Кількість соматичних клітин у молоці корів впливає на якісні показники, ґатунок та безпечність. Рівень в молоці соматичних клітин залежить від стану вим'я у тварин. Тому важливим є вчасно проводити діагностику корів на субклінічний мастит та профілактувати його виникнення.

В результаті проведених досліджень визначений гістологічний спектр соматичних клітин у корів української чорно-рябої молочної породи. Експериментально доведено, що мікроорганізми, виділені з шкіри вим'я корів, ідентичні мікрофлорі, яка викликає у корів захворювання на субклінічний мастит.

В результаті проведених досліджень встановлений спосіб передачі збудників маститної інфекції через гуму молочних стаканів доїльного обладнання.

Також визначений спектр збудників субклінічного маститу та розроблена схема профілактики захворювання і запобігання його поширенню серед дійного стада. В результаті експериментальних досліджень доведена ефективність використання швидкого маститного тесту, який дає змогу своєчасно виділити з стада корів з неякісним молоком.

Поява кетонових тіл у молоці підвищує його кислотність та знижує якість молока. Показник кислотності характеризує харчову цінність молока та контролюється при прийомі на молоказавод. При цьому господарство втрачає гроші на зниженні сортності молока.

Експериментально розроблений спосіб профілактики та лікування кетозу у корів на основі хелатів металів за рахунок покращення обмінних процесів у рубці корів. В результаті проведених досліджень встановлений взаємозв'язок між станом мікрофлори рубця та виникнення ацетонемії у корів. Таким чином, запропоновані заходи дають можливість підвищити якість та безпечність молока корів шляхом зменшення кількості тварин у стаді з ознаками маститу та кетозу

Ключові слова: соматичні клітини, субклінічний мастит, мікрофлора, гума молочних стаканів, якість молока, кетонові тіла

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DEVELOPMENT OF MEASURES TO IMPROVE MILK QUALITY AND SAFETY DURING PRODUCTION

O. Shkromada
Doctor of Veterinary Sciences, Associate Professor*
E-mail: oshkromada@gmail.com
O. Skliar
Doctor of Veterinary Sciences, Professor*
A. Paliy
Doctor of Agricultural Sciences, Associate Professor**
L. Ulko
Doctor of Veterinary Sciences, Professor*
I. Gerun
Postgraduate student*
O. Naumenko
PhD, Professor**
K. Ishchenko
PhD, Senior Lecturer**
O. Kysterna
PhD, Senior Lecturer***
O. Musiienko
PhD, Associate Professor***
A. Paliy
Doctor of Veterinary Sciences, Senior Researcher
Laboratory of Veterinary Sanitation and Parasitology
National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine»
Pushkinska str., 83, Kharkiv, Ukraine, 61023
*Department of Therapy, Pharmacology, Clinical Diagnostics and Chemistry
Sumy National Agrarian University
Herasym Kondratiev str., 160, Sumy, Ukraine, 40021
**Department of Technical Systems and Animal Husbandry Technologies
Kharkiv Petro Vasylenko National Technical University of Agriculture
Alchevskykh str., 44, Kharkiv, Ukraine, 61002
***Scientific Consultant of Veterinary Clinic «Vet Service»
Pershotravneva str., 12 A, Sumy, Ukraine, 40000

1. Introduction

Milk and dairy products occupy one of the significant places in the food chain of people of any age. Cow milk, in addition to main components (fat, protein, carbohydrates), contains about 150 nutrients (vitamins, micro- and micro-elements, etc.) that are essential for the vital activity of the human body. Along with the fact that milk and milk products are necessary for vital activity of people, they are also a good nutrient medium for the development of microorganisms. If sanitary conditions for obtaining and storing are violated, milk can be a dangerous source of infection.

To prevent mastitis at farms, it is necessary to apply modern systems of milking and disinfection of dairy equip-

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ment. In addition, not less important indicator is microbial contamination of the air and the floor the cows lie on. Compliance with the premises disinfection schemes and hygiene of cow udders prevents the pathogens of mastitis from spreading around the farm.

In fact there is a big problem of detection of cows with the signs of sub-clinical mastitis in a herd due to the lack of characteristic symptoms in animals. Some animals may not get sick, but be carriers of mastitis pathogens. Due to this, it is not possible to remove sick animals from a herd by 100 %. Regular milk monitoring for the content of somatic cells will allow prevention of unwanted losses because of milk quality, and the use of an express test for mastitis will help detect sick animals in a herd.

An increase in acetone bodies in cow milk is a sign of ketosis. Diseases are characterized by metabolic disorder that is not diagnosed at the beginning of the development, which leads to the loss of milk quantity and quality followed by rejecting such animals. That is why keeping to a balanced diet for animals with addition of chelate metals will prevent the occurrence of ketosis in cows and will allow retaining the milk quality at a high level. Thus, systematic monitoring and following the scheme of measures will help reduce the risk of low-quality milk occurrence at an enterprise.

Milk quality and safety in case of cows being sick with mastitis is a relevant, however, still not completely solved problem. It is very difficult to monitor the milk quality at a production enterprise because the animal state is constantly changing. There is a constant danger of catching infection and occurrence of sub-clinical mastitis and ketosis in a herd. Given the above, it is necessary to constantly monitor these diseases. The progressive direction in mastitis prevention, above all, its sub-clinical form, is the implementation of technological processes in its production. It is control of sanitary and hygienic parameters of the microclimate in premises where animals are kept, milking technology and the use of dairy equipment.

At present, a significant number of schemes were proposed for the treatment of mastitis, but the use of antibiotics is the main one. In turn, it affects milk quality and safety. The quality and safety of milk at sub-clinical mastitis are directly related to each other. While the milk quality indicator is nutritional value, which changes during the disease, the number of somatic cells and bacterial seeding are safety indicators.

2. Literature review and problem statement

Paper [1] reports results of research into safety and quality of milk. According to data from FAO, milk and milk products are referred to the first category of risks that are caused by food intoxications of microbial etiology. Standards and regulations of the European Union (ISO 13366-2 IDF 148-2, ISO 5725-1, ISO 5725-2) and general health care rules for the trade in milk and milk products for human consumption in the European Union (Council Directive 2002/99/EU) form the legal basis for all animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption. Regulation (EU) No. 178/2002, Regulation (EU) No. 852/2004, Regulation (EU) No. 853/2004, Regulation (EU) No. 854/2004 and Regulation (EU) 882/2004 form the legal base for the public health rules for trade and delivery to the EU, and require monitoring the amount of somatic cells of ketone bodies and microorganisms in milk. In accordance with the international requirements to food products, it is not sufficient to control quality and safety of products at the final stage, because it could not guarantee their safety. Milk that is of high-quality in its physical and chemical composition, obtained in unhygienic conditions can quickly become unsuitable for human consumption or even harmful to the health of consumers. However, as noted in paper [2], high-quality and safe milk can be obtained only from healthy animals. To solve such problems, the modern world food industry implements new systems of product quality control. One of them is HACCP, and such an approach is used in article [3].

Up to now, the problem of controlling milk quality and safety at an enterprise remains not fully resolved. The reason for this may be hidden (symptom-free) forms of cow diseases, which are difficult to diagnose early [2].

Among dairy cow diseases, one of the significant places is occupied by mastitis, especially in sub-clinical (latent) form, the main causes of which are the violation of keeping conditions and milking technology. The non-observance of the technology of milking (breaking the vacuum mode, worn-out rubber, «dry milking», etc.) leads to the occurrence of microtraumas of skin, epithelium of mammary paths, and udder parenchyma. As a result, this causes the impact of negative environmental factors, followed by joining of pathogenic microflora [4].

According to the requirements of the veterinary-sanitary expert examination, it is forbidden to use and sell milk from cows that are sick with mastitis. Milk of sick animals contains a large amount of pathogenic microflora and is not suitable to produce sour milk products, especially cheese [5]. Such milk has elevated acidity, and bacteria destroy valuable substances, including fat and protein, which spoils the taste, smell and consistence of dairy products. Along with this, toxins (products of vital activity of bacteria), including heat-resistant forms, remain in such milk [6].

It is not possible to prevent the problem of occurrence of sub-clinical mastitis in dairy cattle completely. It all depends on the animal breed, propensity to the disease, resistance of the organism, and the climatic conditions of the farm location, milking equipment, modes of disinfection, etc. [7].

That is why, given the created operating conditions and animals keeping, it is appropriate to develop a sequence of measures to prevent and eliminate the problems of sub-clinic mastitis at a dairy farm [8].

The problem of milk safety and the content of somatic cells in it (epithelial cells that were separated from the secrete part of the udder and mammary paths, and blood cells) remains not fully resolved so far. During the inflammatory process in the mammary gland (mastitis), the number of leukocytes increases according to the cell theory of inflammation, and the phagocytosis process starts. This increases the total number of somatic cells, which is a cow health indicator (udder). In addition, not only the number of somatic cells changes, but also the ratio of their species composition [9].

A variant to overcome this problem could be the application of a cytological study, which gives an idea of the nature, level of inflammatory process and mastitis spreading in a herd. In addition, one of the most important measures is the use of a rapid mastitis test for sick cows' rejection from a herd. Even the existence of 2 % of sick cows in a herd can ruin the overall indicator and decrease milk quality, leave alone milk safety [10].

Another problem of milk safety is bacterial contamination, which most closely reflects the sanitary conditions of its production [11]. If the number of somatic cells depends on the state of health of a cow (udder), bacterial infection, to a large extent, depends on a series of technological factors. These include the conditions of animal keeping, hygienic state of the udder skin and bacterial contamination of dairy equipment [12].

The variant to overcome this problem is the disinfection of milking cups before each milking operation and removal the animals that are sick with mastitis from a herd. Such radical measures of animal rejection are necessary due to the fact that even 100 % regular disinfection of the cow udder and milking equipment does not destroy all pathogenic microflora and it can spread throughout a dairy herd [13].

An important milk quality indicator is the absence of ketone bodies in it. But this problem very often arises in highly productive animals through their metabolic disorders [14].

The main requirement in the dairy industry is economic profitability of a dairy herd. According to the latest calculations, it is necessary to receive from cows not less than 9.000–9.500 kg of milk per lactation. In order to increase lactation, fat content and protein content, animals are given a large amount of concentrated feed, which causes metabolic disorders [15]. Ketosis occurs as an independent disease due to the violation of energy imbalances of protein and carbohydrates of feeds. In this regard, it can be attributed to the alimentary factor. A significant number of researchers note the changes in the biochemical reactions of the rumen: an increase in acidity (pH), a change in the ratio of fatty volatile acids and a decrease in the amount (an increase in content of butyric acid) and a decrease in the number of the simplest (Infusoria) [16].

All this makes it possible to assert that it is advisable to use such measures of ketosis prevention as balanced feeding of cows and the treatment with the use of a complex of chelates of iron, zinc and manganese.

3. The aim and objectives of the study

The aim of this study is to develop measures to enhance milk quality and safety at enterprises by reducing the risks of cows catching sub-clinical mastitis and ketosis.

To accomplish the aim, the following tasks have been set:

– to improve the method for determining the quantitative and species composition of somatic cells; to detect the main sources of ways of cow milk contamination with microflora;

– to establish the efficiency of applying chelate metals for the animals that are sick with ketosis; to determine their clinical status; the level of metabolic processes in the organism, the impact on the rumen microflora, to determine acidity and the level of ketone bodies in milk; to develop a system of measures to improve the quality and safety of milk.

4. Materials and methods for the development of measures to enhance milk quality and safety during production

4. 1. Procedure of research into the species composition of somatic cells and microflora in milk

The work was performed at the laboratory of clinical diagnosis at Sumy National Agrarian University (Ukraine) and under industrial conditions at farms in Sumy oblast over 2018. The cows of Ukrainian black-speckled dairy breed of 1–4 lactations were studied. To determine the healthy and diseased quarters of the udder, the Californian mastitis test [17] and the microscopic test for counting somatic cells [18] were used. After investigating the state of the healthy quarter of the udder, the secrete from the one with positive reaction was selected into sterile cups, observing the rules of asepsis. In the lab, the milk smears were performed according to the standard methods for studying dairy products. The total number of somatic cells was counted using the Prescott and Britt method [19] and their species composition was determined.

The composition of the microflora of milk, skin, udder, nipples and dairy equipment was determined using the microbiological methods [20]. Before starting milking, disinfection of dairy equipment was conducted. Bacterial contamination of the rubber of milking cups was studied after each milking.

4. 2. Procedure for clinical examination of animals, for determining acidity and ketone bodies in milk

To detect ketosis, 4 groups, each including 5 heads of cows of similar age having 3–4 lactations, were selected. The control group included a group of healthy cows and those that had sub-clinical ketosis and were not treated for it. At the beginning of the study, the clinical status of the animals was determined. The temperature was determined using a usual maximum medical thermometer, the pulse was studied on the middle caudal artery using the palpation method, respiration was explored by applying the palms of hands to the nasal openings, the rumen contraction – with a ruminograph by Z. S. Gorianova. Throughout the experiment, rumen liquid was taken from all cows from 10 to 11 a.m. through the probe to determine the number, intensity, and direction of Infusoria movement. To determine the intensity and direction of Infusoria motion, the microscope XS-2610 and the digital camera DELTA OPTICAL 2.0 MP were used. The number of Infusoria was counted in the Goryaiev chamber. For the purpose of fixation, a 4 % formalin solution was used. The rumen microflora activity was determined by Dirkens and Hoffrek test with methylene blue [21]. The acidity of the rumen contents was studied by a pH meter [22].

In addition, the blood was taken from the tail artery for morphological and biochemical research. The biochemical indices of blood serum of the experimental and control animals were studied by the following indicators and methods:

– total protein was studied by a refractometer;

– protein fraction was explored using the nephelometric method;

– reserve blood alkalinity was studied using the diffusion method with the help of dual flasks (by I. Kondrakhin);

– carotene was explored using the colorimetric method by G. Koromyslov and L. Kudriavtseva.

The study of ketone bodies was carried out using a ketonometer. The content of hemoglobin was explored using the Sally method. Leukocytic formula was derived by calculation of the relative content of cells in the blood smears stained by Romanovsky-Gimza. To determine the level of natural resistance, the Bredeck index was calculated as the ratio of the number of lymphocytes to stab neutrophils, and the ratio of sum of neutrophils to lymphocytes. The number of red blood cells was determined by the blending method.

5. Results of determining milk quality and safety during production

5. 1. Results of research into the species composition of somatic cells and microflora in milk

The conducted microscopic research into the cow milk smears revealed the species peculiarity and the number of somatic cells. Thus, the study of the NSC showed that their number in the main period of lactation (excluding the colostrum period and the launch period) is within up to 100 thousand/cm^3, which is regulated by DSTU 3662-97 [23]. SC are differentiated as lymphocytes, monocytes, neutrophils (Fig. 1, 2). When a cow is sick with sub-clinical mastitis, the NSC increases by tens, and even hundreds of times, which can reach up to 25,000–30,000 thousand/cm^3.

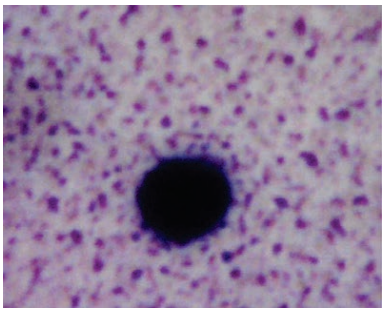

Fig. 1. Lymphocyte (milk of healthy cow) (×1,000)

Thus, Fig. 1 shows the lymphocyte of milk of a healthy cow. It is characteristic of it that at coloring by Levowitz-Weber method (L-W), it is of a round shape, the nucleus has dense consistency, it is intensely colored in dark purple color, a slight membrane of bluish cytoplasm can be clearly seen around the nucleus.

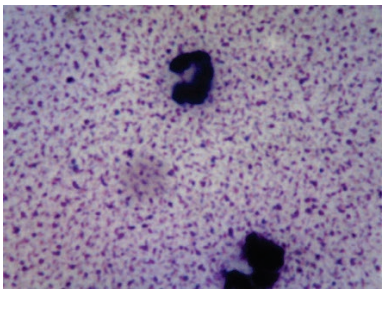

Fig. 2. Mature neutrophil (milk of healthy cow) (×1000)

Fig. 2 shows a mature neutrophil. The studies showed that neutrophils are found in milk of both healthy cows and those that are sick with mastitis. Along with it, it should be noted that in case of being sick with sub-clinical mastitis, their number increases by thousands of times. The number of neutrophils during the disease can take up to 90 % of all cells. Stub neutrophils and immature appear in milk together with mature neutrophils.

Monocytes appear in the secrete of the udder of cows that have sub-clinical mastitis (Fig. 3).

Monocytes (macrophages) have the non-segmented nucleus of irregular shape with significant amount of cytoplasm. Macrophages in large quantities are accumulated in the inflammation centers. Along with this, by Levowitz-Weber, they are colored in intense violet-brown. A cell has a large amount of cytoplasm with a significant number of granules of dark purple to black color (basophile). In the milk of cows with sub-clinical mastitis they perform anti-allergic function.

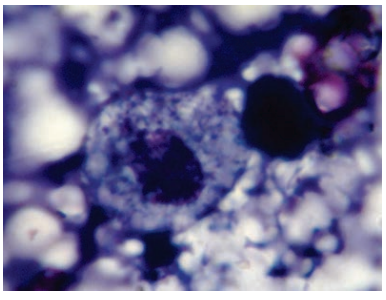

Fig. 3. Monocyte (udder secrete at sub-clinical mastitis) (×1,000)

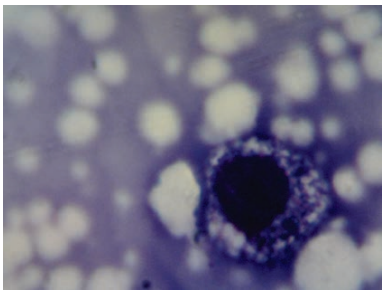

Fig. 4. Basophile (udder secrete at sub-clinical mastitis) (×1,000)

It should be noted that in the milk of cows with sub-clinical mastitis, the outside cell membrane of basophiles is not visible in the light microscope. During the study of the NSC, no relations between their number, morphological structure and the period of disease were detected in the cow milk.

The experimental research revealed the species composition of somatic cells and their number in the cow milk. Thus, the level of leukocytes in healthy quarters of the cow udder is within the determined limits. However, due to the fact that neutrophils perform the udder protection function, they are constantly changing. The level of macrophages in the milk of healthy cows does not exceed 2 % of the total amount, the share of epithelial cells is 60 % (Fig. 5, 6), while the remainder consists of lymphocytes and granulocytes.

The improvement of the method for determining the quantitative and species composition of somatic cells involves the use of alcohol-denaturant to fix the milk smear. The result of this improvement is that the milk smears almost in 100 % are kept on the slide during coloring, whereas the use of the previously proposed methods for fixation of smears was based on stained blood smears. And as it is known, milk smears are almost ten times as fatty as those of blood, which leads to sliding from the slide. It also made it possible to determine mathematically not only the quantity, but also the species composition. At the same time, the coefficient of conversion of determining the number of somatic cells for the microscope SX 2610 was calculated mathematically. Thus, having calculated 25 vision areas of a microscope with an increase by 400 times and knowing the vision area and having derived the average data, we multiply them by the proposed coefficient (120405). Thus, we determine the NSC per cm^3 of milk.

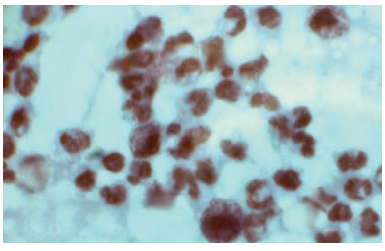

Fig. 5. Somatic cells of secrete of the udder of a cow that has sub-clinical mastitis (×400)

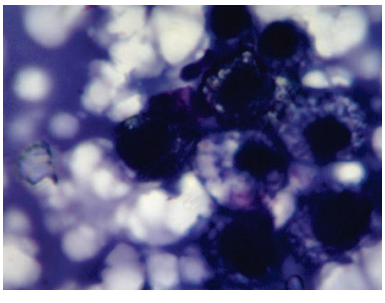

Fig. 6. Somatic cells of the secrete of udder of a cow that has sub-clinical mastitis (×1,000)

Results of studying the cows, affected with sub-clinical mastitis, by using different methods are given in Table 1.

Table 1

Efficiency of research into sub-clinical mastitis

NSC, thousand/cm^3	Research methods			
	Prescott-Brid	«Shalma» test	Mastidine test	Settling test
100	+	–	–	–
500	+	+	–	–
1,000	+	+	+	–
7,000	+	+	+	+

Based on the conducted research, it is possible to conclude that the least effective research method at an early stage was the method of Prescott-Brid. However, it is very time-consuming and not acceptable for using under production conditions. The most sensitive test at the production enterprise turned out to be «Shalma» test. It detects affected animals even at an increase in NSC within 500,000/cm^3. The least effective test turned out to be the settling test as a positive result appears only at an increase in NSC < 7,000 thousand/cm^3.

As a result of production testing, it was found that in 68 % of cases sub-clinical mastitis was caused by pathogenic staphylococci (*S. aureus*). Agalactious streptococcus (*Str. agalactiae*) made up 17 % and associated microflora was found in 15 % of the cases (Fig. 7).

Results of the research into microbial contamination of the skin of nipples and the udder show that on it there are always micro-organisms that can cause sub-clinical mastitis disease. The only difference is that their ratio changes (Table 2).

As Table 2 shows, a primogenital cow has the least amount of *S. aureus* and the largest amount of *Str. agalactiae*. At an increase in animal age, the ratio changes a little, since the amount of *S. aureus* increases and, vice versa, the amount of *Str. Agalactiae* decreases. At the same time, a change in the amount of associated microflora was not detected. It is possible to make an assumption that the microorganisms on the udder skin are antagonists among themselves.

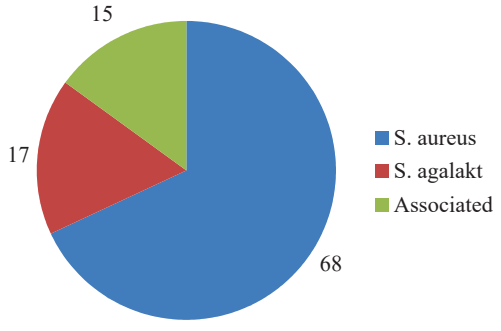

Fig. 7. Ratio of the pathogens of sub-clinical mastitis

Table 2

Microbial contamination of cow udder skin ($M \pm m$)

Age of cows (lactation)	Microflora of nipples skin (%)		
	S. aureus	*Str. agalactiae*	Associated
1 (n=5)	29±1.4	60±3.7	11±2.0
2 (n=7)	38±2.0	56±2.3	6±1.4
3 (n=7)	49±3.8	44±2.9	7±1.3
4 (n=6)	48±3.7	43±4.2	9±1.2

Therefore, pathogens always exist on the udder skin of animals. That is why it is difficult to eliminate completely sub-clinical mastitis in a herd, but it is possible to control it and its spread within 7–8 %.

The main stages of the disease prevention is strict observance of the technology of milking cows, systematic examination of cows using the California mastitis test (test «Shalma») and the separation of the affected animals from the healthy in order to break the epizootic chain. One of the reasons for rapid dissemination of sub-clinical mastitis in a herd is carrying the pathogen, especially with increased pathogeny, from sick animals to the healthy during milking. The main mechanical carrier is the rubber of milking cups through the direct contact with the udder skin of a sick and a healthy animal.

Due to this fact, the research into the dynamics of bacterial contamination of milking cups rubber was carried out. Before starting milking, the rubber of milking cups underwent careful mechanical cleaning, was washed with water and disinfected. After disinfection, it was thoroughly washed with distilled water and dried. Research results are shown in Table 3.

Table 3

Dynamics of bacterial contamination of rubber of milking cups, depending on the number of milked cows ($M \pm m$, n=5)

Age of animal (lactation)	Before starting milking, CFU/cm^2	After milking five cows, thousand, CFU/cm^2	After milking ten cows, thousand, CFU/cm^2
1	2.1±0.1	534.7±29.9*	1,336.7±45.1*
2	2.3±0.2	601.4±23.0*	1,486.3±34.6*
3	2.3±0.3	843.6±45.0*	1,568.4±67.6*
4	2.1±0.2	811.3±48.1*	1,489.9±59.0*

*Note: * – $p \le 0.05$ compared with that before milking*

Microbial contamination of the rubber of milking cups before milking was within 2.1–2.3 thousand CFU/cm^2. After milking five and ten heads of cows, bacterial contamination of the rubber of milking cups in the first lactation increased by 267 and 668 times, in second lactation – by 261.3 and 646.2 times, respectively. The same tendency was observed during other lactations ($p \leq 0.05$). As it can be seen from the research results (Table 2), microbial contamination of the rubber of milking cups has a dynamic character towards increasing.

The main sources of milk seeding are dairy equipment and milk pipeline. In order to study bacterial infection of milk depending on the technologies of its obtaining, experiments were carried out on four farms using different milking systems (Table 4). Before the research, thorough sanitary and hygienic cleaning and disinfection of cow udders were performed. At the same time, dairy equipment (rubber of milking cups, a collector, a milk hose, a milk pipeline and a cooling tank) was prepared. In all cases, milk was taken after its cooling up to 5–6 °C. The DeLaval plant was used at farms 1 and 2. The difference between these plants was the number of milking machines. The plant ADM-8 with the milk pipeline of different length was used at farms 3 and 4.

Table 4

Microbiological seeding of milk at different technologies of its obtaining ($M \pm m$)

Milking technology	Milking equipment, CFU/cm^3					CBO, CFU/cm^3
	rubber of milking cups	milk hose	milk collector	wash water	milking bucket	milk
DeLaval No. 1 $n=24$	24.5±0.90	27.3±1.20	28.8±7.91	67.8±6.90	–	91.3±7.9
DeLaval No. 2 $n=20$	23.8±0.17	26.9±1.30	26.7±5.61	59.3±4.91	–	86.9±3.7
ADM-8 milk pipeline $n=20$	3.7±0.23	8.1±0.31	4.2±0.20	231.8±17.9	–	345.4±38.9
ADM-8 portable milking buckets $n=20$	2.8±0.18	6.7±0.20	3.4±0.20	–	21.9±3.91	67.4±3.9

As Table 4 shows, depending on the technology of obtaining milk and peculiarities of a milking plant, total milk seeding differs significantly (from 67.4±3.9 to 345.4±38.9 CFU/cm^3). Thus, milk is the best by indicators of microbial infection (67.4±3.9 CFU/cm^3) when using milking buckets. This is explained by the fact that in this case all dairy equipment is taken apart, exposed to careful cleaning and disinfection under the control of an operator after each milking. After washing, milking equipment is dried. Although in this case all parts of milk equipment also differ slightly in terms of microbial infection (from 2.8±0.18 to 21.9±3.91 CFO/cm^3).

Most intensive milk seeding was found at a farm with the milk pipeline of the plant ADM-8 – 345.4±38.9 CFU/cm^3. This is due to the fact that this milk pipeline of the outdated model has a number of joints, which accumulate many microorganisms, and are difficult to reach by the disinfecting agent. However, if we compare the results of bacterial contamination of wash water in plants DeLaval, they are almost similar (67.8±6.90 and 59.3±4.91 CFU/cm^3). Although at farm 1, which uses a longer milk pipeline, the CBO of milk is slightly higher (P is unreliable).

Thus, the studies make it possible to affirm that the dairy equipment has the greatest impact on CBO.

To prevent the infection of cows with mastitis pathogens (*S. aureus* and *Str. agalactiae*) during milking, it is necessary to carry out disinfection of rubber milking cups after milking every cow. To do this, it is proposed to use the ozone and air mixture (OAM), which is environmentally friendly. The ozone and air mixture is obtained using an ozone generator. For convenience and time saving in the process of milking, the OAM is fed along rubber tubes to milking cups in between milking.

The production of safe and high-quality milk at dairy farms is possible only at the systematic management based on the NSC control.

The system of control over obtaining high-quality milk is based on two programs and prediction of cows getting sick with a hidden form of mastitis.

The first program determines the correlation dependence of NSC and productivity of animals at a hidden form of mastitis in individual cows in the specified terms. The use of the second program makes it possible to determine the percentage of the cows getting sick with a hidden form of mastitis by determining the NSC in collected milk.

Thus, the proposed *Scor* can be used both on individual farms, and wherever there is significant amount of livestock. NSC in milk at a hidden form of mastitis is expressed in millions per cm^3. In the course of the studies, a simplified system of the ratio was proposed.

Each following count of the *Scor* corresponds to a particular number of somatic cells and the percentage of an increase in dairy efficiency (Table 5). Therefore, the study according to the first program in the system of control over production of safe and high-quality milk indicate a correlation between NSC and productivity of animals.

Table 5

Prediction of milk productivity of cows under *Scor*

Scor	Number of somatic cells (thousand/cm^3)	Losses of milk productivity of cows for a lactation (kg/305 days)			
		1 lactation		2 lactation	
		Mean value	Optimal value	Mean value	Optimal value
1	75	–	–	–	–
2	100	72	75	142	150
3	200	148	150	285	300
4	400	225	225	430	450
5	800	293	300	590	600
6	1,600	375	375	710	750

Note: the developed Scor values correspond to the cows with productivity within 5,000–7,000 kg of milk per lactation

The second program uses the developed indicators of dependence between the NSC in the gathered milk and the percentage of disease of cows in a herd. The correlations between them were established (Table 6).

Table 6

The number of somatic cells in gathered milk depending on percentage of disease incidence

Number of affected cows (%)	NSC in milk, (thousand/cm^3)
7	200–250
12	400–450
20	600–650
30	1,200–1,350
50	1,800–2,000

The data from Table 6 show that there is a direct dependence between NSC in milk and percentage of cow disease incidence in a herd, as well as illustrate the dynamics.

5. 2. Results of studying animals for ketosis and milk for ketone bodies

The experimental studies of milk for ketone bodies were carried out. Thus, after 30 days from the beginning of the experiment, the body temperature of animals on average decreased by 0.7 °C, which corresponds to the healthy state. The conducted research (Table 7) allows stating that the clinical status of the animals that are sick with a hidden form of ketosis changes significantly.

Table 7

Indicators of clinical status and correlation of metabolic processes in organism of cows ($M \pm m$, $n=5$)

Indicators	Examination period	Group of animals		
		control (healthy)	control (sick)	treatment (30 days)
T (°C)	beginning	38.4±0.1	39.1±0.2	39.3±0.2
	ending	38.3±0.1	39.4±0.2	38.6±0.1
Pulse (beats/min)	beginning	61.2±0.9	89.2±1.1*	92.1±2.5*
	ending	61.1±0.8	93.1±1.3*	73.3±0.7
Respiration (motion/min)	beginning	17.4±0.7	33.6±1.6*	34.3±1.7*
	ending	17.3±0.7	36.6±2.2*	23.1±1.1*
Contraction of rumen (motion/5 min)	beginning	9.0±0.3	3.5±0.2*	3.7±0.3*
	ending	9.0±0.3	3.3±0.2*	8.9±0.2
Activity of microflora (min)	beginning	1.9±0.2	6.2±0.3*	6.4±0.9*
	ending	1.9±0.2	8.6±0.3	2.7±0.7
Number of Infusoria (thousand/ml)	beginning	928.9±35.7	342.4±18.8*	367.4±24.3*
	ending	931.7±41.9	296.6±27.4*	753.3±41.0*
(pH) of rumen content)	beginning	6.8±0.4	5.1±.0.3*	4.7±0.2*
	ending	6.8±0.3	4.8±0.4*	6.6±0.2
Milk acidity (°T)	beginning	16.2±0.2	18.2±0.3	18.1±03
	ending	16.2±0.3	20.9±0.7	16.5±0.2

*Note: * – $p \le 0.05$ compared with healthy animals; ** – $p \le 0.05$ compared in relation to the beginning of treatment. At $p \le 0.05$ changes were considered reliable*

The number of pulse waves decreased by 18.8 beats per minute, the frequency of respiratory motions – by 11.2 to the physiological norm ($p \le 0.05$). Rumen contraction became normal and was within 8.9±0.2, which is by 5.2 movements more ($p \le 0.05$) from the beginning of giving chelate metals (iron, zinc, manganese, copper). The activity of microflora also significantly increased. However, it should be noted that the number of Infusoria increased by 385.9 pieces. The acidity of the rumen content was within the 6.7 pH, which corresponds to the state of healthy animals. Titrated milk acidity was within 16.5, which corresponds to the «Extra» grade indicators.

Fig. 8, 9 show a small number (up to 342.4±18.8 thousand/ml) of Infusoria, which was found in the rumen content of the sick animals at the beginning of the study. However, larger forms of Infusoria were not almost observed. According to the results of the experimental studies, the number of large forms of Infusoria in the rumen content increased significantly in 5–7 days after the application of chelate metals.

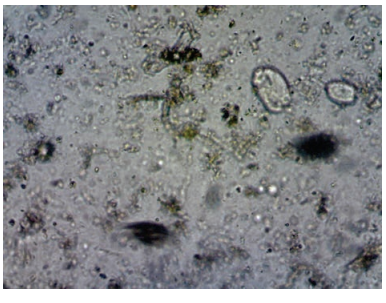

Fig. 8. Infusoria (×400)

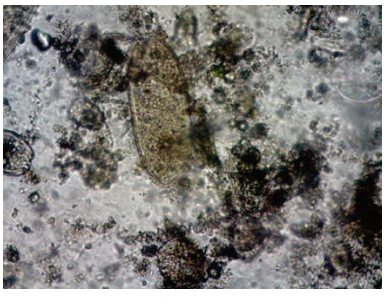

Fig. 9. Small and large forms of Infusoria (×400)

The conducted studies revealed (Table 3) that the simplest after the use of feed supplement with chelate metals became more numerous up to 753.3±41.0 ($p \le 0.05$). In them, the active linear motion of both large and small forms was observed (Fig. 10, 11).

Fig. 10. Infusoria (Holotricha) (×400)

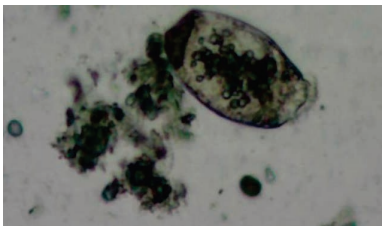

Fig. 11. Infusoria (Spirotricha) (×400)

In Fig. 10, one can see on closer inspection that the internal organs of Infusoria have a bluish shade. This indicates that Infusoria inactivate methylene blue. Thus, it is possible to judge on the level of metabolic processes in the body of cows by the number of Infusoria and their activity. However, for full understanding of the impact of the feed additive based on chelate metal on cows, the studies of the morphological and biochemical composition of venous blood were conducted (Table 8).

Table 8

Morphological and biochemical composition of venous blood of cows

Indicators	Groups of animals (2–5 day after calving)			
	healthy (control)	sick (control)	Examination period	
			beginning 2–4 day	ending (30 days)
Erythrocytes, T/l	5.7±0.3	5.6±0.3	5.6±02	5.9±0.3
Leukocytes, g/l	8.9±0.3	5.6±0.5	5.3±0.4	8.4±0.5*
Total protein, g/l	84.2±4.4	97.2±4.1	96.7±3.1	82.7±4.2*
Glucose, mmol/l	2.5±0.2	1.6±0.2	1.5±0.3	2.6±0.3*
Hemoglobin, g/l	99.1±3.1	98.1±4.1	99.1±3.5	102.2±3.7
Reserve alkalinity (%)	50.1±4.2	30.8±3.4	31.4±2.8	50.1±5.1*
Ketone bodies, mmol/l	2.1±0.2	6.2±0.7	5.9±0.5	1.1±0.2*

*Note: * – $p \le 0.05$ in relation to the data at the beginning of the experiment. At $p \le 0.05$ changes were considered reliable*

Analysis of the data from Table 8 shows positive changes in the organism of the cows. It is known that on the first days after calving the number of leukocytes increases, and at ketosis, vice versa, a decrease was detected (leucopenia). Thus, the number of leukocytes, compared to healthy cows was less by 3.6 T/l. After using the feed additive based on chelates of metals the leukocytes count increased by 3.1 T/l ($p \le 0.05$). The total protein content decreased by 14 G/l ($p \le 0.05$). However, the amount of glucose increased by 1.1 mmol/l ($p \le 0.05$), which corresponds to the minimum indicator of healthy animals. Hemoglobin almost did not differ in indicators. Reserve alkalinity of blood increased by 18.8 % and corresponded to the physiological norm ($p \le 0.05$). The amount of ketone bodies decreased by 4.8 mmol/l ($p \le 0.05$).

6. Discussion of research results and the development of measures to enhance milk quality during production

Experimental research into cow milk has revealed the species composition of somatic cells and their number depending on the udder health. That is, it was found that during occurrence of sub-clinical mastitis, the NSC in milk gradually increases by tens and even by hundreds of times, which can reach up to 25,000–30,000 thousand/cm^3. It was established that this happens at the expense of leukocytes (stab neutrophils). It was proved experimentally that the most accurate method is direct counting the number of somatic cells in raw milk smears – the microscopic method of Prescott-Brid. The disadvantage of this research is its complexity for using at dairy farms. This method also enables the earliest diagnosis, but is quite time-consuming for using on dairy farms. That is why the Californian mastitis test (Shalma test) was used as an alternative at a production enterprise. From all of the above, it gives the most approximated result in comparison with the Prescott- Brid method. It was found to detect affected animals already at an increase in NSC within 500 thousand/cm^3. The prospect of subsequent research is the creation of a more universal mastitis test, which would be less time-consuming and with less error for using at production enterprises.

The improved method for blood smear registration, taking into consideration the milk fat content, makes it possible to determine the species composition of somatic cells. It is necessary to note that the methods for diagnosis of subclinical mastitis have different sensitivity. Researchers believe that the settling test in not effective to detect the cows that are sick with sub-clinical mastitis. The use of dimastine and mastidine, according to certain authors, is not always effective, which leads to researchers receiving an increased number of sick animals [24, 25].

Thus, if at the first stage of milking there is cow that is sick with sub-clinical mastitis, there is a high probability that the causative agent of mastitis will be transferred to the udder skin of other animals. That is why sub-clinical mastitis can occur at the unsatisfactory condition of the udder skin (micro cracks, abrasions, etc.) and decreased resistance of the organism of animals [26].

The method for mathematical calculation of coefficient of conversion of determining the number of somatic cells for microscope SX 2610 was experimentally designed. Thus, we determine the NSC in cm^3 of milk. According to this, we determine the physiological state of the udder.

The species composition of mastitis pathogens was determined and the way of their parsing through the rubber of milk cups during milking with a dynamic increase in the number of microorganisms was proved. That is why the method for disinfection with the ozone and air mixture (OAS) was proposed.

The production of safe and high-quality milk at dairy farms is possible only if there is a systematic management that is based on the NSC control.

The system of control over obtaining high-quality milk is based on two programs and the prediction of cows getting sick with the hidden form of mastitis. The proposed *Scor* can be used both on individual farms and at production enterprises. A simplified *Scor* system was developed, which makes it possible to determine the percentage of the cows with mastitis in a herd by the content of somatic cells in gathered milk and prediction of a decrease in milk productivity.

An animal receives a significant portion of necessary substances to meet the organism's own needs and to generate milk from digestion of microorganisms that are found in the rumen. Microorganisms need satisfactory conditions for the reproduction in the rumen. Dairy cows are capable to convert 30 % of vegetable protein into animal protein [27–29].

During ketosis disease, the physiological state of cows worsens in consequence of metabolic disorders. Ketone bodies and toxins get into milk. It was experimentally proved that the use of chelate metals by animals normalizes metabolic processes in the organism of animals due to the alignment of the sugar-protein ratio. In the rumen, favorable conditions for the growth and reproduction of rumen microflora are created and the level of ketone bodies in milk decreases. Animal feed additive based on chelate metals enables the prevention of ketosis. In terms of using this means as therapeutic, there are some restrictions, depending on the severity of ketosis in animals.

Compared with other methods of dealing with ketosis, one uses propylene glycol, which is necessary to be given to every animal in the form of injections. This method is time-consuming and cost intensive [30].

The additive based on chelate metals can be used together with the feeds for the treatment or prevention of ketosis in cows. Chelate metals (iron, copper, zinc and manganese) are activating agents of the functions of enzymes, hormones and vitamins. They take part in the process of blood formation, growth, reproduction and have antioxidant properties [31, 32].

The study of the activity of Infusoria gives the possibility to determine not only the functional condition of the rumen, but also the state of the organism of animals. The simplest are an indicator of the health of cows. Long-lasting and expensive blood, urine and milk are usually applied at metabolism disorders in ruminant animals. That is why the method for studying Infusoria of the rumen to determine changes in the organism of ruminant animals was proposed.

It should be noted that the proposed measures implemented at a production enterprise make it possible to enhance the quality and safety of milk and to increase its grade up to the «Extra» class. This can be achieved by reducing the number of somatic cells and microorganisms in milk and decreasing the incidence of mastitis in cows. The acidity of milk can be decreased at the expense of prevention and treatment of ketosis in cows.

7. Conclusions

1. It was found that at cows getting sick with sub-clinical mastitis, the number of somatic cells in milk increases and their species composition changes. For early diagnosis of mastitis at a production enterprise, it is recommended to use the Shalma test and to use the Prescott-Brid method to confirm the diagnosis. To prevent cows being infected with mastitis pathogens (*S. aureus* and *Str. agalactiae*) during milking, it is necessary to perform disinfection of the rubber of milking cups with the ozone and air mixture (OAM) after each milking.

2. The effectiveness of using the food additive based on chelate metals was proved to reduce ketone bodies by 4.8 mmol/l, acidity of milk by 4.4 °T and acidity of rumen content, as well as to increase the number of Infusoria compared to the physiological norm ($p \leq 0.05$). The developed system of measures allows the manufacturer to respond timely to changes in animal health by the NSC indicators, and consequently, to improve milk quality and safety indicators.

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