

Complex Evaluation of Biostimulants for Prevention of Immune System Disorders and Highly Productive Cows and Improvement of Milk Quality

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Abstract The article provides data on the study of the effect of biostimulants on milk production and the state of the immune system of cattle. The quality of nutrition of ruminant animals, which were fed using feed additives with various biological properties, such as biostimulants, modifiers, antioxidants, enzymes and phytobiotics, was studied. It is known that the use of industrial technologies significantly increases the load on the cow's body and exacerbates its functionality and metabolic disease occurs. The results of a study conducted by the authors showed that the introduction of biostimulants changes the milk content and affects the intestinal microflora of ruminants. When biostimulants are introduced into the muscles of experimental animals, this indicator in milk increases. Thus, it can be noted that the influence of biostimulants in experimental animals, the lactose content in milk is higher (80.0%, $p < 0.01$) than in animals with normal indicators of biostimulating effect. In animals with normal lactose content in milk, its content exceeded the limit value by 28.57%, and in animals with low biostimulant values, the significant excess was 141.43% ($p < 0.05$).

Keywords Feed additives, Correction, Immunostimulants, Biostimulants, Milk, Livestock, Leukocytes

1. Introduction

In modern Uzbekistan, animal husbandry is considered one of the fastest growing industries. Today, animal husbandry has become an integral part of the country's economy, stably ensuring its food security, supplying markets with high-quality animal protein necessary to maintain human health. The Resolution of the President of the Republic of Uzbekistan Sh.M. Mirziyoyev dated February 08, 2022 No. PP-120 "On approval of the program for the development of the livestock sector and its industries in the Republic of Uzbekistan for 2022-2026" became the main incentive for the further development of the industry. In accordance with the resolution "In order to ensure food security by increasing the production of livestock products, strengthening the feed base and increasing the potential for feed production in the livestock sector and its industries" [1].

Currently, the market economy in the country, which involves competitive relations between producers, requires increasing milk productivity, improving the quality of milk and reducing its cost. According to a number of authors, the economic efficiency of dairy farming can be increased by

breeding highly productive cows with the required adaptive properties that help maintain immunity and increase the duration of economic exploitation [1,2].

In recent years, in a country with an increasing demand for milk and dairy products, scientists in the agricultural sector have been faced with the task of increasing the volume of high-quality milk production. The level of milk productivity, the content of fat and protein in milk are the main indicators of various biostimulants. Therefore, an important task is to identify the highest quality dairy products.

Milk and dairy products are an important source of nutrients in the human diet, as they contain a number of essential substances and other biologically active components. In order to meet the needs of the population for dairy products, it is necessary to have a local healthy and clean dairy product. [2]. Although dairy production, as a rule, has a relatively positive image among the population, animals graze on pastures and live-in rural areas, the practice of dairy farming is increasingly under close public scrutiny [3]. The positive properties of food biological additives are known to many. In animal husbandry, various mineral, vitamin, protein additives, biostimulants, immunostimulants, biologically active substances, etc. have been effectively used for many years [3,4]. In this regard, feed additives with biologically active properties (vitamins, minerals, natural compounds, such as humic acids, etc.), which have a positive effect on the entire body, are also of particular value [5].

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It has been established that the activation of cattle breeding and the use of various industrial technologies significantly affect the body of cattle and contribute to the stress of its functionality [6]. In such modifying and technological conditions, the physiological needs of animals do not always correspond; in some cases, various diseases with metabolic disorders occur [6]. This is due to the fact that in order to obtain high-quality milk and maintain biological value, special attention should be paid to feeding dairy cows.

It should be noted that complementary feeding and various biological additives affect not only milk yield and milk quality, but also affect the immune system of dairy cows [7,8].

2. Purpose of the Research

Today in Uzbekistan, the consumption of milk and dairy products occupies a leading position among the population. Therefore, the solution to the problem of increasing milk yields of cows is the use of biologically active drugs [8]. One of these drugs are tissue biostimulants, these biogenic stimulants enter the animal's body, activate metabolic processes, strengthen motility, and the secretory function of the gastrointestinal tract increases the overall resistance of the body, which leads to an increase in milk productivity of cows, therefore, different doses of biostimulants have an effect. [6]. In this regard, the main objective of the study is to study the effect of biostimulants on milk productivity and the state of the immune system in cattle. In addition, to determine the effect of feed biostimulant on the indicators of milk productivity of cows and the quality of raw milk. To study the biochemical indicators of cows when using a biostimulant. To assess the economic efficiency of introducing biostimulants into the diets of highly productive cows.

3. Materials and Methods

The studies were conducted using clinical-physiological, microbiological, veterinary-sanitary, zootechnical and mathematical methods [7].

To study the effect of biostimulants on milk productivity and the state of the immune system in cattle, experiments were conducted on 32 dairy cows in a farm in the Zangiata district of the Tashkent region. Animals in the control and experimental groups were given wheat straw (0.7 kg), corn silage (24.0 kg), wheat hay (49.0 kg), complete compound feed (14.58 kg), optigen (0.05 kg) and alfalfa hay. 6.50 kg) Animals of the experimental group, in addition to the basic diet (BR), received a mineral nutrition regulator at a dose of 300 g per day as part of a complete feed.

4. Results and Discussion

The studies of the morphological composition of the blood

were carried out according to the generally accepted methods in veterinary medicine: counting the number of erythrocytes and leukocytes - in the Goryaev chamber, the hemoglobin content in the blood using the Sali method, and the erythrocyte sedimentation rate (ESR) - using the Panchenkov method. Also, for these purposes, the URIT-3020 Vet Plus veterinary automatic hematology analyzer (URIT Medical Electronic Co., Ltd, China) was used. With its help, in addition to the above-described indicators, the following were determined: the number of lymphocytes, monocytes, granulocytes; hematocrit; mean corpuscular volume; mean corpuscular hemoglobin content; mean corpuscular hemoglobin concentration; erythrocyte distribution width; accuracy of repetition of the erythrocyte distribution width; platelet count; mean platelet volume; platelet distribution width using reagent kits (diluent – URIT AD-11 Diluent, detergent – URIT D 41 Detergent, lysing reagent – URIT AL-11 Lytic Reagent) from the same manufacturer in accordance with the operating manual.

The content of total protein, albumin, urea, glucose, cholesterol, inorganic phosphorus, iron, α -amylase were determined in the blood serum of animals.

The methods for determining the studied parameters are given in Table 1. The sets of liquid, ready-to-use reagents "DiaVet Test" ("DIAKON-VET", Russia) and a semi-automatic biochemical analyzer with a flow cuvette BS-3000M ("Sinnowa Medical Science & Technology Co., Ltd", China) were used [150].

Table 1. Methods for determining biochemical parameters of blood

Index	Determination method
Total protein	Photometric test according to the biuret method without a serum blank
Albumin	Method with bromocresol green
Urea	Kinetic, urease-glutamate dehydrogenase UV test. Reaction type – fixed time.
Glucose	Enzymatic photometric, lucose oxidase.
Cholesterol	Enzymatic photometric test.
Inorganic phosphorus	Method with ammonium molybdate.
Iron	Photometric method with ferrozine without deproteinization.
α-amylase	Enzymatic photometric test. Reaction type – kinetics.

It is known that the quality of feed directly affects the structure of cow feeding rations. Due to some physiological characteristics, cows experience a period of negative energy balance, which is very often accompanied by metabolic disorders. In this regard, during the transition period, the energy and protein supply of dairy cows should be approached more carefully, especially when choosing a protein source, including hard-to-digest ones.

Most likely, the established trends in the change in the energy and protein nutritional value of feed are largely due to the observed and described features of climate change. Despite the fact that legumes find it more difficult to adapt to such

conditions compared to cereals, the features of photosynthesis and plant physiology in general allow them to accumulate a much larger amount of nutrients (in particular protein) per unit of DM due to more favorable environmental conditions for them. This is largely due to the adaptive capabilities of crops, their plasticity.

The results we obtained complement the previously accumulated knowledge. It is known that the quality of feed directly affects the structure of cow feeding rations. Due to some physiological characteristics, cows experience a period of negative energy balance, which is very often accompanied by metabolic disorders. In this regard, during the transition period, energy and protein provision of dairy cows should be approached more carefully, especially when choosing a protein source, including hard-to-digest ones. The concentration of glucose in the blood serum of animals of all groups on the 1st day of the dry period was significantly lower than the reference values, which indicates a significant use of its reserves by the body as an energy source. Subsequently, its content in the blood of animals continued to decrease. On the 46th day of the dry period, the level of this indicator decreased in cows of the second and third groups by 37.68 ($p < 0.001$) and 29.10 ($p < 0.01$) %, respectively. On the 3rd day of lactation, the tendency for the most pronounced increase in the glucose level in the blood serum was characteristic of individuals of the third group and amounted to 20.14%, against 8.06% in the second group. In the control group, on the contrary, this indicator decreased by 27.95%. During the milking period, the glucose content in the blood of cows of the experimental groups remained at a higher level and exceeded the values in the control by 10.41 and 5.62% on the 30th and 60th days of lactation, respectively. The concentration of cholesterol in the blood serum of animals of all groups on the 1st day of the dry period was slightly lower or was on the border of the lower values of the physiological norm and amounted to 1.91–2.55 mmol/l. On the 46th day of the dry period, a tendency was revealed for the cholesterol content in the blood serum of cows in the control group to increase by 60.20% ($p < 0.05$) and by 36.47 and 42.22 ($p < 0.05$) % in individuals of the experimental groups, respectively. On the 3rd day after calving, an opposite tendency was observed, namely, the most pronounced decrease in cholesterol concentration occurred in cows of the first and second groups - by 34.31 and 30.45%, respectively, against 16.25% in animals of the third group. During the milking period, in animals of all groups, both on the 30th and 60th day of lactation, the cholesterol content slightly exceeded the permissible values of the physiological norm. At the same time, on the 30th day after calving, its content was highest in individuals of the control group. On the 60th day of lactation, the cholesterol content in the blood of cows in the experimental groups exceeded the values of animals in the control group by 23.85 and 22.01%. The concentration of triglycerides in the blood serum of animals tended to increase in all groups as the pregnancy period increased. Thus, if on the 1st day of the dry period it was 0.27–0.28

mmol/l, then on the 46th day in cows of the second and third groups it increased by 35.71 ($p < 0.015$) and 33.33%, respectively, against 18.51% in individuals of the control group. On the 3rd day of lactation, a significant decrease in the concentration of triglycerides in the blood serum of animals of all groups was observed: by 90.62 ($p < 0.001$) and 89.47 ($p < 0.001$) % in cows of the first and second groups, versus 83.33% ($p < 0.001$) in individuals of the third group. On the 30th day of lactation, the value of this indicator significantly increased in animals of all groups and did not differ significantly in animals regardless of the group (0.32–0.33 mmol/l). On the 60th day of lactation, a tendency for the triglyceride content in the blood of cows of the experimental groups to exceed that of the control animals by 14.81% was observed.

According to the accepted scheme of zootechnical analysis of feed, dry matter of feed is the carrier of nutritional value of feed. The higher the content of dry matter in feed, the higher its nutritional value. It is known that consumption of dry matter of bulk feed depends on the concentration of metabolic energy in them and the level of productivity. Animals received biostimulants for 30 days at 10 ml. intramuscularly. Hematological parameters of blood of experimental cows are presented in Table 1. For the entire period of the experiment, the concentration of leukocytes in the control and experimental groups was within the physiological norms and had an average value of $9.5 \cdot 10^9/l$ in the control group and $9.9 \cdot 10^9/l$ in the experimental groups. The content of lymphocytes at the beginning and at the end of the experiment in the control and experimental groups was within the physiological norms and no reliable changes were observed. Control group - average content for the entire period is 33.9%. Experimental groups: I - 27.4%; II - 27.8%; III - 29.01%.

According to the scheme adopted for zootechnical analysis of feed, the carrier of the nutritional value of feed is the dry matter of the feed. The higher the content of dry matter in the feed, the higher its nutritional value. It is known that the consumption of dry matter of bulk feed depends on the concentration of metabolic energy in them and the level of productivity. For 30 days, experimental animals were intramuscularly administered various doses of tissue and peptide biologically active substances of 10 ml. The authors noted that after taking biostimulants, not only milk but also weight increased in the experimental animals. After the introduction of the biostimulant, blood was taken from the experimental cows and the hematological parameters of the blood are presented in Table 2.

During the entire experiment, the concentration of leukocytes in the control animals and experimental groups was within the physiological norm, that is, in the control groups, the concentration of leukocytes was $9.4 \cdot 10^9 / l$ and in the experimental groups it was an average of $9.8 \cdot 10^9 / l$. There were no significant changes in the number of lymphocytes at the beginning and at the end of the experiment. Control group - average composition for the entire period - 34%. Experimental groups: I - 27.5%; II - 27.9%; III - 29.05%.

Table 2. Hematological parameters of the blood of experimental animals

Indicator	Units of measurement	Groups			
		Control	Experienced		
			I	II	III
Beginning of the experiment, day 1 (n=12)					
1	2	3	4	5	6
Leukocytes	10 ⁹ /l	9,21±0,51	9,90±0,59	10,91±0,82	9,76±0,59
Granulocytes	%	51,15±2,72	46,56±2,34	51,77±3,24	50,13±2,94
Basophils	%	10,57±0,67	9,17±0,57	11,19±1,75	9,39±0,81
Lymphocytes	%	28,38±2,18	27,09±2,58	25,84±2,76	28,41±2,19
Erythrocytes	10 ¹² /l	9,42±0,56	8,68±0,28	9,65±0,62	9,17±0,60
Hemoglobin	r/dl	13,77±1,59	10,21±0,21*	10,14*±0,19	13,35±1,65
Thrombocytes	10 ⁹ /l	477,81±73,28	316,57±45,04	358,91±54,25	375,91±80,14
End of the experiment, day 60 (n = 12)					
Leukocytes	10 ⁹ /l	9,85±0,58	9,02±0,43	9,93±0,57	9,78±0,57
Lymphocytes	%	29,52±2,01	27,83±1,95	29,79±2,06	29,05±2,15
Basophils	%	8,88±0,65	9,08±0,31	8,56±0,49	8,62±0,52
Granulocytes	%	50,14±2,33	44,56±1,73	44,85±1,73	43,15±1,67*
Erythrocytes	10 ¹² /l	8,64±0,26	8,06±0,22	8,14±0,20	8,10±0,19
Hemoglobin	r/dl	10,41±0,23	11,12±0,41	10,03±0,23	9,91±0,24
Thrombocytes	10 ⁹ /l	293,25±41,71	340,46±46	294,40±38,74	284,17±35,30

Note: * p<0.05; ** p<0.01; compared to control

Table 3. Rumen fluid indicators of cows

Indicators	Control group	Experimental group		
		I	II	III
pH	6,29±0,22	6,06±0,16	6,01±0,1	6,04±0,1
OMCH, 10⁶	15,55±0,95	13,79±0,77	13,11±0,63	13,64±0,52
Bacillary m/o, 10⁶	90,71±10,50	68,23±13,47	78,12±12,13	85,27±11,64
Lactic acid m/o, 10⁴	0,33±0,15	0,13±0,06	0,16±0,13	0,24±0,09
Yeast-like m/o, 10⁴	1,40±0,62	1,94±1,02	1,82±0,95	1,88±1,02
Molds(fungi, 10⁴	1,75±0,63	0,18±0,08	1,25±0,24	0,67±0,18

Note: p≤0.05

The results of the analyses showed that at the beginning and at the end of the experiment the proportion of basophils tended to decrease, it was seen that in the control group it decreased by 15.7%. Accordingly, in the experimental groups this indicator was 0.99%, 23.3 and 8.2%. In all the experiments the indicators were within the physiological norm, no significant changes were observed. Over the entire period of the experiment the number of granulocytes decreased by 11.2%, 10.5 and 11.9% respectively in the experimental groups compared to the control, but was within the physiological norm. At the beginning of the experiment the composition of the number of erythrocytes was at the maximum values of the physiological norm (5-10*10¹²/l), at the end of the experiment it dropped to the average values, i.e., to the control group - 8.65 (10¹² /l), in the experimental group - 8.12 (10¹² /l). At the beginning of the experiment, the hemoglobin level of the experimental groups was lower than the values of the control group: II - by 25.6%; in second

place - 26.2 percent; in III - by 2.9%. The indicators of the control group and III experimental group were higher than the physiological norms at the beginning of the experiment. At the end of the experiment, all indicators were within the physiological norm and had an average value: the control group - 10.40 g / l and the experimental groups - 10.37 g / l. During the entire period of the experiment, the hematocrit indicators were within the physiological norm (35-45%) and no significant changes were observed. Average indicator: control group - 39.48%; I - 35.5%; II - 40.39%; III - 39.75%. It is evident that in the experimental groups the number of platelets at the beginning of the experiment was within the physiological norm (250-450 10¹² / l), while in the control group the indicators were higher. At the end of the experiment, all indicators were within the physiological norm, no significant changes were observed. Data on milk productivity (MP) of the experimental animals for 90 days of lactation were recorded based on daily records at each milking.

The experimental period was divided into 5 additional periods of 18 days each.

Research has shown that changes in the level of milk productivity and the introduction of a biostimulant into the body of experimental animals are opposite. When the biostimulant acts in accordance with the physiological norm, the amount of biologically active substances in milk is the smallest. With a decrease in the effect of the biostimulant, the concentration of lactose in milk increases, but no increase in the upper limit of the norm was observed. When the concentration of the biostimulant deviates downwards, an average negative significant relationship with the acetone level occurs, and an average positive reliable relationship is established between the content of the biostimulant, regardless of the concentration of the biostimulant. In addition, according to the selected analysis criteria in the studied population of experimental animals, the biostimulant effect corresponded to the optimal values in 17.05% of cows, and in 82.95% of cows it deviated downwards and was 1.10 or 1.10. The biostimulant effect tended to decrease with increasing milk yield of animals, and in the dynamics of milking days it increased as the milk yield of the experimental animals decreased. In the group of experimental animals with a biostimulating effect of 1.10 or less, the lactose content in milk was significantly higher (80.0%, $p < 0.01$) than in animals with normal biostimulating effect values. In cows with normal lactose content in milk, its content exceeded the limit value by 28.57%, and in animals with low biostimulant values, the reliable excess was 141.43% ($p < 0.05$). With a decrease in the biostimulant level, a moderate negative significant relationship was found between this indicator and the acetone level ($r = -0.572$, $p < 0.01$). With a biostimulant value of less than 1.10 and with a biostimulant value within 1.11-1.50, moderate positive reliable relationships were established between the lactose content, and in the first case this relationship was more pronounced.

Analysis of the rumen microflora activity showed that the pH did not change significantly during the experiment, but in the experimental group it decreased to 3.64%, 4.42 and 3.97% compared to the control and meets the requirements of physiological norms. Also, the total number of microbes in the experimental group decreased by 11.25%, 15.55 and 12.21% (Table 3).

The analyses showed that in the experimental group there was an increase of 38.5%, 30 and 35%. When evaluating yeast-like microorganisms, the same pattern is observed. This is explained by the fact that with an increase in the functional activity of the body of experimental animals during lactation, complex processes are formed; a large number of bacteria, fungi in the fermentation of food, the synthesis of nutrients and new substances.

All this provides the animal with the necessary energy and nutrients, and also affects the physiological processes occurring in the body, which in turn contributes to the strengthening of metabolic, production and reproductive processes.

Thus, a significant point in these processes is the ability to control gastric digestion by adjusting the diet with the help of

various biostimulants. It should be noted that biostimulants strengthen the immunity of animals, increase productivity, increase milk production, and also ensure sanitation quality and safety. Also, complete and balanced feeding of dairy cows optimizes metabolism and improves the synthesis of most milk and its components.

5. Conclusions

Thus, the energy balance in the body of dairy cows increases the level of milk productivity. When introducing biostimulants into the muscles of experimental animals, milk productivity increases. According to the results of the analysis, the criterion of 17.03% of cows of the studied animals having biostimulants with optimal values was revealed. The economic efficiency of introducing a biostimulant-feed regulator into the diet of cows consists in calculating the economic feasibility of its use. In this regard, the main indicators of economic assessment are: feed price, average daily milk yield and market price of milk.

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