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New mineral and vitamin feed additive for small cattle

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ABSTRACT

The purpose of the study is to evaluate a new feed additive for the physiological and biochemical parameters of sheep. Studies have shown that sheep of both breeds, whose diet included feed additives based on bentonite with chlorella and essential trace elements, outperformed their peers from the control group in some blood metabolites and morphological parameters. This indicates a higher level of metabolic processes in the body of animals of the experimental groups compared to the control, which is associated with the receipt of additional biologically active substances. It has been investigated that the use of a new feed additive leads to a significant improvement in growth and reduces LPO, namely, it reduces the content of MDA and DC in the blood, and the most positive effect of these complexes on the protein and carbohydrate metabolism of sheep of both groups during feeding. The average daily and absolute gains in live weight in the Hampshire and Kazakh meat wool breeds were 122 g and 6.80 kg and were also higher by 1.5 and 1.7%, respectively, than in the controls of both groups. The feed additive has a good adsorption and antioxidant effect, maintains balance, participates in the inactivation of free radicals and has a protective effect on cell membranes. The feed additive is high in calories, has an antioxidant and adaptogenic effect, increases animal weight, 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.

Article type: Research Article.

Metabolism, Sheep.

INTRODUCTION

Animal husbandry is a priority area of development of agriculture in the Republic of Kazakhstan, the most important source of economic growth of the country and improvement of living standards of the population. In addition, one of the strategic tasks of agriculture is to ensure food security of the country, and today it requires diverse, including scientific support. The potential of the domestic agricultural sector is colossal - there are large areas of agricultural land. However, in almost all sectors, low productivity is observed (Tokayev 2019, 2021). In addition, the physiological functions of animals undergo certain changes with age, depend on productivity and other factors (Bhati *et al.* 2017; Gomez-Miranda *et al.* 2020; Brassard *et al.* 2024). To increase the productivity of farm animals, many biologically active substances are currently used, including various plant extracts (Artuso-Ponte *et al.* 2020; Alem 2024), feed additives, along with feed, are one of the main factors in improving the quality of animal feed, as well as strengthening their health (Kiczorowska *et al.* 2017; Bakhtiyarova *et al.* 2025). Environmental conditions, poor feed quality, high disease levels and weak market infrastructure are all factors that negatively affect livestock production and its products, including feed shortages, seasonal changes and some natural factors. Metabolic diseases are the main factor in reducing animal productivity and agricultural output (Mohamed *et al.* 2024). In recent years, natural products have gained great importance as growth-promoting products, and can have a positive impact on improving livestock production (Demchenko *et al.* 2022; Nastoh *et al.* 2024). Currently, feeding standards are being developed and implemented based on a deep study of the digestive processes and improving the quality of the resulting product (Ronald, *et al.* 2020; Boyko *et al.* 2021). The use of various feed additives, including non-traditional ones, allows us to improve and regulate the composition of feed, as well as improve the metabolism of farm animals and increase the efficiency of their use of products (Caprarulo *et al.* 2020). Feed additives (FA) are substances added to animal feed to improve the taste of the feed and its usefulness, to improve the quality of feed and have a positive effect on animal productivity, increase its growth rate (Placha *et al.* 2022; Neijat *et al.* 2024), as they have anthelmintic, antimicrobial and anti-inflammatory, as well as antioxidant properties and improve microbiological activity, and are aimed at improving the quality of products, livestock production and animal productivity (Chan *et al.* 2018; Silveira *et al.* 2021; Bostami *et al.* 2021). The composition of the FA must correspond to the normative data, for enhanced growth of young small cattle (YSC), vitamins and minerals (Alipour *et al.* 2019; Nigmatyanov *et al.* 2020). Normal vitamin A content is responsible for the normal functioning of the digestive system, prevents miscarriages, improves reproductive function, stimulates the growth of young animals, and prevents obesity (Baker *et al.* 1986; Shastak *et al.* 2024). In addition, iodine and zinc maintain stable milk yields, reproductive function, and are responsible for the normal functioning of the thyroid gland (Mobashar *et al.* 2024; Gelaye 2024; Nhara *et al.* 2025). Potassium and sodium control the water-salt balance, prevent the occurrence of anemia (Henchion *et al.* 2017; Najjar 2023; Yang 2023) in case of cardiovascular disorders. The use of feed additives allows livestock specialists to comprehensively mechanize and automate the processes of preparing and distributing feed. Feed additives are the most effective type of feed that meets the physiological needs of farm animals. According to literarature sources, the result of using additives is not only an increase in the body weight of farm animals, but also an elevation in their productivity, as well as enhance in the economic efficiency of the country (Singh 2015; Pandey *et al.* 2019; Chuang *et al.* 2020). The use of various fortified feed additives improves the functional activity of digestive processes, stimulates the growth and development of young animals, and affects the quality of meat and dairy products. At this time, the study of patterns of changes in blood parameters during growth, development, and formation of productive qualities of animals is of great practical interest in the agro-industrial sector. Studies of blood 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 occurring in the body after the use of various feeds or feed additives, which is necessary to obtain high-quality products. The purpose the research was to study the dynamics of the antioxidant properties of new feed additives in the sheep diet and evaluate their effectiveness.

MATERIALS AND METHODS

Ethics statement

All groups of animals were in the same conditions of feeding and keeping in the vivarium. All experiments with animals were conducted in strict accordance with the rules developed and approved by the local ethics committee of the Institute of Genetics and Physiology, Protocol No. 07-05/158 of October 08, 2024, as well as in accordance with the rules of bioethics approved by the European Convention for the Protection of Vertebrates (Strasbourg, 1986) and the guidelines outlined in the European Union Directive 2010/63/EU of September 22, 2010, "On the protection of animals used for scientific purposes".

Animals and experiment design

The experimental part of the work was carried out on a peasant farm (PF) in Almaty and Zhambyl regions during the winter season. The studies were conducted on 100 ewes of different productivity directions, including PF "Mamed-Khasenov" (Almaty region) - Hampshire breed (50 rams - producers) weighing 64 ± 2.7 kg and PF "Razakhun" (Zhambyl region) - Kazakh meat wool (50 rams - producers) weighing 64 ± 2.7 kg. We formed 2 groups by ram breed - the control group (30 heads) - were on the main feed of the peasant farm and the experimental group of animals (70 heads) on the main feed together took FA1 granules for 60 days (Table 1). The composition of FA1 included chlorella, bentonite, vitamins and minerals. The animals were selected by age and live weight. Live weight changes were determined by weighing the sheep individually every 15 days in the morning before feeding. The conditions of keeping and the general level of feeding of all experimental animals were similar. Animals of all groups were fed with specialized feed FA1; the average daily consumption of compound feed with a FA1 for fattening was 2680–2880 g, balanced in accordance with a detailed system of standardized feeding (Fig. 1).

Table 1. Live weight of sheep.

Group of animals	**«Mamed-Khasenov»** PF, **Hampshire, live weight (kg; n = 50)**		**"Razakhun"** PF, **Kazakh meat and wool, live weight (kg; n = 50)**	
Ewes	Control (n = 15)	Experimental (n = 35)	Control (n = 15)	Experimental (n = 35)
	65.0 ± 0,28	65.6 ± 0.70	52.0 ± 0.17	52.7 ± 0.32

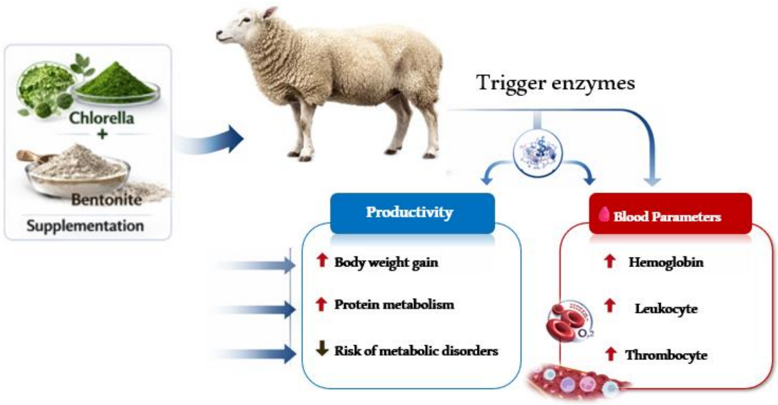

Fig. 1. Sorption complex and effectiveness of feed additives.

Improving the technology of growing young sheep is possible due to the improvement of conditions of maintenance and organization of full feeding. Creation of a complex biologically active additive based on natural components based on liquid chlorella and bentonite in a ratio of 1:3, which additionally contains active substances enriched with vitamins and minerals.

Physiological, biochemical and hematological studies

Physiological indices of animal growth were assessed: weight gain by weighing the sheep individually every 15 days in the morning before feeding and watering. Absolute, average daily and relative gains in live weight of rams were determined. Results of laboratory studies of biological material samples (blood, etc.) obtained from animals of various species delivered from different farms in the country's regions. For the study, blood was collected in vacuum tubes before morning feeding from the jugular vein at the time before and after the experiment: on the 1st day and on the 60th day of the experiment. Peripheral blood samples (serum) were collected by centrifugation for 20 min/1000 rpm. The main biochemical parameters were determined in the blood samples: protein fractions (albumin, α-, β-, γ-globulins) by the nephelometric method. The amount of α-amylase was determined by the amyloclastic method, the activity of lipase and trypsin, glucose, creatinine, urea, alkaline phosphatase, total protein, bilirubin, cholesterol, triglycerides, total lipids, the level of enzyme activity: alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined by generally accepted methods using a set of reagents from the Bio-Lachema-Test company (Czech Republic), their indicators were determined using an automatic biochemical analyzer "COBOS INTEGRA 400" (USA; Abdreshov *et al.* 2015). The cellular composition and physicochemical parameters of the blood, as well as its morphological composition, were studied on the SYSMEX KX-219 hematology analyzer (Japan).

Measurement of lipid peroxidation

Lipid peroxidation (POL) was assessed by the content of malonic dialdehyde (MDA), diene conjugates (DC), catalase, as well as the level of superoxide dismutase (SOD) according to the method of the authors (Behn *et al.* 2007; Li *et al.* 2007; Igbokwe *et al.* 2016a). The optical density was measured at 52 nm. After that, the content of thiobarituric acid – reacting substances (TBARS) was measured according to the method (Wasowicz *et al.* 1994; Jo *et al.* 1998).

Statistical data analysis

The results were statistically processed using the Student's t-test and using the program method (Microsoft Corporation, Washington, DC, USA). Statistical analysis of the data was carried out using methods of variation and comparative statistics. Statistical analysis of the data obtained during the study was performed using the

Student's t-test, and the recorded changes in indicators were considered reliable and were recognized as significant at $p < 0.01$, and $p < 0.05$ using the Fisher criterion.

RESULTS

The composition of the feed additive and effect on the physiological parameters of sheep

In modern conditions of animal husbandry and feeding, animal feed additives often do not meet the needs for microelements, especially in conditions of high environmental loads (meat and milk yield, stress, lactation), which requires the development of complex additives. Also, compared to individual feeding, a combined approach - minerals + plant/algae components + adsorbents - increases efficiency, which is economically viable and improves animal health. In turn, natural bentonites are used as adsorbents for toxins, mycotoxins and other harmful composite compounds. During the experiment, the effect of adding bentonite chlorella premix to the diet of sheep was studied in an experiment conducted on two sheep farms. Bentonites have properties such as hydrophilicity, ion exchange capacity, surface activity, which positively affect the absorption of nutrients in the animal's body, improve the absorption of fatty acids and fat-soluble substances. According to the chemical formula $Al_2[Si_4O_{10}](OH)_2{\bullet}nH_2O$, the composition of aluminosilicate bentonite obtained from the Tagan deposit, according to which it is mainly oxidized aluminum (18-20%) and silicon (56-60%), as well as macro and microelements (Table 2).

Table 2. Chemical composition of bentonite from the Tagan deposit.

Bentonite inclusions (%)		Elements (g; %)		Bentonite inclusions (%)		Elements (g; %)	
$A1_2O_3$	18.76	A_1	4.42	Na_2O	1.03	Na	0.43
SiO_2	61.84	Si	6.24	K_2O	2.19	K	1.07
CaO	7.09	Ca	2.21	P_2O_5	0.19	P	0.14
Fe_2O_3	5.81	Fe	3.9	TiO_2	0.86	-	-
MgO	3.92	Mg	0.75	SO_3	0.21		
MnO	0.92						

Bentonite activates cellular metabolism, it is absolutely harmless and has no contraindications, has anti-inflammatory, analgesic, vasodilating, antioxidant, radioprotective and adsorbing properties. The use of bentonite helps to completely cleanse the body of toxins and toxins, enrich the body with essential minerals, improve the functioning of all internal organs and enhance immunity (Trckova *et al.* 2004; Damato *et al.* 2022; Shin *et al.* 2023). It adsorbs water and digestive juices in the gastrointestinal tract, and also increases the utilization of feed additives. The bentonite–chlorella premix for animal feed was prepared on the basis of local natural bentonite by enriching powdered microalgae *Chlorella vulgaris* with amino acids and vitamins. *Chlorella* is a microalgae known for its rich nutritional composition, including proteins, vitamins, minerals, and antioxidants. It is valued for its complex composition and is used as a dietary supplement to support health, enhance immunity, and detoxify the body. Its composition may vary slightly depending on growing conditions, but the main components and elements are as follows: in general, 100 g of *C. vulgaris* contains protein (58-60 g), fats (9-11 g), carbohydrates (20-23 g), dietary fiber (5-8 g), chlorophyll (2500-4500 mg), and iron (120-150 mg). Among these are many polyunsaturated fatty acids, such as omega-3 and omega-6. This chlorella microalgae, cultivated for animal feed, enriches the premix composition with amino acids, fatty acids, carotene, and vitamins B, D, K, PP, and E, as well as folic acid and biotin. The bentonite–chlorella premix consists of 25-30% bentonite, 1.5-2.0% dried powder of *C. vulgaris*, and 68-70% sunflower meal by mass fraction. As a result, aluminosilicate bentonite minerals are supplemented with the amino acids and vitamins of chlorella (Table 3). The chlorophyll contained in its composition neutralizes toxic compounds formed during digestion, acting as a detoxifying agent. However, current research shows that chlorella is still rarely used in livestock production as a feed ingredient; most studies focus on its use as a valuable biologically active supplement in animal diets. These additives are applied in agricultural livestock and poultry production to regulate metabolism, treat and prevent diseases, improve animal appetite, and increase productivity. When using bentonite-chlorella premix in sheep feed supplements, it was observed that the digestive process of sheep in the experiment improved, and they gained a significant amount of weight. This effect, especially on nitrogen metabolism in the digestive tract, was beneficial for the physiological indicators of sheep. Currently, various methods are being used to improve the productivity of farm animals, one of them is the animal feed supplement diet, which includes microgrowths that optimize the health, growth and efficiency of the livestock industry. In the modern world, among these additives is derived from the green microalgae *C. vulgaris* (CLV), which has significant potential as a sustainable additive to animal feed due to its rich nutritional composition (Martins *et al.* 2022; Gadzama *et al.* 2025). Chlorella is not only rich in essential nutrients, it contains antioxidants, fatty acids, amino acids, vitamins and minerals that promote its health (Spínola *et al.* 2023; Gadzama *et al.* 2024). Thus, the integration of bentonite and *C. vulgaris* into the diets of farm animals seems to be a promising direction that contributes to increasing productivity and sustainability of livestock systems (Fig. 2).

Table 3. Vitamin composition of bentonite-chlorella premix.

Vitamins	Content (μg g^{-1})	Vitamins	Content (μg g^{-1})
Carotene	1000-1500	D	900-1000
B_1	2-17	K	6-8
B_2	20-26	PP	100-170
B_3	10-15	E	10-350
B_6	8-9	Folic acid	480-500
B_{12}	0.025-0.1	Biotin	0.1-0.2
C	1200-5000	-	-

After 60 days of feeding the animals with FA1, the weight increased by 11.14-12.9% (72.91 ± 0.82 and 59.5 ± 0.64), in the control group by 2.1% (66.4 ± 0.71 and 53.09 ± 0.48). One of the main criteria characterizing the adaptation process is the dynamics of the animal's live weight, since it reflects the transition from the catabolic phase to the anabolic direction of metabolism, and also allows us to judge the general condition of the body. For animals after feeding the new FA for 60 days, a significant intensity of growth and development of the body was characteristic, that is, a rapid increase in body weight and body size and individual organs, improvement of all body systems in sheep. The live weight of the Hampshire breed (7.31 ± 0.2 kg), Kazakh meat wool (6.80 ± 0.7 kg) at the end of the experiment showed an increase of 11.14-12.9% ($p < 0.05$) compared to the control groups. The average daily live weight gain of rams was 0.122 ± 0.0007 and 0.113 ± 0.004 kg ($p < 0.01$) and was also higher by 1.3 and 1.5% ($p < 0.05$), respectively, than that of the animals of the control group (Table 4).

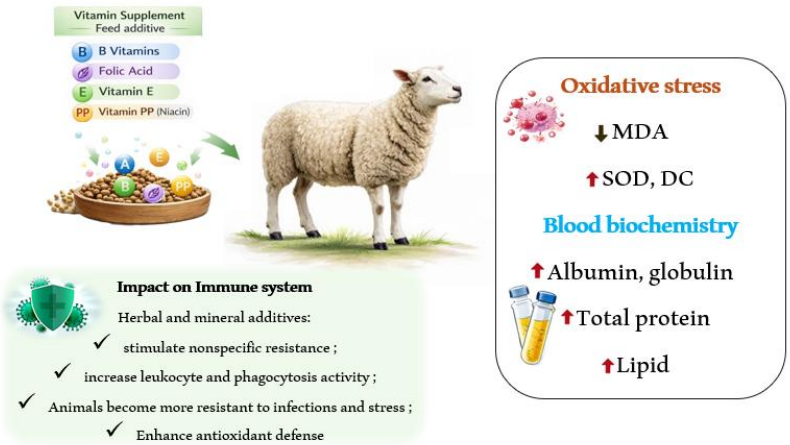

Fig. 2. Bentonite with chlorella as an additive to animal feed. The role of feed additives in improving animal production performance.

Table 4. Live weight and average daily gain data for sheep in the control and experimental groups before and after feeding FA_1.

Sheep breeding indicators	Hampshire (n = 50)			Kazakh meat wool (n = 50)		
	Control (n = 15)	Experimental group FA_1 (n = 35)		Control (n = 15)	Experimental group FA_1 (n = 35)	
		Day 1	60 days		Day 1	60 days
Total weight of animals (kg)	65.0 ± 0.28	65.6 ± 0.70	72.91 ± 0,82	52.0 ± 0,17	52.7 ± 0.32	59.5 ± 0.64
Live weight gain (kg)			7.31 ± 0,2			6.80 ± 0.7
Average daily increase in body weight (kg)	0.047 ± 0.0009	-	0.122 ± 0,0007	0.036 ± 0.0005	-	0.113 ± 0.004

* - p ≤ 0.001 between the control and the experimental group.

The results of average daily gains are shown in Fig. 3. Hampshire ewes had an average live weight of 65.6 ± 0.70 kg, Kazakh meat wool ewes 52.7 ± 0.32 kg. Later, with the increase in the age of both types of animal groups, after the use of the feed additive, the differences in their live weight changed over 15 days. In Hampshire breeds, after 15 days, the daily gains were 1.9 ± 0.014 kg, and in Kazakh meat wool ewes it was 2.5 ± 0.020 kg. Over the 30-day period, these figures were higher and amounted to 4.4 ± 0.015 kg and 4.1 ± 0.009 kg, respectively, by breed.

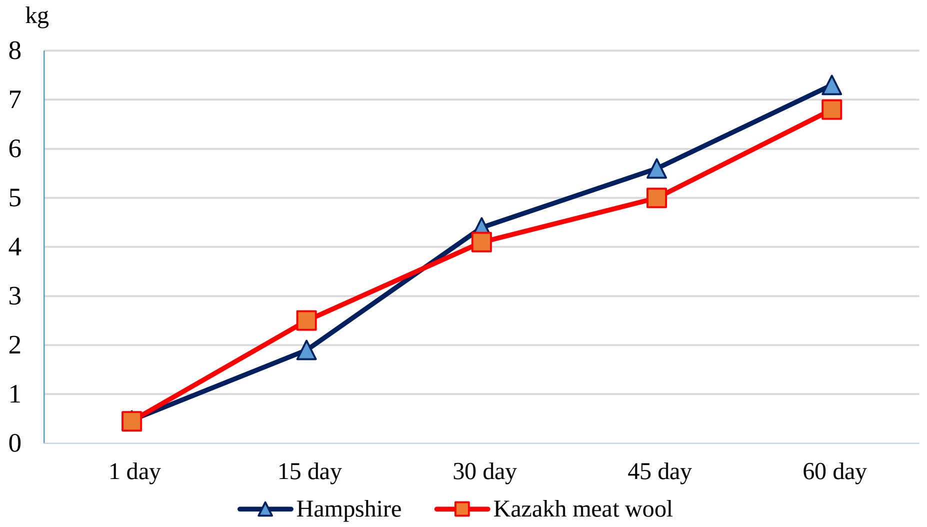

Fig. 3. Dynamics of average daily live weight gains in both breeds of ewes (kg).

Higher weight gains in the experimental Hampshire breeds were observed starting from the 45th day of feeding, their average daily gains were 5.6 ± 0.038 kg. In the Kazakh meat wool breed, the weight indicators were 5.0 ± 0.011 kg. From the 60th day of feeding the feed additive, the maximum average daily gains were noted and were in the Hampshire breed 7.3 ± 0.021 kg. In the Kazakh meat wool breed 6.8±0.014 kg. The effect of the FA1 on live weight in both groups of animals was 7.31 ± 0.2 kg and 6.80 ± 0.7 kg at the end of the experiment. Between the Hampshire and Kazakh meat wool breeds, these differences were statistically significant ($p < 0.05$).

Biochemical and hematological parameters of sheep when feeding a feed additive

When comparing the control data with the parameters of the experimental group taking FA1, it was revealed in the blood that the analysis of the obtained data indicates fluctuations in the activity of transamination enzymes when feeding animals with the FA_1 (Table 5). At the same time, the activity of AST during the period of feeding with the FA in Hampshire sheep increased by 0.11 mmol L^{-1} (7.9%), Kazakh meat wool by 0.08 mmol L^{-1} (6.0%). The increase in ALT activity in the analyzed feeding period was less significant and amounted to 0.05 mmol L^{-1} (7.8%) and 0.04 mmol L^{-1} (6.9%), respectively, in the Hampshire and Kazakh meat wool breeds compared to the control group. The increase in the level of total protein in the blood of Hampshire and Kazakh meat wool sheep during feeding with the new FA by 4.8 ($p < 0.05$) and 2.3% ($p < 0.05$), albumins, α-, β-globulins by 5.4, 15.9 ($p < 0.01$), 21.2% ($p < 0.05$) and 6.9, 20.0 ($p < 0.01$), 21.3% ($p < 0.05$), respectively, characterizes the protein-forming function of the liver and is an integral indicator of animal growth and development. A characteristic feature of the protein composition of the blood of the Hampshire breed is a higher level of globulins by 3.0% due to the β- and γ-globulin fraction by 6.2 and 10.6% ($p <0.05$) compared to the Kazakh meat wool breed, which reflects the state of one of the forms of reactivity of the body, in particular, the synthesis of specific antibodies during the period of low temperatures. After feeding with the FA, the content of amylase, lipase and trypsin in the blood of the Hampshire breed increased by 59%, 38%, 13%, and in the Kazakh meat wool breed, by 55.5%, 45%, 14% in accordance with the control group ($p < 0.01$). The indicators after FA1 HDL cholesterol decreased in the Hampshire breed by 7.1 and in the Kazakh meat wool ewe breed by 7.2% compared to the control ($p < 0.05$). Triglycerides are derivatives of glycerol and higher fatty acids that are obtained from food and are the main source of energy. The content of triglycerides in the blood serum increased significantly by 30% ($p < 0.01$) and by 24.7% ($p < 0.01$) compared to the control group (Table 5). In ewes of both breeds, high concentrations of total protein were recorded 72.5 ± 1.6 and 70.0 ± 1.2 g L^{-1}, respectively, associated with its receipt with a new feed additive.

The globulin fraction was predominant in the blood. The ewes of both breeds exhibited a low albumin level of 35.2 ± 1.8 and 39.9 ± 1.6 g L^{-1}. Moreover, in the ewes of both groups, the albumin concentration was 9-6.9% higher ($p < 0.01$), while globulins differed insignificantly from the control groups. The amount of α-globulins in the blood serum of the Hampshire breed showed an elevation of 32.9%, while in the Kazakh meat wool breed it was 23.1% ($p < 0.01$). The level of β-globulins in the blood serum of both breeds were 23.7% and 11.2% ($p < 0.01$), respectively, higher than in the ewes of the control group (Table 5). The results of the clinical blood test of the sheep showed the following results of the control group on the 60th day after the feed additive, a slight increase in the level of erythrocytes to 4.7 ± 1.3 10^{12} L^{-1} was observed compared to the lower limit of the norm. In animals of all groups, an increase in the hemoglobin level was noted during the experiment. A tendency towards a decrease in the level of platelets was noted in the ewe of both groups; in the Hampshire and Kazakh meat wool breeds after the use of the FA after 60 days it was 221 ± 15.4 × 10^9 L^{-1}. The leukocyte formula was recorded in all animals under study compared to the reference values. An increase in the level of eosinophils was noted in the blood of animals of all studied groups: in the Hampshire breed by 13.5% and in the Kazakh meat wool breed by 18.6%. Before feeding, these indicators were 3.12 ± 0.3% and 2.74 ± 0.92% respectively. The most pronounced increase in the percentage of basophils was noted in the blood of the control of both groups (0.35 ± 0.03% and 0.32 ± 0.07%) and the period of feeding the feed additive in the blood of animals it was 0.47 ± 0.04% and 0.43 ± 0.09%. After feeding the ewes for 60 days, a statistically significant difference of 34.3% of basophils was noted in the blood of both groups, respectively.

Table 5. Changes in biochemical metabolism and blood composition in Sheep after when using FA1.

Indicator parameters	Hampshire		Kazakh meat wool	
	Control	FA1 – 60 days	Control	FA1 – 60 days
Total protein (g L^{-1})	72.81 ± 4.31	80.43 ± 2.11*	74.27 ± 4.89	84.10 ± 5.12*
Albumin (g L^{-1})	35.2 ± 1.8	38.4 ± 1.2	39.9 ± 1.6	42.6 ± 0.07
Globulins (g L^{-1})	32.2 ± 2.2	33.7 ± 2.8	34.1 ± 1.9	34.8 ± 2.5
α-globulins	8.2 ± 0.27	10.9 ± 0.16	9.1 ± 0.16	11.2 ± 0.27
β-globulins	10.1 ± 0.16	12.5 ± 0.16	10.7 ± 0.27	11.9 ± 0.27
γ-globulins	13.8 ± 0.27	15.2 ± 0.27	12.4 ± 0.16	14.8 ± 0.16
Total Amylase (U L^{-1})	12.8 ± 0.38	20.39 ± 3.1**	13.9 ± 0.07	21.62 ± 3.1**
Lipase (U L^{-1})	52.6 ± 3.38	72.6 ± 6.38**	54.2 ± 3.38	78.4±5.17**
Trypsin (U L^{-1})	122 ± 7.4	138 ± 5.6	120 ± 4.9	137±8.1
Glucose (g L^{-1})	3.55 ± 1.1	4.12±0.24*	3.14 ± 0.03	3.91 ± 0.29*
HDL Cholesterol (mmol L^{-1})	2.67 ± 0.02	2.48 ± 0.01*	2.90 ± 0.04	2.69 ± 0.06*
LDL Cholesterol (mmol L^{-1})	0.71 ± 0.04	0.80 ± 0.07	0.72 ± 0.02	0.83 ± 0.08
Bilirubin, (mmol L^{-1})	3.30 ± 0.07	4.04 ± 0.12	3.7 ± 0.15	3.41±0.09
Triglycerides (mmol L^{-1})	0.8 ± 0.03	1.09 ± 0.07**	0.89±0.08	1.11 ± 0.01**
ALT (mmol L^{-1})	89.70 ± 4.9	96.85 ± 5.15	103.8 ± 0.09	112.94 ± 7.79
AST (mmol L^{-1})	118.4 ± 9.3**	148.2 ± 9.23**	120.6 ± 0.16	148.26 ± 9.23**
Alkalinephosphatase (U L^{-1})	264.2 ± 10.4	301.7 ± 9.28*	255.5 ±10.9	297.2 ± 8.36*
Urea (mmol L^{-1})	7.25 ± 0.52	4.47 ± 0.02*	7.81±0.06	4.93 ± 0.49*
Creatinine, (mmol L^{-1})	103.74 ± 5.9	95.89 ± 6.7	104.9 ± 4.8	96.90 ± 8.1
Blood cells				
Leucocyte, (10^9 L^{-1})	9.69 ± 0.47	9.93 ± 0.35	9.79 ± 0.58	9.84 ± 0.58
Erythrocyte (10^{12} L^{-1})	12.34 ± 0.21	12.78 ± 0.78	12.35 ± 0.29	12.91 ± 0.23
Hemoglobin (g L^{-1})	106.9 ± 3.72	110.0 ± 2.36	111.2 ± 4.61	115.4 ±5.91
Hematocrit (%)	42.23 ± 0.47	44.56 ± 1.94	42.67±0.85	45.89 ± 1.94
Platelets, (10^9 L^{-1})	405.4 ± 13.7	509.5 ± 25.8**	421.6 ± 21.5	578.9 ± 22.7**
Leukocyte formula				
Eosinophils (%)	3.12 ± 0.3	3.34 ± 0.3	2.25 ± 0.80	3.25 ± 0.80
Neutrophils (%)	56.1 ± 2.5	58.4 ± 1.9	58.82 ± 6.08	59.82 ± 4.00
Lymphocytes (%)	39.73 ± 3.11	43.16 ± 3.72	45.17 ± 10.24	46.9 ± 3.65
Monocytes (%)	2.9 ± 0.01	2.4 ± 0.05	2.68 ± 0.04	2.91 ± 0.07
Basophils (%)	0.35 ± 0.03	0.47 ± 0.04**	0.32 ± 0.07	0.43 ± 0.09**

* - p <0.05, ** - p < 0.01 between the control and the experimental group.

The number of platelets at the beginning of the experiment in the blood of animals of the control groups was within norms (250-450 × 10^9 L^{-1}), and the indices in both experimental groups after feeding with the FA1 were higher by 26% and 37.3%, respectively. At the end of the experiment, all indices were and corresponded to higher values of the physiological norm, and no reliable changes were observed. During the study, the concentration of leukocytes in all groups was within the physiological norm and had an average value of 9.69×10^9 L^{-1} -9.84×10^9 L^{-1}. In the Hampshire and Kazakh meat and wool, during the experiment after the FA in both groups of ewes no change was observed compared to the control.

Results of analysis determination of lipid peroxidation

The applied FA1 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 has a protective effect on cell membranes. A study of the oxidative activity of blood after the use of a feed additive showed a decrease in the level of diene conjugates and little new dialdehyde, which shows the protective effect of antioxidant components at the cell and tissue levels, contributes to the significant activation of the non-enzymatic link of antioxidant protection. The experiments activity of SOD was significantly higher in ewes of both groups of animals by 29.8 and 31.6%, respectively, compared to the control group. The data obtained show that SOD plays a key role in the body, since an increase in SOD in the blood with a feed additive reduces oxidative stress and improves the antioxidant defense of the body. The detected changes in the body's antioxidant response indicate that the system has achieved the necessary balance of the body. The data obtained showed, a reliable decrease in the level of MD (18.6% and 16.1%) in both breeds of sheep compared to the control group. Studies have shown an increase in diene conjugates in erythrocyte membranes in both the Hampshire breed and the Kazakh meat wool breed by 23.3% -27.9%, respectively (Table 6).

Table 6. Indicators LPO of erythrocyte inbreeds sheep of the Hampshire and Kazakh meat wool after a feed additive.

Animals group	**Indicators**		
	SOD (units mL^{-1})	**Diene conjugates (DC; nmol mL^{-1})**	**MDA (nmol mL^{-1})**
		Hampshire	
Control	1.99 ± 0.03	2.44 ± 0.03	0.131 ± 0.01
Experimental group	2.59 ± 0.07**	3.01 ± 0.05*	0.111 ± 0.07**
		Kazakh meat wool	
Control	2.17 ± 0.08	2.47 ± 0.09	0.139 ± 0.04
Experimental group	2.86 ± 0.050**	3.17 ± 0.02*	0.119 ± 0.06**

Notes: Reliable in comparison with the control group, *$p < 0.05$, **$p < 0.01$.

DISCUSSION

In particular, in ewes, in the diet of feeding a new FA_1, rich in vitamins and easily digestible nutrients, which contributed to an increase in their fatness and provided a higher live weight (4.9 ± 0.19 kg). The live weight of the ewe was 8.2% lower. At the same time, the body of ewes inevitably loses energy when exposed to low temperatures, which affects the development of spring. One of the main criteria characterizing the adaptation process is the dynamics of the animal's live weight, since it reflects the transition from the catabolic phase to the anabolic direction of metabolism, and also allows us to judge the general condition of the body. In animals of both breeds after feeding the feed additive for 60 days, a significant growth rate, rapid increase in body weight and development of all body systems were shown. By the end of the first 60 days, the live weight of the Hampshire breed (10.7 ± 0.37 kg) was higher than that of the Kazakh meat wool breed by 4.6% ($p < 0.05$). The average daily and absolute gains in live weight in the Hampshire and Kazakh meat wool breeds were 193 g and 5.8 kg ($p < 0.01$) and were also higher by 1.5 and 1.7% ($p < 0.05$), respectively, than in the controls of both groups. Scientific studies show that the use of bentonite and chlorella for food and animal feed has shown that they improve the level of metabolism and overall health. Bentonite binds harmful substances, leads to better absorption of nutrients, which indicates more efficient use of feed, promotes detoxification from body toxins and improves overall health (Abdellaoui *et al.* 2019; Kihal *et al.* 2022; Lashkarashvili *et al.* 2025), and chlorella supplements for mammals, including humans, exhibit various activities, including immunomodulatory, antioxidant and other activities, which improves the level of metabolism (Kotrbáček *et al.* 2015; Bito *et al.* 2020). Research indicates that feeding a feed additive to Hampshire and Kazakh meat wool sheep breeds significantly alters protein concentration and biochemical and hematological blood indices, depending on the season. Ewe feeding levels also greatly influence animal live weight development, which is crucial for overall health. The increase in β-globulin in sheep blood may be attributed to their role in lipid metabolism, transport, and regulation, as well as their relatively high specificity and low capacity for binding and regulating sex hormones. Furthermore, winter season increases of 33.5% ($p < 0.01$) and 31.7% ($p < 0.01$) in γ-globulins in both breeds indicate enhanced protective properties and immune status. Biochemical blood parameters in Hampshire and Kazakh meat wool sheep align with the winter season, showing metabolic shifts, including in protein metabolism, aimed at maintaining homeostasis. Evaluation of digestive enzyme activity (amylase, lipase, and trypsin) and blood biochemistry in ewes fed FA_1 suggests these enzymes are crucial for protein and energy catabolism. Supplementation with FA at 100 mg kg^{-1} body weight did not significantly alter blood enzyme activity in either group. Trypsin activity, indicative of pancreatic and liver secretion, correlated with a 1.2-fold increase in alkaline phosphatase, suggesting increased liver workload. Lipid metabolism was altered by increased cholesterol in the blood. While blood glucose remained within normal limits for all animals, FA_1 supplementation resulted in a significant increase in glucose levels (16.5% and 25% higher than controls, respectively), indicating enhanced carbohydrate metabolism. Thus, FA_1 appears to enhance overall metabolism in sheep by improving digestive gland function and nutrient digestibility and absorption. Consistent with previous research demonstrating positive biochemical indicators and restored gastrointestinal metabolism, bentonite, a natural adsorbent, mitigated the harmful effects of metal poisoning (Abdreshov *et al.* 2019). Protein metabolism in Hampshire and Kazakh meat wool sheep changes during winter due to structural and functional shifts in the body. This period coincides with lactation, marked by increased sex hormones. Consequently, vegetative and hormonal mechanisms intensely regulate adaptation, altering the sheep's morphofunctional and physiological state. Winter protein metabolism reflects growth, weight development, and immunobiological reactivity, indicating metabolic adaptation and economic potential. Supplementation with a fatty acid (FA) in both breeds significantly increased leukocytes, erythrocytes, and hemoglobin, with a slight increase in globulin fractions, but did not affect albumin. In ewes of both groups, blood analysis revealed a notable increase in proteins with plastic value, specifically albumins and α, β-globulins. These globulins indicate heightened metabolic activity due to their ability to readily bind carbohydrates, lipids, and transport cholesterol and vitamins. Total protein and albumin levels, which reflect the state of transport systems for nutrients, vitamins, trace elements, hormones, and other metabolic substances, remained relatively stable in both groups after 1-60 days of FA_1 feeding, suggesting effective homeostatic mechanisms and adaptation to environmental conditions. FA_1 feeding significantly altered LDL cholesterol levels, increasing them by 12.6-15.3% compared to the control groups in both breeds and showing a significant rise of 1.1 and 1.2 times ($p < 0.05$). "Total protein" refers to the total protein concentration in blood serum, reflecting the level of protein metabolism during an animal's life. Changes in total protein indicate variations in nutrition, physical activity, liver and kidney function, and metabolic disorders. As a key indicator in sheep blood serum, total protein is crucial for biochemical processes and nutrient transport, supporting growth and development. At the experiment's conclusion, Hampshire and Kazakh meat wool breeds showed 10.5-13.2% higher total protein levels ($p < 0.01$) compared to the control group. Protein levels in all groups increased with age, suggesting greater feed protein hydrolysis. Cholesterol levels are influenced by both dietary intake and endogenous synthesis. Therefore, immediate feed provision following a new diet ensures rapid replenishment of bodily reserves. Elevated blood enzyme activity can result from increased synthesis, reduced excretion, or heightened cell membrane permeability. Physiological and biochemical traits reflect an animal's adaptability and, to some extent, its productive potential. Research indicates a strong correlation between animal weight and growth with serum transaminase activity (Pappas *et al.* 1988). Transaminases (AST and ALT) regulate protein metabolism by mediating the reversible transfer of amino groups from amino acids to keto acids. Urea, the end product of protein metabolism, was within normal physiological limits (7.25–7.81 mmol L^{-1}) in the control groups of both ewe groups, as it is primarily excreted by the kidneys. Following FA_1 feeding, urea levels decreased by 1.6-fold in both groups compared to control, suggesting that blood urea content can be used to assess kidney function. Creatinine, a product of creatinine phosphate breakdown in muscles, is involved in muscle tissue energy metabolism and directly correlates with muscle mass. Clinically, blood creatinine levels assess kidney function and skeletal muscle condition. In this study, the experimental sheep groups exhibited 7.6% lower blood creatinine levels compared to the control group. Creatinine, a key metabolite in protein metabolism, plays an active role in energy metabolism in muscle and other tissues. Its levels are influenced by the adequacy of feeding rations, particularly protein, which promotes average daily weight gain, including muscle mass (Abdreshov *et al.* 2018). In our experiments, blood serum creatinine content ranged from 103.74-104.9 mmol L^{-1}, with no significant differences between groups, and aligned with standard indicators. FA1 supplementation offers advantages due to its high caloric content (glucose) and protein saturation, despite a slight increase in cholesterol and low-density lipids. Triglycerides facilitate protein-carbohydrate metabolism, supporting muscle mass growth. Biochemical parameters changed in experimental groups, with those taking FA_1 approaching control data. However, elevated ALT, AST, and bilirubin levels during the study suggest increased hepatobiliary system activity and cytolytic activity. Thus, FA1 impacts both gastrointestinal and blood parameters. Elevated amylase, lipase, and alkaline phosphatase likely reflect enzyme activity and their presence in circulation, indicating rapid FA_1 uptake in the gastrointestinal tract (Nanji *et al.* 1989; Abdreshov *et al.* 2019). Notably, ALT activity showed the most pronounced changes across all animals, exceeding normal limits. AST activity also changed in sheep receiving FA_1. The presence of trace elements, antioxidants, and other bioactive substances in FA_1 likely contributes to the increased ALT and AST activity, resulting from continuous mineral intake. Malonic dialdehyde levels were measured in control and experimental groups of Hampshire and Kazakh meat wool sheep. In control groups, malonic dialdehyde content was 0.131 ± 0.01 and 0.139 ± 0.04 nmol mL^{-1}, respectively. In experimental groups receiving the FA_1 feed additive, levels were significantly lower at 0.111 ± 0.07 and 0.119 ± 0.06 nmol mL^{-1} (Table 6). These results suggest that FA_1 supplementation benefits the sheep's adaptive capacity by reducing oxidative stress, as evidenced by their increased activity. The FA_1 components appear to contribute to this reduction in oxidative stress. FA_1 application in experiments led to reduced red blood cell antioxidant properties and malondialdehyde levels, coupled with increased SOD activity. This aligns with findings that antioxidant compounds generally suppress oxidative reactions (Muñoz-Cuautle *et al.* 2022; Piao *et al.* 2023). Data analysis revealed insights into how sheep blood cells adapt to environmental conditions when fed a novel feed mixture. Feed additives activated lipid peroxidation (LPO), causing minor homeostatic changes that suggest the maintenance of body balance in animals. The SOD activity observed during FA feeding in sheep appears sufficient to neutralize reactive oxygen species and alter intracellular membrane permeability. In both blood groups of sheep, the feed additive induced destructive changes in erythrocyte membrane resistance and activated LPO. Hematological indices were studied during these experiments, with results presented in Table 4. Hematocrit levels remained within the normal physiological range (35-45%) throughout the experiment and showed no significant variations. Control groups exhibited values of 42.48% and 42.67%, while experimental groups showed 44.56% and 45.89%, respectively. Lymphocyte and neutrophil levels remained within normal physiological ranges in both control and experimental groups throughout the experiment, with no significant changes observed. Basophil ratios were also within normal limits at the start but showed a 34% increase in both ewe groups by the experiment's end; however, all values remained within physiological norms, and these changes were not statistically significant. Therefore, this study suggests that the new FA_1 feed additive positively influences protein and carbohydrate metabolism in sheep of both groups during the feeding period. The use of this feed additive demonstrates benefits in farming and holds a significant role in animal nutrition science. Furthermore, it is shown to improve productivity, digestibility, growth, immunity, and antioxidant status in animals. Further research is needed to elucidate the mechanisms of action of plant-based feed additives and their application in ruminant nutrition.

CONCLUSION

Fortified feed additive (FA) with natural bentonite from chlorella maintains physiological parameters and influences metabolism, promoting weight gain. Dietary FA positively affects ewe health and productivity. FA_1 proved most effective in the experimental group, improving blood biochemistry, hematology, and overall physiological status. Metabolic indices in Hampshire and Kazakh meat wool sheep blood vary with age and feeding period due to genetic factors and seasonal influences, reflecting structural and functional body changes. FA_1 exhibits hydration and antioxidant effects, protecting cell membranes and neutralizing free radicals. This new FA_1 plays a significant role in metabolism, regulating body function and facilitating vitamin and mineral transport.

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