Evaluation of the quality and safety of cows' milk when using the mineral elements

Liliya Sufyanova[1], *Sergey* Smolentsev[1*], *Nadezhda* Kislitsyna[1], *Damir* Aleev[2], *Andrey* Malanev[2], *Kadria* Khalikov[2], *Guzaliya* Yamalova[2], *Gulnara* Gabitova[2], and *Almaz* Valiev[2]

[1]Mari State University, Lenin Square 1, Yoshkar-Ola city, 424000, Russia
[2]Federal center for toxicological, radiation and biological safety, Scientific town 2, Kazan city, 420075, Russia

Abstract. A successful feeding program should solve the following problems: achieving planned milk production, increasing dry matter intake and improving ruminal microbiota. As an integral part of the diet, feed additives have an impact on the animal's body, which is expressed, for example, in an increase in average daily weight gain, a decrease in conversion, an improvement in metabolic metabolism and so on. However, it should be noted that feed additives alone do not guarantee of high productivity or profitability of milk production. The purpose of these studies was to evaluate the effect of the multicomponent feed additive Felucen on the sanitary quality and safety of cows' milk. The studies were conducted on the basis of training and experimental farm, where two groups of dairy cows, 40 heads each, were formed according to the principle of analogues. All cows of both groups received the basic diet accepted on the farm. Additionally, the first group received Felucen feed additive at the rate of 5 g per 1 kg of body. Analysis of the milk composition and productivity was carried out on the 10th, 30th, 60th and 90th day. The use of Felucen provided an increase in the milk productivity of cows by 11% and an increase in the quality indicators of milk: the mass fraction of fat increased by 0.11%, protein - by 0.21%, lactose - by 0.20%.

1 Introduction

At present, the issues of raw milk quality have gone far beyond professional analysis and have become a strategically significant social problem. The modern approach to dairy cattle breeding requires rationalization not only in terms of increasing the gross production of milk, but also ensuring its sanitary quality and safety [1, 2].

Among the complex of external conditions that affect the physicochemical parameters and biological value of milk, the feeding of dairy livestock is of particular importance since feeds not only have a direct impact on milk productivity and quality, but also indirectly affect the body of cows through microbiological processes occurring in the rumen, and other indicators of cicatricial digestion. In addition, a complete and balanced feeding of dairy livestock optimizes metabolism and therefore intensifies the synthesis of milk and its components [3, 4].

* Corresponding author: Smolentsev82@mail.ru

In recent years, much attention has been paid to the development of various feed additives that can increase milk productivity, milk fat content, its saturation with vitamins, microelements and other nutrients, as well as increase feed digestibility and stimulate metabolic processes in cow. From this point of view additives of plant origin are the most valuable due to their natural origin.

Over the past few decades, biologically active substances of natural origin have been mentioned as an important component of the feed rations of productive animals. A certain number of plants, as well as products of their processing and waste products of these industries, are rich in such compounds. By-products of the processing of vegetable raw materials are more often used in the feeding of farm animals both in the form of mono-feed and in combination with various natural and synthetic components [5-7].

In Russia, there are conditions for the development of new forage crops, as well as large reserves of unused plant raw materials, which can serve as a source for the production of high-quality biologically valuable feed. In particular, stevia and yacon processed products are considered. Feed composition based on these plants can fully meet the requirements for new generation supplements, which should be biologically active, harmless and bioavailable, and also be able to improve the palatability of basic feeds, ensure a balanced diet, increase the digestibility and use of nutrients, improve the physiological status of livestock and contribute to the prevention of stress and metabolic diseases, and, as a result, have a positive impact on the quality of the products, in particular milk [8-10].

That is why the study of using phyto-feed compositions from non-traditional raw materials in the diet of lactating cows in order to improve the veterinary and sanitary indicators of milk quality and safety is relevant.

The aim of the work was a veterinary and sanitary assessment of the quality and safety of cows' milk when using Felucen multicomponent feed additive in the diet.

2 Materials and methods

The main experimental and laboratory part of the work was carried out at the Department of Veterinary and Sanitary Expertise. Felucen feed additive was introduced into the basic diet of lactating cows at the rate of 5 g per 1 kg of live weight per feeding 3 times a day.

We used 40 red-and-white cows of the 1st and 2nd calvings, divided into experimental and control groups according to the principle of paired analogues taking into account age, calving time, number of lactations, live weight and productivity, fat and protein content in milk, with identical conditions of keeping, milking and feeding. The first group received the main diet (control group); the second group received the main diet + 5 g/ of a multicomponent feed additive per 1 kg of live weight. According to the design of the experiment, the examined parameters were determined during four studies in both groups of animals (control and experimental): the first study - background indicators at the beginning of the studies (10th day of lactation), then the 30th, 60th and 90th day of the experiment.

Animal productivity was assessed using the method of control milking every month from the 10th day of lactation.

Hematological studies of venous blood from the jugular vein were carried out on the 1st (background), 30th, 60th and 90th day of the experiment in the amount of 160 samples before the morning feeding of animals. The studies included indicators of natural resistance: lysozyme, complementary, bactericidal activity of blood serum, the level of phagocytosis; biochemical indicators: the amount of total protein (including the ratio of albumin and globulin fractions), urea, total lipids, cholesterol, phospholipids, ketone bodies, glucose levels, as well as the activity of alkaline phosphatase, ALT (alanine aminotransferase) and AST (aspartate aminotransferase); indicators of the body's antioxidant defense system and lipid peroxidation products: activity of SOD (superoxide

dismutase), catalase, GPO (glutathione peroxidase), GR (glutathione reductase), antioxidant activity of blood plasma, as well as the amount of MDA (malonic dialdehyde) and vitamin E.

Veterinary and sanitary indicators of milk quality and safety were determined in accordance with the Technical Regulations of the Customs Union "On the safety of milk and dairy products" (TR CU 033/2013), "On food safety" (TR CU 021/2011) during the first 100 days lactation. The mass fraction of fat, protein, lactose was determined on the 10th day of lactation (background), then on the 25th, 50th, 75th and 100th day. In addition, the structural characteristics of fat and protein were studied. The fatty acid composition of milk and technological indicators (rennet-fermentation test, heat resistance, acidity, density) were studied in triplicate: background, the 50th and 100th day of lactation. Microbiological indicators of milk (total microbial count, the number of somatic cells and pathogenic microorganisms) were examined every decade, starting from the 10th day of lactation (background), for three months. The content of antibiotics and potentially hazardous substances in milk was determined during four replicates: background (the 10th day of lactation), the 30th, 60th, 90th day of the experiment.

3 Results and discussion

The level of metabolism in the animals can be assessed using such indicators as the concentration of total protein, the ratio of albumins and globulins, urea. The studies showed an increase in the concentration of total protein by 14.32% ($P \leq 0.001$) in the experimental group, including an increase of the albumin fraction by 42.7%; by the 90th day it was 44.39±1.21 g/l ($P \leq 0.001$). At the same time, the concentration of albumin did not change significantly in the control group. Most likely the increase in protein concentration was governed by the stimulating effect of Felucen as a source of microbial protein for cicatricial microflora. Based on these data, it can be concluded that protein and albumin synthesis in the liver and milk protein synthesis proceed more intensively in cows fed by Felucen as part of the diet.

To get a more complete picture of the intensity of protein metabolism, the concentration of urea in the blood serum was studied. Analysis of its content in the blood serum of cows of the experimental group showed a decrease in urea concentration; the urea concentration remained at the initial level in the control group. Thus, the established growth of total protein level due to albumins with a simultaneous decrease in the level of urea in the blood of the cows of the experimental group indicated a more effective absorption of dietary nitrogen in general.

The nature of carbohydrate-fat metabolism in animals can be assessed by the concentration of total lipids, cholesterol, total phospholipids and glucose in the blood serum.

During the experiment, an increase in the level of glucose up to 2.55±0.01 mmol/l ($P \leq 0.001$) was observed in the blood of the cows of the experimental group, which was equal to 8.97%. In the control group, this indicator did not change significantly throughout the experiment. In our opinion, the increase in glucose level to the middle of the reference values is explained by the presence of additional source of easily digestible carbohydrates in the form of Felucen, which indicates an increase in the level of carbohydrate metabolism, and, accordingly, an intensification of bioenergetic processes.

The amount of total lipids increased by 24.13% ($P \leq 0.001$), the greatest increase by 13.48% was observed from 30 to 60 days. This indicator had no significant fluctuations in the control group. As for the concentration of cholesterol in the blood serum, it decreased by 4.57% ($P \leq 0.001$) in the experimental group by the 90th day of the study; the level of total phospholipids increased by 4.86% ($P \leq 0.01$) compared with the control group.

The activity of alkaline phosphatase in the blood of cows of the experimental group decreased by 12.8% ($P \leq 0.01$) compared to the initial level. The difference between the final values of the control and experimental groups was 14.46% in favor of the latter ($P \leq 0.001$). This indicates that the process of catalyzing the hydrolysis of phosphoric acid esters and organic compounds, as well as the transport of phosphorus, was successful, thus, Felucen may serve as an additional source of calcium and phosphorus.

The decrease in the concentration of ALT and AST in the blood of cows of the experimental group was 19.4% ($P \leq 0.001$) and 8.66% ($P \leq 0.001$), respectively. A gradual decrease in the activity of these enzymes within the normal range indicates the normalization of their release into the bloodstream, as well as the absence of damage or destruction of cells with high level of these enzymes (liver, kidneys, myocardium, skeletal muscles, nervous tissue, etc.).

In the experimental group of cows, whose diet included a phyto feed additive from biologically active raw materials, the lysozyme activity of blood serum (LABC), the complementary activity of blood serum (CABC), the bactericidal activity of serum blood (BABC) increased by 10.05%, 14.41% and 7.78% ($P \leq 0.001$) in relation to the background values by the end of the experiment. These indicators did not change significantly in the control group of cows. Phagocytic activity, phagocytic index and phagocytic number in the experimental group tended to increase, while in the control group they did not change significantly.

All this indicates an improvement of the characteristics of nonspecific resistance of the body.

The activity of the enzymatic part of antioxidant defense increased by 17.5% for SOD (superoxide dismutase) ($P \leq 0.05$), by 20.37% for GPO (glutathione peroxidase) ($P \leq 0.05$), by 5.37% for GR (glutathione reductase) ($P \leq 0.05$) and by 17.65% for catalase ($P \leq 0.001$). These data indicate that upon entering the body of animals, the feed additive had an antioxidant effect and increased the activity of the enzymatic part of the antioxidant defense system of the body, on the one hand, and reduced the amount of free radicals, on the other.

The activity of free radical lipid oxidation was assessed by the accumulation of lipid peroxides, which were determined in the form of malondialdehyde (MDA). The concentration of MDA in the experimental group significantly decreased by 16.09% compared to the background values ($P \leq 0.001$) and was 0.73 μmol/l. The difference in the content of MDA between the control and experimental groups was 27% ($P \leq 0.001$) by the fourth study.

The concentration of vitamin E in the blood serum of cows of the experimental group increased by 9.36% ($P \leq 0.001$) by the end of the study. The result obtained indicates a sufficient amount of tocopherols in the feed, their normal assimilation and a sufficient body supplement with lipoproteins responsible for the transport of tocopherols.

In addition, the antioxidant activity of blood plasma was studied. It increased by 37.75% ($P \leq 0.001$) in the experimental group of cows in contrast to the control group, where this value almost did not change during the entire experiment.

Thus, we can say that Felucen helps to increase the antioxidant activity of the blood of cows of the experimental group, increases the body's ability to withstand the factors that activate free radical oxidation.

The milk productivity of experimental animals that received feed additive as part of the diet for the entire lactation period significantly increased by 11.0% compared to the control group. It should be noted that the physiologically determined decrease in milk yield from the 6th to the 9th month of lactation occurred more smoothly in the experimental group, and the difference in milk yield over this period was 26.7%. On average, milk yield in the experimental group increased by 2.2 kg per day.

Quantitative and qualitative indicators of the most important components of milk directly depend on the level of metabolism. Increase in the content of fat, protein and lactose due to metabolism intensification was confirmed by the data obtained during the research. Background values in both groups were at the same level, small difference between them were not significant. In the experimental group, the mass fraction of fat, protein and lactose increased by 0.13%, 0.22% and 0.24%. In the control group, changes in these parameters were not statistically significant.

Studies have shown that the mass fraction of fat has increased due to an increase in the number of fat globules, but not their size. In the experimental group, the number of fat globules increased by 24% ($P \leq 0.001$) in relation to the background values by the 100th day of the experiment while in the control group it remained approximately at the same level. An important criterion that determines the properties of milk protein is its fractional composition. A redistribution of protein fractions due to an increase in the proportion of casein fractions by 11.79% ($P \leq 0.001$) was established. At the same time, there was a trend towards a decrease in the proportion of whey proteins. In the control group of cows, these changes were insignificant.

The increase in the percentage of fat, protein and lactose in milk can be explained by the fact that upon entering the body of cows Felucen contributed to the growth of microflora activity, the intensification of fermentation processes and, accordingly, the increase in the amount of VFA in the rumen, in particular acetic and propionic acids, which are precursors of milk fat, protein and lactose.

In addition, studies have shown that the use of Felucen in the diet of cows provided an increase in the proportion of unsaturated fatty acids in milk by 10% by the 100th day of the experiment. The proportion of saturated fatty acids gradually decreased, so the difference between the final and background values was 5% by the end of the experiment.

Proper implementation of the technological process and obtaining a standard dairy product that meets the requirements of regulatory documents ensure the technological characteristics of milk, such as rennet clotting, heat resistance, density and acidity. In this regard, the effect of Felucen on these milk indicators was studied. The results obtained are presented in table 1.

Table 1. Technological indicators of milk of experimental animals.

Groups of animals	Rennet-fermentation test, class	Thermal stability, group	Density, kg/m^3	Acidity, ^{o}T
Background				
Control	2.0±0.02	2.0±0.03	1027.9±0.20	16.9±0.17
Experiment	2.0±0.02	2.0±0.01	1028.0±0.18	16.8±0.14
50th day				
Control	2.0±0.03	2.0±0.04	1027.6±0.16	17.2±0.11
Experiment	1.9±0.02	1.5±0.06	1027.5±0.25	17.1±0.08
100th day				
Control	2.0±0.05	2.0±0.01	1027.6±0.13	17.0±0.09
Experiment	1.7±0.02*	1.6±0.02*	1027.6±0.30	16.9±0.23

An increase in the rennet-fermentation test class by 15% was noted in the experimental group by the 100th day of lactation, which, apparently, was associated with an increase in the proportion of the casein fraction of milk protein. Also, an increase in the heat resistance group by 20% in relation to the control group was noted. Most likely, it was associated with the additional intake of carbohydrates in the composition of Felucen and the optimal protein content in the diet. The density and acidity of milk did not change after the introduction of the feed additive into the diet and remained normal in both groups of

animals throughout the experiment, which indicated a balanced diet in terms of mineral composition.

The results of the microbiological analysis of milk from cows that received the feed additive, as well as the determination of the number of somatic cells, are presented in Table 2.

Table 2. Veterinary and sanitary indicators of milk of experimental animals.

Groups of animals	**Total microbial count CFU/g (maximum allowable level no more than $5*10^5$)**	**Somatic cells, in 1 sm^3 (maximum allowable level no more than $7,5*10^5$)**	**Pathogenic microorganisms, including salmonella, in 25 g**
Background			
Control	$0.36*10^5$	$2.9*10^5$	Not detected
Experiment	$0.36*10^5$	$2.7*10^5$	Not detected
30th day			
Control	$0.22*10^5$	$3.2*10^5$	Not detected
Experiment	$0.21*10^5$	$2.5*10^5$	Not detected
60th day			
Control	$0.28*10^5$	$3.7*10^5$	Not detected
Experiment	$0.28*10^5$	$1.7*10^5$	Not detected
90th day			
Control	$0.28*10^5$	$3.1*10^5$	Not detected
Experiment	$0.27*10^5$	$1.6*10^5$	Not detected

As can be seen from the data presented in the table, the total microbial count in the experimental and control groups decreased by 25% and 22.2% by the 90th day, and was equal to 0.27*105 CFU/g and 0.28*105 CFU/g, respectively. The number of somatic cells in the group of cows receiving the feed additive decreased by 40.74% by the 90th day of the experiment. The number of somatic cells in general increased by 6.9% In the control group of cows. No pathogenic microorganisms, including salmonella, were found in the milk of both groups. This indicates that Felucen did not affect the level of bacterial contamination, and the difference in the number of somatic cells in the milk of cows from the experimental and control groups may be an indirect indicator of feed additive ability to influence the body's natural resistance which provides higher milk quality. At the next stage of research, indicators that determine the safety of milk were studied: the content of antibiotics, toxic elements (lead, arsenic, cadmium, mercury), radionuclides, pesticides, mycotoxins and inhibitory substances.

No antibiotics of the tetracycline group, streptomycin and penicillin were found in the milk of cows of the control and experimental groups. Levomycetin was found during the first study in the control and experimental groups, but its concentration did not exceed the maximum allowable level.

The content of lead, arsenic, cadmium and mercury in the milk of cows of both groups was less than the allowable values. The amount of pesticides, mycotoxins and radionuclides also did not exceed the normal value, there were no inhibitory substances.

Thus, our studies have shown that feed additive has a positive effect on the quality of milk, increasing the amount of fat and protein, does not affect the safety of milk according to the main criteria and can indirectly affect microbiological indicators, reducing the number of somatic cells. The milk obtained from the cows of the experimental group is safe in terms of all veterinary and sanitary indicators and can serve as a good raw material for the production of safe dairy products.

4 Conclusion

1. Hematological parameters of animals of the experimental group were characterized by an increase in total protein in blood serum by 14.32%, including albumin fraction - by 42.7%, glucose levels - by 8.97%, total lipids - by 24.13%; a decrease in the concentration of urea - by 25.6%, ketone bodies - by 3.6%, cholesterol - by 5.7%; activity of alkaline phosphatase - by 12.8%, ALT - by 19.4%, AST - by 8.6%. Nonspecific indicators of cellular and humoral immunity increased: LABC - by 10.05%, CABC - by 14.41%, BABC - by 7.78%. The concentration of MDA decreased by 32.36%, the activity of SOD increased by 17.5%, catalase - by 17.65%, GPO - by 20.37%, GR - by 5.37%, the concentration of vitamin E increased by 9.36%, antioxidant activity of blood plasma - by 37.75%.
2. The use of Felucen provided an increase in the milk productivity by 11% and an increase in the quality indicators of milk: the mass fraction of fat increased by 0.13%, protein - by 0.22%, lactose - by 0.24%. An increase in the number of fat globules by 24% and the proportion of casein fraction by 11.79% was noted. The volume of milk belonging to class I according to rennet-fermentation test and heat resistance increased by 15% and 20%, respectively.
3. Veterinary and sanitary assessment of the milk of experimental animals showed a decrease in total microbial count and somatic cells by 25% and 40.74%. Safety indicators, such as the content of antibiotics, toxic elements (lead, arsenic, cadmium, mercury), pesticides, mycotoxins and radionuclides did not exceed the maximum allowable level when using Felucen. There were no inhibitory substances in milk.

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