

EXPERIMENTAL EVALUATION OF THE IMMUNOMODULATORY ACTIVITY OF A NEW COMPLEX FEED ADDITIVE OF NATURAL ORIGIN

Koibasova L.U.*, Candidate of Biological Science, Associate Professor
laura.koibasova74@mail.ru, <https://orcid.org/0000-0002-9898-3580>

Demchenko G.A., Doctor of Medical Sciences, Professor
georgiidemchenko@mail.ru, <https://orcid.org/0000-0001-9906-2700>

Makashev E.K., Doctor of Biological Sciences, Professor
e_makashev@mail.ru, <https://orcid.org/0000-0001-9177-1000>

Abdreshov S.N., Candidate of Biological Science, professor
SNABDRESHOV@mail.ru, <https://orcid.org/0000-0002-8527-921X>

Mussayeva A.S., Candidate of Biological Science, Associate Professor
aimus@mail.ru, <https://orcid.org/0000-0003-2871-1552>

Yessenova M.A., PhD, Senior Researcher
esenova_makpal@mail.ru, <https://orcid.org/0000-0002-2436-0620>

Institute of Genetics and Physiology CS MSHE RK, Almaty, Kazakhstan

Annotation. This paper reports an experimental evaluation of the metabolic and immunomodulatory effects of two complex feed additives of natural origin based on bentonite and chlorella with added potassium iodide and selenium (FA1 and FA2). The additives were fed to Sprague-Dawley rats (n = 62) for 21 days, after which cellular, biochemical and immunological parameters were measured in blood and lymph. FA2 was additionally evaluated in 18 cows under farm conditions, using intraruminal boluses to record rumen pH, temperature and locomotor activity.

Both additives raised lymphocyte counts in lymph by 10–25% and produced moderate shifts in blood cell composition that remained within physiological limits. Albumin, total protein and glucose increased by 11–26%; ALT, AST, creatinine and urea also rose, while bilirubin, triglycerides and alkaline phosphatase were unchanged, indicating activation of protein and energy metabolism without loss of hepatic or renal function. Lipid peroxidation products increased only slightly, whereas catalase activity rose by 29.1% (FA1) and 11.6% (FA2), indicating mobilisation of antioxidant defence rather than oxidative damage. IgM, IgG and IgA increased in both blood and lymph, most markedly for IgM in lymph (up to 48.2%). In cattle, FA2 did not disturb rumen pH or temperature and was well tolerated.

The results indicate a predominantly immunomodulatory action of both additives, with FA2 producing more moderate metabolic and oxidative changes than FA1. This supports further evaluation of FA2 as a feed supplement for farm animals.

Keywords: feed additive, livestock nutrition, lymph, blood, immunomodulatory effect.

Introduction. Today, more attention is being paid to feed additives that are safe for animals and help maintain their normal physiological condition and ability to adapt to different housing conditions. This is especially important in animal production, where maintaining animal health and resistance to unfavorable environmental conditions is closely related to welfare and productivity [1,2]. Natural biologically active substances are increasingly being studied as components of feed additives because they may help regulate metabolism, immune function, and oxidative balance.

Bentonite clays are used in feed because of their high sorption capacity: they bind mycotoxins, heavy metals and other toxic metabolites in the gastrointestinal tract and reduce their absorption [3,4,5]. Beyond this direct sorption effect, bentonite has been reported to influence mineral balance and digestive function.

The microalga Chlorella supplies protein, polyunsaturated fatty acids, vitamins and minerals, together with carotenoids and other compounds with antioxidant activity [6,7]. In feeding trials it has been shown to modify immune responses and to reduce lipid peroxidation. Selenium and iodine act through defined mechanisms: selenium is a component of glutathione peroxidase and other selenoenzymes that protect cells against oxidative damage [8], while iodine is required for thyroid hormone synthesis and thus for the regulation of metabolic rate [9]. Combining a sorbent

with these three biologically active components addresses toxin load, antioxidant status and endocrine regulation at the same time, which is not achieved by any of them alone.

The lymphatic system has received far less attention in feed additive research than blood. It maintains fluid balance, transports lipids and immune cells, and provides the environment in which humoral immune responses develop [10]. Because lymphatic function depends on microcirculation, rheological properties and tissue metabolism, measuring lymph composition alongside blood gives a more direct view of immune and metabolic status than blood alone.

The combined effect of bentonite, Chlorella, iodine and selenium on lymph composition has not been reported. The present study therefore examined how two such additives affect the cellular and biochemical composition of blood and lymph, lipid peroxidation and immunoglobulin levels in rats, and assessed the tolerance of FA2 in cattle under farm conditions using continuous intraruminal monitoring.

Materials and methods. The study was conducted on 62 white Sprague-Dawley (SD) rats weighing 277 ± 17 g. The animals were divided into three groups. The control group ($n = 20$) received the standard vivarium diet. The experimental group ($n = 20$) received a feed additive (FA1, consisting of bentonite, chlorella, potassium iodide, and selenium) (patent no. 7945, April 14, 2023) [10]; and the third group of animals ($n = 22$) received a new feed additive (FA2). They were given this additive for 21 days. The additive consisted of bentonite powder and liquid chlorella in a ratio of 1:3, ground into a homogeneous mass, then dried and mixed to a powder state, and supplemented with KI and selenium. After feeding FA1 and FA2, the animals were weighed and selected for the experimental study.

The animals were anesthetized with ether inhalation using a mask with a cotton ball soaked in ether. After anesthesia, an incision was made along the linea alba of the abdominal muscles. The thoracic lymphatic duct was exposed near the diaphragm. A graduated microcannula was inserted into the duct to measure lymph flow and collect lymph samples. After the lymph was collected, the abdominal aorta was exposed in the caudal part of the abdominal cavity. A Teflon catheter was inserted into the aorta for blood collection. Urine was collected by bladder puncture, and its volume was measured. Blood pressure (BP) and heart rate (HR) were recorded with a Dräger surgical monitor (model GAMMA). The viscosity and coagulation of blood and lymph were measured with a VK-4 viscometer (ZMSO, Russia). The cellular composition of blood, lymph, and urine was analyzed using a SYSMEX KX-219 hematology analyzer (Japan). Electrolytes in lymph and blood plasma were measured with an AVL 9190 analyzer (Roche Diagnostics, Austria, 2012).

Blood and lymph biochemical parameters included total protein (TP), albumin (Alb), triglycerides (Tri), cholesterol (Chol), glucose (Glu), bilirubin (Bil), ALT, AST, and alkaline phosphatase (ALP). Phosphorus (P) and magnesium (Mg) were also measured. These analyses were performed with an A-25 BIOSYSTEMS biochemical analyzer (Spain) using the appropriate test kits according to standard procedures. Lipid peroxidation analysis included determination of conjugated dienes (CD) using an Apel PD-303UV spectrophotometer (Apel Co., Saitama, Japan), analysis of malondialdehyde (MDA) using a CECIL CE 1021 UV-VIS spectrophotometer (Cecil Instruments, Cambridge, UK), and titration of catalase (CAT) activity [11]. Immunoglobulins IgM, IgG, and IgA were determined by enzyme-linked immunosorbent assay using commercial T-systems (Wuhan Fine Biotech Co., China).

A practical evaluation of the FA-2 feed additive was conducted at the EPI farm in the Almaty region. Eighteen clinically healthy cows weighing 320–400 kg participated in the study. The animals were divided into two groups: control ($n = 9$) and experimental ($n = 9$). Before the experiment, animals were weighed, and blood samples were collected for subsequent biochemical and hematological analysis.

To continuously monitor the physiological state of some animals from both groups, intrarumen boluses were injected into the rumen. These devices were used to monitor selected physiological parameters and animal behavior during the experiment. The results were analyzed using Microsoft Excel. Changes in the measured parameters were assessed with the unpaired Fisher–Student test. Statistical significance was set at $p \leq 0.05$.

For the animal studies, the requirements of the local Ethics Committee of the Institute of Human and Animal Physiology (IGAP), SC MSHE RK, were followed. The study was covered by Protocol No. 3 (3), dated October 8, 2024, outgoing No. 07-05/158.

Results and Discussion. The study examined the effects of the new feed additives FA1 and FA2 on the functional state of blood, lymph, and key hemodynamic parameters in rats. Analysis of hemodynamic parameters revealed moderate changes in the cellular blood composition when the feed additives were included in the rat diet. In the FA1 group, the total white blood cell (WBC) count increased by 5.9% compared to the control (3.02 vs. $2.85 \times 10^9/L$; $p < 0.05$), and by 4.9% in the FA2 group. The absolute lymphocyte count (LYM) increased by 5.9% in FA1 and by 4.4% in FA2 relative to the control, while their relative content increased by approximately 5–6%, without significant differences (Table 1). The most noticeable changes were observed in the fraction of the total number of "medium" leukocytes (MID): in the FA1 group, the absolute value increased by approximately 128%, and in the FA2 group - by 171% compared to the control ($p < 0.001$). The relative content of MID increased by 45.6% in FA1 and by 61.1% in FA2. The proportion of granulocytes (GRA%) in the FA2 group increased more than 4 times compared to the control ($p < 0.001$).

Regarding the red blood cell component, hemoglobin (HGB) increased by 5.5% in the FA1 group, and the red blood cell (RBC) count by 3.0%. The RDW-SD index increased by 4.4% in the FA2 group. The platelet count (PLT) decreased by 5.5% in FA1 and by 6.6% in FA2 compared to the control group ($p < 0.05$). Overall, these changes are within physiological norms and demonstrate a moderate effect of feed additives on blood composition without signs of pathological changes.

Analysis of lymph cellular composition showed that the use of FA 1 and FA2 was accompanied by changes in certain hematological parameters compared to the control group (Table 2). The total white blood cell (WBC) count in the lymph of animals receiving FA1 increased by 1.3%, while in the group receiving FA2 it increased by 4.6% compared to the control group.

Table 1 – Blood cellular composition of animals receiving FA1 and FA2, compared to the control group

	Control group	FA 1	FA 2
WBC ($10^9/L$)	4.85 ± 0.1	$5.02 \pm 0.2^*$	4.99 ± 0.20
LYM ($10^9/L$)	2.71 ± 0.3	2.87 ± 0.3	2.83 ± 0.06
LYM %	55.5 ± 3.4	58.7 ± 6.7	58.5 ± 5.1
MID ($10^9/L$)	0.07 ± 0.001	$0.16 \pm 0.003^{**}$	$0.19 \pm 0.004^{**}$
MID %	3.75 ± 0.021	$5.46 \pm 0.09^{**}$	$6.04 \pm 0.051^{**}$
GRA ($10^9/L$)	2.0 ± 0.010	$2.5 \pm 0.03^*$	2.4 ± 0.05
GRA %	2.35 ± 0.22	11.21 ± 0.09	$10.10 \pm 0.08^{**}$
HGB g/L	150.0 ± 3.0	$158.3 \pm 4.0^*$	$159.1 \pm 5.2^*$
MCH pq	19.65 ± 1.1	19.7 ± 1.8	19.88 ± 2.2
MCHC	40.4 ± 2.1	42.45 ± 1.8	42.9 ± 2.5
RBC ($10^{12}/L$)	7.4 ± 0.2	$7.62 \pm 0.5^*$	7.7 ± 0.5
MCV fl	48.6 ± 5.1	48.9 ± 4.8	49.1 ± 4.4
HCT %	45.0 ± 3.2	$44.2 \pm 3.4^*$	45.6 ± 4.3
RDWcfl	36.1 ± 0.8	35.25 ± 1.7	37.7 ± 1.3
RDWs %	13.7 ± 0.5	$17.75 \pm 0.6^*$	$17.7 \pm 0.5^*$
PLT ($10^9/L$)	525 ± 14	$496.3 \pm 29^*$	$490.3 \pm 29^*$
MPV	7.4 ± 0.5	$7.3 \pm 0.4^*$	7.3 ± 0.6
PDWaf1	10.35 ± 0.7	$10.25 \pm 0.4^*$	10.3 ± 0.6
PDWc %	38.65 ± 0.7	39.4 ± 3.2	39 ± 3.5
PCT %	0.50 ± 0.04	$0.48 \pm 0.06^*$	$0.49 \pm 0.06^*$
Note: significant compared with the control, $-p < 0.05^*$, $-p < 0.001^{**}$			

The red blood cell (RBC) count in the lymph of animals receiving FA2 significantly

increased, but only minimally, indicating the absence of a significant effect of the supplements on the red blood cell count in the lymph.

Table 2 – Lymph cellular composition of animals receiving FA1 and FA2 compared to the control group in the experiment

Parameters	Lymph		
	control	FA 1	FA 2
WBC ($\times 10^3/\mu\text{L}$)	15.2 $\pm$ 0.3	15.4 $\pm$ 0.4	15.9 $\pm$ 0.6*
RBC ($\times 10^6 / \mu\text{L}$)	0.02 $\pm$ 0.003	0.02 $\pm$ 0.003	0.02 $\pm$ 0.004
LYM (%)	85.0 $\pm$ 0.8	94.5 $\pm$ 0.8*	94 $\pm$ 0.7*
LYM ($\times 10^3/\mu\text{L}$)	13.0 $\pm$ 0.4	16.1 $\pm$ 0.5	16.2 $\pm$ 0.4**
Note: significant compared with the control, -p <0.05*, -p<0.01**			

The most pronounced changes were also observed in the lymphocyte component. The relative lymph content (LYM%) increased by 11.2% in the FA1 group and by 10.5% in the FA2 group compared to the control.

The absolute lymph count (LYM $\times 10^3/\mu\text{L}$) increased by 23.8% in the FA1 group and by 24.6% in the FA2 group compared to the control. Thus, the use of FA1 and FA2 promoted an increase in lymphocyte counts in the lymph by 10-25% compared to the control, which may indicate immune system activation.

Analysis of blood plasma biochemical parameters showed that the use of FA1 and FA2 was accompanied by changes in certain metabolic and enzymatic parameters. The use of FA2 caused changes in blood plasma biochemical parameters (Table 3). Albumin and total protein levels increased by 11% compared to controls, likely due to increased protein metabolism. Blood glucose increased by 23%, indicating increased energy supply.

Cholesterol and LDL increased by 8% and 7.7%, respectively, which apparently indicates a moderate activation of lipid metabolism. Sample parameters of ALT and AST increased by 31% and 39%, respectively, compared to controls. Creatinine increased by 16% and urea by 52%, while bilirubin, triglycerides, and alkaline phosphatase remained at control levels, with no significant changes in other metabolic parameters.

Table 3 – Biochemical parameters of blood plasma after administration of FD2 in the experiment

Parameters	Blood plasma		
	control	FA 1	FA 2
Albumin, g/L	23.9 $\pm$ 0.10	25.4 $\pm$ 0.09*	26.5 $\pm$ 0.09*
Total protein, g/L	63.4 $\pm$ 2.11	69.9 $\pm$ 3.71*	70.2 $\pm$ 3.40*
Glucose, mmol/L	12.55 $\pm$ 0.81	15.41 $\pm$ 0.99*	15.39 $\pm$ 0.92*
Cholesterol, mmol/L	0.48 $\pm$ 0.03	0.54 $\pm$ 0.02*	0.52 $\pm$ 0.03*
LDL cholesterol, mmol/L	0.39 $\pm$ 0.01	0.45 $\pm$ 0.02*	0.42 $\pm$ 0.01*
ALT, U/L	154.7 $\pm$ 6.17	211.3 $\pm$ 9.21*	183.0 $\pm$ 9.49*
AST, U/L	118.4 $\pm$ 5.27	143.2 $\pm$ 6.88*	134.7 $\pm$ 5.66*
Bilirubin, $\mu\text{mol/L}$	15.3 $\pm$ 0.41	16.5 $\pm$ 0.66*	15.8 $\pm$ 0.60
Creatinine, $\mu\text{mol/L}$	45.1 $\pm$ 4.22	55.6 $\pm$ 4.51*	52.3 $\pm$ 4.15*
Triglycerides, mmol/L	0.84 $\pm$ 0.03	0.88 $\pm$ 0.04	0.83 $\pm$ 0.03
Alkaline phosphatase, U/L	264.2 $\pm$ 10.41	278.5 $\pm$ 11.43	265.0 $\pm$ 11.34
Urea, mmol/L	3.00 $\pm$ 0.05	4.44 $\pm$ 0.06*	4.56 $\pm$ 0.05*
Note: significant compared with the control, -p<0.05*, -p<0.01**			

The use of FA2 caused changes in lymph biochemical parameters (Table 4). Albumin increased by 15.8%, total protein by 16.3%, which is associated with an increase in protein metabolism. Glucose significantly increased by 26.1%, and cholesterol levels increased by 5.1%,

indicating activation of energy and lipid metabolism. Liver enzyme activity (ALT) remained virtually unchanged, while AST levels increased by 17.3%, bilirubin by 8%, creatinine by 26%, and urea by 17.9%, reflecting the response of the kidneys and nitrogen metabolism. Triglyceride levels increased by 8%, alkaline phosphatase by 9.4%, while the changes were moderate to minor.

Table 4 – Biochemical parameters of lymph after administration of FA2 in the experiment

Parameters	Lymph		
	control	FA1	FA 2
Albumin, g/L	10.1±0.07	10.7±0.11*	11.69±0.10*
Total protein, g/L	39.3 ±4.4	45.0±4.8	45.7±5.2
Glucose, mmol/L	6.35 ±0.4	7.22±0.8	8.8±0.7*
Cholesterol, mmol/L	0.39±0.02	0.40±0.03	0.41±0.03
Lipids LDL, mmol/L	0.37± 0.04	0.38±0.05	0.39±0.07
ALT, U/L	150.0 ±10.8	154.6±12.1	151.9±12.4
AST, U/L	80.0 ±11.5	120.5±8.4*	93.8±7.4
Bilirubin, µmol/L	1.50±0.04	1.65±0.05*	1.62±0.06*
Creatinine, µmol/L	42.4±3.8	55.4±5.3*	53.4±4.2*
Triglycerides, mmol/L	3.7 ±0.05	3.9±0.06	4.0±0.08
Alkaline phosphatase, U/L	180 ±17.0*	194±16.7	197±17.1*
Urea, mmol/L	3.90± 0.20	4.8±0.4	4.60±0.5*
Note: significant compared with the control, -p<0.05*, -p<0.01**			

Thus, the use of FA2 resulted in activation of protein and energy metabolism, moderate changes in lipid metabolism, and enzymatic activation of the liver, while maintaining stability of other metabolic parameters.

Our studies also examined lipid peroxidation (LPO), which reflects the metabolic and toxicological impact of the studied feed additives (Table 5). For example, dichloroacetic acid (DCA) levels increased by 1.2% in the FA1 group and by 3.7% in the FA2 group. Malondialdehyde (MDA) increased in the FA1 group and by 7.6% in the FA2 group compared to the control. This indicates significant activation of lipid peroxidation processes with FA1 feeding. Hydrogen peroxidase (HP) increased by 29.1% with FA1 feeding and by 11.6% with FA2 feeding.

Table 5 – Lipid peroxidation parameters in the blood of animals treated with FA1 and FA2

№ п/п	Groups	Parameter		
		Conjugated dienes (DC), nmol/L	Malondialdehyde (MDA), nmol/L	AKrH ₂ O ₂ /ml
1	Control (n=20)	2.44±0.03	0.131±0.01	1.99±0.03
2	FA-1 (n=22)	2.47±0.04	0.144±0.004	2.57±0.07*
3	FA-2 (n=20)	2.53±0.03	0.141±0.01	2.22±0.02
Note: * Significant differences compared with the control group (p<0.05)				

Thus, FA1 caused a more pronounced increase in lipid peroxidation than FA2, which was manifested by a significant increase in MDA and AA activity compared to the control. FA2 also activated these processes, but to a moderate degree.

The use of FA1 and FA2 had a significant effect on immunoglobulin levels in the blood and lymph of animals. IgM levels in the blood increased by 26.7% in the group of animals receiving FA1 and by 33.6% in the FA2 group, while in the lymph, their levels increased to 271.5% with FA2 and to 247.7% with FA2. IgG levels in the blood increased by 30.5% with FA1 and to 26.5% with FA2, while in the lymph, these indicators increased by 59.1% with FA1 and 28.5% with FA2. IgA

levels showed the most pronounced changes. Thus, in the blood level increased by 168.6% with feeding FA1 and by 48.4% with FA2, in the lymph this indicator increased by 129.2% with FA1 and by 62.6% with FA2 (Table 6).

Animal weight remained within normal limits throughout the study. At baseline, the average weight was 277 ± 17 g and increased to 281 ± 19 g after 30 days. After feeding FA1, animal weight was 273 ± 18 g and reached 295 ± 19 g after 30 days. With FA2, a more pronounced increase in body weight was observed: the average weight was 276 ± 16 g at baseline and increased to 303 ± 18 g after 30 days. These results indicate good tolerability of FA2 and its effectiveness in supporting normal animal growth, with significant weight gain over the 30-day feeding period.

Use of Intraruminal Boluses to Monitor the Physiological State of Cattle.

To assess the effect of the feed additive on the functional state of the animals, continuous physiological monitoring was used in addition to laboratory analyses.

Table 6 – Blood and lymph immunoglobulins after FA-1 и FA-2 administration

	Parameters	Control group	FA 1	FA 2
IgM, ng/mL	blood	125.85 ± 0.67	$139.5 \pm 7.25^*$	$142.4 \pm 8.11^*$
	lymph	44.75 ± 3.84	$66.3 \pm 4.23^*$	$61.5 \pm 3.02^*$
IgG, ng/mL	blood	212.35 ± 9.44	$247.20 \pm 9.71^*$	$251.8 \pm 8.99^*$
	lymph	144.14 ± 10.65	$169.53 \pm 12.05^*$	$171.3 \pm 11.75^*$
IgA, ng/mL	blood	66.55 ± 7.09	$78.73 \pm 6.25^*$	$84.8 \pm 5.84^*$
	lymph	76.55 ± 8.64	$91.34 \pm 7.11^*$	$89.42 \pm 9.21^*$

Note: *Significant differences compared with the control group ($p < 0.01$)

Electronic intraruminal boluses were placed in the rumen of selected animals from both the control and experimental groups. The boluses automatically recorded the main physiological parameters. The data were transmitted through a receiving device to a computer, allowing continuous monitoring throughout the observation period.

The boluses were used to monitor changes in rumen temperature, pH, and animal activity at different times of the day. The data showed clear daily changes in these parameters. At night, animal activity decreased, while rumen pH increased slightly and rumen temperature gradually decreased. After feeding and when the animals went to pasture, their activity increased markedly. Rumen pH remained within the normal physiological range, while rumen temperature gradually increased as digestive activity increased.

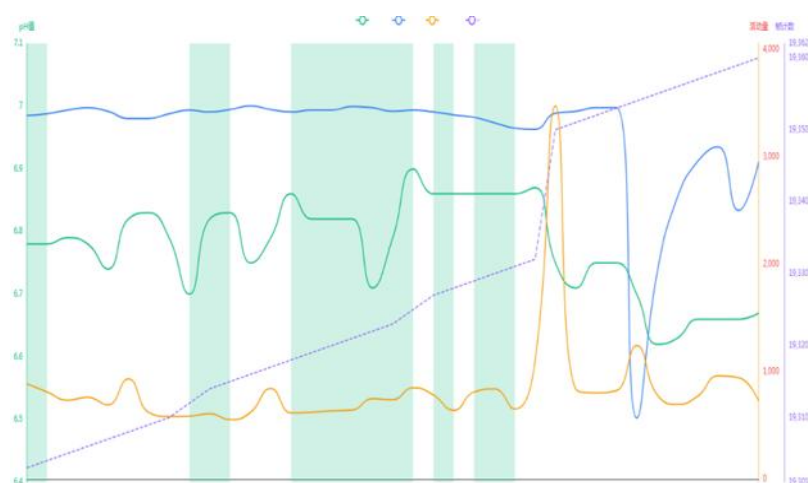


Figure 1 – Physiological and behavioral parameters of cattle in the control group: green line – pH; blue line – temperature; yellow line – locomotor activity

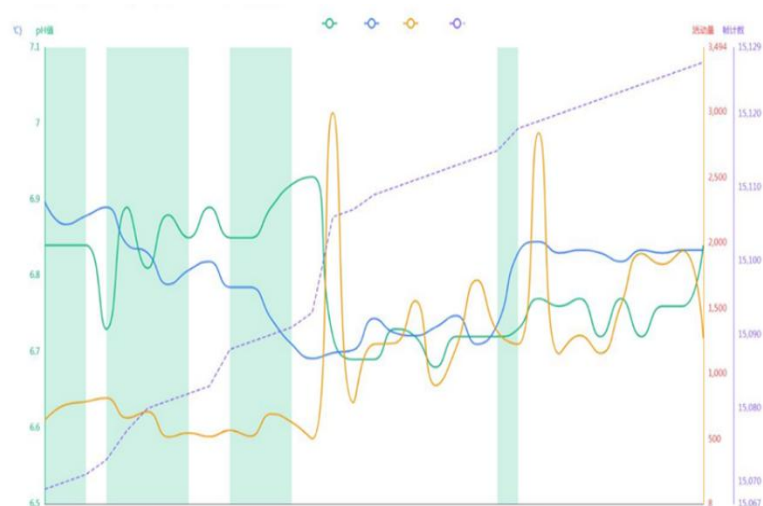


Figure 2 – Physiological and behavioral parameters of cattle receiving FA-2: green line – pH; blue line – temperature; yellow line – locomotor activity

Analysis of the continuous monitoring data showed that the use of the FA2 feed additive did not cause any changes indicating disturbances in rumen function. Animals in the experimental group showed increased locomotor activity, while rumen temperature and pH remained within the normal physiological range. These findings indicate good tolerance of the feed additive, no adverse effects on rumen digestion, and its potential for safe use in cattle diets.

Table 7 – Blood immunological parameters in cows

Parametrs	Control	Parametrs after FA2 administration
IgM, ng/ml	125.85	159.50
IgG, ng/ml	212.35	277.20
IgA, ng/ml	66.55	178.83

Evaluation of the immune status showed a positive change in humoral immunity after the use of the additive FA2 feed. The levels of IgM, IgG, and IgA increased, indicating activation of the body's protective mechanisms and improved immune response. The most noticeable changes were observed for IgA, which plays an important role in the local immune protection of mucous membranes. These results suggest that FA2 may have an immunomodulatory effect and contribute to increased natural resistance in cattle.

Thus, the use of FA1 and FA2 feed additives activated humoral immunity, with FA1 causing a more pronounced increase in lymph and blood immunity, indicating significant stimulation of the immune response. The use of FA1 and FA2 had a significant effect on hematological, biochemical, and immunological parameters, as well as on lipid peroxidation processes in laboratory animals. The observed increase in lymphocyte counts by 10–25% indicates activation of the immune system, which is likely due to the immunomodulatory effect of the drugs and stimulation of lymphocyte proliferation [11]. These data confirm the role of FA1 and FA2 in enhancing the cellular component of immunity, which may be particularly important for enhancing the body's defenses under experimental conditions.

An increase in albumin, total protein, and glucose by 10–26% reflects activation of protein-energy metabolism, which may indicate increased metabolic activity and readiness to adapt to the action of the test substances. An increase in liver enzymes (ALT, AST) indicates a moderate load on hepatocytes; however, the stability of bilirubin and alkaline phosphatase indicates the maintenance of liver function [12]. Concurrently, the increase in creatinine and urea reflects the activation of nitrogen metabolism and the kidneys' adaptive response to increased metabolic load, which is consistent with data on renal physiology and protein metabolism [13].

Lipid peroxidation parameters (MDA and hydrogen peroxidase activity) indicate that FA1 induces a more pronounced activation of these processes than FA2. Moderate activation of lipid peroxidation can be considered an adaptive response to biologically active substances, as it is accompanied by mobilization of the antioxidant system and enhanced detoxification mechanisms [14,15,16]. FA2, on the other hand, induces less pronounced changes, indicating a milder effect on the body's redox balance and a reduced potential load on antioxidant systems.

Changes in IgM, IgG, and IgA immunoglobulin levels in the blood and lymph indicate that both drugs activate humoral immunity. A particularly noticeable increase in IgM in the lymph (up to 271% with FA1) indicates stimulation of the primary immune response, while the increase in IgG reflects an enhanced secondary immune response. The increase in IgA indicates enhanced barrier protection of the mucous membranes, consistent with the mechanisms of humoral immunity described in classical literature [17]. These data highlight the ability of FA1 and FA2 to improve the body's immunological readiness and enhance resistance to potential pathogens.

Thus, FA1 has a more pronounced effect on immune and antioxidant systems, as well as metabolic processes, while FA2 causes moderate but significant changes without compromising the functional stability of the liver and kidneys. The obtained data demonstrate that both drugs are capable of stimulating immunity and metabolism, with FA2 being preferable when a mild modulating effect is desired. These results have practical implications for the development of dietary supplements and the evaluation of their safety in experimental models.

Conclusion. Feeding rats the bentonite- and chlorella-based additives FA1 and FA2 for 21 days produced no signs of toxicity: haematological parameters remained within physiological limits, bilirubin and alkaline phosphatase were unchanged, and body weight increased in all groups, most in the FA2 group. Both additives activated protein and energy metabolism, as shown by higher albumin, total protein and glucose, and both increased IgM, IgG and IgA in blood and lymph, with the largest change in lymph IgM. Lipid peroxidation products rose only slightly while catalase activity increased, indicating that the antioxidant system was mobilised rather than overwhelmed; this response was stronger with FA1 than with FA2. In cattle, FA2 did not alter rumen pH or temperature over the monitoring period and was well tolerated. FA2 therefore combines a moderate metabolic effect with a clear immunomodulatory one, and merits evaluation in longer production trials with farm animals.

Financing. This research has been funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No BR24993004 «Development of innovative methods to increase the productivity of livestock animals using physiological and genetic approaches») (for 2024-2026).

References

- [1] Hashemi, S. R., & Davoodi H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Veterinary research communications, 2011, 35(3), 169–180. <https://doi.org/10.1007/s11259-010-9458-2>
- [2] Windisch, W., Schedle K., Plitzner C., Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. Journal of animal science, 2008. 86(14 Suppl), E140–E148. <https://doi.org/10.2527/jas.2007-0459>
- [3] Saadaoui, I., Rasheed R., Aguilar A., Cherif M., Al Jabri H., Sayadi S., Manning S.R. Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. Journal of animal science and biotechnology, 2021. 12(1), 76. <https://doi.org/10.1186/s40104-021-00593-z>
- [4] Gouveia L, Batista AP, Miranda A, Empis J, Raymundo A. Chlorella vulgaris biomass used as colouring source in traditional butter cookies. Innov Food Sci Emerg Technol. 2007;8(3):433–436. <https://doi.org/10.1016/j.ifset.2007.03.026>
- [5] Abdel-Wahhab MA, Aly SE. Antioxidant property of bentonite in aflatoxin-contaminated diet. J Appl Toxicol. 2003;23(6):395–401. [https://doi.org/10.1002/\(sici\)1099-1263\(199905/06\)19:3<199::aid-jat558>3.0.co;2-d](https://doi.org/10.1002/(sici)1099-1263(199905/06)19:3<199::aid-jat558>3.0.co;2-d)
- [6] Panahi, Y., Darvishi B., Jowzi N., Beiraghdar F., Sahebkar A. Chlorella vulgaris: A Multifunctional Dietary Supplement with Diverse Medicinal Properties. Current pharmaceutical design,

2016. 22(2), 164–173. <https://doi.org/10.2174/138161282266615112145226>

[7] **Niccolai A**, Zittelli GC, Rodolfi L, Biondi N, Tredici MR. Microalgae of interest as food source: biochemical composition and digestibility. *Algal Res.* 2019; 42:101617. <https://doi.org/10.1016/j.algal.2019.101617>

[8] **Rayman**, Margaret P. “Selenium and human health.” *Lancet* (London, England) vol. 379,9822 (2012): 1256–68. [https://doi.org/10.1016/S0140-6736\(11\)61452-9](https://doi.org/10.1016/S0140-6736(11)61452-9)

[9] **Zimmermann**, Michael B, and Kristien Boelaert. “Iodine deficiency and thyroid disorders.” *The lancet. Diabetes & endocrinology* vol. 3,4 (2015): 286–95. [https://doi.org/10.1016/S2213-8587\(14\)70225-6](https://doi.org/10.1016/S2213-8587(14)70225-6)

[10] **Rockson SG**. The lymphatics and the inflammatory response: lessons learned from human lymphedema. *Lymphat Res Biol.* 2013 Sep;11(3):117-20. <https://doi.org/10.1089/lrb.2013.1132>

[11] **Janeway, C A Jr**. “How the immune system protects the host from infection.” *Microbes and infection* vol. 3,13 (2001): 1167–71. [https://doi.org/10.1016/s1286-4579\(01\)01477-0](https://doi.org/10.1016/s1286-4579(01)01477-0)

[12] **Guyton A.C.**, Hall J.E. *Textbook of Medical Physiology* (14th ed.). Elsevier. 2021

[13] **Halliwell, Barry**, and John M. C. Gutteridge, *Free Radicals in Biology and Medicine*, 5th edn (Oxford, 2015; online edn, Oxford Academic, 22 Oct. 2015), <https://doi.org/10.1093/acprof:oso/9780198717478.001.0001>

[14] **Sies, H**. “Oxidative stress: oxidants and antioxidants.” *Experimental physiology* vol. 82,2 (1997): 291–5. <https://doi.org/10.1113/expphysiol.1997.sp004024>

[15] **Fridovich, I**. “The trail to superoxide dismutase.” *Protein science: a publication of the Protein Society* vol. 7,12 (1998): 2688–90. <https://doi.org/10.1002/pro.5560071225>

[16] **Ayala, A.**, Muñoz M.F., Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, 2014, 360438. <https://doi.org/10.1155/2014/360438>

[17] **Abbas A.K.**, Lichtman A.H. (2021). *Basic immunology: Function and Disorders of the immune system* (16th ed.). Elsevier. [101312426\[nlmid\]](https://doi.org/10.1016/B978-0-323-98565-1)

ЖАҢА ТАБИҒИ КЕШЕНДІ ЖЕМДІК ҚОСПАНЫҢ ИММУНОМОДУЛЯЦИЯЛЫҚ БЕЛСЕНДІЛІГІН ТӘЖІРИБЕЛІК БАҒАЛАУ

Қойбасова Л.Ұ., биология ғылымдарының кандидаты, қаумдастырылған профессор

Демченко Г.А., медицина ғылымдарының докторы, профессор

Макашев Е.К., биология ғылымдарының докторы, профессор

Абдрешов С.Н., биология ғылымдарының кандидаты, профессор

Мусаева А.С., биология ғылымдарының кандидаты, қаумдастырылған профессор

Есенова М.А., PhD, аға ғылыми қызметкер

ҚР ҒЖБМ ҒК Генетика және физиология институты, Алматы қ., Қазақстан

Аңдатпа. Бұл мақалада бентонит, хлорелла, калий йодиді және селенді қамтитын жаңа табиғи кешенді жемдік қоспаның иммуномодуляциялық және метаболикалық әсерлерін тәжірибелік бағалау ұсынылған. Зерттеудің мақсаты – қоспаның лимфа айналымына, гемодинамикаға, қанның негізгі биохимиялық көрсеткіштеріне және гуморалдық иммунитетке әсерін бақыланатын эксперименттік жағдайда анықтау.

Нәтижелер көрсеткендей, 21 күндік қоспаны қолдану лимфа жүйесінің функционалды жағдайын айтарлықтай жақсартуға, лимфа ағымын арттыруға және тамырлық параметрлерді тұрақтандыруға ықпал етті. Қанның бірнеше биохимиялық көрсеткіштерін маңызды түрде қалыпқа келтіру байқалды, бұл ақуыз және липид метаболизмінің жақсарғанын көрсетеді. Сонымен қатар, липидтердің пероксидтік тотығу қарқыны айтарлықтай төмендеп, антиоксиданттық жүйенің белсенділігін білдірді. Қан мен лимфада IgM, IgG және IgA иммуноглобулиндерінің деңгейінің артуы гуморалдық иммундық жүйенің ынталандырылуын растайды.

Бұл нәтижелер қоспаның кешенді метаболикалық, антиоксиданттық және иммуномодуляциялық қасиеттерін көрсетіп, оны жануарлардың жалпы физиологиялық және иммундық жағдайын жақсартуға арналған функционалды жемдік қоспа ретінде қолдану әлеуетін дәлелдейді. Зерттеу табиғи қосылыстарды жемдік қоспаларда пайдалану арқылы жануарлардың денсаулығын оңтайландыру, гомеостазды қолдау және өнімділік пен ауруларға төзімділікті арттыру мүмкіндіктерін түсінуге үлес қосады.

Тірек сөздер: жемдік қоспа, мал азығы, лимфа, қан, гуморалдық иммунитет, иммуномодуляциялық әсер.

ЭКСПЕРИМЕНТАЛЬНАЯ ОЦЕНКА ИММУНОМОДУЛИРУЮЩЕЙ АКТИВНОСТИ НОВОЙ КОРМОВОЙ ДОБАВКИ ПРИРОДНОГО ПРОИСХОЖДЕНИЯ

Койбасова Л.У., кандидат биологических наук, ассоциированный профессор

Демченко Г.А., доктор медицинских наук, профессор

Макашев Е.К., доктор биологических наук, профессор

Абдрешов С.Н., кандидат биологических наук, профессор

Мусаева А.С., кандидат биологических наук, ассоциированный профессор

Есенова М.А., PhD, старший научный сотрудник

Институт генетики и физиологии КН МНВО РК, Алматы, Казахстан

Аннотация. В исследовании изучали действие новой комплексной кормовой добавки, состоящей из бентонита, хлореллы, йодида калия и селена. Оценивали ее влияние на лимфоток, гемодинамику, биохимические показатели крови и показатели гуморального иммунитета у крыс и сельскохозяйственных животных в условиях эксперимента.

После 21 дня применения добавки у животных отмечалось увеличение лимфотока и улучшение показателей работы сосудов. Изменения биохимических показателей крови указывали на улучшение белкового и липидного обмена. При этом уровень перекисного окисления липидов снижался, что может свидетельствовать об усилении антиоксидантной защиты организма. В крови и лимфе также отмечалось повышение содержания иммуноглобулинов IgM, IgG и IgA.

Полученные результаты показывают, что добавка положительно влияет на обмен веществ, антиоксидантную защиту и иммунные показатели животных. Она может быть использована как дополнительный компонент рациона для поддержания нормального физиологического состояния и иммунной защиты. Полученные данные также показывают перспективность использования природных компонентов в составе кормовых добавок для поддержания здоровья животных и их устойчивости к неблагоприятным факторам.

Ключевые слова: кормовая добавка, питание животных, лимфа, кровь, гуморальный иммунитет, иммуномодулирующий эффект.