RESEARCH PAPER

Biochemical Indicators of Cow Blood Using Nanostructured Bentonite in Combination with a Probiotic in Their Diet

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ABSTRACT
This article presents the results of a study examining the effects of a combined feed additive consisting of nanostructured bentonite and a probiotic on blood biochemistry in cows. Experiments were conducted on five groups of animals (one control group and four experimental groups) over a period of 120 days. The cows' basal diet was supplemented with various combinations of bentonite and an acidifier, allowing for the evaluation of their effects on hematological and biochemical parameters. The results showed that cows fed nanostructured bentonite in combination with a probiotic showed a significant improvement in blood parameters: an increase in hemoglobin levels by 1.93-4.21%, erythrocytes by 1.23-6.54%, and leukocytes by 1.30-8.41%. There was also an increase in total protein content by 2.99-6.22%, glucose by 9.12-10.71%, inorganic phosphorus by 10.00-10.49%, and total calcium by 9.13-9.89%. The concentration of trace elements: iron, copper, and zinc also increased significantly. The study confirmed that the use of nanostructured bentonite in combination with a probiotic helps stimulate hematopoiesis, improve protein and carbohydrate metabolism, and normalize mineral homeostasis in cows. The data obtained may be useful for the development of effective feed additives aimed at improving the productivity and health of farm animals.
Keywords: cows, acidifier, bentonite, hematology, biochemistry, protein, microelements, hematopoiesis.
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INTRODUCTION

Livestock products are nutritionally balanced. They provide consumers with protein, minerals, micro- and macroelements, enzymes, and vitamins.

According to Directive 2002/32/EC, the quality and safety of products intended for animal feed must be assessed before their use to ensure that they do not pose a risk to human health, animal health or the environment [1,2].

Research into new natural, balanced feed additives to improve animal productivity, particularly for cattle, plays a significant role in the development of livestock farming. Various acidifiers offer effective alternatives to antimicrobials. In addition to acidifiers, promising areas in feed production include the use of enzyme preparations, probiotics, and prebiotics. Enzymes improve the digestibility of feed nutrients by breaking down complex carbohydrates, proteins, and fats. Probiotics, which contain live microorganisms, normalize the intestinal microflora, promoting better digestion and strengthening the animal's immune system. Prebiotics, on the other hand, serve as a nutrient medium for beneficial bacteria in the intestine, stimulating their growth and activity [3,4].

Particular attention should be paid to the quality of silage and haylage—the main feeds for cattle during the winter. Proper harvesting technology, including optimal harvesting timing, the use of preservatives, and proper storage conditions, maximizes the preservation of nutrients and minimizes losses. Poor-quality feed can cause various diseases and reduce animal productivity [5,6].

In today's livestock development environment, the implementation of innovative feeding technologies based on scientific research and practical experience is essential. The development and use of balanced diets that take into account the age, physiological condition, and productivity of animals is the key to producing high-quality products and increasing production profitability [7,8].

The implementation of modern feed quality control methods, including rapid analysis of nutritional value and toxicity, will enable prompt adjustments to diets and prevent negative impacts on animal health. Digitalization of feeding processes, along with the use of sensors and monitoring systems, will enable real-time tracking of feed consumption and animal health, and prompt response to any deviations.

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Providing animals with high-quality, balanced feed is therefore a key factor in increasing productivity and improving health. The use of modern technologies and scientific advances in nutrition will significantly improve the efficiency of livestock farming and provide consumers with high-quality products [9,10,11].

The aim of this work is to study the effect of a biologically active feed additive - nanostructured bentonite in combination with a probiotic on the biochemical parameters of cows' blood.

MATERIALS AND METHODS

Five groups of 10 cows each were created (control group 1; experimental groups 2, 3, 4, and 5). Animals were selected for the groups based on similar live weight, productivity, and body condition.

Table 1. Experimental scheme

Group	Number of animals, heads	Feeding pattern
I – control	10	The basic diet (BR) used on the farm
II – experienced	10	OR+ bentonite (50 g/head)
III - experimental	10	OR + acidifier (2.0 ml / kg body weight)
IV - experimental	10	OR + acidifier (1.5 ml / kg live weight) + nanostructured bentonite (50.0 g / head)
V- experienced	10	OR + acidifier (2.0 ml / kg live weight) + nanostructured bentonite (50.0 g / head)

The experiment lasted 120 days. All animals were kept under identical conditions and fed standard farm diets. Subsequently, biologically active feed supplements were added to the main diet, according to the experimental design (Table 1). Analysis of the main diet revealed deficiencies in iron, copper, manganese, calcium, and zinc.

The cows' housing and feeding conditions were assessed using standard methods. Serum biochemical parameters, including total protein, albumin, glucose, urea, cholesterol, calcium, inorganic phosphorus, alkaline phosphatase, and aspartate and alanine aminotransferase (AST and ALT), were determined using a Siemens Expressplus analyzer.

Statistical processing of the obtained data was performed using the variation statistics method using Microsoft Excel for Windows 10 software. The significance of differences in the obtained results was determined using Student's t-test. Differences in the numerical data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Over the course of the four-month study, the clinical condition of cows in the experimental groups did not differ from that of control animals. Blood parameters are important for assessing the animals' overall health. Feeding was monitored using key blood parameters, which, when combined with other parameters, provide insight into the functional status of both individual organs and the entire body.

Table 2. Hematological parameters of experimental cows, M±m

Indicators	Deadlines research, month	Experimental groups, n=50				
		1 control	2	3	4	5
Hemoglobin g/l	beginning of the experience	85.37±0.52	85.30±0.65	87.00±1.30	85.22±1.23	87.50±0.76
	in a month	85.50±1.25	87.10±1.42	88.10±0.99	89.30±1.08*	89.10±1.21*
	Through 4 months	86.73±0.82	87.12±1.61	88.20±1.15	86.30±0.98	88.40±0.85*
Red blood cells $x10^{12}$/l	beginning of the experience	5.40±0.27	6.10±0.19	5.78±0.16	6.15±0.31	5.91±0.53
	in a month	6.48 ±0.18	5.81±0.23	5.94±0.35	6.78±0.43*	6.56±0.36*
	Through 4 months	6.57±0.21	5.52±0.11	5.90±0.34	6.80±0.30*	7.00±0.55*

Leukocytes 109/l	beginning of the experience	8.63±0.04	8.56±0.05	8.61±0.06	8.62±0.05	8.60±0.06
	in a month	8.44±0.32	8.11±0.16	8.13±0.29	8.55±0.48*	9.15±0.51*
	Through 4 months	8.56±0.33	8.32±0.16	8.53±0.20	9.05±0.39*	9.27±0.36*

*P<0.05

Animals in groups 4 and 5, which received nanostructured bentonite in combination with a probiotic at the recommended doses, demonstrated a positive effect, as reflected in hematological parameters. Table 2 shows that hemoglobin levels, compared to the control group, increased by 1.93-4.21% on days 30 and 120 of the study, erythrocyte levels by 1.23-6.54%, and leukocyte levels by 1.30-8.41%. These results suggest that nanostructured bentonite in combination with a probiotic stimulates hematopoiesis in cows' diets.

Table 3. Content of total protein and its fractions in the blood serum of experimental cows, g/l, M±m

Indicators	Deadlines research month	Experimental groups, n=50				
		1 control	2	3	4	5
1	2	3	4	5	6	7
General protein	beginning of the experience	82.42±0.48	80.80±0.80	82.72±0.57	82.90±0.81	83.20±1.13
	in a month	80.12±1.08	80.60±0.95	81.30±0.98	81.30±0.92	80.90±0.92
	Through 4 months	80.40±0.82	80.92±0.95	82.10±1.14	84.60±0.57*	82.80±0.78*
Albumins	beginning of the experience	31.90±0.55	32.73±0.65	32.64±0.59	33.70±0.57*	33.90±0.46*
	in a month	31.70±0.83	32.30±0.62	33.23±0.38	33.54±0.41	32.62±0.46
	Through 4 months	32.20±0.38	32.63±0.94	32.92±0.48	34.20±0.41*	33.10±0.37
Alpha globulins	beginning of the experience	15.06±0.12	15.17±0.09	15.27±0.30	15.32±0.65	15.26±0.56
	in a month	15.17±0.41	15.08±0.64	15.43±0.13	15.19±0.47	15.14±0.30
	Through 4 months	15.15±0.29	14.97±0.27	15.10±0.19	15.60±0.47	15.22±0.46
Beta-globulins	beginning of the experience	24.40±0.36	25.06±0.12	25.32±0.71	25.02±0.13	25.06±0.14
	in a month	25.12±0.70	25.10±0.16	25.32±0.18	25.44±0.10	25.12±0.23
	Through 4 months	24.86±0.18	25.08±0.18	25.20±0.10	25.54±0.58	25.44±0.68
Gamma-globulins	beginning of the experience	10.58±0.18	11.02±0.16	11.18±0.21	11.50±0.10	11.18±0.25
	in a month	11.22±0.18	10.94±0.29	11.10±0.12	11.60±0.09	11.26±0.11
	Through	11.20±0.25	11.00±0.18	11.18±0.63	11.60±0.04	11.44±0.65

	4 months					

*P<0.05

Proteins play a vital role in the synthesis of many enzymes and vitamins, participate in the body's protective and adaptive responses, and provide humoral factors for specific and nonspecific resistance. The inclusion of dietary supplements in cow diets in various combinations increased total protein content by 2.99-6.22% compared to control values. No significant differences were found in other protein components.

Table 4. Biochemical parameters of blood of experimental cows, M±m

Indicators	Deadlines research month	Experimental groups, n=50				
		1 control	2	3	4	5
Glucose, mmol/L	beginning of the experience	2.70±0.07	2.64±0.12	2.70±0.08	2.66±0.07	2.78±0.04
	in a month	2.74±0.09	2.52±0.09	2.79±0.07	2.66±0.12	2.70±0.03
	in 4 months	2.52±0.08	2.41±0.06	2.64±0.05	2.79±0.03*	2.75±0.05*
Phosphorus inorganic mmol/L	beginning of the experience	1.68±0.06	1.46±0.07	1.60±0.04	1.78±0.04	1.70±0.07
	in a month	1.60±0.05	1.68±0.04	1.72±0.04	1.76±0.03*	1.80±0.04*
	in 4 months	1.62±0.04	1.65±0.05	1.72±0.04	1.79±0.05*	1.79±0.04*
Total calcium, mmol/L	beginning of the experience	2.54±0.06	2.68±0.09	2.58±0.06	2.68±0.06	2.64±0.07
	in a month	2.60±0.09	2.62±0.06	2.66±0.05	2.88±0.12	2.66±0.05
	in 4 months	2.62±0.03	2.66±0.04	2.68±0.03	2.87±0.07*	2.89±0.11*
Carotene, mmol/l	beginning of the experience	0.72±0.06	0.67±0.05	0.68±0.06	0.74±0.07	0.78±0.06
	in a month	0.73±0.03	0.67±0.09	0.68±0.06	0.75±0.03*	0.76±0.04*
	in 4 months	0.74±0.04	0.66±0.03	0.77±0.06	0.82±0.04*	0.80±0.04*
Copper, mmol/L	beginning of the experience	7.38±0.14	7.58±0.11	7.52±0.07	7.60±0.12	7.56±0.08
	in a month	7.44±0.14	7.84±0.12	7.78±0.19	7.80±0.20	7.68±0.13
	in 4 months	7.56±0.20	7.48±0.11	8.62±0.09*	8.84±0.10*	9.06±0.10*
Zinc, mmol/L	beginning of the experience	14.02±0.25	14.74±0.36	14.90±0.18	14.92±0.26	14.96±0.14
	in a month	15.04±0.40	15.20±0.34	15.34±0.35	15.20±0.22	15.52±0.24
	in 4 months	14.78±0.31	17.14±0.27*	17.56±0.10*	18.40±0.25*	19.02±0.17*
Manganese, mmol/l	beginning of the experience	1.60±0.05	1.62±0.10	1.58±0.04	1.60±0.05	1.64±0.08
	in a month	1.60±0.0	1.72±0.08	1.80±0.05*	1.82±0.05*	1.88±0.04*

		6				
	in 4 months	1.70±0.08	1.66±0.06	1.72±0.07	1.76±0.04	1.92±0.03
Iron, mmol/L	beginning of the experience	227.50±8.19	283.28±7.89	289.86±6.27	287.90±9.82	286.88±13.48
	in a month	278.50±5.87	302.08±3.55*	307.66±2.75*	316.70±4.78*	319.26±6.65*
	in 4 months	279.23±5.58	306.66±1.67*	312.32±1.79*	320.12±3.27*	325.28±2.84*
Alkaline reserve, vol/% CO2	beginning of the experience	55.05±0.53	54.94±0.27	54.70±0.50	54.80±0.52	55.10±0.72
	in a month	54.80±0.58	53.70±0.58	55.10±0.37	55.50±0.36*	55.40±0.36*
	in 4 months	55.60±0.55	54.52±0.53	54.50±0.17	54.80±0.62	55.70±0.65*

Note: *P<0.05

Macro- and microelements play a significant role in metabolic processes in animals. Deficiency or excess of minerals in animal diets leads to decreased productivity, fertility, and deteriorates product quality. They are essential for animal growth, development, and reproduction, affect hematopoiesis, regulate metabolism, participate in protein biosynthesis, influence the functioning of the gastrointestinal microflora, etc. Analyzing the data in Table 4, it can be noted that blood glucose levels in the experimental cows exceeded control values by 9.12-10.71%. When nanostructured bentonite was included in the cows' diets in combination with a probiotic, the concentration of inorganic phosphorus on days 30 and 150 increased by 10.00-10.49%, total calcium by 9.13-9.89%, and carotene by 2.74-10.81%. Blood iron levels in cows ranged from 283.28±7.89 to 325.28±2.84 mmol/L, within the reference limits. Iron levels in cows in the experimental groups receiving nanobentonite in combination with the probiotic (Groups 3 and 4) were significantly higher (P>0.05) than in the control group on day 30 of the study by 38.20-40.76 mmol/L, or 13.72% and 14.62%, respectively. Blood alkaline reserve levels in the experimental cows did not differ significantly from those in the control group.

CONCLUSION

Adding biologically active feed additives—nanostructured bentonite combined with a probiotic—to cow diets stimulates protein and carbohydrate metabolism, improves physiological and biochemical status, enhances absorption of biologically active feed additives, and normalizes mineral homeostasis.

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