

Infectious and non-infectious factors are influenced greatly on the somatic cells count in goat milk, unlike in cow milk. It was established that somatic cell count did not correlate with the index of bacterial contamination in goat milk.

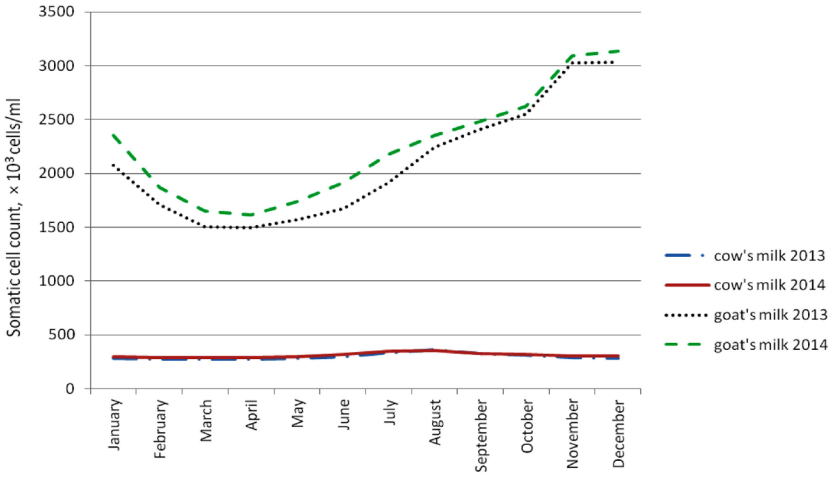

Figure 7. Somatic cell count in milk of cows and goats for two years (× 10^3 cells/ml, n ~ 293 thousand samples per month, according to the data of LILCO)

In France, the best goat milk is considered with somatic cell count ≤ 1 million/ml. But dairy plants can take for processing goat milk with somatic cells count up to 3 million/ml at a reduced price. According to Ukraine requirements it is permitted to 400 × 10^3 cells/ml for the highest quality cow milk, and for the goat milk – up to 500 × × 10^3 cells/ml. Perhaps we have to review Ukraini-an requirements for somatic cell count of goat milk.

Bacterial contamination of cow milk was on average 22–24 × 10^3 CFU/ml during the period of two years (Fig. 8).

Requirements for cow milk indexes in France are stricter than EU Directive concerning bacterial contamination and somatic cell count.

The fluctuations of indexes of microbial contamination in goat's milk were from 17 to 35 × 10^3 CFU/ml during two years ac-cording to the data of LILCO. The indexes of somatic cell count of milk did not always correlate with microbial contamination, espe-cially in goat's milk.

Freezing temperature of cow's milk was about –0,52 °C, of goat's milk – about –0,55 °C (Fig. 9).

The level of lipolysis in cow's milk varied within 0,55–0,71 mg equivalent/100 g fat. The lipolysis indexes in goat's milk increased from March to June (to the 0.67 mg equivalent/100 g fat), and then insensibly decreased until September.

The parameter of milk density is not determined in the laboratory LILCO because this indicator is considered non-informative. To determine the falsification of milk with water, they use a freezing point. The acidity of milk is also not determined, because all farmers have cooling tank, and milk collection is in accordance with the schedule.

Comparative analysis of indicators of goat and cow milk in Ukraine: significant variations in acidity, density, somatic cell count of goat milk during the year were noted (Table 2).

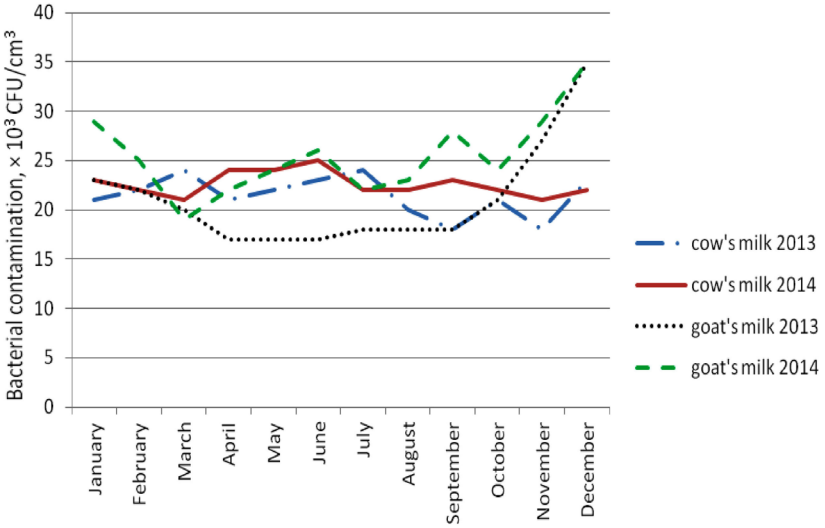

Figure 8. Bacterial contamination in milk of cows and goats for two years (× 103 CFU/ml, n ~ 16 thousand samples per month, according to the data of LILCO)

Indicators of the acidity in goat milk vary from 14 °T to 27 °T, of the density – from 25.6 °A to 35.4 °A. In milk of cows the average index of acidity is 17.6 ± 1.7 °T, of density – 28.6 ± ± 1.3 °A. The smallest somatic cell count in the goat's milk was observed in the autumn 265 ± 41 × 103 cells/ml, the highest – in the winter – 451 ± 46 × 10^3 cells/ml (P < 0.05) (Table 2). These indexes corre-spond to the "higher" grade of goat's milk in accordance with "Goat's milk. Raw. Specifications: DSTU7006: 2009" – up to 500 × 10^3 cells/cm^3.

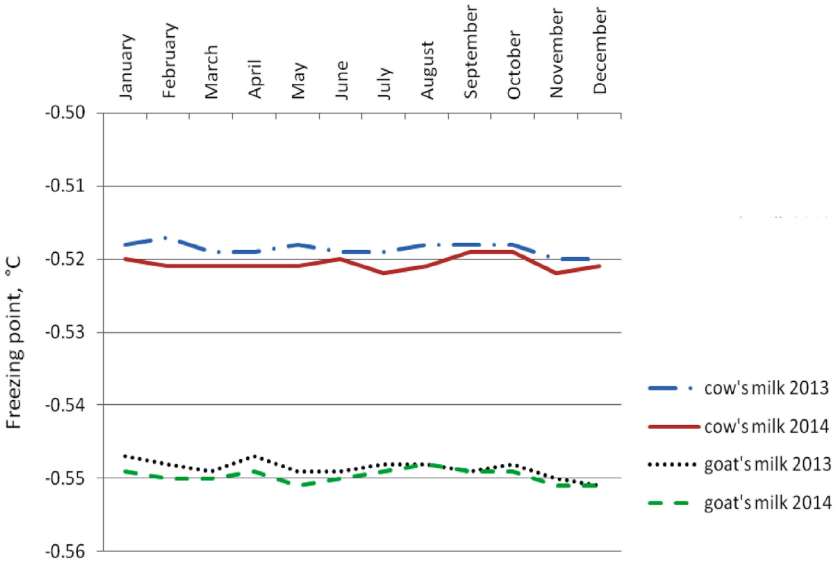

Figure 9. Freezing temperature in milk of cows and goats for two years (°C, n~18 thousand samples per month, according to the data of LILCO)

In cow milk the biggest somatic cell count was observed in winter 205 ± 67 ×10^3 cells/ml and in summer – six times less (P < 0.05, tab. 3). These values of somatic cells correspond to the "extra" grade of cow's milk – up to 400 × 10^3 cells/cm^3 (Zazharska, 2014; Zazharska & Pryadka, 2015).

Table 2. – Results of seasonal study of goat's milk in Ukraine (x ± SE)

Season, number of samples	Acidity, °T	Density, °A	Somatic cell count (by viscosimetric method), × 10^3 cells/ml
Spring, n = 7	17.3 ± 2.7	29.7 ± 1.5	336 ± 77^{ab}
Summer, n = 12	17.6 ± 2.8	29.2 ± 1.2	388 ± 50^{ab}
Autumn, n = 53	21.8 ± 3.2	29.8 ± 1.9	265 ± 41^{a}
Winter, n = 6	21.3 ± 1.6	30.5 ± 1.0	451 ± 46^{b}

Note: different letters within the column correspond to the selections which had significant differences between one another according to the results of Tukey's test (P < 0.05) with Bonferroni correction

Table 3. – Results of seasonal study of cow's milk in Ukraine (x ± SE)

Season, number of samples	Acidity, °T	Density, °A	Somatic cell count (by viscosimetric method), × 10^3 cells/ml
Spring, n = 15	17.2 ± 1.0	28.7 ± 1.1	111.7 ± 29.5^{ab}
Summer, n = 14	15.1 ± 1.3	27.9 ± 1.1	34.1 ± 8.8^{a}
Autumn, n = 12	16.8 ± 1.2	28.4 ± 1.4	164.8 ± 17.5^{ab}
Winter, n = 8	18.2 ± 1.3	28.9 ± 1.6	204.9 ± 67.5^{b}

Note: different letters within the column correspond to the selections which had significant differences between one another according to the results of Tukey's test (P < 0.05) with Bonferroni correction

The objective of the study was to estimate the effect of the number of lactation on the indexes of milk of cows and goats by breeds. The material of research were milk samples from 10 cows of Holstein

breed and 17 goats of German white, Anglo-Nubian and Alpine breeds. The samples were analyzed on the somatic cell count, the content of fat, protein during the period of four lactations. The biochemical indexes of milk were determined by means of "Ekomilk" and "Dairy Spec Bentley Instruments". The somatic cell count in milk samples was determined by flow cytometr "SomaCount Bentley Instruments". It was established that the indexes of milk such us fat and protein depend on the quality of feeding basically, and not on the quantity of lactations. The indexes of somatic cell count in milk of cows of the 2nd, 3rd, 4th lactation was more at 5,2, 4,6 and 4,9 times respectively, according to the parameter of the first lactation ($P < 0,05$). The smallest indexes of somatic cell count throughout the life of the animal was observed in the milk of the first lactation (in cows – 37 ± 7, in goats – 712 ± 174 × 10^3 cells/cm^3, fig. 10). In cows from the second to the fourth lactation, somatic cell count was recorded at the level of 169–192 × 10^3 cells/cm^3, in goats – 880–1092 × × 10^3 cells/cm^3 (flow cytometry method).

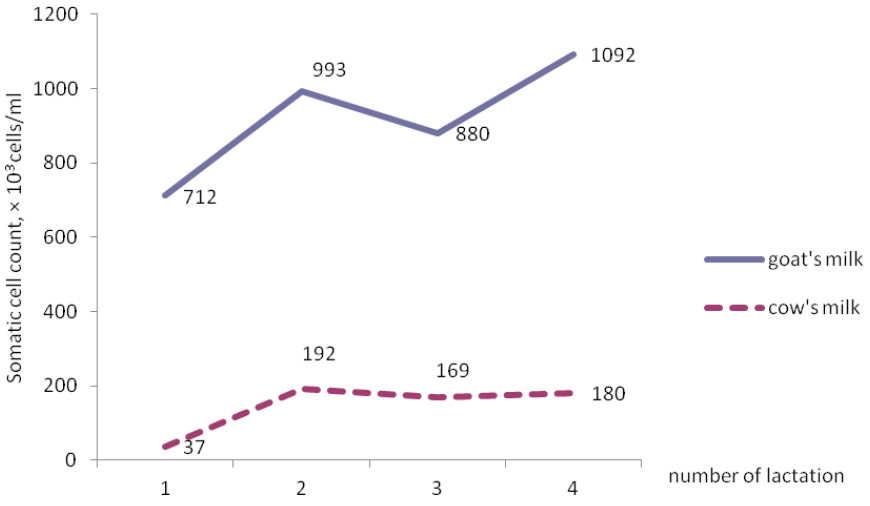

Figure 10. The somatic cell count in milk of cows (n = 10) and of goats (n = 17) depending on the number of lactation

In general, we did not find interconnection between increasing of the somatic cell count and the age of cows or goats. The index of somatic cell count in second lactation increased in three times compared with the first in Alpine breed goats, while in the German white and Anglo-Nubian breeds it had decreased by 5.1 and 31.7% respectively, which proves the great variability of this indicator (Zazharska et al., 2017 a).

Effect of exogenous and endogenous factors on the quality and safety of goat milk

Bacterial contamination of milk at different temperatures and shelf life

The main problem of the Ukrainian dairy industry is adaptation to European standards in quality parameters of milk. Milk in Ukraine has big bacterial contamination (second grade of DSTU – to 3 million CFU/cm^3). The aim of the study was to determine bacterial contamination and physical-chemical parameters in goat milk depending of different temperatures and periods of storage.

Analyses were conducted in the laboratory LILCO (Laboratoire Interprofessionnel Laitiere du Centre Ouest – Interprofessional milk laboratory of center and west), Surgères, France. All biochemical indexes of milk were determined by devices FossomaticTM FC and MilkoScanTM FT$^+$. Bacterial contamination of milk was determined by epifluorescence microscopy (FOSS Integrated Milk Testung BactoScanFC).

The data were analysed in Statisica 6.0 (StatSoft Inc., USA). The data in the tables are presented as x ± SE (x ± standard error). The differences between the values in groups were determined using Tukey test, where the differences were considered significant at $P < 0.05$ (with taking into account the Bonferroni correction).

For the first experiment, 2 liters of bulk tank goat milk was selected from the farmer – the client of laboratory LILCO. Milk was divided in 45 flacons: the first 15 samples were cooled immediately and stored at 4 °C. Second 15 samples were stored at 8 °C, the last 15 flacons – at 12 °C. 5 samples of milk from every refrigerator were

analyzed at 18 hours after milking, at the second and third day. The enterprises do not collect milk from farms every day, so the samples were kept for several days for research.

All indexes of milk were at the same level at different temperature of storage after 18 hours of milking (Table 4). The length of bactericidal phase depends on temperature of milk storage. Bactericidal properties were in milk during the first day, so bacterial contamination was at one level, even at 12 °C.

Table 4. – Results of the analysis of bulk tank goat's milk samples, (x ± SE, n = 5)

Storage time	Bacterial contamination at different temperature storage of milk samples, $\times 10^3$ CFU/cm^3		
	4 °C	8 °C	12 °C
First day (18 hours after milking)	23.2 ± 0.4	23.2 ± 0.8	22.6 ± 0.8
Second day	20.6 ± 0.7^a	20.4 ± 0.5^a	151.6 ± 3.4^b
Third day	21.8 ± 0.4^a	28.4 ± 0.5^b	1236.0 ± 55.6^c

Note: different letters within the line correspond to the selections which had significant differences between one another according to the results of Tukey's test (P < 0.05) with Bonferroni correction

All indexes of milk have not changed at the second day of storage compared to the first day besides a total plate count of milk which was kept at a temperature of 12 °C. Bactericidal phase finished and microbes began to multiply in milk.

The bacterial contamination of milk kept at a temperature 12 °C was higher 7.4 times compared with milk stored at temperatures 4 °C and 8 °C at the second day after milking (P < 0.001). A total plate count of goat milk stored at temperature 8 °C (requirement for the delivery of milk to enterprises) was much higher than when stored at temperature 4 °C (21.8 ± 0.4 × 10^3 CFU/cm^3) at the third day after milking (P < 0.05).

If the milk has not been collected within 2 hours after milking, it should be cooled to a temperature of 8 °C or lower, or 6 °C

and lower if the collecting lasts more than a day (Regulation (EC) № 853/2004).

It was established that bacterial contamination in goat milk stored at temperature 8 °C was much higher than in milk stored at temperature 4°C at the third day after milking ($P < 0.001$). So, it is not enough to cool the milk to 8 °C after milking: at third day bacterial contamination of milk is too much for producing dairy products. The maximal continuation of bactericidal phase is possible only with the rapid cooling of milk after milking to 4 °C. Every farmer in Ukraine has to milk animals only mechanically with closed supply milk to the cooling tank. Then bacterial contamination of milk will correspond to European requirements. It was proved that to ensure high quality milk it should be the rapid cooling of milk after milking to 4 °C.

For the second experiment, 24 samples of cooled bulk tank goat milk were selected be transported within 2–3 hours at different temperatures. Then all samples were stored day at 4 °C. The indicators of bacterial contamination, fat, protein, freezing point, somatic cell count, urea were similar for different temperatures of transporting milk samples. It was noted the big somatic cell count ($> 2000 \times 10^3$ cells/ml) at low bacterial contamination (19.6×10^3 CFU/ml) in goat milk. It was proved that milk samples can be transport to the laboratory at a temperature of 2, 10 or 20 °C during 2–3 hours if the milk after milking was cooled immediately and stored at 4 °C.

For the third experiment, 5 samples of non-cooled cow's milk were analyzed at 3 hours after milking, 5 samples of cooled cow's milk – after a day. A total plate count of milk which was cooled and stored one day at 4 °C was in 4.6 times less ($P < 0.01$) than non-cooled milk, which has been analyzed in 3 hours after milking. This proves that bacterial contamination of milk in Ukraine accordance with European requirements (up to 100×10^3 CFU/ml) is possible only when rapid cooling to 4°C and storing in the cooling tank (Zazharska, 2016 a).

Comparative characteristic of milk quality of German White, Alpine and Anglo-Nubian breeds of goats

The comparative characteristic of milk indexes of different breeds of goat was conducted. The material for research was individual milk samples from 21 goats of Alpine, German White and Anglo-Nubian breeds. The best milk quality indexes were observed in the Anglo-Nubian breed (fig. 11) – the highest fat content (3.81%), protein (3.52%), lactose (5.25%), solids (13.33%), but the goats of the Alpine and German white breeds are characterized by large milk yields.

A significant increase in the fat content in milk was found in the Anglo-Nubian and Alpine goats ($P < 0.05$).

The indexes of calcium content ranged from 94.7 to 169.8 mg/100 g, the significant difference was found between the milk of German White (the highest calcium content – 169.8 ± 28.1 mg/100 g), and Alpine breed ($P < 0.05$). The content of calcium in goat's milk is on average 124 mg/100 g (Greppi et al., 2008).

The somatic cell count of milk of all goat breeds corresponded to a higher grade of DSTU7006:2009.

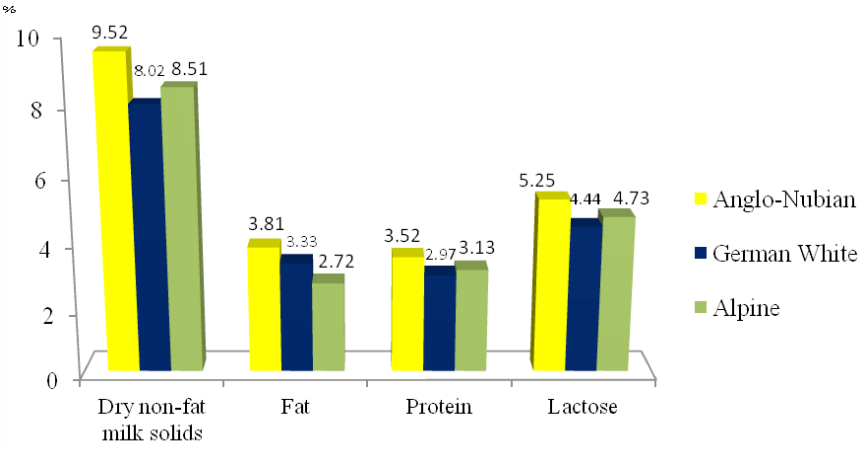

Figure 11. Physic-chemical indicators of milk of different breeds of goat

The best suitability of milk for cheesemaking was marked in Alpine goats. The smallest somatic cell count (271×10^3 cells/cm^3) was noted in German White goats. Therefore, it is expedient to breed all three breeds of goats (Zazharska & Gramma, 2016).

Influence of diet on the productivity and characteristics of goat milk

We assessed the relationship between the milk quality indexes of goats of different breeds and their diets. There were used in the experiment: Anglo-Nubian (7 animals), German White (7) and Alpine goats (7). We investigated the influence of two diets: a routine diet (hay and concentrates) and a diet improved by granulated alfalfa hay, mixed feed. The volume of the morning milk yield and biochemical indexes of the milk of each goat after machine milking were measured.

It was established that the milk yield of the Alpine goats increased 3 times; and that of the German White goats increased more than 2.5 times when goats got improved diet (added granulated alfalfa hay and concentrate feed). Feeding improved diet resulted in significant increase in fat content of milk of all breeds from 2.06–2.62% to 3.76–5.69 ($P < 0.05$). A significant increase ($P < 0.05$) in the protein was observed in Anglo-Nubian (from 3.02 to 3.31%) and German White goats (from 2.88 to 3.03%) when they got the improved diet.

Compared with the German White and Alpine goats, the highest figures for the fat, protein and lactose were found in milk of the Anglo-Nubian goats with the routine and improved diet. With all breeds under study the freezing point and electrical conductivity of the milk decreased when they were fed the improved diet. An inverse relationship was found between the protein content and the freezing temperature in the goats' milk: when the protein content increased, the freezing point decreased.

Content of protein in milk of Saanen and Alpine goats from 27.0 to 29.2 g/kg (on average 2.8%), and fat content equal to 30.2–34.1 g/kg (on average 3.2%) (Maurer et al., 2013). According to the other data the protein content in milk of the British Saanen goat is 2.6%, of Nubian in Great Britain – 3.6%, Alpine and Saanen in France – 3.2%, and fat content of 3.5%, 4.9%, and 3.6%, respectively

(Yangilar, 2013). In our studies, a significant increase of fat and protein content in goat's milk was recorded after improvement in the diet (Zazharska et al., 2018).

Physical and chemical composition of goat and sheep milk depending on the altitude of grazing

The quality and safety parameters of milk were compared, depending on the altitude of grazing of goats in the valleys of Zakarpattya (251, 309, 341, 376, 394, 524, 580 and 750 m above sea level). The milk samples were studied from 5 goats at each height.

It was determined, that acidity of milk ranged from 13 to 17 °T, the density of the goat – from 24.4 °A to 30.5 °A in goats, grazed in the valleys of Zakarpattya. The protein content of goat milk was – 2.90 ± 0.07 to 3.19 ± 0.15%, fat content – 2.60 ± 0.50 to 5.61 ± 0.66%, somatic cell count – up to 359 ± 226 $\times 10^3$ cells/cm^3. At the highest altitude of the grazing – 750 m above sea level, a lowest fat content in the milk of goats (2.6 ± 0.5%) was marked that in 1.4–2.2 times was less than the values of other altitude of the grazing. The lowest concentration of somatic cells in goat milk was 82 ± 29 × 10^3 cells/cm^3 (by viscosimetric analyzer) at this altitude, that in 1.9–4.4 times was less than the values of other heights (Fig. 12).

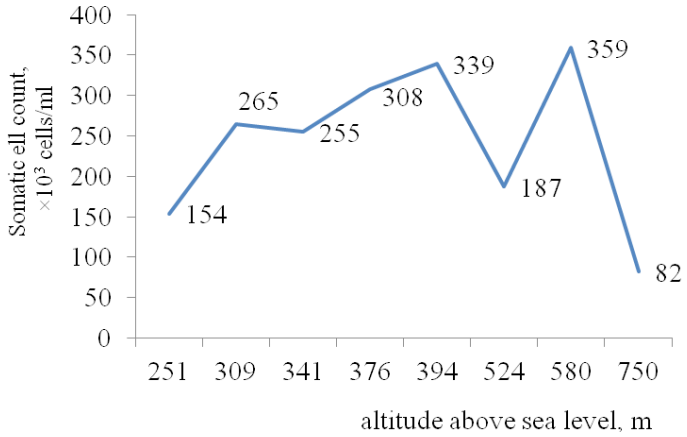

Figure 12. Somatic cell count in goat milk depending on the altitude of grazing (by viscosimetric method)

It was proved that if the fat of goat's milk was more, then its density was less. An inverse relationship was found between the protein content in the goats' milk and the freezing point: with increase in the protein content, the freezing point was reduced. It was revealed that somatic cell count in goat milk (359×10^3 cells/ml) was much less than requirements in Europe ($< 1000 \times 10^3$/ml). The high fat content in the milk of goats (5.61%) was observed at an altitude of 341 m above sea level ($P < 0.01$). The highest freezing point of milk, and at the same time – low figures of protein and density were marked in the same animals. At the highest altitude of grazing – 750 m above sea level, a lowest fat content (2.6%) was marked along with highest figures of dry non-fat milk solid and protein content in goat's milk. The lowest somatic cell count in milk of goats at this altitude indicates a high sanitary quality of milk (Fotina & Zazharska, 2016).

Study of indicators of safety of goat's milk for intense man-caused pollution

The hazard of grazing dairy goats near highways and racing them along the roadside of the highways to the pasture has been proved. Content of plum (0,23 ± 0,08 mg/kg) in milk from the goats of the city of Dnipro exceeds a maximally possible level in accordance with Ukrainian requirements more than in 2 times, and maximally possible level for the milk used for production of child's and dietary products more, than in 4 times. The content of plum and cadmium in a cottage cheese in 2.1–2.4 times is higher, and in whey in 1.5–1.6 times below as compared to milk (Fig. 13).

The purpose of the study was to estimate changes of goat's milk depending on the season and lactation period.

Monitoring study of the milk from 4 goats of 1–2 lactation and 4 goats of 4–5 lactation from the village Mar'yanske, Apostol district of Dnepropetrovsk region were conducted (total 211 samples). The quality and safety indexes of goat's milk depending on seasons were determined by analyzer of milk "Ekomilk". The somatic cell count depending on lactation period, portions of milk during milking was determined by viscosimetric analyzer "Somatos".

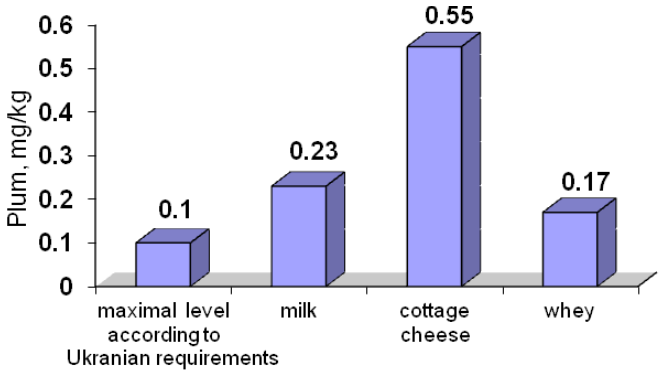

Figure 13. The content of plum in goat milk and dairy products, mg/kg

Effect of lactation period, yield time, season on goat milk indexes

Cheese, dangerous for the content of salts of plum and cadmium is produced from such milk in milk processing enterprises.

It is proved, that protein and lactose in goat milk in autumn increased on 17.5% and 13.5% accordingly compared to the summer ($P < 0.05$). During summer fat (3.70 ± 0.18%) in milk was lower in 1.8 times compared with the winter period ($P < 0.05$), in 1.5 times – with spring ($P < 0.05$), on 16% – with autumn period ($P < 0.05$). The lowest freezing point marked in winter, accompanied by a high content of lactose, fat and the largest somatic cell count. In autumn and winter the somatic cell count was 3.7 ($P < 0.05$) and 5 ($P < 0.05$) times accordingly more than the spring-summer figure (96 ± 14 $\times 10^3$ cells/cm^3).

In the first month of lactation goat milk contained very low somatic cells count from 33 to 107 $\times$ 10^3 cells/cm^3. Low figures of somatic cell count (15 to 63 $\times$ 10^3 cells/cm^3) was marked in milk of goats of first lactation, but it lasted only seven months.

The somatic cell count in milk of goats of 1–2 lactation is slightly lower than in animals of 4–5 lactation, but no statistical difference was detected (Fig. 14).

During the year, the indexes of milk yield increased in May and September, decreased in summer months – because of the heat, and to the end of lactation – in 7–9 months.

While studying the milk of eight goats for more than one and a half years, each animal noted an increase in the somatic cell count in the evening and in the morning. The interconnection of the index of somatic cell count and yield time was not found. The index can change twice and more at morning and evening milking during the day (Zazharska & Kostyuchenko, 2015; Shapovalov et al., 2015; Fotina et al., 2018).

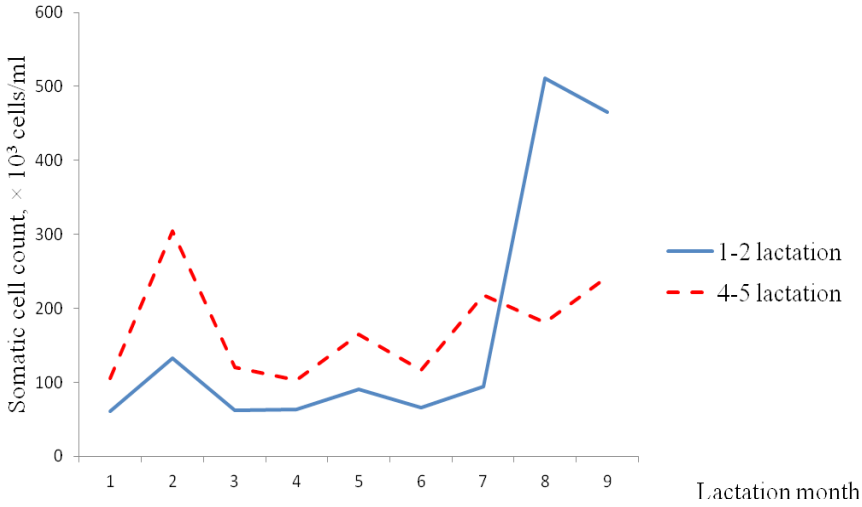

Figure 14. Somatic cell count in goat milk depending on lactation month, × 10^3 cells/cm^3

Colostrums contain large concentration of immunoglobulin G, which is particularly important in the first hours and days of a newborn. It is very important to be sure that there are no colostrums in the total yield because of the high acidity of colostrums. That milk cannot be pasteurized or sterilized.

The changes of organoleptic, physical-chemical parameters and concentration of immunoglobulin G were studied in 44 samples of goat milk and colostrum depending on the period of lactation. The concentration of immunoglobulin G was analyzed by means of IDRing Plate-Caprine IgG Test, France (simple radial immunodiffusion).

The organoleptic indicators (colour, consistency, taste, smell) of goat's colostrums at first day after lambing were significantly different from milk of other days. Colostrums colour in the first 3 days was creamy-yellow, texture was viscous, especially at the first day. The maximum acidity 56 °T was in the first colostrums yield, due to the maximum concentration of immunoglobulin, at the second and third day acidity was 15–16 °T.

All main parameters of colostrums significantly decreased at the second day of lactation. Fat and lactose of colostrums at the second day after lambing fell more than 2 times, dry non-fat milk solids – by 42.6%, density – 40% of total protein – 42%, the freezing point – 44% compared to first day. Conductivity and pH on the contrary increased at the second day of lactation in 44.9 and 5.7% accordingly, but these were only parameters where significant difference compared to the first colostrums yield wasn't found for seven days. Determined, that fat, dry non-fat milk solids, density, protein, lactose of colostrums during the period from second to seventh day of lactation were significantly below from the same parameters of first milk yield ($P < 0.05$), which proved the value of the first colostrums

Somatic cell count in colostrums on the second day of lactation decreased twice, and on the seventh – in ten times compared to the first milk yield ($680 \pm 217 \times 10^3$ cells/cm^3) ($P < 0.05$).

According to data of Spanish scientists, Ig G content in goat colostrums was 19.97 g/l. The average concentration of Ig G after the seventh milking was less than 2 g/l, and after the eleventh – less than 1 g/l (Fernandez et al., 2006). The same pattern was observed in our study. The Ig G content in goat's colostrums in the first yield was 15.79 g/l, the day after lambing – 16.8% less. The sharp decline of Ig G concentration compared with the first milk yield was from the 3rd day of colostric period – in 6 times less ($P <0.05$). From the 6th day of lactation the concentration of immunoglobulin G in milk wasn't exceed 1 g/l, which indicates the possibility of adding such milk to the total yield for further processing. The system for obtaining high quality goat's milk has been developed. System is due to the

perfect cleaning of milking equipment, proper milking and care of udder health in goats.

Also, in order to improve the hygienic state of the udder and to reduce the amount of somatic cells in milk, it is recommended to use mean for pre-milking washing "MolSan" and the means for udder care: ointment "Fitosept", "Dbayliva doyarochka", "Zorka" cream, gel of "Nizhnodiy" (Fotina et al., 2015; Fotina & Zazharska, 2015; Zazharska & Ryaba, 2016).

References

1. *Bagnicka E., Łukaszewicz M., & Ådnøy T.* (2016). Genetic parameters of somatic cell score and lactose content in goat's milk. Journal of Animal and Feed Sciences, 25(3), 210–215.
2. *Barth K.* (2009). Eutergesundheitsüberwachung bei Milchschafen und Milchziegen – welche Methoden sind geeignet. Landbauforsch SH, 332, 89–95.
3. *Czopowicz M., Strzalkowska N., Rzewuska M., Winnicka A., Jozwik A., Kosciuczuk E., Kaba J., Jarczak J., Krzyzewski J., Bagnicka E.* (2013). Factors influencing technological properties of goat milk. Goat Milk Quality Regional IGA Conference, Norway. Abstractgbs. 14.
4. *Fernandez A., Ramos J. J., Loste A., Ferrer L. M., Figueras L., Verde M. T., Marca M. C.* (2006). Influence of colostrum treated by heat on immunity function in goat kids. Comparative Immunology, Microbiology & Infectious Diseases, 29, 353–364.
5. *Fotina T. I., Fotina H. A., Ladyka V. I., Ladyka L. M., Zazharska N. M.* (2018). Monitoring research of somatic cells count in goat milk in the eastern region of Ukraine. Journal of the Hellenic Veterinary Medical Society, 69(3), 1101–1108.
6. *Fotina T. I., Zazharska N. M., Kostyuchenko V. Y.* (2015). Influence of facilities for milking on sanitary quality of goat's milk. Visnik Sumskogo nacionalnogo agrarnogo universitetu, 37, 59–65.
7. *Fotina T., & Zazharska N.* (2016). Physical and chemical composition of goat and sheep milk depending on the altitude of grazing. The Animal Biology, 18(4), 106–112.
8. *Fotina T. I., & Zazharska N. M.* (2015). Influence of drugs for udder hygiene on sanitary parameters of goat milk // Materials of the V International Congress of Veterinary Pharmacology and Toxicology, Vitebsk, Belorus, 380–384.
9. *Greppi G. F., Roncada P., Fortin R.* (2008). Protein components of goat's milk. In: Pulina G.; Cannas A. (Eds.) Dairy goats feeding and nutrition. 2.ed. Bologna: CAB International, 71–94.

10. *Maurer J., Berger T., Amrein R., Schaeren W., Stierli M.* (2013). Critères de qualité pour le lait de chèvre et de brebis: exigences et valeurs indicatives ainsi que propositions pour un paiement du lait selon des caractéristiques qualitatives. ALP Forum, 97, 1–16.
11. *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. 38.
12. *Park Y. W.* (1991). Interrelationships between somatic cell counts, electrical conductivity, bacteria counts, percent fat and protein in goat milk. Small Ruminant Research, 5(4), 367–375.
13. *Schüppel H. & Schwope M.* (1999). Zum Gehalt somatischer Zellen und zur mikrobiologischen Beschaffenheit der Milch von Ziegen mit klinisch unauffälligem Euterbefund. Milchwissensch, 54, 13–16.
14. *Shapovalov S., Fotina T., Kalachnikov V.* and Zazharska N. (2015) Composition physico-chimique du lait de chèvre de l'Est de l'Ukraine. Revue Écologie-Environnement, Algérie 11: 70–73.
15. *Yangilar F.* (2013). As a potentially functional food: Goats' milk and products. J. Food Nutr. Res., 1(4), 68–81.
16. *Zazharska N. & Kostyuchenko K.* (2015). Influence of lactation period, yield time, season on the somatic cells count in goat milk. Problems of zooengineering and veterinary medicine, 31(2), 179–184.
17. *Zazharska N.* (2015). Organization of work and analysis at the milk laboratory in France. Materials of the international scientific-practical conference, Ganja Azerbaijan, 1, 480–484.
18. *Zazharska N. M.* (2014). Somatic cell count of cow and goat milk. News of Sumy National Agrarian University, 1 (34), 89–92.
19. *Zazharska N. M.* (2016a). Bacterial contamination of milk at different temperatures and shelf life. Scientific Messenger of LNU of Veterinary Medicine and Biotechnology, 70, 108–112.
20. *Zazharska N. M.* (2016 b). Comparative characteristics of cow`s and goat`s milk, according to the data of laboratory LILCO. Scientific Bulletin of the National University of Life and Environmental Sciences of Ukraine, 237, 297–308.

21. *Zazharska N. M.* (2018). Criteria for safety and quality assessment of goat's milk. The thesis for the scientific degree of doctor of the veterinary science by specialty 16.00.09 – veterinary and sanitary expertise. Sumy National Agrarian University.
22. *Zazharska N. M., & Gramma V. O.* (2016). Comparative characteristic of milk quality of German white, Alpine and Anglo-Nubian breeds of goats]. News of Zhytomyr National Agroecological University, 1 (53), 214–220.
23. *Zazharska N. M., & Pryadka E. V.* (2015). Influence of lactation period, yield time, season on the somatic cell count in cow milk. Science and Technology Bulletin of SRC for Biosafety and Environmental Control of AIC, 3(1), 107–112.
24. *Zazharska N. M., & Ryaba A. A.* (2016). Sanitary quality of goat milk in the application of the homeopathic preparations for milking. Scientific and technical bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology, 17 (1), 72–77.
25. *Zazharska N. M., Kurban D. A., & Holubyeva O. V.* (2017 a). Contain of fat, protein, somatic cells in cow's and goat's milk depending on number of lactation. Theoretical and Applied Veterinary Medicine, 5(4), 17–24.
26. *Zazharska N. M., Neverkovets N. Y., & Danyliuk V. O.* (2017 b). Parameters of subclinical mastitis in goats. News of Dnipropetrovsk State Agrarian and Economic University, 45, 77–81.
27. *Zazharska N. N., & Rosenko S. O.* (2018). Study on the prevalence of subclinical mastitis in goat milk. Theoretical and Applied Veterinary Medicine, 6(3), 50–53.
28. *Zazharska N. N., & Zharko L.* (2016). Comparison of methods efficiency for determination of somatic cells count in goat milk. Science and Technology Bulletin of SRC for Biosafety and Environmental Control of AIC, 4(4), 29–35.
29. *Zazharska N. N., Boyko O., & Brygadyrenko V.* (2018). Influence of diet on the productivity and characteristics of goat milk. Indian Journal of Animal Research, 52(5), 711–717.