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The effect of chlorella and spirulina on hematological and biochemical blood parameters of working German shepherd dogs

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Abstract

The study aimed to investigate the effects of individual and combined microalgae supplements on the biochemical status of working dogs fed a complete dry diet. The experiment was conducted at the cynological club "DOGARM COMPANY" (Kyiv) on 20 working German Shepherd dogs with a body weight of 30 kg, which were divided into four groups: a control group (basal diet Josera Premium) and three experimental groups (with the addition of 1.5 g/day of chlorella, spirulina, or their combination at a 1:1 ratio). The study lasted 60 days. The results demonstrated a clear positive effect of microalgae supplementation on hematological and biochemical blood parameters. Dogs in the experimental groups showed increases in erythrocyte count, hemoglobin concentration, hematocrit, albumin level, and platelet count compared with the control group. The most pronounced changes were observed in the group receiving the combined chlorella and spirulina supplement: erythrocyte count increased by 0.6×10^{12}/L (9 %), hemoglobin by 13.2 g/L (8.5 %), hematocrit by 3.5 % (7.8%), albumin by 2.5 g/L (6.9 %), while platelet count increased by 30.1×10^{9}/L (12 %). Biochemical analysis revealed a significant decrease in liver enzyme activity, with ALT reduced by 27.4 % and AST by 19.1%, along with a 16 U/L (8 %) decrease in LDH activity, indicating reduced cellular damage and improved metabolic stability. In addition, total protein levels increased by 4.6 g/L (7.1 %), confirming enhanced protein metabolism and liver synthetic function. These findings highlight the multifaceted biological effects of chlorella and spirulina, including stimulation of hematopoiesis, improvement of oxygen transport capacity, hepatoprotective and immunomodulatory actions, and stabilization of biochemical homeostasis. The combined use of both microalgae proved to be the most effective strategy, improving endurance, adaptive capacity, and overall functional performance of working dogs under high physical load conditions.

Keywords: dietary supplement (DS); dogs, microalgae; blood parameters; metabolism.

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1. Introduction

In modern science, the search for alternative sources of protein, vitamins, minerals, and bioactive compounds to enhance productivity and improve animal health remains highly relevant. The use of microalgae as feed additives in animal husbandry and aquaculture is a subject of intensive research (Cabrita et al., 2025). Due to their high biological value, microalgae of the genera *Chlorella* and *Spirulina* are recognized as promising cultivation objects (Grishko et al., 2024a; Bondarenko et al., 2024).

Over the past decade, scientific interest has increased in the potential of the microalgae *Chlorella vulgaris* and *Spirulina platensis* as sources of biologically active compounds capable of modulating hematopoiesis, blood biochemical profile, immune responses, and antioxidant status in animals (Andrade et al., 2018; Souza et al., 2021; Spínola et al., 2024).

Microalgae *Chlorella* and *Spirulina* are rich in complete protein containing all essential amino acids, polyunsaturated fatty acids (especially omega-3), vitamins (B-group, A, E, C), macro- and microelements, as well as a complex of antioxidants (β-carotene, astaxanthin, phycocyanin). This rich chemical composition determines a wide range of biological effects, including immunostimulatory, antioxidant, anti-inflammatory, hepatoprotective, and detoxifying activities (Grishko et al., 2024b).

Chlorella vulgaris is characterized by a high content of polysaccharides (including β-glucans), carotenoids, and chlorophyll, which confer immunomodulatory and antioxidant properties (Andrade et al., 2018; Gadzama et al., 2025).

Spirulina platensis, in contrast, is rich in pigments, particularly C-phycocyanin, which exhibit anti-inflammatory and antioxidant effects through activation of the Nrf2/HO-1 signaling pathway and inhibition of NF-κB (Souza et al., 2021; Liu et al., 2022).

Studies conducted on dogs have shown that spirulina supplementation significantly enhances the immune response to vaccination (higher antibody titers), increases fecal sIgA levels, and improves intestinal microbiota stability (Satyaraj et al., 2021).

Similar findings have been reported for the digestive system: microalgae reduced defecation frequency, improved functional intestinal parameters, and were well tolerated by animals during long-term use (Cabrita et al., 2023; Stefanutti et al., 2023).

Regarding biochemical blood parameters, studies in dogs have not revealed significant changes in routine markers (Satyaraj et al., 2021), likely due to the immunotropic nature of the effects or the limited duration of supplementation.

However, mechanistic studies of C-phycocyanin have demonstrated its ability to reduce the activity of liver enzymes ALT and AST by suppressing lipid peroxidation (Souza et al., 2021; Liu et al., 2022). These findings are consistent with our study, which showed that combined supplementation with chlorella and spirulina significantly reduced ALT and AST activity and normalized the De Ritis ratio.

Although direct evidence of erythropoiesis stimulation in dogs is lacking, available data confirm that microalgae contain bioavailable iron, folic acid, vitamin B_{12}, amino acids, and chlorophyll–key components for hemoglobin synthesis and red blood cell formation–which may explain the observed trends toward increased erythrocyte count, hemoglobin, and hematocrit in working dogs (Andrade et al., 2018; Spínola et al., 2024).

Studies in other animal species have shown that *Chlorella* reduces malondialdehyde (MDA) levels, decreases oxidative stress markers, and activates antioxidant proteins (in a rabbit stress model) (Andrade et al., 2018).

Similarly, spirulina reduces triglycerides in overweight dogs while improving overall antioxidant defense (Stefanutti et al., 2024).

In poultry, spirulina use reduces the negative effects of toxic agents (e.g., OTA) on hematological and antioxidant parameters (Salah et al., 2025).

Thus, the analysis of literature sources confirms the importance of investigating the effectiveness of using dried microalgae powder of chlorella and spirulina, as well as their combination, in the feeding of working dogs.

2. Materials and methods

The present experimental study aimed to evaluate the feasibility of supplementing the daily diet of German Shepherd dogs with complex biological additives of chlorella and spirulina produced by biotechnological methods in order to improve diet balance, enhance the biological value of feed, and support the physiological state of the animals during intensive training.

The organization and implementation of the study on the effects of feeding biologically active supplements to working German Shepherd dogs were carried out at the service dog breeding kennel “DOGARM COMPANY” (Kyiv).

The dogs were housed in enclosures measuring 2 m × 2.5 m, equipped with removable-roof kennels, feeders, and drinking bowls (Fig. 1 and Fig. 2).

The study was conducted on 20 clinically healthy working German Shepherds aged 3 years, with a body weight of 30 ± 2 kg. The animals were divided into four groups of five dogs each using the method of analog pairs (Table 1). The equalization period lasted 10 days, and the experimental period lasted 60 days.

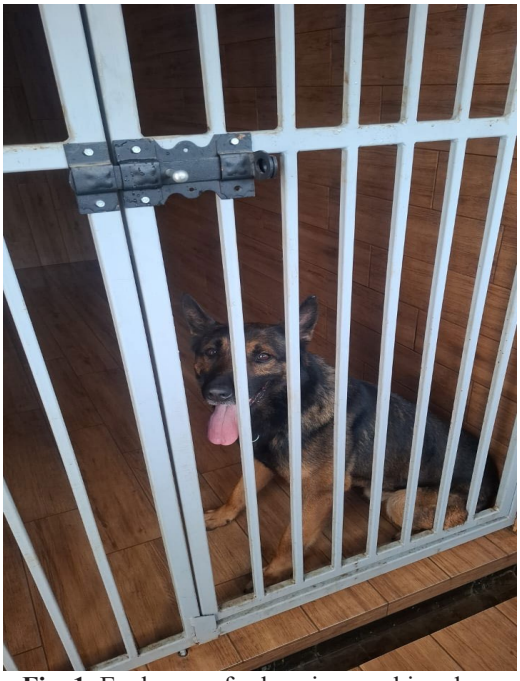

Fig. 1. Enclosures for housing working dogs

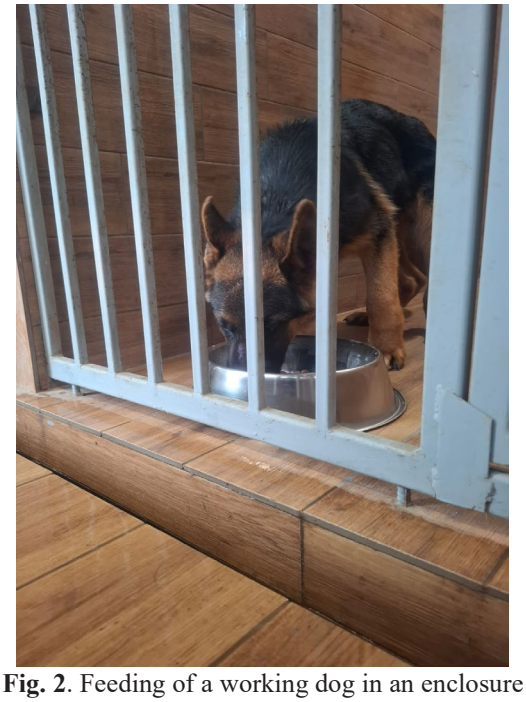

Fig. 2. Feeding of a working dog in an enclosure

Table 1
Scheme of the scientific and production experiment

Group	Number of animals	Feeding conditions	
		equalization period	experimental period
Control (Group 1)	5	CD *	CD *
Experimental (Group 2)	5	CD *	CD* + 1.5 g/day chlorella powder
Experimental (Group 3)	5	CD *	CD* + 1.5 g/day spirulina powder
Experimental (Group 4)	5	CD *	CD* + 1.5 g/day mixture (chlorella 50 % + spirulina 50 %)

Note: CD – complete diet

Feeding of German Shepherd dogs weighing 30 kg was carried out using Josera Premium feed, taking into account physical activity. Dogs from all groups were subjected to the same average daily workload for training exercises. Each animal received 400 g of complete dry feed twice a day. Thus, by consuming 800 g of feed daily, the dogs fully met their energy and nutrient requirements.

Watering of the experimental animals was organized according to the widely accepted practical recommendation that dogs be provided with approximately 2.5–3 times the volume of water as the amount of dry feed consumed. During the autumn–winter period, the daily water intake was 2.5 L, and during the spring–summer period, 3 L/day.

The housing system ensured constant access to fresh, clean water for the animals in the enclosures. The studied supplements were dissolved in drinking water; this did not affect water intake in the dogs.

Animal condition was monitored every 10 days by visual examination. During examination, it was noted that the ribs were easily palpable but not visible. All dogs were in good body condition, lean, and energetic.

On day 60 of the experiment, blood samples were collected in the morning on an empty stomach from the subcutaneous vein of the forearm. Biochemical analysis of blood serum was performed using a semi-automatic biochemical analyzer "Clima" (Spain) with standard reagent kits. The following parameters were determined: total protein, albumin, total bilirubin, urea, creatinine, glucose, triglycerides, enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), α-amylase, and lactate dehydrogenase (LDH).

All manipulations with animals were carried out in accordance with the principles regulated by the European Convention for the Protection of Pet Animals (1987) and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (2006).

The obtained numerical data were processed using methods of variation statistics with the software packages "Microsoft Excel" and "SPSS Statistics". The arithmetic mean (M) and standard error of the mean (±m) were calculated. The significance of differences between groups was assessed using Student's t-test. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Results

Chlorella and spirulina exhibit diverse biological effects. The results of the experiment demonstrated a positive effect of these biologically active supplements on hematological parameters in the experimental dogs (Tables 2 and 3).

Table 2
Hematological parameters of dogs (M ± m, n = 5)

Parameter	Control group 1	Experimental group 2 (Chlorella)	Experimental group 3 (Spirulina)	Experimental group 4 (Complex)
Erythrocytes, $\times 10^{12}$/L	6.8 ± 0.30	7.0 ± 0.24	7.1 ± 0.37	7.4 ± 0.22*
Hemoglobin, g/L	155.3 ± 5.84	160.1 ± 4.47	162.3 ± 5.21	168.5 ± 4.32*
Hematocrit, %	45.0 ± 1.55	46.5 ± 1.28	47.0 ± 1.49	48.5 ± 1.16*
Leukocytes, $\times 10^{9}$/L	10.5 ± 0.61	11.2 ± 0.54	11.5 ± 0.70	12.0 ± 0.63
Neutrophils, %	65.0 ± 4.15	64.2 ± 3.16	63.7 ± 4.22	62.2 ± 3.13
Lymphocytes, %	25.5 ± 3.16	27.7 ± 2.37	28.6 ± 3.25	30.8 ± 2.11*
Platelets, $\times 10^{9}$/L	250.6 ± 15.39	260.5 ± 12.51	265.2 ± 14.36	280.7 ± 10.27*

Note: * – significant differences compared to the control group ($P < 0.05$)

The use of chlorella and spirulina supplements, especially in combination, demonstrates a positive effect on the hematological status of dogs, manifested by stimulation of hematopoiesis (erythropoiesis) and a moderate immunostimulatory effect.

The study results revealed a clear tendency toward higher erythrocyte counts, hemoglobin concentrations, and hematocrits in the experimental groups compared with the control. In terms of hemoglobin concentration, dogs of the 4th experimental group, whose diet included 1.5 g/day of a mixture of spirulina and chlorella powders, showed the highest values compared to the control group. The difference amounted to 13.2 g/L (8.5 %) and was statistically significant. This increase may be attributed to the high content of iron, vitamin B_{12}, and folic acid in microalgae, which are essential cofactors for hemoglobin synthesis and erythrocyte formation in the bone marrow.

Improvement in overall protein metabolism, confirmed by increased albumin levels in the biochemical analysis, directly contributes to the synthesis of the protein component of hemoglobin. This indicates an enhancement of the blood's oxygen-transport function, a key factor for endurance and performance in working dogs.

Changes in the leukocyte profile of the experimental animals indicate a moderate immunostimulatory effect of the studied supplements. This is confirmed by a slight increase

in total leukocyte count and the proportion of lymphocytes. This phenomenon can be explained by the presence of bioactive polysaccharides (e.g., β-glucans) and other immunostimulatory components in chlorella and spirulina, which may activate immune system cells.

An increase in the percentage of lymphocytes may also indicate enhanced specific immune defense (both humoral and cellular), suggesting a strengthening of the body's natural defense mechanisms without signs of inflammation (e.g., neutrophilia), which is particularly important for animals exposed to stress and a higher risk of infection.

Platelets play an essential role in blood coagulation; therefore, an increase in their number improves hemostatic capacity. During the experiment, platelet concentrations in the experimental groups' dogs were significantly higher than in the control group. Specifically, animals of the 2nd experimental group (chlorella) exceeded the control by 9.9 $\times 10^9$/L (4.0 %), while dogs of the 3rd group (spirulina) showed an increase of 14.6 $\times 10^9$/L (5.8 %). The largest and statistically significant difference was observed in the 4th experimental group (combined supplement), where platelet count increased by 30.1 $\times$ 10^9/L (12.0 %).

The process of platelet formation is influenced by the liver, which synthesizes thrombopoietin—a hormone regulating platelet production. In this study, liver function in dogs was assessed by ALT and AST levels. Improved liver enzyme activity contributes to better tissue repair and overall hemostasis in working dogs.

All the above-mentioned homeostasis parameters in dogs of the experimental groups remained within physiological norms during supplementation with the complex biologically active additives obtained by biotechnological methods, indicating their safety of use.

Thus, based on hemoglobin concentration, erythrocyte, leukocyte, and platelet counts, it can be concluded that the combined use of chlorella and spirulina is more effective than the use of individual microalgae.

The results of the biochemical analysis of blood serum in working dogs are presented in Table 3. Analysis of the collected data indicates that the studied supplements have a positive effect on the animals' biochemical status.

In dogs of all experimental groups, a tendency toward increased levels of total protein and albumin was observed compared to the control. The most pronounced effect was recorded in animals of the 4th experimental group. Consumption of the combined feed additive increased total protein by 4.6 g/L (7.1 %) and albumin by 2.5 g/L (6.9 %), with the differences being statistically significant.

This may indicate an improvement in the synthetic function of the liver and better provision of the organism with plastic material when consuming chlorella and spirulina supplements, both individually and in combination, which is particularly important for dogs with increased energy demands.

Table 3

Biochemical parameters of blood serum in dogs (M ± m, n = 5)

Parameter	Control group 1	Experimental group 2 (Chlorella)	Experimental group 3 (Spirulina)	Experimental group 4 (Complex)
Total protein, g/L	65.2 ± 1.41	67.1 ± 1.82	68.5 ± 1.60	69.8 ± 1.24*
Albumin, g/L	36.0 ± 1.51	37.2 ± 1.12	37.8 ± 0.92	38.5 ± 0.82*
Urea, mmol/L	5.1 ± 0.14	4.9 ± 0.19	4.8 ± 0.12	4.7 ± 0.13*
Creatinine, μmol/L	105.0 ± 6.08	98.0 ± 5.24	97.5 ± 4.83	95.0 ± 3.50
ALT, U/L	33.2 ± 2.17	28.5 ± 1.79*	27.8 ± 1.50*	24.1 ± 1.13*
AST, U/L	34.6 ± 3.41	30.1 ± 2.12	29.4 ± 1.88*	28.0 ± 1.53*
De Ritis ratio (AST/ALT)	1.04	1.06	1.06	1.16**
Alkaline phosphatase, U/L	37.0 ± 3.47	40.1 ± 2.83	42.5 ± 3.12	45.2 ± 2.89*
LDH, U/L	201.1 ± 10.24	195.2 ± 8.71	190.4 ± 9.14	185.0 ± 7.53

Note: significant compared to the control group: * ($P < 0.05$); ** ($P < 0.01$)

The concentrations of urea and creatinine are specific indicators of renal function. In dogs from the experimental groups, the levels of these compounds in blood serum were slightly lower than in the control animals. However, a statistically significant difference was observed only between the 4th experimental group and the control for urea concentration. The consumption of the spirulina and chlorella mixture resulted in a decrease in urea concentration by 0.4 mmol/L (7.8 %) and in creatinine by 10.0 μmol/L (9.5 %). This may be attributed to improved utilization of nitrogenous compounds and more efficient use of dietary protein, as well as the antioxidant and hepatoprotective effects of microalgae.

Clinical and laboratory assessments of enzyme activity and liver function revealed that the most significant changes were observed in aminotransferase activity. In animals of all experimental groups, ALT and AST activities were markedly reduced. Statistically significant differences in ALT levels were observed between all experimental groups and the control. In contrast, significant differences in AST activity were observed between the 3rd and 4th experimental groups and the control. The most pronounced decrease in these parameters was recorded in dogs receiving the combined spirulina and chlorella supplement, with reductions of 19.1 % for AST and 27.4 % for ALT compared to the control. This is a key indicator of improved functional status of hepatocytes and reduced intensity of catabolic processes in muscle tissue under physical stress.

The De Ritis ratio (AST/ALT) in dogs of the experimental groups showed a slight increase compared to the control, with a statistically significant difference in the 4th experimental group. This indicates restoration of enzyme balance and reflects improved regenerative processes in the liver.

During the experiment, an increase in alkaline phosphatase levels was observed in dogs of the experimental groups. The greatest and statistically significant difference was

found between the control animals and those receiving the combined spirulina and chlorella supplement, with an increase of 8.2 U/L (22.2 %). The elevation in alkaline phosphatase activity may be associated with activation of metabolic processes in bone tissue and the liver under the influence of vitamins and minerals contained in the supplements, remaining within physiological limits.

Lactate dehydrogenase (LDH) is an enzyme present in most tissues, and its blood level is a sensitive indicator of cellular damage, commonly used to assess the functional status of the heart, liver, muscles, and other organs. In dogs of the experimental groups, LDH levels decreased compared to the control. The largest difference – 16 U/L (8.0 %) – was observed between the control animals and those receiving the combined chlorella and spirulina supplement.

This indicates a positive effect of chlorella and spirulina supplements, especially their combination, on metabolic processes and cellular membrane stability. Such results may reflect the organism's enhanced adaptive capacity under physical stress and confirm the feasibility of using these supplements to maintain overall biochemical homeostasis.

3.2. Discussion

The results of studies on the effects of feeding chlorella and spirulina to dogs are consistent with data reported by other researchers obtained in similar experiments, although conducted on different animal species. Thus, Emmanuel Opoola et al. (2021) reported that feeding broiler chickens diets containing 12 and 6 g of spirulina per 1 kg of complete feed resulted in increased hemoglobin concentration, erythrocyte, and leukocyte counts. The levels of total protein, globulin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were also significantly higher in chickens fed spirulina-containing diets compared to those fed diets without spirulina.

In similar studies conducted by a group of scientists from Egypt, Japan, and Slovakia, led by Rasha I. M. Hassan (Hassan et al., 2022), it was also noted that inclusion of *Spirulina platensis* in compound feeds at doses of 0.5% or 1.0% resulted in significant ($P < 0.05$) increases in total protein, serum globulins, and lymphocyte counts.

A study conducted by a group of Indian researchers (Merin et al., 2022) on broiler chickens investigated the effects of an aqueous-alcoholic extract of *Chlorella vulgaris* on blood biochemical properties. The results showed that poultry receiving 0.4 % *C. vulgaris* extract in the diet exhibited significantly increased leukocyte ($P < 0.001$), erythrocyte ($P < 0.01$), and hemoglobin levels ($P < 0.05$), while total protein, albumin, globulin, and the A: G ratio remained similar to control values.

The results of another experiment conducted on broiler chickens (El-Gogary et al., 2023) demonstrated a significant increase in erythrocyte count and hemoglobin levels with supplementation of 6.0 g/kg *Spirulina platensis* and 4.0 g/kg *Chlorella vulgaris*. Feeding diets containing different types and levels of algae had no significant effect on ALT or total protein; however, AST levels were significantly lower in broilers fed *Chlorella vulgaris* at 6 g/kg.

Sameh A. Abdelnour et al. (2019) concluded that supplementation of *Chlorella vulgaris* in the diet of growing rabbits improves health status, as evidenced by increased platelet count, hematocrit, and hemoglobin concentration when 0.5 g/kg of chlorella powder was added to the diet. At the same time, inclusion of chlorella at 1.5 g/kg significantly reduced alanine aminotransferase activity.

Mohamed Mohamed El-Deeb et al. (2023) reported that in lambs supplemented with spirulina, hemoglobin levels, hematocrit percentage, erythrocyte count, total protein, and urea were significantly higher ($P < 0.01$). Meanwhile, albumin levels and liver enzymes (AST and ALT) did not differ significantly. In other studies (Alharthi et al., 2024), an increase in leukocyte levels in lambs fed spirulina was also observed.

In experiments on common carp (*Cyprinus carpio* L.), Mohsen Abdel-Tawwab et al. (2022) reported significant increases in leukocyte and erythrocyte counts, hemoglobin, hematocrit, and total protein levels in fish fed diets enriched with chlorella, daphnia, and their combination. At the same time, the activity of AST, ALT, and alkaline phosphatase did not differ significantly. Based on their findings, the authors recommended using a combination of chlorella and daphnia at 25 g of each per 1 kg of diet as feed additives.

Supplementation of spirulina in the diet of Nile tilapia (Youssef et al., 2023) resulted in a statistically significant ($P < 0.05$) increase in hemoglobin, albumin, erythrocyte, and leukocyte levels, indicating stimulation of hematopoiesis and improved protein metabolism. In addition, spirulina contributed to a significant ($P < 0.05$) reduction in creatinine and urea levels, which may be associated with more efficient utilization of nitrogenous compounds and reduced renal load.

4. Conclusions

The use of chlorella and spirulina biological supplements had a positive effect on the morphological and biochemical blood parameters of working German Shepherd dogs, with the most pronounced effect observed when combined. In animals of the 2nd experimental group receiving chlorella, erythrocyte count increased by 0.2×10^{12}/L, hemoglobin by 4.8 g/L, and platelets by 9.9×10^{9}/L compared to the control, indicating stimulation of erythropoiesis and improvement of blood coagulation function. In dogs of the 3rd experimental group receiving spirulina, changes in hematological parameters were more pronounced: erythrocytes increased by 0.3×10^{12}/L, hemoglobin by 7.0 g/L, and platelets by 14.6×10^{9}/L. Additionally, significant decreases in AST (15.0 %) and ALT (16.3 %) activity were observed, indicating a hepatoprotective effect. The most significant changes were recorded in the 4th experimental group receiving the combination of chlorella and spirulina: erythrocyte count increased by 0.6×10^{12}/L, hemoglobin by 13.2 g/L, hematocrit by 3.5%, platelet count by 30.1×10^{9}/L, total protein by 4.6 g/L, and albumin by 2.5 g/L, while ALT and AST activity decreased by 27.4 % and 19.1 %, respectively. In addition, LDH levels decreased by 16 U/L, indicating reduced cellular damage and improved metabolic stability. Thus, the combined use of chlorella and spirulina appears to be the most effective approach, providing a comprehensive positive effect on hematopoiesis, immune status, liver function, and overall biochemical homeostasis in working dogs.

Conflict of interest

The authors declares no conflict of interest.

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