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JOURNAL OF
Food Science and Nutrition Therapy
OPEN ACCESS JOURNAL

ISSN: 2641-3043 DOI: https://dx.doi.org/10.17352/jfsnt

Research Article

Biochemical applications and application of indicators in blood and lymph after a new feed additive based on bentonite

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Received: 24 December, 2021
Accepted: 01 February, 2022
Published: 02 February, 2022

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Keywords: Feed additive; Nutrition of agricultural animals; Biochemistry of blood and lymph

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Abstract

The aim of the study was to assess the physiological and biochemical action the new feed additive.

The studies were carried out on white laboratory rats of the Spraque Dawley line. The additive consisted of four ingredients - bentonite, barley, wheat, calcium phosphate, in the ratio (2: 8: 8: 2). The use of fortified, enriched with vegetable fat-protein components, feed additive with natural montmorillonite (bentonite) affects the protein, carbohydrate and fat metabolism in the body, contributes to an increase in the weight of the animal. After prolonged intake of the feed additive, enzymatic activity and an increase in the content of protein, glucose, and lipids in the blood were observed. The feed additive has a good adsorptive and antioxidant effect, reducing toxic products, participating in the inactivation of free radicals, and exerting a protective effect on cell membranes. When feeding with a fodder additive, the protein content in the diet increased, as compared with the control, it should be noted that the phosphorus content in the blood and lymph increased, the increase in energy costs increased with an increase in muscle mass. The supplement is high in calories, has an antioxidant and adaptogenic effect, maintains a balance of biochemical and oxidative processes and can be recommended as a safe and effective supplement to the daily diet of farm animals.

To increase the productivity of farm animals, new feed additives are being developed and introduced that improve the processes of digestion and absorption, ensuring the safety and quality of products [1]. The composition of the Feed Additive (FA) must comply with the regulatory data for any diet when feeding farm animals. In order to ensure the safety of meat and dairy products, the use of montmorillonites is becoming increasingly popular, since clayey layered minerals have a number of properties, such as sorption, complexing, biocompatibility, which allows them to be widely used as components of feed additives that meet the basic principles of "green" chemistry [2]. Tagansorbent, bentonite, zeolites, silica gel, activated carbon, shungite, mined in Kazakhstan, are able to stop various intoxications, adsorb harmful substances in the food supply of farm animals [3]. It should be noted that many montmorillonites exhibit high complexing activity with ions of various elements, which reduces the level of toxic substances in the cells and tissues of the body and improves its blood

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supply [4]. The main montmorillonites, tagansorbents and zeolites, are not absorbed into the blood, but adsorb all harmful substances in the blood, carrying out a general detoxification of the body, which improves the quality and nutritional value of agricultural products [5]. For enhanced growth of young animals, vitamins and microelements, such as calcium, zinc, iodine, fluorine, potassium, sodium, and phosphorus are also needed [6,7]. For example, the normal content of vitamin A is responsible for the normal functioning of the digestive system, prevents miscarriages, improves reproductive function, stimulates the growth of young animals, and prevents obesity [8]. Like iodine, zinc maintains stable milk yield, reproductive function, which is responsible for the normal functioning of the thyroid gland [9]. Potassium, sodium regulates the water-salt balance, prevent the occurrence of anemia [10] in case of dysfunction of the cardiovascular system. The use of various fortified feed additives based on montmorillonite components improves the functional activity of digestive processes, stimulates the growth and development of young animals, and affects the quality of meat and dairy products. Studies of blood and lymph circulation, as the internal environment of any organism, anatomically and functionally interconnected with the digestive system of animals, are necessary to identify water-salt, biochemical and enzymatic processes that occur in the body after the use of various feeds or feed additives, which is necessary to obtain high-quality products.

The aim of the study was to evaluate the physiological and biochemical effects of a new feed additive on linear rats with subsequent use on farm animals.

Materials and research methods

The studies were carried out on 55 white laboratory rats of the Sprague-Dawley (SD) line, weighing 277±17 grams, divided into 2 groups - the control group (25) - were on the main food of the vivarium and the experimental group of animals (30) took FA in granules for 21 days. The additive consisted of four ingredients - bentonite, barley, wheat, calcium phosphate, in the ratio (2: 8: 8: 2). The composition of bentonite also includes, %: SiO_2 -57.84; Al_2O_3-14.29; Fe_2O_3-5.93; FeO-1.14; CaO-0.99; MgO-2.79; K_2O-2.10; Na_2O-1.15; SO_3-0.62. The content of radionuclides in bentonite samples does not exceed the permissible limits. The bentonite deposit is located in the Saryagach district of the Chimkent region of the Republic of Kazakhstan.

Anesthesia of animals was carried out by inhalation with ether through a mask, into which with ether was placed. After anesthesia, an incision was made along the white line of the abdominal muscles, then the thoracic lymphatic duct was dissected at the diaphragm into which a graduated microcannula was inserted, and through it, the lymph flow was determined and the lymph was collected for research. After collecting lymph, the abdominal aorta was dissected in the caudal part of the abdominal cavity, and a Teflon catheter was inserted into it to collect blood. Diuresis from the urinary bladder BP and heart rate were recorded by the sensors of the Dreiger surgical monitor.

The cellular composition of blood, lymph, urine was determined using a hematological analyzer (SYSMEX KX-219). Electrolytes in lymph and blood plasma were analyzed using an AVL9190 analyzer (ROSHE DIAGNOSTICS, Austria, 2012). Blood pressure, heart rate (HR) in animals were recorded through a sensor of a surgical monitor (DREGOR, modelGAMMA). Biochemical studies of blood and lymph included determination of the level of total protein, albumin, triglycerides, cholesterol, glucose, bilirubin, ALT and AST enzymes, alkaline phosphatase, trace elements phosphorus and magnesium on a biochemical analyzer A-25 BIOSYSTEMS (Spain) using test kits according to the standard methodology. The state of oxidative activity in the blood was determined by the level of malondialdehyde, diene conjugates, and the level of catalase. The results obtained were statistically processed using the Microsoft Excel program and changing the parameters taking into account the unpaired Fisher-Student test and were considered reliable at $p \leq 0.05$.

In studies on laboratory animals, we were guided by the decision of the LEK - an extract from the protocol of the Local Ethical Commission at the Republican State Enterprise "Institute of Human and Animal Physiology" (IFCJ) KN MES RK No. 3 (3) dated 10/08/2020 ref. No. 07-05 / 158.

Research results

After 21 days of feeding the animals, the FD weight increased by 6.6% (269±17 and 288±21), in the control group by 2.1% (277±17 and 281±19).

Lymphatic flow in control rats was 0.0031±0.0002ml/min. per 100g of animal weight, and after feeding, FD, corresponding to 0.0056±0.0005ml/min. The blood coagulability in the control animals was within 3.09±0.5 min, and after feeding, the FD corresponded to 3.48±0.4 min. In the lymph of the control animals, 3.1±0.5 minutes, and after feeding, the FD was 3.68±0.4 minutes. The blood viscosity in the control groups was 4.5±0.5P units, and after feeding, FD was 5.3±0.4P units. In the lymph of the control group, the animals corresponded to 3.9±0.5 units, and after feeding, the FD was 4.1±0.6 P. The volume of plasma by hematacrit in the control was 45.0±3.2% and in the experimental 48.0±4.0%. Heart rate in control animals is 481±11 beats per minute. Blood pressure in control animals corresponded to 103±7 and in experimental animals 105±9. Diuresis in control animals is 0.0018±0.0001ml/min. per 100g of body weight, and after feeding, FD 0.0029±0.0001ml/min per 100g.

Studies of the cellular composition of the blood and lymph of experimental animals showed that the level of leukocytes, lymphocytes and erythrocytes fluctuated at the level of control values, there was a decrease in platelet concentration and an increase in hemoglobin after application of FD (Table 1).

After the application of FD in the blood serum, the level of Alb and OB increased by 11%, the protein level in the lymph was maintained in comparison with the control, and the glucose level, both in the blood and in the lymph, increased 1.5 times. The concentration of High-Density Cholesterol (HDL) in blood plasma increased by 30% compared to the control, and in the

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lymph by 28%, and LDL with low density increased by 28%, in the control group by 13%. Biochemical parameters of lymph in terms of proteins and lipids corresponded to those of blood plasma. Consequently, the use of FD showed an increase in the level of total protein due to albumin, as well as an increase in the concentration of glucose, triglycerides and cholesterol, especially with a low density. Elevated high-density HDL cholesterol levels indicate increased metabolic processes in the liver and utilization of excess cholesterol Table 2.

An increase in the activity of such enzymes as (ALT), (AST), (ALP) in blood plasma and lymph to a greater extent reflects the state of permeability of cell membranes, liver and myocardium.

When comparing the control data with the indicators of the experimental group taking FD, an increase in the ALT level by 44% in the blood was revealed, in the AST level by 59%, and an increase in the ALP level by 59%, the bilirubin level increased by 46%, the level of creatinine by 26%, urea by 55%.

In the lymph, almost all indicators of enzymatic activity, as well as the level of pigment bilirubin and creatinine, showed an increase of 20-50%. It is likely that the increase in bilirubin and creatinine is associated with a saturated protein diet and an increase in the body weight of animals when fed with FD.

Table 1: Cellular composition of blood and lymph of animals taking FD. Compared with the data of the control group.

Indicators	Blood		Lymph	
	control	Feed Additive (FD)	control	Feed Additive (FD)
WBCx10^3/μL	7.50± 0.10	6.90±0.20*	15.2± 0.32	14.9±0.51
RBCx10^6/μL	7.40± 0.20	7.73±0.45	0.02±0.003	0.01±0.002*
HGB g/dL	15.0±0.30	14.88±0.43	-	-
Hct% гематокрит	45.0± 3.20	48.04±4.01	-	-
PLTx10^3/μL	425.0±14.0	395.0±19.2		-
LYM %	55.50±3.40	54.25±3.82	85.0± 0.85	88.0±0.91
LYM x 10^3/μL	2.70± 0.31	2.70±0.52	13.0±0.41	12.6±0.42

Note: reliable compared to control. -p<0.05*

Table 2: Biochemical parameters of blood plasma and lymph after application of FD.

Indicators	Blood plasma		Lymph	
	control	FA	control	FA
Albumin. g/l	23.90±1.6	26.72±1.8	10.10±0.7	11.09±0.9
Total protein g/l	63.41±10.1	71.40±10.0	39.32±4.0	39.52±6.1
Glucose. mmol/l	12.55±1.1	19.29±1.5*	9.35±0.7	13.92±0.8*
HDL cholesterol mmol/l	0.48±0.03	0.62±0.01*	0.39±0.02	0.50±0.03*
LDL cholesterol mmol/l	0.39±0.01	0.50±0.01*	0.37± 0.04	0.42±0.06
ALAT. units/l	154.70±17.0	223.01±16.6*	150.01±10.8	141.3±3.4
AsAT. u/l	118.40±9.3	188.73±10.4*	80.02±11.5	92.92±7.1*
Bilirubin. μmol/l	15.30±0.7	22.43±0.9*	1.50±0.04	2.32±0.05*
Creatinine. μmol/l	45.10±0.22	56.82±0.15*	42.43±3.8	69.32±4.3*
Triglycerides.mmol/l	0.84±0.03	0.93±0.01*	3.72±0.5	4.12±0.7
Alkaline phosphatase. U/l	264.20±10.4	305.01±12.2*	180.23±14.0	217.23±19.0*
Urea. mmol/l	3.00±0.5	5.58±0.5*	3.90± 0.20	5.00±0.16

Note: reliable compared to control.-p<0.05*

In general, the analysis of biochemical data in the blood and lymph showed that after feeding with FD, a general tendency was observed - an increase in the concentration of indicators of protein, fat and carbohydrate metabolism in the blood of animals, namely, an increase in the concentration of total protein, albumin and glucose, as well as cholesterol, enzymatic activity, which indicates a high level of protein-carbohydrate-fat supply of the body when feeding FD. It should be noted that after the use of FD, the level of triglycerides increased by 12%, the concentration of low-density cholesterol and bilirubin, ALT and AST increased by 2 times, which indicates an increase in the activity of lipid metabolism and the regulatory role of the liver with an increase in the live body weight of animals.

The results of studies of ions of trace elements in blood plasma, lymph and urine of animals are presented in Table 3.

As shown in Table 3, the level of Na + ions in the blood and lymph during feeding with FD was slightly higher than the control values (2%), but Na + was washed out with urine after taking FD by 8.5% higher than in the control data. It is likely that the components of FD bind sodium ions due to bentonite and excrete them with urine, that is, they have a diuretic effect.

After taking FD, the level of both magnesium and phosphorus increased by 28% and 35%, respectively.

Lipid Peroxidation (LPO) is a vital link in metabolic metabolism. The results of studies of lipid peroxidation in the blood of animals taking FD are presented in Table 4.

After the application of FD, the level of catalase activity was 25% lower than the control values, which characterizes the degree of inhibition of metabolic processes on the membranes and an increase in the processes of hepatic dysfunction. At the same time, the concentration of diene conjugates (DCs) of fatty acid hydroperoxides was 7% less than the control values, MDA as a final product - by 2%, that is, after the application of FD activation of LPO processes is not observed, but inhibition of antioxidant activity is observed (Table 4).

Discussion

The study showed that FD components enhanced the drainage and transport functions of the lymphatic system. FD did not affect cardiac activity - the heart rate of the animals fluctuated within the control values. Changes in the diuresis of animals showed a 1.5-2.0 -fold increase after the application of FD, which is a consequence of a slight increase in the liquid part of blood and lymph in the vessels and shows an increase in tissue hydration and the activity of metabolic processes in the body, after the application of FD.

The use of natural sources of mineral substances greatly facilitates the organization of the mineral nutrition of animals, contributes to a better provision of their needs for macro- and microelements, and has a positive effect on the body's homeostasis [11].

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Table 3: Content of ions of trace elements in blood plasma. Lymph and urine of animals after application of FD.

Electrolytes	Control	FA
Ca^+ in plasma (mmol/l)	0.58±0.03	0.62±0.05
Na^+ in plasma (mmol/l)	140.5±5.40	144±6.50
K^+ in plasma (mmol/l)	3.16±0.30	3.08±0.50
Mg^+ in plasma (mmol/l)	0.39±0.01	0.50±0.01
P^+ in plasma (mmol/l)	1.66±0.03	2.16±0.04*
Ca^+ in lymph (mmol/l)	0.40±0.03	0.45±0.04
Na^+ in lymph (mmol/l)	135.1±4.5	139±8.50
K^+ in lymph (mmol/l)	3.52±0.20	3.6±0.40
Mg^+ in lymph (mmol/l)	0.35±0.10	0.45±0.01
P^+ in lymph (mmol/l)	1.68±0.10	1.9±0.01*
Ca^+ in urine	-	-
Na^+ in urine (mmol/l)	16.41±1.02	17.8±2.00
K^+ in urine (mmol/l)	3.14±0.10	3.16±0.30

Note: reliable compared to control. -p<0.05*. -p<0.01**

Table 4: Indicators of lipid peroxidation in the blood of animals taking FD.

№ п/п	Group	Indicator		
		DC nmol/l	MDA nmol / l	$AKgH_2O_2$ / ml
1	Control (n=15)	2.44±0.03	0.131±0.01	1.99±0.03
2	FA (n=25)	2.27±0.02	0.128±0.01	1.50±0.02*

Note: *- P≤0.001 compared to the control data.

The use of FD has shown the advantages of its use glucose and its protein saturation against the background of a low level of low-density cholesterol and an increase in high-density cholesterol utilized in the liver. Triglycerides show mainly the activity of protein-carbohydrate metabolism, which contributes to the growth of muscle mass without affecting hepatocytes. The revealed changes in the biochemical activity in the blood serum and lymph of the experimental animals taking FD are closer to the control data and physiological norm, while the indices of the enzymes ALT, AST, bilirubin in the group of animals taking FD were higher than the norm, which shows a tendency to develop deviations in the work of the hepatobiliary system, such as fatty liver - hepatosis, increased cytolytic activity [12].

Compound feed with carbohydrate-vitamin-mineral feed concentrate "Felutsen" K-6 contributed to an increase in the digestibility of nutrients in the diets of bulls, the growth rate of bulls by 16.13% with more rational use of feed and labor per unit of production [13].

Since the composition of feed additives includes such important trace elements as magnesium and phosphorus, studies were carried out for the presence of these substances in the blood of animals. Magnesium with calcium are responsible for the state of the skeletal system, magnesium with phosphorus regulate muscle activity and participate in the formation of vitamins B6, D and E. Deficiency of magnesium and phosphorus electrolytes leads to disruption and reduction of carbohydrate-fat metabolism of non-enzymatic components of the antioxidant defense system and the formation of oxidative stress [14]. It has been established that the use of non-traditional additives of khongurin zeolite and Kempendyai salt in the composition of feed rations contributed to an increase in milk productivity, and also improved the metabolism and physiological parameters of cattle [15].

Enzymatic oxidation serves to renew the phospholipid bilayer of cell membranes, participates in the formation of biologically active substances, detoxification of the body, and metabolic reactions. Non-enzymatic oxidation leads to the accumulation of peroxides, which significantly reduces the activity of the antioxidant system and destroys the body [16]. The applied FD does not provoke an increase in lipid peroxidation in the blood, which is associated with the antioxidant effect of the components used to inactivate free radicals and have a protective effect on cell membranes. The study of the oxidative activity of blood after the use of the feed additive showed a decrease in the level of diene conjugates and malonic dialdehyde, which shows the protective effect of antioxidant components at the level of cells and tissues, contributes to a significant activation of the non-enzymatic link of antioxidant protection.

It is likely that the insufficient content of retinol in the composition of FD worsened the antioxidant properties of this complex. So, as the diet of animals consisted entirely of FD. The use of our FD provoked a slight increase in low molecular weight cholesterol, as well as ALT, AST, alkaline phosphatase and bilirubin, therefore, it should be used as a feed supplement, but not completely replaced it with the main diet.

Studied according to the effect of the feed additive on physiological and biochemical parameters, it showed sufficient activity and can be recommended for widespread use as an additional feed with antioxidant and nutritional properties in the practice of animal husbandry.

Conclusion

Feed additive with natural montmorillonite (bentonite) preserves physiological parameters by influencing the protein, carbohydrate and fat metabolism in the body, contributing to weight gain. The feed additive has good hydration, enhances lymph circulation and urination, and removes toxic products. It has an antioxidant effect used to inactivate free radicals and has a protective effect on cell membranes.

Thus, the supplement is high-calorie, has an antioxidant and adaptogenic effect, maintains a balance of biochemical and oxidative processes, and can be recommended as a safe and effective supplement to the daily diet of farm animals, but after further research in real farm conditions.

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