International Journal of Agriculture and Biosciences 2025 14(6): 1196-1205.
https://doi.org/10.47278/journal.ijab/2025.107
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RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Effect of Walnut-based Preparation on the Treatment of Dyspepsia in Calves

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ABSTRACT

The study examined the effect of a preparation made from walnut leaves and fruits on treating dyspepsia in calves. The primary research method was a comparative analysis of morphological, biochemical, and immunological parameters of the blood of calves with dyspepsia. The calves were divided into experimental (10 specimens) and control groups (11 specimens). The results showed that using the walnut-based preparation helped reduce the level of leukocytes, increase the number of erythrocytes and hemoglobin, improve metabolic processes, and increase immunity. The most pronounced effect in the experimental group was observed by the 10th day of the study when the number of erythrocytes increased by 24.7%, the hemoglobin content by 10.3%, and the total protein by 8.1%. There was also a significant decrease in residual nitrogen and catalase, indicating an acceleration in the elimination of toxic metabolic products. Including a walnut-based phytopreparation in the treatment regimen for calves with dyspepsia accelerated their recovery by 5-7 days compared with the control group. Thus, plant-based medicines can become an effective alternative to traditional antibiotic therapy. The obtained data confirm the prospects of using phytopreparations in veterinary medicine and the need for further research to optimize their dosage and application methods.

Keywords: Productivity, Component, Erythrocytes, Leukocytes, Total protein, Albumin.

Article History
Article # 25-077
Received: 20-Feb-25
Revised: 17-Apr-25
Accepted: 21-Apr-25
Online First: 16-Jul-25

INTRODUCTION

Using a wide range of antibiotics is essential in improving the effectiveness of treating diseases of agricultural animals caused by microorganisms (Belousov and Leonova 2002; Belova, 2011; Tanbayeva et al., 2024). However, there are disadvantages to antibiotic therapy. Frequent use of a group of antimicrobial preparations in medicine and veterinary medicine increases the incidence of resistant strains of microorganisms (Bochkarev and Sergeev 2000; Borsuk et al., 2011; Gizinger et al., 2024; Jakupov et al., 2024). In addition, taking antibiotics can lead to various side effects, including allergic reactions, decreased immunity, and increased fungal infections in the body (Zhumatayeva et al., 2022). Therefore, searching for new antibacterial preparations for treating farm animals is an important and urgent problem of modern pharmacy (Budulov and Magomedov 2011; Esxodjaev & Moldağwlov, 2016; Soimala et al., 2024).

The productivity of animals is directly related to their genetic ability to deliver food substances that enter their bodies into organs and tissues, convert them into nutrients, and then use them as a product. The effectiveness of this transformation is determined by the metabolism level (Moldağwlov et al., 2004; Molağwlov et al., 2007; Khonyoung et al., 2025).

In the proper course of metabolism, medicinal components, vitamins, and minerals contained in plants occupy a special place (Burkov et al., 2003; Aitzhanov & Zamanbekov 2016; Tharwat et al., 2024; Ugwu et al., 2024). The discrepancy between their deficiency, excess, or imbalance is accompanied by metabolic disorders, decreased performance, intolerance to diseases, slower growth and development of young animals, decreased reproductive function and fertility of adult animals, and a specific pathology characterized by nonviable offspring

Cite this Article as: Zhylgeldiyeva A, Baiberekov N, Korabaev Y, Orynkhanov K, Omarov K, Terlikbayev A, Nussupova S and Tuganbay A, 2025. Effect of walnut-based preparation on the treatment of dyspepsia in calves. International Journal of Agriculture and Biosciences 14(6): 1196-1205. https://doi.org/10.47278/journal.ijab/2025.107

A Publication of Unique Scientific Publishers

(Vinnikov et al., 2012; Tenaya et al., 2024; Pazla et al., 2024). The normal growth and development of a calf's body requires many minerals. Their deficiency leads to slow development, decreased performance and a weakened immune system (Volkov, 2000; Coniglio et al., 2023).

The lack of these substances explains one of the most common cattle diseases, dyspepsia of young animals. This disease causes significant economic damage to agriculture in Kazakhstan. Diseases of the digestive system, including dyspepsia and enteritis, are widespread in all farms, and the treatment and prevention of these diseases are urgent problems of veterinary medicine (Vorontsova et al., 2007; Korotkiy et al., 2024).

The factors causing digestive system diseases in young animals have a complex etiology. The pathogenesis of the disease is based on profound functional and compositional changes that can exacerbate the foci of the disease and form lesions in intestinal tissues. The disease can last long and progress to death (Gamidov, 2004). Among all non-communicable diseases, the incidence of digestive system diseases ranges from 32 to 64%. Digestive system diseases are most often observed in young cattle, especially dyspepsia. In the context of the modern functioning of the pharmaceutical industry, many medicines are used for treatment. In the entire range of medicines, a special place belongs to the group of plant-based medicines (Aleksandrov & Antipov, 2004; Darwish and Salama, 2024; Ryandini et al., 2024). A broad therapeutic effect and a low risk of side effects characterize this category of pharmaceutical products. However, the current state of pharmaceutical science in the field of pharmacognosy is characterized by incomplete study of medicinal plants (Bedoeva 2006; Akhmedyarova et al., 2007; Anjum et al., 2024; Shode et al., 2025). A reason for this is the insufficient attention of researchers working in the field of pharmacognosy to new and little-studied types of plant raw materials. Representatives of walnut trees are valuable plants widely used in the food industry. However, nut raw materials have not yet been commonly used in evidence-based medicine.

In veterinary practice, medicines used to treat digestive system diseases, including dyspepsia, have a slightly lower effectiveness. In most cases, their therapeutic result is small, and they cannot eliminate the consequences of the disease. Since the microflora is less sensitive to antibiotics and is susceptible to damage to the enzymatic nerve, the harm is more significant than its benefit against digestive system diseases. Therefore, in treating dyspepsia, a plant-based preparation has a high therapeutic efficacy (Qorabaev and Zamanbekov 2022; Korotkiy et al., 2024).

Recently, in veterinary medicine, using plant-based medicines to treat diseases caused by metabolic disorders has yielded results. The walnut tree is one of such plants (Bochkarev and Sergeev 2000). The composition of walnuts and products made of them (leaves and fruits) includes oil (15%), carbon (30g/kg), lysine (3.0%), multienzyme complex (1.5%), calcium (26.0%), phosphorus (8.2%), sulfur (24g/kg), magnesium (35 mg/kg), zinc (950mg/kg), copper (158 mg/kg), manganese (13mg/kg), cobalt (44 mg/kg), iodine (38 mg/kg), mercury (0.9mg/kg), vitamin A (400,000 international units (IU)), vitamin D3 (200,000IU), vitamin E (200mg/kg), phospholipids (not less than 5%) and essential oils (Gamidov 2004; Gorkovenko et al., 2009). The substances in walnuts bind heavy metals to each other, promote excretion from the body, normalize the endocrine system, and help treat respiratory and digestive tract diseases. Regular consumption of nuts has a beneficial effect on the cardiovascular system.

Thus, the chemical composition of walnut leaves and fruits has been studied intensely. However, the results of such studies are based on isolated substances and the degree of phytochemical development of other representatives of the genus *Juglans* (*J. nigra* L. and *J. cinerea* L. bark and leaves). They are insufficient to be used as raw materials for new plant-based medicines. Further phytochemical studies of the species of representatives of this family of walnuts are needed.

The variety of chemical composition, including many phenolic compounds, determines the range of pharmacological activity of representatives of the walnut family (English walnut, black walnut, and butternut). Medicinal forms obtained from nuts can treat microbes, viruses, and pathogenic fungi as an anti-inflammatory and antioxidant substance and as preparations that affect cardio, neuro-, hepatoprotective, hypolipidemic, and cognitive functions. For example, the aqueous pulp extract of *J. regia* unripe fruits can suppress the growth of Gram-positive bacteria, as well as inhibit the work of three main enzymes: cystatin-γ-synthase (HpCGS), cyl-transporting protein transacylase (HpFabD), and β-hydroxyacylase-dehydratase (HpFabZ) isolated from nuts, and *H. pylori* bacteria. *J. regia* leaves essential oil and its main components (β-pinene, α-pinene, limonene, caryophyllene) exhibit potent antibacterial activity against *S. epidermidis*, *S. typhi*, *S. aureus*, *B. subtilis*, *P. vulgaris*, *E. coli*, and *S. dyssenteriae*. It has been found that aqueous and ethanol extracts of *J. regia* leaves have anti-inflammatory activity.

Based on the need to replace antibiotics with plant-based medicines described in the scientific literature and the potential applicability of walnuts and their derivatives in the treatment of dyspepsia, one of the most common diseases among livestock (Batrakov et al., 2010), we evaluated the reaction to walnut-based phytopreparations in cattle in more detail. Based on the walnut tincture, which was used as a medicinal product, the immune parameters of calves in hematological and various functional conditions were studied for the first time. As a result, the possibility of using the preparation for the functional activation of nonspecific immune system factors was confirmed, and the optimal dosage and method of administration of the preparation were determined.

To study the effect of a walnut-based preparation on the course of dyspepsia and the effectiveness of its use as a treatment. To achieve this goal, the following tasks were set:

- Manufacture of a new preparation from a collection of various medicinal plants for the treatment of dyspepsia;
- Study of clinical, morphological, biochemical, and immunological changes in the blood that occur in calves with various types of dyspepsia;

- Study the therapeutic effect of a preparation made from medicinal plants on a sick animal in acute dyspepsia;

Study the effect of a preparation made from medicinal plants on preventing dyspepsia.

MATERIALS & METHODS

Ethical Approval

The study was carried out in compliance with international guidelines for the ethical use of animals in research, including the principles outlined in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the OIE (World Organization for Animal Health) Terrestrial Animal Health Code (European Parliament and Council of the European Union, 2010).

Conditions of the Study and Raw Materials

Following the research plan, farms in the Turkestan and Almaty regions (Kazakhstan) were involved in the research period from 2020 to 2023. The laboratory research complex uses the capabilities of the Faculty of Veterinary Medicine and its veterinary clinic and laboratories of the Department of Clinical Veterinary Medicine. The collection and harvesting of raw materials (*J. regia* L.) were carried out from March to September 2022 to 2023 at sites in the Turkestan region.

Methods

The samples of plant raw materials were dried using a natural air-and-shade method.

The samples were examined visually with a magnifying glass. The organoleptic evaluation of these raw materials was carried out according to the parameters of the studied objects' size, color, smell, and taste.

The samples were examined using laboratory equipment and experimental methods.

Experiment

The physiological state was determined by studying the clinical, morphological, biochemical, and immuno-logical parameters of the blood of calves with dyspepsia.

The experiment involved 21 calves with dyspepsia: 11 in the experimental group and 10 in the control group. For the experimental group, 250-300mL of a medicinal plant-based preparation was used twice a day until clinical recovery, while euphyllin (0.021g/kg) and ampicillin (0.2g/kg) were used for the control group. Vital signs were measured on the 3rd, 5th, 7th, and 10th day of using the preparation.

The number of erythrocytes, leukocytes, and hemoglobin, along with traditional methods, was calculated on a MEK-6550K automatic hematology analyzer. Biochemical parameters in blood serum were determined using a Stat Fax 1904 Plus biochemical analyzer and generally accepted methods.

The number of erythrocytes and leukocytes was further determined in Goryaev's chamber. The erythrocyte sedimentation rate (ESR) was determined using Panchenkov's apparatus, and the amount of hemoglobin – using the Sahli hemoglobinometer. The alkaline blood reserve was determined using the Kondrakhin method, the amount of total blood catalase activity – the Bakh & Zubkova (1937) method, the amount of total protein – the Lowry (1984) method, and the number of immunoglobulins – McEwan (1983) method. The albumin content was determined using the approaches developed by Hutapea et al. (2023) and bilirubin – using the techniques of Tjiong et al. (2005).

We determined the total amount of protein using an automatic AMS FT-2 biochemical analyzer. To determine the total protein using the AMS FT-2 analyzer, a test serum or plasma, special ready-made standards, and reagents are required to operate the device following this program. Sampling, mixing, comparison with a standard solution, and further differentiation are done automatically. The test duration is 18 minutes.

We used the AMS FT-2 analyzer to determine the quantitative and qualitative composition of immunoglobulins by classes (M (IgM), G (IgG), and A (IgA)). The data required to determine the amount of immunoglobulins was previously entered into the automatic immunoanalyzer computer.

Several standard solutions were used and manufactured at the factory for every class to determine the different classes of immunoglobulins. Sampling, mixing, comparison with a standard solution, and further differentiation are done automatically. The duration of testing for IgM is 18 minutes; for IgA, 14 minutes; and for IgG, 26 minutes.

If necessary, blood serums were stored in micro-samples of 0.3-0.5mL before the study at -200°C. The serums were not frozen again.

When leukocytes were isolated, blood was taken into a heparin tube (approximately 100 units of heparin per 1-1.5mL of whole blood). Lymphocytes from peripheral blood A. According to the Boyum method, ficoll verografin was separated using a density gradient.

The formation of the number of T-lymphocytes (E-CFT (complement fixation test)) with sheep erythrocytes was determined following Jondal et al. (1972). We used 1% of sheep erythrocytes as a marker. To detect T-lymphocytes, we mixed 0.1mL of lymphocytes with sheep erythrocytes and kept them in a thermostat for 45 minutes at 25°C.

After that, they were centrifuged for 3 minutes at 2,000 revolutions and placed in a refrigerator at 4.0°C for 18 hours. The tincture of erythrocytes was dropped on a slide, dried in the air, fixed, and stained using the Romanowsky-Giemsa method. We counted 200 lymphocytes under the immersion of altered cells (CFT).

The lysozyme activity of blood plasma was determined using a non-photometric method using *M. lysodeikticus* microbes (Dorfeychuk 1968) with quantitative indicators of the percentage of lysozyme activity. The phagocytic activity was determined using the method of Berman and Slavskaya (1958) (the degree of phagocytes, the number of phagocytosis parameters, i.e., the active percentage of leukocytes that killed microbes).

Data Analysis

Experimental data was processed using modern

computing tools, such as the variation statistics method in Microsoft Office Excel. The reliability of the obtained data, the main provisions, and scientific conclusions are substantiated by the large volume of preclinical and clinical studies performed on certified equipment, a wide range of methodological methods, and the results of statistical processing of quantitative material.

Processing

The experimental data were processed using the methods described by Gannushkin et al. (1974). Statistical processing of the results was carried out using the Student-Fisher test. At $P<0.05$, it is considered valid. The mathematical analysis of the number of indicators was carried out using the Sadovsky method. The economic efficiency of using the walnut tincture in the system of pathogenetic therapy for dyspepsia was calculated using the Shaikhamanov method (1987).

RESULTS

In a general blood test, we determined the number of erythrocytes, leukocytes, hemoglobin, total protein, immunoglobulins, albumins, and ESR. Special tests also determined the alkaline reserves of albumin and blood plasma. These indicators reflect the physiological state of the digestive system. Table 1 shows the general clinical parameters in calves with dyspepsia.

Table 1: Clinical parameters of calves with dyspepsia (M±m, n=21)

Groups of sick animals	Temperature, °C		Heart rate, bpm		Respiration, rpm	
	Morning	Evening	Morning	Evening	Morning	Evening
Experimental	42.3±5.4	42.9±2.6	94.2±8.1	98.4±6.3	38.2±2.1	42.4±3.4
Control	41.1±3.6	41.9±6.3	83.1±5.8	87.1±5.2	33.6±5.2	37.2±7.4

Note: M – arithmetic mean (average value), m – standard error of the mean (SE), n – number of animals in the group. The data are expressed as M±m, indicating the reliability and variability of the measurements.

Table 2 presents the morphological and biochemical parameters of the blood of calves with dyspepsia. The number of leukocytes during dyspepsia was 14.1% higher than in the control group. The number of erythrocytes was 15.6% lower, and the hemoglobin content was 14.2% lower compared to the control group. While the alkali content was 24.5% lower than in the control group, the total protein in the blood was 18.8% lower, the catalase content was 11.4% higher, and the residual nitrogen content was 19.9% higher. In case of the chronic course of dyspepsia, the percentage of phagocytosis was 12.0% lower than in adult animals with the acute form and 21.0% lower than in calves with the acute form.

The phagocytic index was 6.8% lower in animals with dyspepsia than in the control group. The phagocytic index was 29.2% lower, and the lysozyme activity was 45.3% lower (Table 3).

As for immunoglobulins, in the experimental group, immunoglobulins were 38.0% lower than in the control group, and albumins were 26.5% lower. Studies showed that the longer the disease lasts, the lower the physiological parameters. When treating sick animals, the need to apply measures to restore disturbed homeostasis should be considered.

Table 2: Morphological and biochemical blood parameters of calves with dyspepsia (m±M, n=21)

Indicators	Types of the course of the disease	
	Experimental	Control
Leukocytes, x 10^9/L	9.8±2.5	10.7±3.5
Erythrocytes, x 10^{12}/L	6.4±0.7	5.8±2.1
Hemoglobin, g/L	96±2.7	89±5.6
ESR, mm/hour	1.1±0.2	1.2±0.2
Alkaline reserve, CO^2%	38.7±2.3	31.9±3.1
Total protein, g/L	64±13	56±16
Catalase unit, mg/%	8.8±0.5	9.4±0.2
Residual nitrogen mg/%	41.2±1.7	46.3±2.4

Leukocytes (×10^9/L): total white blood cell count, indicating immune system activity; Erythrocytes (×10^{12}/L): red blood cell count, responsible for oxygen transport; Hemoglobin (g/L): concentration of hemoglobin, which carries oxygen in the blood; ESR (mm/hour): erythrocyte sedimentation rate, reflecting inflammatory processes; Alkaline reserve (CO_2%): percentage of carbon dioxide in the blood buffer system, indicating acid-base balance; Total protein (g/L): overall protein content in the blood, reflecting metabolic and immune status; Catalase (mg/%): activity of the catalase enzyme, related to oxidative stress response; Residual nitrogen (mg/%): concentration of nitrogenous waste, indicating kidney function and protein metabolism; Note: Data are presented as M±m, where M – arithmetic mean, m – standard error of the mean, n=21 – number of animals included in the measurements.

Table 3: Immune blood parameters in calves with dyspepsia (m±M, n=21)

Indicators	Groups of sick animals	
	Experimental	Control
Percentage of phagocytosis	59.4±2.1	46.9±5.8
Phagocytosis index	1.32±0.1	1.23±0.5
Absolute phagocytic index x10^9/L	6.5±0.4	4.6±0.1
E-CFT, %	45.8±1.4	34.5±0.6
Lysozyme activity, %	48.3±2.4	26.4±3.2
Immunoglobulins, %	16.3±2.2	10.1±1.7
Albumins, %	8.3±2.4	6.1±0.9

Note: M – arithmetic mean (average value), m – standard error of the mean (SE), n – number of animals in the group. The data are expressed as M±m, indicating the reliability and variability of the measurements

Table 4 presents the general clinical results on the preparation's effect on the clinical, morphological, biochemical, and immunological parameters. In the first days, the animals of both groups had a higher body temperature than normal.

Table 4: General clinical parameters of calves with dyspepsia in the experimental and control groups (m±M, n=21)

Day of the study	Temperature, °C		Heart rate, bpm		Respiration, rpm	
	Groups		Groups		Groups	
	experimental	control	experimental	control	experimental	control
1	39.9±0.4	40.2±0.6	98±3.1	95.1±2.6	34.2±1.1	32.5±0.4
3	40.3±0.2	41.1±0.8	87±1.2	91.8±0.3	28.5±2.8	29.2±0.6
5	38.8±0.6	41.2±0.3	74.3±2.4	86.3±0.2	25.4±0.2	28.2±1.4
7	38.4±1.3	39.9±1.4	71.7±3.2	83.5±1.6	24.3±0.5	27.2±0.8
10	38.5±0.4	38.2±0.1	67.1±2.2	80.9±1.7	23.7±1.6	25.4±0.5

Note: M – arithmetic mean (average value), m – standard error of the mean (SE), n – number of animals in the group. The data are expressed as M±m, indicating the reliability and variability of the measurements.

The heart rate in the control group was 16.2% more frequent on day 1, 16.5% more frequent on day 3, and 20.8% more frequent on day 5 than in the experimental group. In the control group, the most frequent heart rate was observed between days 3 and 5, which was 26.9-31.2% higher than the average normal physiological parameters. Thus, in the control group, the heart rate was back to normal on the 10th day, and in the experimental group, the heart rate was back to normal on the 5th-7th day.

The respiratory rate in the control group was 11.0% higher on day 3, 11.9% on day 5, and 7.2% higher on day 7 compared to the experimental group. Thus, in the control group, the respiratory rate reached complete physiological normality on the 10th day, and in the experimental group, on the 5th-7th day. Morphological and biochemical parameters of blood in the experimental group when using a walnut-based preparation are shown in Table 5 and 6.

Several key trends can be noted when analyzing the dynamics of blood parameters in the experimental group compared with the control group. The number of leukocytes in the experimental group consistently decreased throughout the entire period, with a particularly pronounced decrease observed on day 5 (26.4%). On the contrary, the level of erythrocytes and hemoglobin steadily increased, reaching a maximum increase on the 10th day (24.7 and 10.3%, respectively), indicating an improvement in oxygen metabolism. The alkaline reserves and catalase content in the experimental group remained lower than in the control group, which may indicate the peculiarities of metabolic adaptation. The total protein volume steadily increased, which means a higher level of metabolic processes, and the remaining nitrogen content decreased significantly, especially by the 10th day (by 43.4%), which may indicate an improvement in the excretion of protein metabolism products. The experimental group generally showed positive changes in blood counts, suggesting a more efficient metabolism and adaptation. The immune parameters of the blood when using a plant-based preparation in the experimental group are shown in Table 7 and 8.

Table 5: Morphological and biochemical parameters of blood when using a plant-based preparation in the experimental group (M±m, n=11)

Indicators	Day of the study				
	1	3	5	7	10
Leukocytes, x10^9/L	13.3±0.4**	11.5±0.5	10.2±0.3**	9.2±0.2	8.3±0.3
Erythrocytes, x10^{12}/L	6.1±0.3*	7.9±0.7*	8.3±0.3*	8.9±0.4*	9.6±0.4*
Hemoglobin, g/L	70.2±2.2*	74.6±2.3*	78.2±3.1**	81.2±1.6*	87.5±2.6*
ESR, mm/h	1.3±0.2*	1.2±0.1*	1.1±0.2*	0.9±0, *2	0.9±0.1*
Alkaline reserve, CO^2%	27.2±3*	29.6±2*	34.2±2*	38.6±3*	42.3±2.0*
Total protein, g/L	5.7±1.3*	5.8±1.2*	6.1±1.4*	6.4±0.8*	6.7±1.1*
Catalase unit, mg/%	9.9±0.6*	8.4±0.3**	6.6±0, 5 *	5.6±0.5*	4.3±0.2*
Residual nitrogen, mg/%	44.5±3.4*	38.2±0.4*	32.4±1.3*	28.1±0.6**	25.2±0.8*

Note: *reliability in comparison with the control population; x; *P<0.05; xx; **P<0.01; xxx; ***P<0.001; M – arithmetic mean (average value), m – standard error of the mean (SE), n – number of animals in the group. The data are expressed as M±m, indicating the reliability and variability of the measurements.

Table 6: Morphological and biochemical blood parameters in the control group (m±m, n=10)

Indicators	Day of the study				
	1	5	7	10	21
Leukocytes, x10^9/L	12.4±0.1	14.5±0.7	13.5±0.4	12.5±0.6	9.8±0.2
Erythrocytes, x10^{12}/L	7.2±0.4	7.1±0.6	7.3±0.4	7.5±0.4	7.7±0.3
Hemoglobin, g/L	61.7±4.1	66.3±1.3	72.5±3.1	72.6±2.7	79.3±2.2
ESR, mm/hour	0.9±0.2	1.3±0.5	1.9±0.1	1.5±0.2	1.3±0.1
Alkaline reserves, CO^2%	36.6±4	39.9±1.3	40.3±0.8	44.6±2	47.3±1
Total protein, g/L	5.3±1.3	5.5±0.2	5.6±1.1	5.9±1.6	6.2±0.1
Catalase unit, mg/%	9.6±0.3	9.2±1.5	8.9±1.2	8.7±0.4	7.6±0.5
Residual nitrogen, mg/%	43.8±1.5	40.5±2.1	38.5±4.5	37.8±1.2	34.5±2.3

Table 7: Immune blood parameters in the experimental group when using a walnut-based preparation (m±m, n=11)

Indicators	Day of the study				
	1	5	10	15	20
Number of neutrophils, x10^9/L	5.2±0.2**	6.8±0.4***	7.1±0.7*	7.4±0.4**	7.9±0.8**
Percentage of phagocytosis	59.4±2.2**	64.2±4.5*	68.3±6.3**	72.5±5.8**	76.4±3.9**
Phagocytic index	1.32±0.1*	1.42±0.4**	1.52±0.3X **	1.64±0.1X **	1.62±0.4***
Absolute phagocytic index, x10^9/L	3.5±0.5*	3.8±1.2***	5.3±1.7***	6.1±2.3***	7.4±0.8***
E-CFT, %	22.8±1.4**	28.7±4.6**	32.4±0.8**	38.5±0.5**	42.8±4.3**
Lysozyme activity,%	28.3±3.4*	35.5±4.5****	37.2±2.6***	39.5±0.4**	41.6±4.7**
Albumins, %	64.1±0.4**	58.8±0.6*	54.4±0.3*	51.1±0.3*	45.1±0.3*

Note: *reliability in comparison with the control population; x; *P<0.05; xx; **P<0.01; xxx; ***P<0.001.

Table 8: Immune blood parameters in the control group (m±m, n=10)

Indicators	Day of the study				
	1	5	10	15	20
Number of neutrophils, x10^9/L	5.1±0.6	6.1±0.1	6.3±0.12	6.5±0.7	6.9±0.1
Percentage of phagocytosis	61.3±3.2	63.0±6.5	64.6±5.1	67.6±8.2	68.7±9.6
Phagocytic index	1.29±0.6	1.31±0.5	1.34±0.5	1.37±0.3	1.41±0.8
Absolute phagocytic indicator, x10^9/L	3.3±0.2	3.5±0.7	3.7±1.5	4.2±0.3	4.8±0.4
E-CFT,%	25.8±2.1	27.3±3.8	29.8±0.6	31.5±3.9	35.8±2.8
Lysozyme activity, %	29.2±0.7	31.5±3.2	32.5±6.1	35.4±3.1	36.6±4.1
Albumins, %	59.7±±0.3	56.5±0.1	53.8±0.4	49.6±0.4	45.3±0.2

The experimental group had 11.8% more neutrophils on day 3, 10.7% on day 5, 13.9% on day 7, and 14.6% on day 10 compared with the control group. In the experimental group, compared with the control group, the percentage of phagocytosis was 1.9% higher on day 3, 5.3% on day 5, 7.3% on day 7, and 11.2% on day 10. The phagocytic index in the experimental group was 8.4% higher on day 3, 13.4% on day 5, 19.7% on day 7, and 14.9% on day 10 compared with the control group. The experimental group had an absolute phagocytic index that was 8.6% higher on day 3, 43.3% higher on day 5, 45.2% higher on day 7, and 54.2% higher on day 10 compared with the control group.

The experimental group E-CFT index was 5.1% higher on day 3, 8.7% on day 5, 22.2% on day 7, and 19.6% on day 10 compared with the control group. The experimental group had a 6.4% higher level of lysozyme activity on day 3, 14.5% on day 5, 11.6% on day 7, and 13.7% on day 10 compared with the control group. The experimental group had 16.9% higher immunoglobulin content on day 3, 31.8% on day 5, 18.1% on day 7, and 16.5% on day 10 compared with the control group. The experimental group had an albumin content of 5.4% on day 3, 12.5% on day 5, 13.1% on day 7, and 18.6% more on day 10 compared with the control group (Fig. 1 and 2).

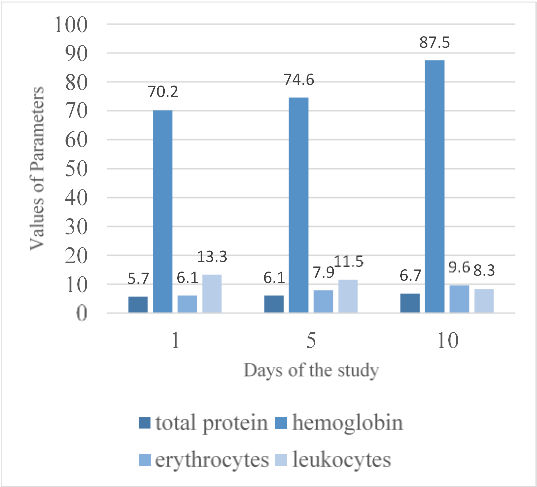

Fig. 1: Morphological parameters of blood (experimental group).

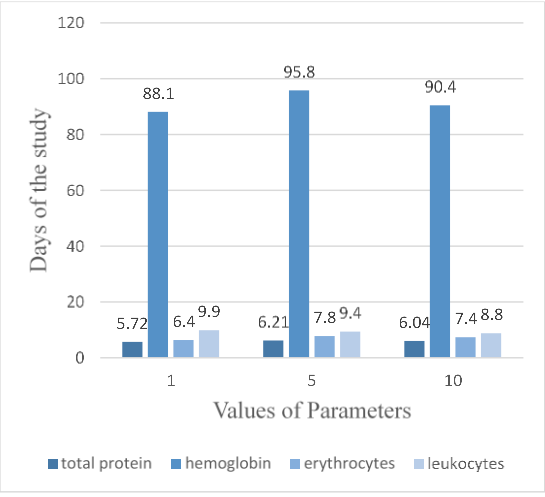

Fig. 2: Morphological parameters of blood (control group).

Table 9 shows that the walnut tincture significantly increases the level of immunoglobulins.

Table 9 and Fig. 3-5 demonstrate the dependence of the level of immunoglobulins (the main component of humoral immunity) on the walnut-based preparation. The following classes of immunoglobulins were dependent: IgA (they have very high bactericidal properties and protect from bacteria that can be transmitted through the digestive tract), IgM (they have very high agglutination, precipitation, bactericidal and hemolytic properties and are involved in the formation of immunity against cellular infections), and IgG (they are considered the main protective protein). The serum levels studied after administering walnut tincture were approximately the same in both groups. Noticeable changes became apparent after the preparation was administered.

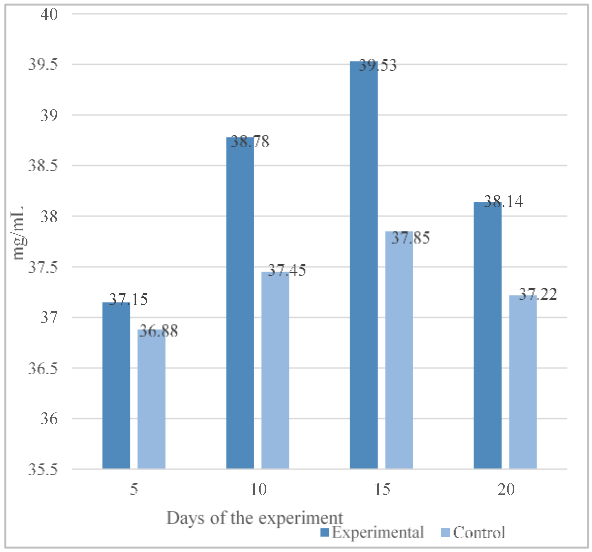

Fig. 3: IgA concentration.

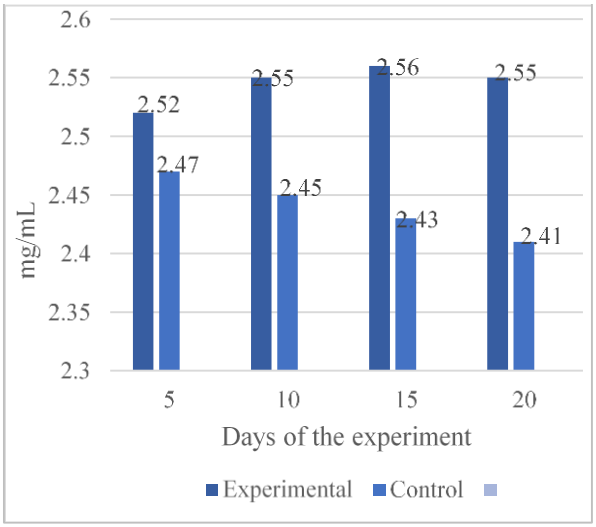

Fig. 4: IgM concentration.

In the days after the walnut preparation was administered, the concentration of immunoglobulins in the experimental group began to increase slightly compared to the day of the administration. For example, IgA increased by 4.9%, IgM by 2.3%, and IgG by 7.2% ($P<0.05$).

Table 9: Effect on the dynamics of immunoglobulins contained in blood serum at each stage of walnut tincture (m±m; n=10), mg/mL

Indicators	Groups	Background indicator	Days of the study				
			1	5	10	15	20
IgA	Experimental	0.63±0.05	0.65±0.06	0.68±0.05	0.70±0.07	0.70±0.09	0.67±0.04
	Control	0.63±0.04	0.62±0.05	0.61±0.04	0.59±0.08	0.58±0.06	0.55±0.05
IgM	Experimental	2.49±0.12	2.52±0.12	2.52±0.17	2.55±0.15	2.56±0.16	2.55±0.19
	Control	2.50±0.14	2.50±0.11	2.47±0.12	2.45±0.14	2.43±0.18	2.41±0.20
IgG	Experimental	25.31±1.15	25.92±1.12	26.47±1.3	27.24±1.18	28.31±1.24	27.18±1.17
	Control	25.33±1.21	25.18±1.15	24.62±1.13	24.30±1.22	24.09±1.19	23.56±1.20
∑Ig	Experimental	28.43±1.32	29.09±1.30	29.67±1.53	30.49±1.40	31.57±1.49	30.40±1.40
	Control	28.45±1.39	28.30±1.31	27.70±1.29	27.34±1.44	27.10±1.43	26.52±1.45

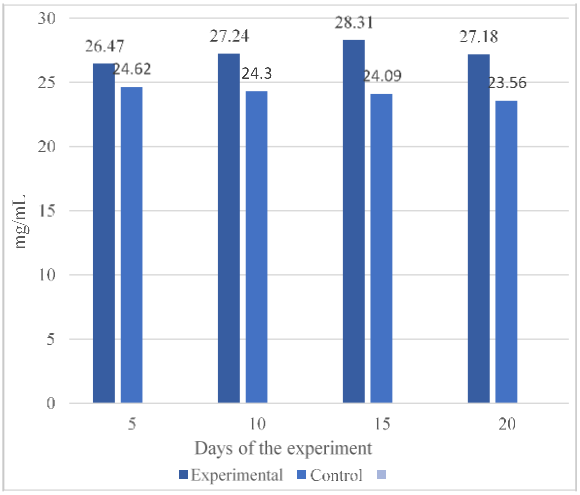

Fig. 5: IgG concentration.

In the last days of treatment, immunoglobulin levels decreased slightly in both groups. At the same time, the parameters in the experimental group were significantly higher than in the control group. The concentration of IgA in the experimental group increased by 91.2%, gradually decreasing during the remaining study periods. In the control group, compared with baseline data (0.63±0.04 mg (mL)) a decrease of up to 1.40% can be observed. The concentration of immunoglobulins in the IgM group increased by 1.2% after 5 days, by 2.4%, compared with the background value of the preparation on day 1, and began to decrease gradually during the rest of the study. For example, after 10 days, this indicator equaled 2.56±0.16 mg/mL, and in the control group, this indicator was only 2.43±0.18 mg/mL ($P<0.05$).

IgG increased by 4.6% after the preparation was administered, and on the 5th day, their level rose to 11.8%. The level of the obtained indicators in the experimental group was significantly higher than in the control group, indicating increased livestock immunity. The highest levels of all immunoglobulins were observed on the 10th day of treatment (31.57±1.49 mg/mL), and their number was significantly lower in the control group (27.10±1.43mg/mL). Thus, the data indicate that walnut tincture effectively affects the quantitative and qualitative parameters of immunoglobulins, increasing the immune status of sick young animals and positively affecting the course of their life after illness in favorable conditions.

DISCUSSION

The present study investigated the therapeutic potential of a walnut-based preparation for treating dyspepsia in calves, with a focus on its impact on hematological and biochemical parameters. The findings indicate that administration of the walnut-based preparation resulted in significant improvements in key physiological markers, supporting its potential efficacy as a natural therapeutic intervention. These findings align with the numerous studies on the inclusion of walnuts in the diets of farm animals as a way of alleviating dyspepsia symptoms. It also agrees with the study of Zhylgeldieva et al. (2024), who concluded that phytopreparations made from walnuts achieved positive results in dyspepsia therapy and balanced hematological parameters.

A notable outcome of the study was the observed increase in erythrocyte count and hemoglobin concentration in the experimental group by the 10th day of treatment. Specifically, erythrocytes increased by 24.7% and hemoglobin levels by 10.3%. These findings suggest an improvement in oxygen transport capacity, which is essential for metabolic homeostasis and overall animal health. This finding agrees with the studies of Li et al. (2021), who studied the anti-hypoxic effects of walnut (*in vitro*) and observed a positive increase in hematological parameters as well as oxygen capacity. The enhancement in erythropoiesis in our results may be attributed to the rich composition of bioactive compounds present in walnuts, including polyphenols and flavonoids, which have been documented in *in vitro* studies for their antioxidative effects (Rusu et al., 2020).The results reveal an increase in total protein levels with a decrease in residual nitrogen and catalase activity, suggesting an enhancement in protein metabolism and accelerated removal of toxic metabolic products.

The increased protein levels confirm established principles of walnuts being protein-rich nuts, as documented by Wen et al. (2023) and Si et al. (2023). Our finding also agrees with Zhou et al. (2022) who observed a similar reduction in serum urea nitrogen levels. The improvement in protein metabolism may be linked to the hepatoprotective properties of walnut-derived compounds, which could contribute to better nutrient assimilation and detoxification processes. Similar hepatoprotective effects have been documented in previous studies investigating walnut-based therapeutics (Yusuff, 2022; Al-Nadaf et al., 2024). An interesting result from this study is the reduction in leukocyte count, indicating a potential anti-inflammatory or immunomodulatory effect of the walnut-based preparation. Our hypothesis on the anti-inflammatory effects of walnuts is confirmed by the studies of Fizeșan et al. (2021) and Tan et al. (2022). Also, studies from Mao et al. (2020) highlight the immunomodulatory effect of the walnut-based preparation. Elevated leukocyte levels are indicative of systemic inflammation, which is commonly observed in dyspeptic conditions. Bioactive compounds

found in walnuts may exert their effects by modulating cytokine responses and reducing oxidative stress, ultimately contributing to improved immune function.

Despite the positive results, it is important to acknowledge inherent constraints in the study. A relatively small sample size can have limitations on the degree to which the results can be extrapolated. In addition, the study focused primarily on immediate changes in the blood count and biochemistry, thus the need for inquiry into long-term effects. Future studies should use larger subject cohorts and longer follow-up intervals so as to comprehensively assess the long-term efficacy and safety of walnut-containing interventions. The findings provide convincing evidence that a walnut treatment favorably affects hematology and biochemical parameters in calves experiencing dyspepsia, thus placing it firmly as a viable natural replacement for conventional treatment. Increased erythropoiesis, as well as enhanced protein metabolism and immunity, emphasizes the therapeutic potential of walnut-based compounds. Further studies are needed to confirm these findings and define standard treatment protocols, enabling progress in the field of natural therapies with clinical practice in the animal sciences.

Conclusion

1. A new preparation from walnut leaves was developed for preventing and treating dyspepsia in calves, including the clinical condition of the walnut tincture or decoction in newborn calves.
2. The resistance of calves with dyspepsia significantly decreased, confirmed by their blood morphological and biochemical parameters. The erythrocytes were reduced to 24.1%, hemoglobin to 27.9%, total protein to 19.6%, and catalase to 26.3%, while leukocytes and ESR increased to 29.5 and 15.7%.
3. Deficiency of general and immune resistance in calves with dyspepsia and morphological, biochemical, and immunological parameters of their blood was reduced to erythrocytes 34.3%, hemoglobin 32.5%, total protein 23.7%, catalase 35.7%, E-CFT 28.3%, albumins 28.1%, immunoglobulins 35.6%, and leukocytes 33.7%. ESR increased to 23.5%.
4. Compared with preparations widely used to treat dyspepsia in calves, our preparation improved their general clinical condition 5-7 days earlier and increased the morphological and biochemical parameters of the blood to 14-27%.

Compared with preparations widely used for the treatment, the preparation, in addition to affecting the general and immune resistance, improves their calves' deviant indicators 5-7 days earlier.

Funding: This paper was prepared as a result of scientific research in the framework of research project AR19177538 "Development and pharmacotherapeutic justification of a walnut phytopreparation for the treatment and prevention of dyspepsia in young animals" (contract No. 168-ZHG4, 17.05.2023), approved at the competition for grant financing of research by young scientists under the Zhas Galym project for 2023-2025 with an implementation period of 35 months.

Acknowledgement: The authors would like to thank the editorial team for attentive and accurate reading and checking our article.

Conflict of Interest: The authors declare no conflict of interest.

Author Contributions: The contribution of each author is as follows: Assel Ayatkhanovna Zhylgeldiyeva – conceptual supervision. Nurjan Baiberekov, Yerganat Korabayev and Kanat Orynkhanov collected all the required data and created experimental design. Overall onceptualization and data analysis: Askar Terlikbayev, Saltanat Nussupova and Almas Tuganbay.

Generative AI statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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