IJAS Iranian Journal of Applied Animal Science

New Synbiotic-Mineral Complex in Lactating Cows' Diets to Improve Their Productivity and Milk Composition

Research Article

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Received on: 20 Feb 2019
Revised on: 5 Jun 2019
Accepted on: 15 Jun 2019
Online Published on: Mar 2020

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Online version is available on: www.ijas.ir

ABSTRACT

The aim of this study was to investigate the effectiveness of a new experimental complex in diets of lactating cows. The scientific and economic experiments were conducted in conditions of a large industrial complex for milk production from cows of Holstein breed (Russia). Four groups of Holstein cows (third calving), 20 animals each, were formed to conduct an analog method research. The cows in the control group were fed with a basal diet. The cows in other groups were fed with the experimental complex at various doses. All indices have been determined by wide-known methods, such as extracting, capillary electrophoresis, chromatography, quantitative titrimetry, atomic-emission spectrometry and others. To synthesize milk, the organisms of cows in experimental groups spent more digested nitrogen on the average by 14.85%; nitrogen excreted from the bodies of cows with urine was less on average by 4.13%. The increase in milk production averaged 3.8%. In comparison with the control group, the cows of experimental groups had higher content of dry matter in milk on average by 0.4%; fat by 0.17%; and protein by 0.1% (including α-lactalbumin by 31.8% and β-lactoglobulin by 36.6%). The milk protein in experimental groups contained more essential amino acids on average by 9.83%, and the milk fat contained more unsaturated fatty acids on average by 1.46%. The milk in experimental groups was noted for a high content of vitamins (on average by 16.3%, compared with the control group), in particular B_2 by 10.8%, B9 by 28.6% and D_3 by 39.8%. In addition, the weight fraction of trace elements in milk has been found to increase, i.e., calcium by 17.3%, iodine by 36.4%, potassium by 20.6%, magnesium by 18.5%, manganese by 66.7%, phosphorus by 20.3% and silicon by 54.2%. The increase in the milk productivity of cows and higher fat content in milk made it possible to increase the sale profit of 1 ton of milk on average by 12% and increase the level of profitability of production by 6.5%. The study performed has reliably proved that the proposed premix promoted nutrient availability and digestibility, improved digestibility of feeds and their effective consumption, improved blood hematology, increased milk production and improved the milk composition, which led to an increase in the profitability of production.

KEY WORDS bioavailability, dairy cows, feeding, profitability, synbiotic-mineral, yield.

INTRODUCTION

The problem of producing milk and dairy products in sufficient quantities to meet the recommended norms has remained urgent so far. Moreover, an essential field of research in livestock species is studying important production traits (Pasandideh *et al*. 2015). The increase in the volume of milk production and improvement of its quality largely

depend on the adequate rations of lactating cows (Gorlov *et al.* 2014; Gorlov *et al.* 2016b; Titov *et al.* 2016; Martin *et al.* 2017). Mineral nutrition has paramount importance for a fortified diet of productive animals. In recent years, special attention has been paid to the use of mineral substances in organic form in feeding cattle, since they are the safest and most bioavailable (Spears, 1996; Prasad and Gowda, 2005; Overton and Yasui, 2014). To date, there has been found high effectiveness of clays, bentonite and zeolite as sources of the organic forms of micronutrients in the livestock and poultry industry to eliminate their deficiency in diets, and also as adsorbents for removing toxic substances from the body (Mighoratil *et al.* 2007; Jones *et al.* 2017; Valpotic *et al.* 2017; Gorlov *et al.* 2018). At the same time, the issue of the effectiveness of feed additives based on organic silicon remains relevant.

The increase in the dairy cattle breeding productivity is known to be achieved by increasing the share of concentrated feedstuff in rations, but the feed of nonindustrial production does not always meet the necessary requirements. However, the excess of starch in the mixed feed promotes the development of *Streptococcus bovis* that converts starch into lactic acid. Its high level, in turn, reduces the amount of cellulolytic microorganisms, so the animal digests fiber worse (Russell and Wilson, 1996). To reduce the negative effects of starch excess is possible due to cellulolytic probiotics in the diet of cattle. For this purpose, we have chosen the "*Bacillus* spp." natural complex of living bacteria that has an increased thermostability and functions as a feed enzyme and probiotic, which may help restore the cellulose activity of the rumen and make more complete use of the dietary fiber.

The living yeast culture in the livestock diets is known to promote the development of enzymes that accelerate the process of fermentation in the rumen, increase the feed palatability and synthesis of bacterial protein, accelerate the synthesis of free fatty acids and reduce the level of ammonia in the rumen. Getting into organisms of cattle and small ruminants, living yeast creates an anaerobic environment in the rumen, which promotes the growth of cellulolytic bacteria. Due to the yeast, less lactic acid is formed in the rumen, which makes it possible to control the pH level in the rumen. The result of the influence of yeast on fermentation in the rumen is an increase in milk yield and muscle mass (Aquilina *et al.* 2015).

Silicon-containing preparations of natural origin possess not only sorption and catalytic properties (due to the large nanoporosity of microscopic shells of diatomaceous algae), they are also sources of mineral substances due to the content of biogenic silicon (up to 75-88%), aluminum, iron, potassium, sodium, calcium, magnesium, barium, titanium and etc. In addition, diatomite adsorbs and removes mycotoxins, pesticides, toxic metals and radionuclides from the body, enhances the activity of a number of its enzyme systems and is the source of water-soluble silicon that is necessary for the stable functioning of the smooth muscles of the intestine and stomach of animals and improving the assimilation of calcium (Fernandez *et al.* 1998).

Taking into account the biological properties of the aforementioned feedstuffs, the team of authors set a goal to study the efficiency of a new premix on the basis of experimental complex in feeding lactating cows of Holstein breed and reveal the patterns of its influence on the productive indices of animals and milk composition. To achieve this goal, there were accomplished the tasks:

- to determine the effect of different doses of the developed premix on the consumption and level of nutrient digestibility and availability in the rations
- reveal the dynamics of hematological indices
- study the milk production of animals
- evaluate the profitability of milk production when using premix in feeding

The experimental complex (%) contained Coretron diatomite (63.8); enzymatic probiotic "Cellobakterin®-T" (5.4), Biosprint® preparation (1.8); glucose (3.6); and propylene glycol (5.4).

MATERIALS AND METHODS

Animals and sampling

The scientific and economic experiment was conducted in conditions of one of the largest of Russia industrial complexes for breeding dairy cattle of Holstein breed (3.9 thousand animals) – the limited liability company Agricultural Enterprise Donskoe (Gorlov *et al.* 2016a). Furthermore, this company is one of the leading raw milk producers in the Russian Federation.

The animals were in a loose housing barn. Separate milking was done in special areas using milking parlors. The dairy complex had two milking parlors equipped with two types of milking machines, i.e. Herringbone parlor "Impulsa" (2006) containing 16 places with throughput up to 120 animals per hour and Rotary Milking Parlor "Westfalia Surge" (2010) containing 36 places with throughput up to 200 animals per hour.

Four groups of aged Holstein cows (third calving), 20 animals each, to conduct an analog method research, was formed with respect to the similar age, health status, calving, insemination time, live weight and productivity (from March 2017 up to November 2017, during the entire lactation period). The similarity of animals was established on the data in the farm record books, determination of the live weight of animals and registration of daily milk yields.

The cows in the control group were fed with a standard diet adopted in the household. The cows in treatment 1 were fed with the premix studied at a dose of 8 g per animal a day in their diet, in treatment 2- 10 g and treatment 3- 12 g. The developed premix was being introduced into the ration of cows for 240 days of lactation in the composition of concentrated fodders. The main period of the experiment was preceded by a preliminary one (10 days). The ration developed in the farm was designed to receive a daily milk yield of 25 to 27 kg. The average daily diet consisted of 20 kg of corn silage, 4 kg of alfalfa hay, 10 kg of combined silage (cereals, legumes, etc.), 6 kg of concentrated feedstuff (wheat, barley, etc.), 1 kg of soybean cake, and 10 kg of brewer's grains. The feeding standards per head a day were at least 19 energetic feed units during days in milk (the expected producing ability was 9000 kg of milk per lactation) and depended on the weight and the age of the animal. The rations were developed on the basis of the requirements established (Dunham and Call, 1989; DeLaval, 2001; NRC, 2001; Kalashnikov *et al.* 2003) with the "Korm Optima Expert" program complex ("KormOptima", Russia) used. The preparation of analysed samples of animal feeding stuffs was carried out according to the GOST ISO 6498-2014 "Feeds, compound feeds. Preparation of samples for testing". The chemical analysis of the feed was conducted by the SpectraStar 2400 infrared analyzer (Unity Scientific, USA).

Control feeding and balance experiments were conducted according to the recommendations. Three animals in each group were evaluated with respect to the nutrient digestibility of the ration and balance of nitrogen, calcium and phosphorus in their bodies by the digestion trials (Ovsyannikov, 1976; Nevalainen, 2014). During the experiment, animals received accurately counted amount of feed, whose composition was previously analyzed. Then, the amount of feces excreted for the experiment was accurately taken into account, and its chemical composition was determined. The amounts of consumed and excreted nutrients were calculated based on the data on the weight and chemical composition of the feed consumed and feces excreted. The amount of digested substances was identified by their difference. The digestibility coefficient was found by the formula:

$$DC= (n-v/n) \times 100$$

Where:
n: amount of the nutrients consumed.
v: amount of the nutrients excreted.

Food consumption in each group of cows was tested by weighing the fodder and its orts every ten days during two adjacent days. Morphological and biochemical compositions of blood were monitored using Medonic CA 620 (Ireland) and Cormay (Poland) analyzers. Blood samples were taken monthly from the jugular vein. The state of natural resistance was determined by the tests characterizing the phagocytic activity of the white blood cells (Kondrakhin, 2004; Day and Schultz, 2014). The milk productivity level of cows was determined based on the results of the daily control milking operations with the fat and protein content registered. Sampling of milk and dairy products for physical and chemical analyzes was carried out in accordance with the GOST 26809-86 "Milk and milk products. Acceptance regulations, methods of sampling and preparation for testing".

Milk composition and quality evaluations

The quality indices of milk were analyzed once a month in accordance with the GOST requirements: titratable acidity according to the GOST R 54669-2011 "Milk and milk products. Methods for determination of acidity", density according to the GOST R 54758-2011 "Milk and milk products. Methods for determination of density", determination of weight fraction of moisture and dry skim milk residue according to the GOST R 54668-2011 "Milk and milk products. Methods for determination of moisture and dry substance mass fraction" and GOST R 54761-2011 "Milk and milk products. Methods for determination of dry skim dairy residue mass-fraction", respectively, fat content according to the GOST R ISO 2446-2011 "Milk. Method of determination of fat content", protein according to the GOST 23327-98 "Milk and milk products. Determination of mass part of total nitrogen by Kjeldahl method and determination of mass part of protein, amino acid composition – using capillary electrophoresis system "KAPEL®-105M" (Russia) and fatty acid composition by gas chromatography according to the GOST 32915-2014 "Milk and milk products. Determination of fatty acid content by gas chromatography method.

The safety examination of the dairy products was based on the following standards: SanR&S 2.3.2.1078-01 "Hygienic requirements for safety and nutritional value of foods," technical regulations of the Customs Union "On the safety of milk and dairy products" (TR TC 033/2013) and Guidelines GL 1.2.2961-11 "Scientific rationale of the maximum permissible levels for contaminants of the chemical nature and food additives in food products".

Determination of the chemical elements concentration in fermented milk products was performed on the quadrupole mass spectrometer Elan 9000 (PerkinElmer Instruments LLC, USA, by the inductively coupled plasma mass spectrometry: MS-ICP) and on the atomic emission spectrometer Optima 200 DK (PerkinElmer Instruments LLC, USA, by the atomic-emission spectrometry with inductively cou-

pled plasma: NPP-ISP). The content of vitamins was determined on a chromatograph "Agilent 1200 Series" (Agilent Technologies, USA) by the high-performance liquid chromatography (HPLC). The cost-effectiveness of the raw milk production was counted in accordance with the methodology of determining the economic efficiency of the research and design work results, new facilities, inventions and innovations in agriculture, as well as the annual actual and intrafarm economic effect using the following formulas (Ministry of Agriculture of the USSR and All-Union Academy of Agricultural Science, 1983; Minakov, 2015), per animal:

Cost price of 1 kg of standard fat content milk (€)= farm inputs (€) / milk of standard fat content (kg)

Milk sales proceeds (€)= milk of standard fat content (kg) × sales price 1 kg of standard fat milk (€)

Profit (€)= milk sales proceeds (€) – farm inputs (€)

Profitability level (%)= (profit (€)/farm inputs (€)) × 100

Statistical analysis

The data on different variables, obtained from the experiment, were statistically analyzed by Statistica 10 package (StatSoft Inc.). The significance of differences between the indices was determined using the criteria of nonparametric statistics for the linked populations (differences with P < 0.05 were considered significant: ***P<0.001; **P<0.01; *P<0.05; ns: non significant at P>0.05) (Johnson and Bhattacharyya, 2010). The mean of a set of measurements was calculated according to the formula:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Where:

$\bar{x}$: mean value.

$\sum_{i=1}^{n} x_i$: sum of all x_i with i ranging from 1 to n.

n: number of measurements.

The residual variation is expressed as a root mean square error (RMSE):

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} \left(x_i - \bar{x}\right)^2}{n-1}}$$

The standard error of mean (SEM) was calculated using the formula:

$$s.e.m.\left(\bar{x}\right) = \frac{\sigma}{\sqrt{n}}$$

The reliability of a sample difference (*Student's t-distribution*) was estimated by the test of the difference validity, which is the ratio between the sample difference and the non-sampling error. The test of the difference validity was determined by the formula:

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{s.e.m._1^2 + s.e.m._2^2}} \geq t_{st.}(d.f. = n_1 + n_2 - 2)$$

Where:

t: student's tdistribution.

$\overline{x_1} - \overline{x_2}$: difference of the sample mean measurements.

$\sqrt{s.e.m._1^2 + s.e.m._2^2}$: sample difference error.

$s.e.m._1$ and $s.e.m._2$: nonsampling errors of the compared sample statistics.

t_{st}: standard criterion according to the t-Table for the probability threshold preset depending on degrees of freedom.

n_1 and n_2: numbers of measurements in the samples compared.

$d.f.$: degrees of freedom for the difference of two mean measurements.

RESULTS AND DISCUSSION

Feed consumption and nutrient digestibility

As Hall (1997) showed, specialists who provided nutritional consulting services to cattle owners had to ensure that the feed samples for nutrient analysis were properly subsampled and then used their knowledge of the feedstuffs to evaluate the validity and usefulness of the results. Therefore, all samples of feedstuffs were estimated before and during this study. The introduction of the fodder additive in the diet of cows contributed to the increase in palatability and nutrient digestibility. So, over a period of 305 days of lactation the cows in treatment 1, 2 and 3 consumed more hay legume than the control cows by 1.69, 9.63 and 4.21%, respectively; Sudan grass hay by 1.56, 3.69 and 4.23%; haylage by 2.60, 3.15 and 3.21%; and silage by 2.72, 3.54 and 3.99% (in accordance with results of control feeding and balance experiment). Compared to the control group, the consumption of dry matter in Treatment 1, 2 and 3 was higher by 1.56 (P<0.05), 2.55 (P<0.001) and 3% (P<0.001); crude protein by 0.91 (P>0.05), 2.24 (P<0.05) and 3.13% (P<0.001); and crude fat by 2.95 (P<0.001), 4.36 (P<0.001) and 6.16% (P<0.001).

This regularity was also observed in terms of consumption of organic matter, fiber and nitrogen-free extractable substances. The nutrient digestibility and accessibility are important in the nutrient metabolism in the bodies of cattle (Hall, 1997). The animals fed with the additive at a dose of 8 g per animal exceeded their analogs in the control group with respect to the amount of digested dry matter by 3.93% (P<0.001), at a dose of 10 g by 5.50 (P<0.001) and 12 g by 6.68% (P<0.001); crude protein by 2.19 (P>0.05), 4.34 (P<0.01) and 5.57% (P<0.001), respectively; and crude fat by 4.73 (P<0.001), 7.49 (P<0.001) and 11.50% (P<0.001). Accordingly, more organic matter, neutral detergent fiber (NDF) and nitrogen-free extractable substances were digested in the organisms of cows in treatment 1, 2 and 3 (Table 1). Due to more intensive digestion of nutrients in the bodies of cows consuming the additive studied, the indices of their digestibility coefficient were higher (Figure 1).

Table 2 shows that the cows in treatment 1, 2 and 3 digested more nitrogen than in control group by 2.17% (P<0.01), 4.34% (P<0.001) and 5.55% (P<0.001). To synthesize milk, the organisms of cows spent more digested nitrogen by 9.11% (P<0.001); 14.79% (P<0.001) and 20.66% (P<0.001), compared with the control group. In the bodies of cows, there was more dry matter stored than in the control group by 12.33% (P<0.01), 17.81% (P<0.001) and 20.55% (P<0.001). In this case, in Treatment 1, 2 and 3 the nitrogen excreted from the animals' organisms with urine was less than in the control group by 3.12% (P<0.01), 3.55% (P<0.01) and 5.72% (P<0.001), respectively. There were no significant differences in the amount of nitrogen excreted with feces.

Compared with the control group, digested calcium secreted with milk was higher in treatment 1, 2 and 3 by 4.46% (P<0.01), 8.27% (P<0.001) and 11.02% (P<0.001), respectively. In bodies of that cows, there was more calcium stored, than in the control group by 12.19% (P<0.001), 19.51% (P<0.001) and 24.39% (P<0.001). There were no significant differences in the amount of calcium excreted with feces and urine.

The cows in treatment 1, 2 and 3 digested more phosphorus than in the control group by 6.50% (P<0.001), 9.05% (P<0.001) and 9.51% (P<0.001). There were no significant differences in the amount of phosphorus secreted with urine. To synthesize milk, there was consumed more phosphorus than in the control group by 7.01% (P<0.001), 9.55% (P<0.001) and 10.51% (P<0.001), respectively. In the bodies of the cows consuming the additive under study, there was stored more phosphorus than in the control group by 12.5% (P<0.001), 21.87% (P<0.001) and 25.0% (P<0.001).

Jones *et al.* (2017) hypothesized that the ion exchange capacities of zeolites may serve as a buffering agent within the rumen and improve performance in a dose dependent fashion. The found data indicated, that under the conditions of that experiment, the addition of zeolites to steam flaked corn-based finishing diets does not impact final body weight or feed efficiency and tended to improve dry matter intake and average daily gain of feedlot cattle when zeolites were included at 1% of diet dry matter.

Hematologic and natural resistance indices

An important exposure indicator of environmental factors on the animal organism is changes in their blood composition. Being the main link between metabolic processes in the body, i.e. delivery of nutrients and oxygen to the cells of organs and tissues, removal of metabolic wastes, direction and intensity of metabolism and physiological state of an organism are most accurately determined on the basis of a biochemical and morphological analysis of blood, because while maintaining a constant composition, the blood, nevertheless, is a sufficiently mobile system that reflects the changes in the body under normal and pathological conditions. When categorized for experiment, hematological indices of the cows were almost equal (in accordance with analog method research). After 90 days of feeding, that is, in the period of intensive increasing in the milk yield of the lactating cows, there was registered a tendency of erythrocytes and hemoglobin in blood to increase in favor of the treatment 1, 2 and 3 (Table 3). So, the blood of that cows contained more erythrocytes than in the control group by 2.80 (P>0.05), 6.07 (P>0.05) and 7.32% (P<0.05), respectively; and more hemoglobin by 0.92 (P>0.05), 2.27 (P>0.05) and 2.84% (P>0.05). At the end of lactation, a similar situation persisted the concentration of erythrocytes in blood of cows consuming the additive was higher than that in the control group by 1.87 (P>0.05), 5.47 (P<0.05) and 6.41% (P<0.01). Hemoglobin was contained in blood of the cows in treatment 1, 2 and 3 more than in the control by 0.83 (P>0.05), 2.51 (P>0.05) and 3.15% (P>0.05). According to the leukocytes content in blood of the cows, certain regularity was not revealed. It should be noted that all the analyzed parameters of the morphological blood composition varied within the biological norm.

It should be noted that the parameters of the biochemical blood composition varied with more significant intervals in groups. So, after 90 days of feeding, the blood serum of the cows in treatment 1, 2 and 3 contained more total protein than in control group by 0.64 (P<0.05), 1.29 (P<0.001) and 1.35% (P<0.001), respectively. Furthermore, the blood serum contained more albumins by 2.76 (P<0.001), 8.10 (P<0.001) and 8.49% (P<0.001).

Table 1 The level of consumption and digestibility of nutrients in rations (g)

Indices (Mean±SE)	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
Nutrients received				
Dry matter	20017.8±87.5	20331.4±81.4*	20527.9±90.2***	20618.4±73.6***
Organic matter	18816.7±82.3	19009.8±78.5^{ns}	19419.4±69.3***	19340.1±80.1***
Crude protein	3578.6±23.2	3611.3±21.8^{ns}	3658.9±24.3*	3690.7±18.8***
Crude fat	587.2±3.6	604.5±2.8***	612.8±3.1***	623.4±2.9***
NDF	3532.5±7.9	3638.1±9.4***	3695.4±8.1***	3737.3±8.5***
NFC	8442.7±23.1	8622.3±30.6***	8721.1±25.4***	8945.5±31.6***
Nutrients digested				
Dry matter	12891.5±84.3	13398.4±91.5***	13601.0±101.4***	13752.5±93.6***
Organic matter	12212.0±79.8	12641.5±86.4**	13069.3±93.1***	13112.6±89.2***
Crude protein	2268.8±20.6	2318.5±21.2^{ns}	2367.3±22.6**	2395.2±17.6***
Crude fat	374.0±2.9	391.7±3.1***	402.0±2.3***	417.0±3.7***
NDF	1932.3±6.3	2015.5±7.5***	2062.0±5.9***	2090.9±7.0***
NFC	5825.5±19.6	6104.6±23.8***	6235.6±21.2***	6431.8±25.4***

NDF: neutral detergent fiber and NFC: non fibrous carbohydrates.
*** (P<0.001); ** (P<0.01) and * (P<0.05).
NS: non significant.
SE: standard error.

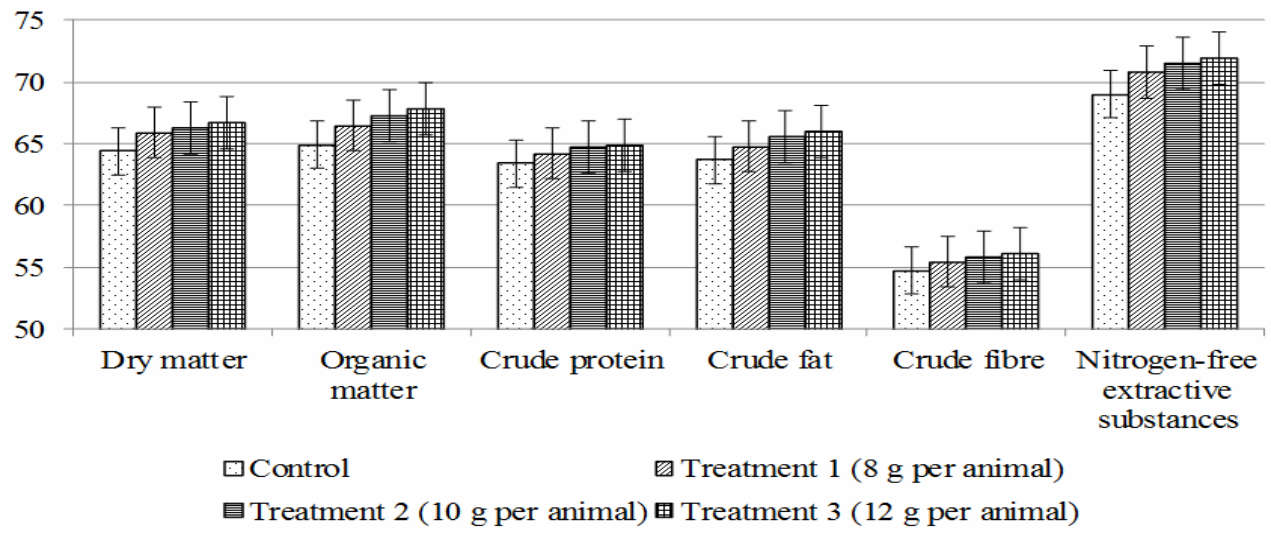

Figure 1 Feed digestibility ratios (%)

At the end of the lactation, that cows had more total protein in the blood serum than the cows in the control group by 0.19 (P>0.05), 0.82 (P<0.01) and 0.98% (P<0.01). The albumin fraction was contained in the serum of their blood more than in the control group by 1.44 (P<0.01); 6.44 (P<0.001) and 6.62% (P<0.001), respectively.

An increase of these indices of blood serum in experimental groups indicated a better assimilation of nitrogen in feed as a result of an increase in the enzymes activity in their bodies. Being in close relationship with proteins of various tissues, protein fractions of blood serum subtly reacted to the changes in chemical and physicochemical processes in the organs of animals. The changes in the immunobiological reactivity of the body indicated the intensity and lability of metabolic processes, which affected the constituent protein fraction of blood serum.

Higher productivity of animals is known to cause higher blood saturation with proteins and especially with albumins. Albumins play an important role in colloid osmotic pressure and carry out a transport function consisting in binding and transfer of fatty acids, cholesterol and a number of other substances.

Only the cows fed with the premix in their diet were established to have increased the content of mineral elements in their blood. So, in blood of that cows, there was more silicon than in the control group by 9.29 (P<0.05), 18.59 (P<0.001) and 19.33% (P<0.001), respectively; calcium by 10.78 (P>0.05), 18.97 (P<0.01) and 20.69% (P<0.001); phosphorus by 1.42 (P>0.05), 3.79 (P>0.05) and 4.26% (P>0.05); iron by 1.70 (P>0.05), 3.71 (P>0.05) and 4.07% (P>0.05); and sodium by 4.98 (P>0.05), 8.65 (P>0.05) and 8.87% (P>0.05).

It should be noted that a similar trend in blood was seen with respect to copper, zinc and magnesium. At the same time, the content of lead and cadmium decreased in blood of the cows in treatment 1, 2 and 3. The lead content decreased by 6.10 (P>0.05), 13.82 (P<0.001) and 12.60% (P<0.001), respectively; and cadmium by 10.45 (P>0.05), 20.90 (P<0.05) and 23.88% (P<0.001), which confirmed the sorption properties of the premix.

In the course of the research, we investigated the effect of the premix on the indices of phagocytic activity of leukocytes (Table 3). At the start of the experiment, the indicators characterizing the natural resistance of the organisms of lactating cows varied in groups within the sampling error. However, after 90 days, the phagocytic activity of leukocytes of the cows in treatment 1, 2 and 3increased, in comparison with the control group by 1.68 (P<0.05), 3.41 (P<0.001) and 3.52% (P<0.001); phagocytic number increased by 0.24 (P<0.05), 1.07 (P<0.001) and 1.14 (P<0.001); and phagocytic capacity by 2.14 (P>0.05), 5.86 (P<0.01) and 7.55% (P<0.001). The phagocytic index was observed to have a tendency to increase as the doses of the feed additive in rations enhanced. At the end of the lactation, the phagocytic activity of leukocytes of that cows was higher in comparison with their analogs in the control group by 1.29 (P>0.05), 2 (P>0.05) and 2.17% (P>0.05); phagocytic number was higher by 0.69 (P<0.05), 1.15 (P<0.001) and 1.26 (P<0.001); phagocytic capacity by 3.34 (P>0.05), 5.56 (P<0.05) and 6.82% (P<0.05); and phagocytic index was higher by 0.27 (P>0.05), 0.76 (P<0.001) and 0.87 (P<0.001). The indices of the phagocytic activity of blood leukocytes of cows in treatment 1, 2 and 3 indicated a positive effect of the experimental complex on the natural resistance of their organisms. In this study, it is concluded that this supplementation can boost the immunity. This finding was in agreement with the finding of De *et al.* (2014). They found out the effect of micronutrient supplementation on the immune function, reproductive performance, milk yield and milk quality of crossbred cows.

Thus, the dynamics of hematological indices reliably indicated a more intensive metabolism, which was explained by the positive effect of the constituent components of the experimental additive on the digestion of animals.

Lactation performance of cows

As reported earlier (Aquilina *et al.* 2015), there was a statistically significant improvement in average daily weight gain in feeding based supplementation gut flora stabilizer. We have studied the relationship between the experimental complex that included the drug specified by Aquilina *et al.* (2015) and milk productivity. Mighoratil *et al.* (2007) showed that the addition of 1% on dry matter of the diet for lactating cows of non-nutritional adsorbents (clinoptilolite+sepiolite) have not negatively affected milk yield, milk composition, or cheese-making features. No significant difference was found between treatments in milk yield, milk fat, protein, and lactose concentrations, milk protein yield, pH, or titratable acidity. Valpotic *et al.* (2017) showed potentials and limitations of zeolites in cattle regarding metabolic and endocrine status, oxidative stress and systemic/local inflammatory responses involved in reproductive and metabolic disorders of dairy cows. Their findings confirmed the usefulness of zeolites as a novel feed additive able to maintain and improve health, fertility and performance in cattle production. Considering that diatomaceous earth that was included in our premix is a unique type of sand that consists of fossilized algae and different from zeolites, we expected a positive effect of this supplementation using in feeding of dairy cows. Over a period of 90 days of lactation (the period of increasing the milk yield), the milk yield from cows that received the developed premix in the amount of 8, 10 and 12 g per animal a day was higher than in the control group by 7.15% (P<0.001), 8.20% (P<0.001) and 13.45% (P<0.001), respectively (Table 4); the fat content in milk was higher by 0.05 (P>0.05), 0.09 (P>0.05) and 0.10% (P<0.01); and the protein by 0.07 (P<0.01), 0.12 (P<0.01) and 0.15% (P<0.001). In terms of the total amount of milk fat, the cows in treatment 1, 2 and 3 outperformed their control analogs by 8.56% (P<0.001), 10.80% (P<0.001) and 16.58% (P<0.001), respectively; and the protein by 9.48% (P<0.001), 12.15% (P<0.001) and 18.71% (P<0.001).

For the first half of the lactation (over a period of 150 days), the control group cows produced 4107.7 kg of milk, whereas their analogs in Treatment 1, 2 and 3 produced more by 5.18% (P<0.001), 9.70% (P<0.001) and 11.91% (P<0.001), respectively. The weight fraction of fat in milk from the cows in that groups was higher than in the control group by 0.11 (P>0.05), 0.19 (P<0.001) and 0.21% (P<0.01); and weight fraction of protein by 0.06 (P > 0.05), 0.11 (P<0.001) and 0.13% (P<0.001), respectively. With respect to the total amount of milk fat, that cows outperformed their control analogs by 8.29% (P<0.001), 15.33% (P<0.001) and 18.22% (P<0.001), respectively; and protein by 7.11% (P<0.001), 13.40% (P<0.001) and 16.36% (P<0.001).

It should be noted that over a period of the whole lactation (for 305 days) the yield from the cows in the control group was 7757.8 kg or less than from their analogs in treatment 1, 2 and 3 by 2.92% (P<0.01), 3.75% (P<0.001) and 4.73% (P<0.001). The total amount of fat in milk from the cows in that groups was higher than in the control group by 7.09% (P<0.001), 8.80% (P<0.001) and 10.37% (P<0.001); and the amount of protein by 4.48% (P<0.001), 6.85% (P<0.001) and 8.53% (P<0.001).

Table 2 Balance of nitrogen, calcium and phosphorus in the body of experimental animals (g)

Indices (Mean±SE)	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
Nitrogen				
Received	573.5±1.79	578.7±2.14^{ns}	586.4±1.65***	591.5±2.03***
Secreted with feces	209.9±1.16	207.2±1.35^{ns}	207.0±1.02^{ns}	207.7±1.20^{ns}
Digested	363.6±1.40	371.5±1.81**	379.4±1.34***	383.8±1.67***
Secreted with urine	208.2±1.52	201.7±1.30**	200.8±1.42**	196.3±1.29***
Secreted with milk	148.1±0.98	161.6±0.79***	170.0±1.03***	178.7±0.90***
Stored in body	7.3±0.15	8.2±0.21**	8.6±0.17***	8.8±0.19***
Calcium				
Received with fodder	294.3±2.63	301.1±2.74^{ns}	305.8±2.41**	306.9±3.04**
Secreted with feces	202.5±2.18	206.0±2.30^{ns}	206.9±1.98^{ns}	205.6±2.40^{ns}
Digested	91.8±0.98	95.1±1.04*	98.9±1.22***	101.3±0.94***
Secreted with urine	11.5±0.09	11.3±0.11^{ns}	11.5±0.08^{ns}	11.6±0.09^{ns}
Secreted with milk	76.2±0.84	79.6±0.90**	82.5±1.09***	84.6±1.07***
Stored in body	4.1±0.02	4.6±0.03***	4.9±0.02***	5.1±0.03***
Phosphorus				
Received	143.8±1.62	147.5±1.84^{ns}	150.2±1.38**	151.9±2.12**
Secreted with feces	100.7±0.86	101.6±0.78^{ns}	103.2±0.91^{ns}	104.7±0.83**
Digested	43.1±0.39	45.9±0.45***	47.0±0.26***	47.2±0.32***
Secreted with urine	8.5±0.06	8.6±0.08^{ns}	8.7±0.11^{ns}	8.5±0.07^{ns}
Secreted with milk	31.4±0.27	33.6±0.31***	34.4±0.25***	34.7±0.38***
Stored in body	3.2±0.02	3.61±0.03***	3.9±0.02***	4.0±0.03***

*** (P<0.001); ** (P<0.01) and * (P<0.05).
NS: non significant.
SE: standard error.

Table 3 Dynamics of hematological indices of cows during the experiment

Indices (Mean±SE)	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
After 90 days of lactation				
Erythrocytes, 10^{12} L^{-1}	6.42±0.14	6.60±0.12^{ns}	6.81±0.15^{ns}	6.89±0.12*
Leukocytes, 10^{9} L^{-1}	7.11±0.19	7.36±0.25^{ns}	7.20±0.18^{ns}	7.32±0.27^{ns}
Haemoglobin, g L^{-1}	109.57±1.96	110.58±2.18^{ns}	112.06±2.27^{ns}	112.68±2.73^{ns}
Total protein, g L^{-1}	87.23±0.19	87.79±0.15*	88.36±0.12***	88.41±0.16***
Albumins, g L^{-1}	37.67±0.14	38.71±0.11***	40.72±0.08***	40.87±0.10***
Globulins, g L^{-1}	49.56±0.21	49.08±0.17^{ns}	47.64±0.19***	47.54±0.12***
Phagocyte activity, %	63.42±0.57	65.10±0.49*	66.83±0.52***	66.94±0.42***
Phagocyte number	3.84±0.06	4.08±0.07*	4.91±0.05***	4.98±0.06***
Phagocyte capacity, thousands cells	30.86±0.40	31.52±0.51^{ns}	32.67±0.43**	33.19±0.34***
Phagocyte index	5.10±0.14	5.26±0.11^{ns}	5.31±0.15^{ns}	5.38±0.17^{ns}
At the end of experiment				
Erythrocytes, 10^{12} L^{-1}	6.40±0.10	6.52±0.17^{ns}	6.75±0.13*	6.81±0.08**
Leukocytes, 10^{9} L^{-1}	7.06±0.21	7.42±0.16^{ns}	7.17±0.19^{ns}	7.36±0.12^{ns}
Haemoglobin, g L^{-1}	108.93±1.78	109.84±2.40^{ns}	111.66±2.17^{ns}	112.36±2.40^{ns}
Total protein, g L^{-1}	87.29±0.16	87.46±0.18^{ns}	88.01±0.13**	88.15±0.20**
Albumins, g L^{-1}	37.44±0.13	37.98±0.11**	39.85±0.15***	39.92±0.16***
Globulins, g L^{-1}	49.85±0.19	49.48±0.21^{ns}	48.16±0.12***	48.23±0.15***
Phagocyte activity, %	66.74±0.91	68.03±0.85^{ns}	68.74±0.60^{ns}	68.91±0.74^{ns}
Phagocyte number	4.22±0.17	4.91±0.24*	5.37±0.15***	5.48±0.13***
Phagocyte capacity, thousands cells	34.15±0.62	35.29±0.54^{ns}	36.05±0.44*	36.48±0.60*
Phagocyte index	5.16±0.13	5.43±0.11^{ns}	5.92±0.09***	6.03±0.17***

*** (P<0.001); ** (P<0.01) and * (P<0.05).
NS: non significant.
SE: standard error.

Table 4 Indices of milk productivity of experimental animals

Indices (Mean±SE)	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
Over a period of 90 days of lactation				
Milk yield, kg	2547.4±26.9	2729.6±30.1***	2756.3±27.5***	2890.1±26.3***
Fat, %	3.67±0.03	3.72±0.03ns	3.76±0.04ns	3.77±0.02**
Fat, kg	93.5±0.91	101.5±0.88***	103.6±0.97***	109.0±1.24***
Protein, %	3.23±0.02	3.30±0.01**	3.35±0.03**	3.38±0.02***
Protein, kg	82.3±0.84	90.1±0.99***	92.3±0.92***	97.7±1.06***
Over a period of 150 days of lactation				
Milk yield, kg	4107.7±28.1	4320.5±30.8***	4506.1±34.2***	4597.0±38.5***
Fat, %	3.70±0.03	3.81±0.05ns	3.89±0.03***	3.91±0.05**
Fat, kg	152.0±0.88	164.6±0.83***	175.3±0.99***	179.7±1.40***
Protein, %	3.29±0.01	3.35±0.03ns	3.40±0.02***	3.42±0.02***
Protein, kg	135.1±0.74	144.7±0.89***	153.2±0.96***	157.2±1.36***
Over a period of 305 days of lactation				
Milk yield, kg	7757.8±48.7	7984.5±54.2**	8048.4±42.6***	8124.6±49.0***
Fat, %	3.69±0.04	3.84±0.03**	3.87±0.04**	3.89±0.03***
Fat, kg	286.3±0.91	306.6±0.98***	311.5±1.03***	316.0±1.57***
Protein, %	3.31±0.03	3.36±0.02ns	3.41±0.02**	3.43±0.03**
Protein, kg	256.8±0.60	268.3±0.69***	274.4±0.55***	278.7±0.58***

*** (P<0.001); ** (P<0.01) and * (P<0.05).
NS: non significant.
SE: standard error.

Table 5 Qualitative indices of milk (over a period of 5 months of lactation)

Indices (Mean±SE)	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
Average daily milk yield, kg	26.07±0.17	26.70±0.24*	27.11±0.18***	27.48±0.29***
Fat, %	3.70±0.03	3.81±0.05ns	3.89±0.03***	3.91±0.05**
Protein, %	3.29±0.01	3.35±0.03ns	3.40±0.02***	3.42±0.02***
Incl. casein	2.68±0.03	2.73±0.02ns	2.76±0.02*	2.78±0.03*
Whey protein	0.61±0.01	0.62±0.01ns	0.64±0.01*	0.64±0.01*
β- lactoglobulin, mg ml^{-1}	18.68±1.48	21.85±1.62*	27.32±1.54***	27.38±1.51***
α- lactalbumine, mg ml^{-1}	10.23±1.24	12.35±1.31ns	13.93±1.19*	14.17±1.28*
Density, kg/m^3	1029.56±0.78	1031.14±0.82ns	1032.67±0.90**	1032.73±0.92*
MSNF, %	8.73±0.03	8.86±004*	9.00±0.03***	9.03±0.05***
Milk solids, %	12.43±0.04	12.67±0.07**	12.89±0.05***	12.94±0.06***
Carbohydrates, %	4.68±0.05	4.73±0.04ns	4.81±0.05ns	4.82±0.06ns
Ash, %	0.76±0.01	0.78±0.01ns	0.79±0.01*	0.79±0.01*
Acidity, °T	17.30±0.28	17.41±0.30ns	17.49±0.19ns	17.50±0.23ns
Rennin coagulation property, min.	38.67±1.54	37.04±1.70ns	32.27±3.11*	32.11±1.96*

MSNF: milk solids-not-fat.
*** (P<0.001); ** (P<0.01) and * (P<0.05).
NS: non significant.
SE: standard error.

Determination of the qualitative composition of milk showed that in comparison with the control group, cows in Treatment 1, 2 and 3 had higher weight fraction of fat in milk by 0.11 (P>0.05), 0.19 (P<0.001) and 0.21% (P<0.01); and the protein by 0.06 (P>0.05), 0.11 (P<0.001) and 0.13% (P<0.001), including casein by 0.05 (P>0.05), 0.08 (P<0.05) and 0.10% (P<0.05) (Table 5).

At the same time, there was established a tendency of the whey proteins to increase. So, the content of β-lactoglobulin that performs a transport role increased by 16.97 (P<0.05), 46.25 (P<0.001) and 46.57% (P<0.001), and α-lactalbumin required for the synthesis of lactose in milk from UDP-galactose and glucose – increased by 20.72 (P>0.05), 36.16 (P<0.05) and 38.51% (P<0.05), respectively.

Due to the higher content of fat, protein, carbohydrate and ash in milk from the cows in treatment 1, 2 and 3, the indices of milk solids were higher by 0.24 (P<0.01), 0.46 (P<0.001) and 0.51% (P<0.001), respectively; and the milk solids non fat by 0.13 (P<0.05), 0.27 (P<0.001) and 0.30% (P<0.001), in comparison with the cows in the control group. The rennin coagulation property of milk was also higher in treatment 1, 2 and 3, i.e. the coagulation time was reduced by groups by 1.63 min. or 4.22% (P>0.05), 6.40 min. or 16.55% (P<0.05) and 6.56 min. or 16.96% (P<0.05), respectively.

In milk protein from the cows in treatment 1, 2 and 3, essential amino acids were contained more than in the analogs that did not consume the premix by 2.65% (P>0.05), 12.09% (P<0.05) and 14.75% (P<0.001), respectively (Figures 2A, B).

The increase in the content of essential acids was not the same as the doses of premix enhanced. With respect to the content of essential amino acids in milk, here should be noted a trend in favor of the cows in that groups. In milk from the cows that consumed the premix, there was an increase in the amino acid index from 0.813 (control) to 0.912 (treatment 3).

The biological value of milk and dairy products largely depends on the content of fatty acids and the ratio between saturated and unsaturated acids and the type of ration could have caused the changes in the content of the particular fatty acids in milk fat (Pieszka *et al.* 2015). Our study has found a tendency of the fatty acid content to increase as the dosage of the additive increased (Figure 3A, B). The increase in saturated fatty acids in Treatment 1, 2 and 3 compared with the control was 1.58 (P<0.05), 2.28 (P<0.01) and 2.93% (P<0.001) and unsaturated fatty acids – 0.42 (P>0.05), 1.67 (P<0.01) and 2.28% (P<0.001), respectively.

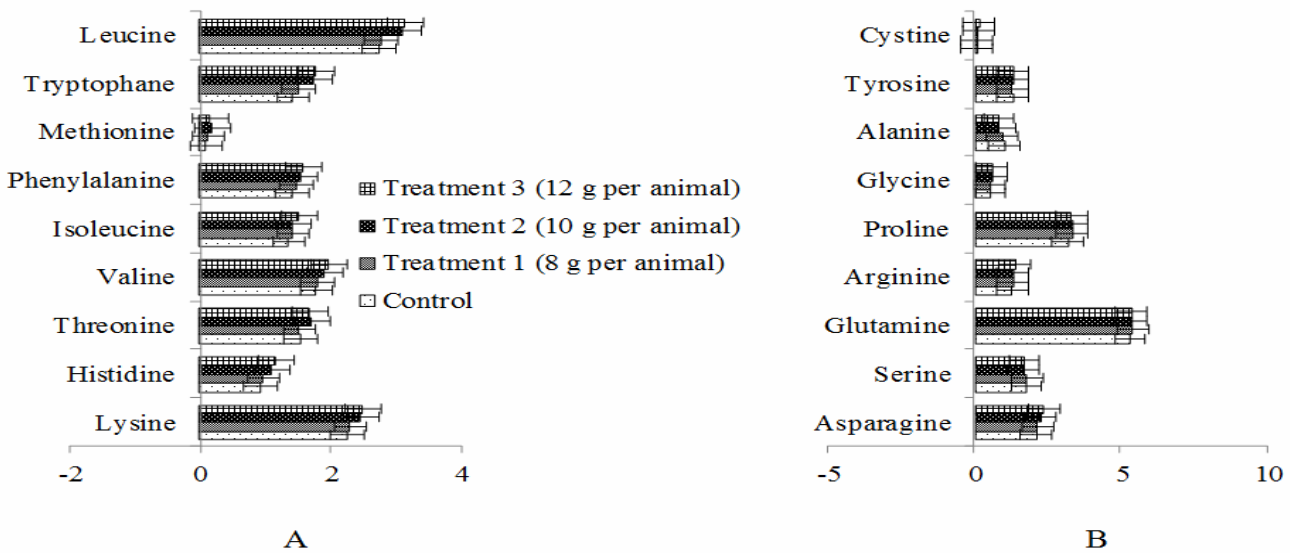

Figure 2 Amino acid composition of milk, g per kg
A: for essential amino acids and **B**: for nonessential amino acids

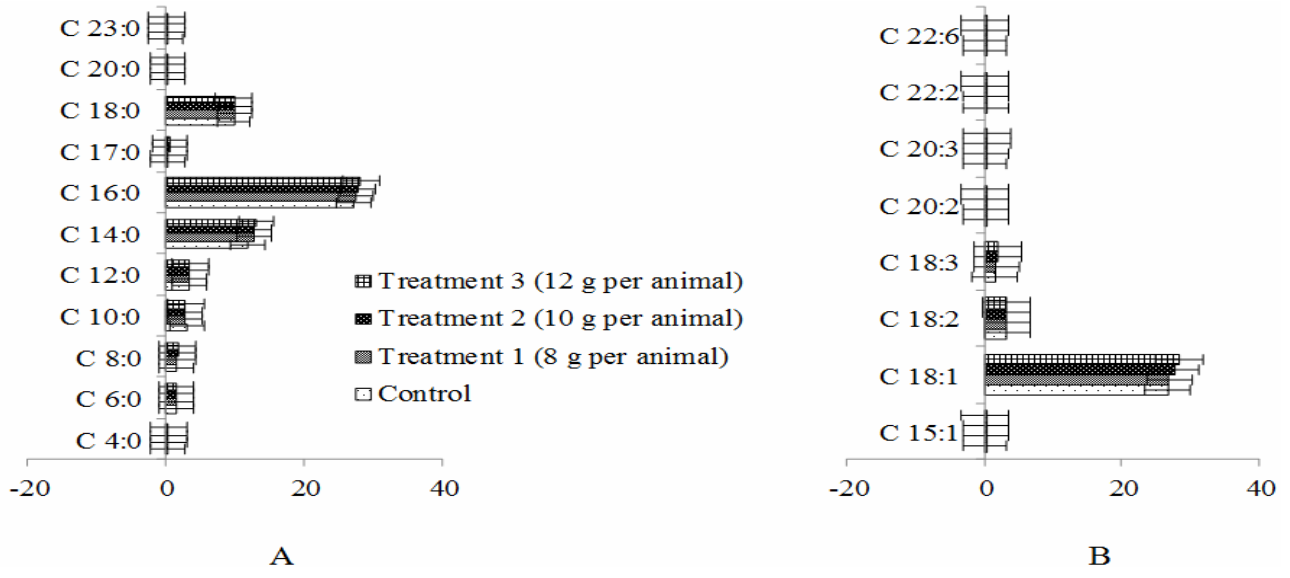

Figure 3 The content of fatty acids in milk fat, %
A: for saturated (C 4:0 oily, C 6:0 caproic, C 8:0 caprylic, C 10:0 capric, C 12:0 lauric, C 14:0 myristic, C 16:0 palmitic, C 17:0 margarine, C 18:0 stearic, C 20:0 arachine, C 23:0 tricosanoic) and **B:** for unsaturated (C 15:1 cis-10-pentadecanoic, C 18:1 oleic, C 18:2 linoleic, C 18:3 linolenic, C 20:2 eicosadienoic, C 20:3 cis-11,14,17-eicosatriene, C 22:2 docosadienoic, C 22:6 docosahexaenoic)

It should be noted that the content of the number of fatty acids (oily, caproic and stearic) did not change significantly as the doses of the feed additive increased, and some acids (capric and linoleic) decreased.

The increase in the weight fraction of lauric acid was 0.21 (P<0.01), 0.25 (P<0.001) and 0.27% (P<0.01); myristic acid – 1.09 (P<0.001), 1.16 (P<0.001) and 1.33% (P<0.001); palmitic acid – 0.23 (P>0.05), 0.48 (P>0.05) and 0.95% (P<0.05); margarine – 0.05 (P>0.05), 0.22 (P<0.001) and 0.19% (P<0.001); cis-10-pentadecanoic acid increased by a factor of 5-13 (P<0.001); oleic acid – by 0.23 (P>0.05), 1.08 (P<0.01) and 1.63% (P<0.001); linolenic – by 0.14 (P>0.05), 0.29 (P<0.01) and 0.32% (P < 0.01); cis-11,14,17-eicosatriene acid increased by a factor of 4-15 (P<0.001).

In milk obtained from the cows in treatment 1, 2 and 3, there was an increase in Al (26.5%), B (33.3%), Ca (17.3%), I (36.4%), K (20.6%), Mg (18.5%), Mn (66.7%), P (20.3%), Sr (13.1%) and Zn (17.9%), compared to the control analogs. It should be noted that the content of silicon (Si) in milk increased by 54.16% (P<0.01).

When studying the vitamin composition (Figure 4) in milk from the cows in treatment 1, 2 and 3, in comparison with their analogs in the control group, an increase in the vitamin B2 content was observed on average by 5.0 (P>0.05), 12.4 (P<0.05) and 14.9% (P<0.001); vitamin B9 – by 18.1 (P>0.05), 33.0 (P<0.001) and 34.6% (P<0.01); vitamin D_3 – by 19.4 (P>0.05), 45.2 (P<0.05) and 54.8% (P<0.01).

Lactation curves characterizing the dynamics of cows' productivity are shown in Figure 5. In general, the lactation characters of the cows in control and treatment 1, 2 and 3 were identical. However, the level of the average daily milk yield of cows for all months of lactation was significantly higher.

Economic effect of milk production

The mean values were calculated as economic parameters in autumn of 2017, the RUR/EUR exchange rate was 68.0. The actual realization value of milk in 2017 in the household was at the level of 0.44 EUR for 1 kg. Due to the fact that the milk yield and its fat content were higher in treatment 1, 2 and 3, the amount of milk with a basic fat content (3.4%) was higher than in the control group by 598.3, 741.4 and 876.0 kg, respectively. The milk sales proceeds received from the cows in that groups were higher by 263.2, 326.2 and 385.4 EUR. The amount of profit was more than in control group by 179.7, 221.9 and 260.1 EUR. The level of profitability of its production was higher by 5.4, 6.5 and 7.6%, respectively (Table 6).

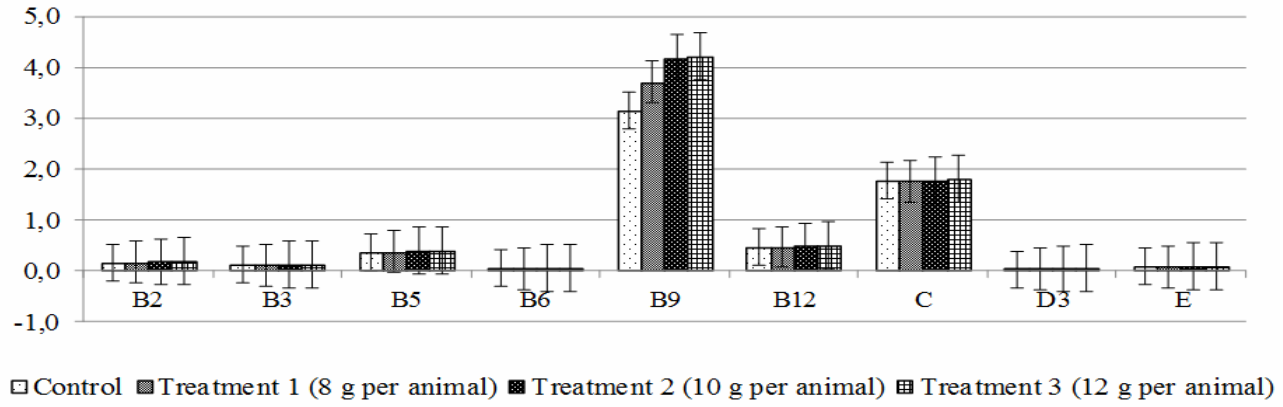

Figure 4 Content of vitamins in milk, mg per 100 g

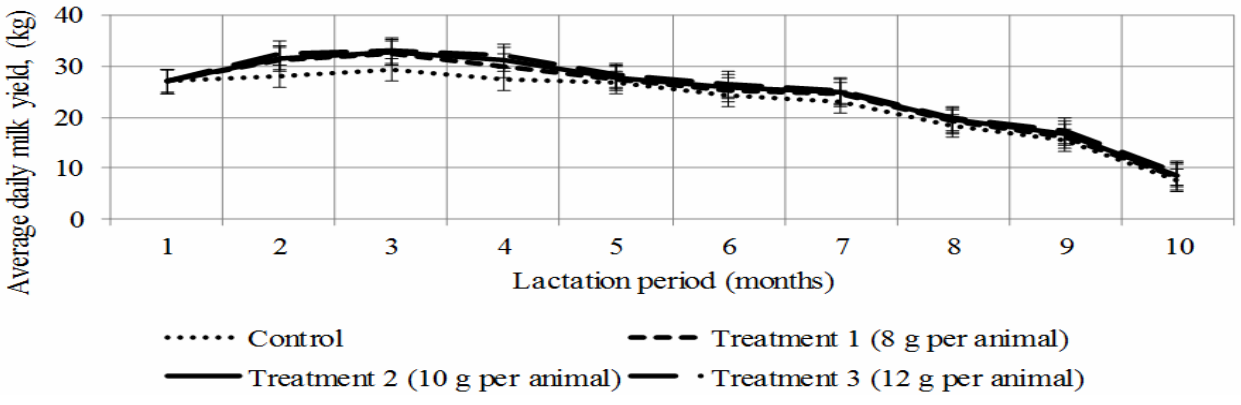

Figure 5 Lactation curves of experimental cows

Table 6 Cost-effectiveness of raw milk production. The average values calculated as economic indicators up to autumn 2017, the RUR/EUR exchange rate of 68.0

Indices	Control	Treatment 1 (8 g per animal)	Treatment 2 (10 g per animal)	Treatment 3 (12 g per animal)
Milk obtained from the full lactation (for 305 days), kg	7757.8	7984.5	8048.4	8124.6
Milk of standard fat content (3.4%), kg	8419.5	9017.8	9160.9	9295.5
Sales price 1 kg of 3.4% fat milk, EUR	0.44	0.44	0.44	0.44
Milk sales proceeds, EUR	3704.6	3967.8	4030.8	4090.0
Farm inputs, EUR	2695.6	2779.1	2800.0	2820.9
Including additional costs for premix	-	83.6	104.5	125.4
Profit, EUR	1009.0	1188.7	1230.8	1269.1
Level of profitability, %	37.4	42.8	43.9	45.0

CONCLUSION

The proposed feed additive used in the rations of lactating cows in doses of 8, 10 and 12 g per animal provided an increase in milk yield by 2.92, 3.75 and 4.73%, the fat content in milk by 0.11, 0.19 and 0.21%, and protein by 0.06, 0.11 and 0.13%, respectively. The level of profitability of milk production from cows fed with the studied additive increased by 5.4, 6.5 and 7.6%, respectively. This study discovered the new experimental complex that can be beneficial for dairy cattle. This study will help the researchers to uncover the critical areas of use and conservation of natural resources that many researchers were not able to explore earlier. Thus a new theory on synergistic effect of this supplement may be arrived at.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Science and Higher Education of the Russian Federation for the financial support in the implementation of this research according to the state assignment of NIIMMP.

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