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EFFECTS OF DIETARY SPIRULINA ON THE GROWTH AND HEALTH STATUS OF CARP (*Cyprinus carpio*, Linnaeus 1758)

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Abstract

The study aimed to investigate the influence of dietary supplementation with S. platensis on the growth performance of carp (Cyprinus carpio, Linnaeus, 1758), reared in a recirculating aquaculture system, and on its health status, assessed by primary hematological indicators and blood biochemical parameters. For this purpose, three experimental variants were used, differentiated by the concentration of spirulina added to the feed: V0 – control, without spirulina supplementation; V1 – diet supplemented with 4% spirulina powder; V2 – diet supplemented with 6% spirulina powder. At the end of the study, it was found that all biotechnological indicators analyzed showed higher values in the experimental variant in which the diet was supplemented with 4% spirulina. The main conclusion derived from the evaluation of hematological parameters is that supplementation with 4% spirulina enhances the process of erythropoiesis, as evidenced by increased hematocrit values and erythrocyte counts. The data obtained indicate that spirulina is a safe and effective protein additive, and that including 4% in the diet is the optimal option for stimulating growth, improving protein utilization, and increasing feed conversion efficiency without compromising fish health and while ensuring a favorable cost-benefit ratio.

Key words*: carp, growth, health status, RAS, spirulina.*

INTRODUCTION

The growing demand for aquaculture as a major source of high-quality animal protein, combined with fish's high protein requirements and the high cost or limited availability of fishmeal and soybean meal, is driving intensified research to identify affordable and sustainable alternative protein sources (Youssef et al., 2023).

Over the last few years, increased attention has been paid to dietary supplements such as probiotics, microalgae, and medicinal plants, to improve growth performance, nonspecific immune responses, and feed efficiency in aquatic animals, as these supplements are inexpensive and easy to prepare, are effective, have fewer side effects during disease treatment, and do not pose environmental or safety concerns (Watanuki et al., 2006; Milad et al., 2016). Spirulina is the dried biomass of microscopic filamentous cyanobacteria of the genus Arthrospira, particularly *A. platensis*, *A. fusiformis*, and *A. maxima*, which are widely cultivated for human and animal consumption. Since the cell wall of spirulina does not contain cellulose, 85–95% of the substance is absorbed by the body (Babadzhanov et al., 1999). *Spirulina platensis* is a microalga (filamentous cyanobacterium) recognized as an excellent dietary supplement because of its high content of protein, vitamins, minerals, and carbohydrates, including vitamins (B, C, D, A, E) and minerals (potassium, calcium, iron, magnesium, copper, etc.) (Milad et al., 2016; Hayashi et al., 1998). For animals - including dogs, cats, and fish - spirulina offers significant benefits: it supports immune, skin, and coat health (Iatrou et al., 2023), improves digestion, aids in detoxification, and enhances fish coloration. Spirulina is often given in powder or tablet form, mixed with the animals' regular feed (Selmi et al., 2011). Including spirulina in the diet has immunostimulatory effects on the

innate immune system of carp (Watanuki et al., 2006).

Previous studies have shown various beneficial effects of spirulina, especially on growth performance, immunity, and disease resistance (Hirahashi et al., 2002; Sayed & Fawzy, 2014), through dietary supplementation with *S. platensis* in different fish species, such as the Siberian sturgeon, *Acipenser baeri* (Milad et al., 2016); sturgeon *Huso huso* (Adel et al., 2016); common carp, *Cyprinus carpio* (Javeed et al., 2021); rainbow trout, *Oncorhynchus mykiss* (Yeganeh et al., 2015); and Nile tilapia, *Oreochromis niloticus* (Lu & Takeuchi, 2004).

The study focused on the common carp, which is considered one of the most economically valuable species due to its rapid growth rate, the superior organoleptic quality of its meat, its high natural resistance, its ability to adapt to cold water, and the ease with which it can be farmed in closed aquaculture systems (Javeed et al., 2021).

It is well known that fish have a close relationship with their aquatic environment; therefore, their blood can indicate health issues in their bodies long before any visible signs of disease appear (Musa & Omoregie, 1999; Okechukwu et al., 2007). Hematological and biochemical parameters serve as indicators of health and physiological responses that can be affected by various stressors (Sayed & Authman, 2018; Sayed et al., 2020, 2021). Hematological indices are strongly connected to an animal's response to its environment, suggesting that the environment where fish live may influence hematological characteristics (Gabriel et al., 2004).

The main goal of this study was, based on the well-known benefits of spirulina (*S. platensis*), to assess the effects of dietary supplementation with this microalga on the growth performance of carp (*Cyprinus carpio*, Linnaeus, 1758), as well as on their health status, indicated by key hematological and biochemical blood parameters.

MATERIALS AND METHODS

Experimental design

The study was performed in an intensive recirculating aquaculture system (RAS) achieved of side glass tanks, each with a capacity of 60 liters. To maintain water quality in the culture system, Tetratec EX 1500 external filters were used equipment effective at keeping optimal water parameters. These filters have technical features that enable efficient removal of solid particles and control of other parameters essential for the aquatic environment. A total of six such filters were used, with one for each tank. The biological material consisted of 5-month-old carp juveniles with an average weight of 89.5 grams per fish.

The experiment was conducted from September 22, 2025, to October 31, 2025, spanning 40 days. It was designed to assess the impact of spirulina powder supplementation on the biological and physiological parameters of the subjects. The main parameters were chosen to enable a clear comparison between a control group and two experimental groups, differentiated by the amount of spirulina added to their diet. Consequently, the following experimental groups were established:

- V0 – control group without added spirulina, used as a reference for evaluating effects, consisting of two rearing units, B1 and B2. Ten carp juveniles, averaging 87±14.3 g per fish, were placed in each rearing unit;
- V1 – the experimental treatment with 4% Spirulina powder added, designed to highlight the impact of a moderate level of supplementation, comprises two rearing units, B3 and B4. Ten carp juveniles, with an average weight of 89.9±21 g per fish, were introduced into each grow-out unit;
- V2 – the experimental treatment with 6% Spirulina powder added, designed to analyze the effect of a higher level of dietary supplement, comprises two rearing units, B5 and B6. Ten carp juveniles, with an average weight of 89.9±25.3 g per fish, were introduced into each rearing unit.

Throughout the experiment, all treatment groups experienced the same environmental conditions, basic diet, and care routine to ensure comparable results.

Diet preparation

The biochemical and granulometric characteristics of the feed administered are shown in Table 1. For all experimental groups, the feeding rate was 2.5% of body weight per day (2.5% BW/day), ensuring a consistent diet

and allowing for supplementation. The feeding schedule consisted of four meals per day, with pellets administered manually at 8:00 a.m., 11:00 a.m., 2:00 p.m., and 4:00 p.m., based on the subjects' daily needs and feeding behavior. The food was evenly distributed across meals to promote optimal digestion and efficient nutrient uptake.

Table 1. The biochemical composition of Aqua Start 1 mm basic feed and spirulina-enriched feed

Nutrients	Measurement unit	Aqua Classic 2.5 mm	Variant with 4% spirulina	Variant with 6% spirulina
Crude protein	%	34	35	35,5
Crude fat	%	9	8,92	8,88
Cellulose	%	4	4.12	4,17
Digestible energy	MJ/kg	16,8	16,9	17

For this experiment, spirulina powder from the "Pilot Research and Development Station for Algae Cultivation" was used; it was cultivated in modern photobioreactors operated in a fully controlled environment at the Research and Development Institute for Aquatic Ecology, Fisheries, and Aquaculture in Galați. Growing spirulina in photobioreactors guarantees a stable and nutritionally consistent biomass, which directly helps produce high-quality feed with superior biological value and positive effects on fish growth. The spirulina used in this experiment has the following composition: crude protein 60%, crude fat 7%, crude fiber 7%, and digestible energy 19 MJ/kg.

The protocol for adding spirulina into feed was designed to maintain its physicochemical properties and was carried out as follows: dissolving and homogenizing spirulina powder in a 2% gelatine solution; incorporating the resulting solution into the pelleted feed; drying the pellets in an oven at $T^0 = 25°C$ for 2 hours.

Throughout the experimental period (40 days), environmental conditions in the rearing units were monitored through physicochemical analysis of water samples taken from the carp juvenile rearing units.

Water temperature, dissolved oxygen (DO), and pH were recorded daily, while the other water quality parameters (concentrations of NH_4-N, NO_2-N, and NO_3-N) were monitored through sampling twice a week.

Growth parameters

At the beginning and end of the experiment, the juvenile carp were weighed, and the following parameters were calculated: actual growth rate [Sr], daily growth rate [GR], specific growth rate [SGR], feed conversion ratio [FCR], and protein conversion ratio [PER], using equations already established in the literature.

Hematological and biochemical blood parameters

To evaluate the effect of supplementing the feed with spirulina and to determine whether the health status of the biological material was influenced, blood samples (approx. 0.3 ml) were collected at the end of the experiment from 10 fish in each experimental group, selected at random. The sampling was performed on previously sedated carp juveniles with clove oil. After collecting blood samples, the main hematological parameters (hematocrit, hemoglobin, red blood cells, and erythrocyte constants) were determined using methods already established in the scientific literature and veterinary medicine (Blaxhall & Daisley, 1973; Ghergariu et al., 1985; Svobodova et al., 1991; Dima et al., 2022). Various blood biochemical parameters (TP, ALT, AST, ALB, GLO, A/G, glucose) were analyzed using a hematology analyzer (automated blood analyzer), specifically the Seamaty SMT-30V model, employing a reagent/test disc preloaded with reagents for biochemical analysis.

Hemoglobin was measured using the colorimetric method with Drabkin reagent on a SPECORD 210 spectrophotometer (Analytik Jena, Jena, Germany) at 540 nm. Hematocrit was determined by centrifuging blood in heparinized microhematocrit capillary tubes with the Haematokrit 210 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany) at 12,000 rpm for 5 minutes. The red blood cell count was performed using the classical method, with Vulpian's solution as the diluent (prepared in the laboratory from sodium citrate, potassium iodide, and metallic iodine, Sigma-Aldrich, St. Louis, MO, USA), a Neubauer counting chamber, and a Potain pipette for erythrocytes. Based on the results of hemoglobin, hematocrit, and red blood cell count measurements, erythrocyte constants such as MCV (mean corpuscular volume), MCH (mean corpuscular

hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) were calculated. These are important parameters in characterizing the physiological state of fish.
The study was conducted in accordance with the guidelines of the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes and the Romanian legislation (Low 43 of 11 April 2014), approved by the Ethical Commission of Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu Sișești", Department of Veterinary Medicine (Decision No 2048 of 04 April 2023).

Statistical analysis
Statistical analysis of data was performed using Excel 2019 and SPSS Statistics 26 software. Single-factor ANOVA and t-test were used to compare the experimental groups. Significant differences between the experimental groups were assessed using paired post-hoc comparisons with the Tukey HSD test. Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The physicochemical parameters of water
The fundamental biological principle of aquaculture is the principle of the unity of the organism with its environment. According to this principle, every organism and every species as a whole can exist and develop only under the conditions of a specific environment to which that species has adapted during its evolution. The primary technological objective in a recirculating aquaculture system is to ensure environmental conditions that closely correspond to the ecophysiological characteristics of the cultured species (Cristea et al., 2002).
By analyzing the values recorded for each water quality parameter during the experimental period, the following observations were made:
- Water temperature, which is closely connected to dissolved oxygen the key water characteristic influencing fish health averaged 24.5±0.90°C during the experiment. The water temperature patterns across different experimental groups are naturally similar due to the recirculating system, and they stayed within the species' optimal range. The juvenile carp displayed active feeding behavior, consuming all the feed provided.
- Concentration of dissolved oxygen, measured at the grow-out units, recorded average values of 6.55±0.58 mg/L, which is optimal for the cultured species. Some warm-water species, such as tilapia and carp, are better adapted to survive under low DO conditions, whereas cold-water species cannot survive (Svobodova et al., 1993).
- The pH range where carp can survive is between 5 and 10, but they grow best at a pH of 6 to 9. During the experiment, the pH stayed within the optimal range needed by the species' biological functions.
- Throughout the experiment, nitrogen compounds remained within the optimal range (<0.1 mg/L for nitrites and 15-23 mg/L for nitrates) by replacing 20-30% of the total water volume in the grow-out unit and leveraging the filters' ability to treat the process water. Assessing nitrogen compounds is especially important in aquaculture, as some decomposition products mainly ammonia and nitrites are toxic. To a lesser extent, nitrates also become toxic when they build up to high concentrations (Romano & Zeng, 2013; Zhang et al., 2016).

Growth parameters
From the analysis of the growth equations, plotted in Figures 1, 2, and 3 for each experimental treatment, it can be observed that the coefficient of determination (R^2) in the control group where the fish were fed without added spirulina was 0.8274 (Figure 1). Although this value indicates a strong correlation, it still highlights the presence of considerable biological variability, likely caused by factors such as individual differences among fish, uneven feed intake, or microenvironmental variations.
Adding 4% spirulina to the feed significantly raised the R^2 value to 0.948 (Figure 2). This indicates an extremely strong correlation between dietary supplementation and growth response, with the model explaining 94.8% of the variation observed. This notable improvement suggests that spirulina helps reduce individual variability and stabilize the metabolic processes involved in growth, resulting in much more consistent outcomes.

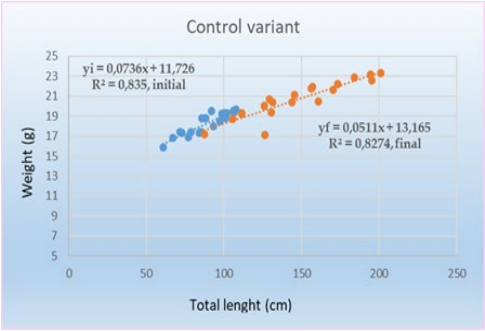

Figure 1. Length-weight regression (initial and final) for juvenile carp from the control variant

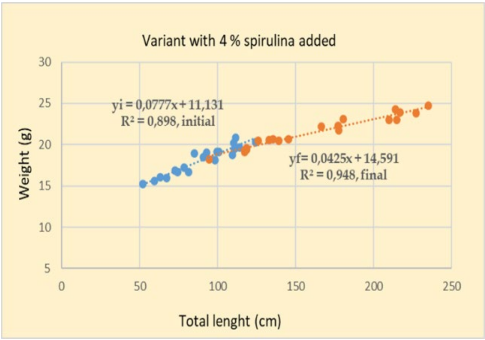

Figure 2. Length-weight regression (initial and final) for juvenile carp in the variant with 4% spirulina added

In the treatment with 6% spirulina supplementation, the R^2 value obtained was 0.9331 (Figure 3). Although it is slightly lower than the value recorded at the 4% spirulina level, it remains very high, indicating a strong correlation between spirulina supplementation in the diet and growth performance. The result suggests that higher levels of spirulina continue to support a consistent biological response, even though maximum uniformity was achieved with the 4% treatment.

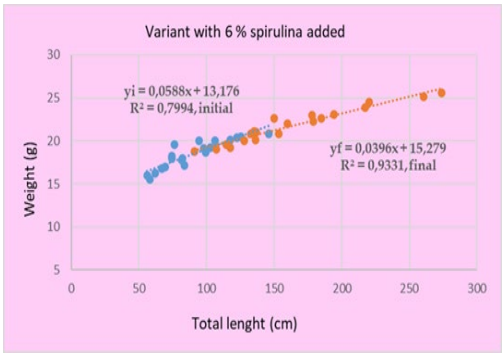

Figure 3. Length-weight regression (initial and final) for juvenile carp in the variant with 6% spirulina added

This upward trend in R^2 values shows that spirulina supplementation not only boosts growth but also lowers individual variability, with the 4% concentration being the most effective in producing a consistent and predictable growth response. The present study aimed to evaluate the effect of adding spirulina (4% and 6%) to fish feed on growth performance, feed conversion, and protein efficiency, compared to a control group without supplementation. The results emphasize the positive impact of spirulina on growth indicators, with variations depending on the dose, and it is evident that the experimental group with 4% spirulina achieved a higher growth rate than the other two experimental groups (Table 2).

Table 2. Biotechnological indicators for juvenile carp reared in a recirculating aquaculture system

Experimental Variant	**Control variant**	**The 4% spirulina variant**	**The 6% spirulina variant**
Tank indicator	**B1, B2**	**B3, B4**	**B5, B6**
Initial number of fish	20	20	20
Final number of fish	20	20	20
Survival (%)	100	100	100
Initial biomass (g)	1776.7	1798	1798.4
Initial biomass (kg/m³)	3.55	3.6	3.6
Final biomass (g)	2941.1	3267.4	3245.6
Final biomass (kg/m³)	5.88	6.53	6.49
Biomass gain (g)	1164.4	1469.4	1447.2
Biomass gain (kg/m³)	2.33	2.94	2.89
Initial average weight (g/fish)	88.84	89.9	89.92
Final average weight (g/fish)	147.06	163.37	162.28
Individual weight gain (g)	58.22	73.47	72.36

Experimental Variant	Control variant	The 4% spirulina variant	The 6% spirulina variant
Growth days	40	40	40
Crude protein in feed (CP %)	34	34	34
Daily ration (% biomass)	2.5	2.5	2.5
Total feed distributed (g)	1929.6	2045.7	2187.6
GR (Daily growth rate) (g/day)	29.11	36.74	36.18
SGR (specific growth rate)(%/day)	1.26	1.49	1.48
FCR (feed conversion ratio) (g feed/g biomass gain)	1.66	1.39	1.51
PER (protein efficiency ratio) (g/g)	1.77	2.11	1.95

Analysis of the data shows that survival was 100% across all experimental groups, indicating that spirulina has no toxic effects at the levels used and that stocking density and rearing conditions were optimal. This result confirms earlier research indicating that spirulina can serve as a protein supplement without harming fish health (Habib et al., 2008; Nuhu, 2013; Palmegiano et al., 2005).

The SGR and individual growth rates were significantly higher in the spirulina-treated groups, indicating superior metabolic efficiency and uniform fish growth. A similar observation was made using *Spirulina platensis* at different concentrations in *C. carpio* by Ramakrishnan et al. (2008). Although 6% spirulina yielded good results, the highest values were observed at 4%, suggesting that a higher dose does not necessarily yield maximum performance and that a ceiling effect may exist.

The lower FCR values seen in the spirulina-supplemented groups indicate more efficient feed utilization. PER increased significantly at 4% spirulina, confirming that the feed's protein is used more effectively for body growth. The results of this study on carp juveniles align with data from Al-Sulivany (2023). Similarly, Sayed et al. (2021), reported comparable results for catfish, emphasizing that growth parameters are optimal at a 2.5% spirulina concentration and decline at 5%. Unlike the results for carp juveniles, tilapia showed higher growth parameter values at a 10% spirulina concentration, which is above the optimal level for carp juveniles (Youssef et al., 2023).

Hematological and biochemical blood parameters

Knowledge of the hematological profile is an essential tool for monitoring physiological and pathological changes in fish (Kori-Siakpere et al., 2005).

Hematological indices, including hemoglobin concentration, hematocrit, and erythrocyte count, are particularly significant, reflecting the blood's oxygen-carrying capacity and overall physiological condition (Witeska, 2013; Youssef et al., 2023). By integrating hematological and biochemical data, scientists can gain a comprehensive understanding of fish physiology and the impact of nutritional strategies or environmental stressors on aquaculture species (Hrubec & Smith, 2010; Abdel-Tawwab et al., 2008). The values of the blood parameters and erythrocyte constants are presented analytically in Table 3.

Table 3. Hematological indices values

Experimental variant	RBC ($\times 10^6$cel/µl) (media±st. dev)	Hb (g/dl) (media±st. dev)	Ht (%) (media±st. dev)	MCV (µm^3) (media±st. dev)	MHC (pg) (media±st. dev)	MCHC (g/dl) (media±st. dev)
V0	1.58±0.20^a	10.82±0.35^a	27.1±2.44^a	173.14±13.05^a	69.77±9.82^a	40.25±9.82^a
V1	1.64±0.17^a	9.99±0.26^b	29.7±1.30^a	184.08±23.51^a	61.65±6.74^a	33.59±1.85^b
V2	1.55±0.19^a	10.5±0.88^a	25.6±3.96^b	166.6±30.08^a	68.68±9.64^a	41.92±7.14^a

Different letters (a,b) in the same column represent significant differences ($p<0.05$).
RBC- red blood cells; Hb - Hemoglobin; Ht - Hematocrit; MCV - Mean corpuscular volume; MCH - Mean corpuscular hemoglobin; MCHC - Mean corpuscular hemoglobin concentration.

In the experiment that studied the effects of spirulina supplementation on carp juveniles, the observed changes in erythrocytes reflect specific, dose-dependent physiological

responses. In variant V1, where the feed was supplemented with 4% spirulina, the erythrocyte count showed a slight, statistically insignificant increase ($p > 0.05$), reaching 1.64×10^6/µl, compared with the control group. This increase may suggest a moderate stimulation of erythropoiesis, possibly due to the bioactive compounds in spirulina, such as phycocyanin, chlorophyll, B-complex vitamins, and trace elements involved in heme synthesis. Therefore, a moderate supplemental intake of spirulina could enhance the efficiency of hematopoietic processes and the body's ability to adapt to increased metabolic demands. These results are also consistent with the data obtained by Abdulrahman et al. (2019), who observed no significant differences for added spirulina concentrations of 3% and 5%.

In contrast, in the 6% spirulina group, the red blood cell count decreased from not significantly ($p>0.05$) to 1.55×10^6/µl, indicating a change opposite to that seen at the lower concentration. This decrease may suggest metabolic overload caused by increased intake of bioactive nutrients, which could indirectly inhibit erythrocyte synthesis or redirect metabolic resources toward other physiological processes. Additionally, high concentrations of spirulina may impact osmotic balance, erythrocyte turnover, or alter endocrine parameters involved in regulating erythropoiesis (Abdel-Tawwab et al., 2008; Witeska, 2013; Hrubec & Smith, 2010; Promya & Chitmanat, 2011).

A decrease in red blood cell count at high spirulina levels may indicate a disturbance in physiological and metabolic balance, as numerous studies have shown that spirulina affects hematological parameters in a dose-dependent way, capable of both stimulating and modifying erythropoiesis depending on the level of inclusion in the diet (Rasheed et al., 2024). In experiments studying the effect of spirulina supplementation on the hematological parameters of carp juveniles, hemoglobin (Hb) concentration is a key indicator of physiological status, oxygen transport efficiency, and the organism's metabolic capacity (Javeed et al., 2021). A comparative analysis of Hb values between the control group and the experimental groups reveals subtle but biologically relevant changes.

In the experimental group where the feed was supplemented with 4% spirulina, the average hemoglobin level showed a statistically significant ($p<0.05$) decrease (9.99 g/dL) compared to the control group (10.82 g/dL). The same effect of spirulina concentration was also observed by Abdulrahman et al. (2019) in carp juveniles. In the variant where supplementation was increased to 6% spirulina, the hemoglobin level (10.5 g/dL) was considerably closer to that of the control group but still showed statistically significant differences compared to variant V1 ($p<0.05$). Overall, the results indicate that moderate spirulina supplementation (4%) can cause temporary changes in the hematological profile (Al-Sulivany, 2023), while a higher level (6%) appears to support the stabilization of hemoglobin, possibly through a gradual adaptation of fish metabolism to increased intake of functional nutrients. These findings highlight the importance of determining the optimal spirulina dose to maintain hematological balance and promote the overall health of carp juveniles.

Hematocrit (Ht) values increased in the experimental group treated with 4% spirulina (29.7%), but this was not statistically significant ($p>0.05$), compared to the control group (27.1%). The 6% spirulina group showed a statistically significant decrease ($p<0.05$) to 25.6%. This change may relate to variations in plasma volume or differences in erythrocyte size and volume, indicating a distinct adaptive physiological response based on the level of spirulina supplementation. Compared to the current study, Javeed et al. (2021) reported higher hematocrit values at a 7.5% spirulina concentration but observed decreases in hematological parameters at a 10% concentration in the feed.

The erythrocyte constants are vital hematological markers for evaluating red blood cell quality and function, offering additional insight into the physiological mechanisms behind changes in red blood cell count. Analysis of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) enables a comprehensive understanding of how spirulina supplementation affects erythrocyte maturation and efficiency in carp juveniles.

The mean corpuscular volume (MCV) showed a significant increase in the experimental group receiving 4% spirulina, reaching 184.08 µm³, compared with the control group. This increase suggests that erythrocytes formed in the presence of a moderate intake of spirulina are larger, possibly as a result of intensified erythropoiesis or accelerated erythrocyte maturation. At the same time, an increased erythrocyte volume may indicate increased availability of nutrients involved in red blood cell formation. In the 6% spirulina group, MCV decreased to 166.6 µm³, falling just below the control value, which may reflect the appearance of smaller erythrocytes, potentially due to incomplete cellular maturation, rapid nutrient utilization, or metabolic stress caused by excessive supplementation.

MCH (mean corpuscular hemoglobin) followed a different pattern, being lower at 4% spirulina (61.65 pg), suggesting that, although the red blood cells are larger in this treatment group, they contain less hemoglobin per cell. Similar data for carp were also obtained by Macoveiu et al. (2024). This combination (increased MCV, decreased MCH) may indicate a temporary imbalance in hemoglobin synthesis, possibly due to the redistribution of iron and other micronutrients toward other metabolic processes. At the 6% spirulina level, the MCH value increased to 68.68 pg, approaching control levels, suggesting a normalization of hemoglobin synthesis as the body adapts to higher supplementation.

The mean corpuscular hemoglobin concentration (MCHC) was highest in the 6% spirulina group, reaching 41.92 g/dL. This increase indicates a clear improvement in the hemoglobin content of erythrocytes, suggesting that, despite a slightly reduced erythrocyte volume in this variant, the hemoglobin density within the cells is significantly higher. Thus, the erythrocytes become more efficient at transporting oxygen, which may represent an important physiological advantage for fish under intensive rearing conditions.

Blood biochemical parameters are essential indicators of the physiological and metabolic status of fish, providing valuable information about their health, nutritional condition, and responses to environmental or experimental factors. These parameters include glucose, total proteins, albumin, liver enzymes (AST, ALT, ALP) (Hrubec & Smith, 2010; Abdel-Tawwab et al., 2008).

Supplementing the diet with spirulina resulted in significant changes in the analysed serum biochemical parameters, indicating a direct influence on protein metabolism, liver function, immunity, and physiological stress levels. The results highlight clear differences between the experimental groups, suggesting a dose-response relationship for some parameters.

Table 4. Blood biochemical parameters in the three experimental variants

Parameters	UM	Experimental variants		
		V0	V1	V2
Total serum protein (TP)	g/dl	3.08±0,01^{a}	3,02±0,01^{b}	3,61±0,02^{c}
Serum albumin (ALB)	g/dl	1.20±0,01^{a}	1,22±0,01^{b}	1,33±0,01^{c}
Globulin (GLO)	g/dl	1,87±0,01^{a}	1,80±0,01^{b}	2,29±0,01^{c}
ALB/GLO	-	0,64±0^{a}	0,68±0^{b}	0,58±0^{c}
Glucose (GLU)	mmol/l	13,36±0,40^{a}	8,06±0,09^{b}	7,98±0,08^{b}
Aspartate aminotransferase (AST)	U/l	133±2,46^{a}	106±2,57^{b}	210±3,02^{c}
Alanine aminotransferase (ALT)	U/l	46±2,21^{a}	47,5±0,48^{a}	34±2,22^{b}

Different letters (a,b,c) in the same column represent significant differences ($p<0.05$).

Total protein, albumin, and globulin levels showed consistent changes, particularly in the 6% spirulina variant, with statistically significant differences among the three variants. A significant increase in total protein and albumin content confirms that spirulina, because of its high crude protein, essential amino acid, and bioactive compound content, can stimulate protein synthesis and metabolic efficiency in fish. The ALB/GLO ratio decreased in the 6% spirulina variant, mainly due to a significant increase in globulins. A lower ALB/GLO ratio

is typical of organisms with heightened immune activity, indicating stimulation of defense mechanisms. This finding aligns with the literature emphasizing spirulina's role in activating macrophages, boosting antibody production, and modulating the nonspecific immune response (Hayashi et al., 1994). Serum glucose decreased significantly in both spirulina variants, indicating reduced metabolic stress and a potential improvement in stress tolerance (Macoveiu et al., 2024). Spirulina is known for its antioxidant properties. Analysis of liver enzymes reveals a clear and significant difference between variants. At 4% spirulina, the notable statistically significant decrease ($p < 0.05$) in AST levels indicates a hepatoprotective effect, likely due to the antioxidants and anti-inflammatory compounds in spirulina (Belay et al., 1993; Khan et al., 2005; Wu et al., 2016). Conversely, at 6% spirulina, AST levels increased significantly statistically ($p<0.05$), implying either metabolic overload or an adaptive response related to an increased overall metabolic rate (Ambroise et al., 2008; Holman & Malau-Aduli, 2013).

It is important to highlight that ALT levels, the more specific marker of liver health, decrease significantly ($p<0.05$) in the 6% group, indicating good liver function regardless of the increase in AST levels. Similar findings have been reported in common carp and other fish species, where spirulina supplementation reduced or maintained ALT levels even when AST levels varied, suggesting preserved liver health and potential metabolic adaptation (Abdalla et al., 2014). Furthermore, studies on fish like tilapia and catfish show that spirulina supplementation generally lowers ALT and AST under stress, highlighting its liver-protective and antioxidant effects (Sayed et al., 2021; Abdel-Daim et al., 2020). These findings support the idea that decreased ALT, even with elevated AST, may indicate that liver integrity remains intact but could also reflect increased metabolic activity or the release of enzymes outside the liver.

CONCLUSIONS

The experimental infrastructure, supported by advanced water quality control techniques, maintained optimal conditions for fish biomass growth across all three experimental variants, lending scientific credibility to the results.

Overall, the length-weight regression analysis shows that adding spirulina to feed enhances growth uniformity in fish. The 4% spirulina level is the most effective for producing a consistent and predictable response, while the 6% level continues to yield excellent results, confirming the beneficial role of spirulina in fish nutrition.

Analysis of key biotechnology indicators shows that spirulina is an effective and safe protein additive. A 4% spirulina dose is optimal for maximizing growth, protein utilization, and feed efficiency, without any negative effects on fish survival, thus making the feed cost-effective.

The findings align with existing scientific research, which emphasizes spirulina's role as a functional nutritional supplement with antioxidant, hepatoprotective, and immunostimulatory properties. The obtained results emphasize the importance of determining the optimal spirulina dose to maintain hematological, biochemical, and immune balance, as well as to promote the overall health of juvenile carp.

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