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Research Article

Effect of different levels of dietary *Arthrospira platensis* or prebiotic on growth performance, intestinal microflora, digestive enzyme activity, and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study was conducted to evaluate the effects of dietary *Arthrospira platensis* or a prebiotic (Mannan-oligosaccharide, MOS) on growth performance, survival, intestinal microflora, digestive enzyme activity, and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). A total of 400 rainbow trout (initial body weight: 10.0±0.2 g) were distributed into tanks in a completely randomized design with five treatments and four replicates. The experimental diets included a control (no additive), three levels of *A. platensis* (1, 1.5, and 2 g/kg), or a prebiotic diet (1 g/kg) as a positive control. The fish were fed with the experimental diets for 56 days. Results indicated that dietary supplementation of 2 g/kg *A. platensis* or the prebiotic significantly improved final body weight, survival rate, and decreased feed conversion ratio compared to the control (p<0.05). Furthermore, the inclusion of 1.5 and 2 g/kg *A. platensis* significantly increased the intestinal *Bacillus subtilis* population while decreasing coliforms (p<0.05). Digestive protease activity was significantly elevated in fish fed 2 g/kg *A. platensis* compared to the control and prebiotic groups. Biochemical analysis revealed that serum malondialdehyde levels significantly decreased in fish fed 1.5 and 2 g/kg *A. platensis* (p<0.05), indicating enhanced antioxidant status, whereas other hematological parameters remained unaffected. In conclusion, dietary *A. platensis* (particularly at 2 g/kg) exhibits prebiotic – like benefits, improving growth, gut health, and antioxidant capacity in rainbow trout.

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Introduction

The rapid intensification of global aquaculture has highlighted the need to adopt environmentally responsible and sustainable production strategies that reduce reliance on conventional antimicrobial treatments (Abdel-Latif *et al.*, 2025; Siddik *et al.*, 2024; Irabor *et al.*, 2026). Rainbow trout (*Oncorhynchus mykiss*) is one of the most commercially valuable cold-water salmonid species globally; however, intensive rearing conditions may expose this species to nutritional stress, pathogen challenges, and disturbances in intestinal microbial communities, potentially compromising fish health and production performance (Abdel-Latif *et al*., 2025; McLean *et al.*, 2025). To address these challenges, researchers have focused on natural dietary supplements, including prebiotics and single-cell protein sources, as potential strategies to support intestinal health, improve growth performance, and maintain fish health under intensive aquaculture conditions. (Jouga *et al.*, 2026; Naderi Farsani *et al.*, 2021).

Arthrospira platensis, commonly known as *Spirulina*, has emerged as a promising functional feed ingredient (Mohammadian *et al.*, 2025; Spínola *et al.*, 2024). This cyanobacterium is a protein-rich source containing all essential amino acids and a range of bioactive compounds, including carotenoids and phycocyanin, a pigment associated with antioxidant and immunomodulatory properties (Braune *et al.*, 2025; Mabrouk *et al.*, 2024; Hajati and Zaghari, 2019). Dietary inclusion of *A. platensis* has been investigated for its potential effects on growth performance, antioxidant status, and intestinal health in various aquaculture species (Islam *et al.*, 2025; Abdo *et al.*, 2024). However, responses to dietary supplementation may vary according to fish species, inclusion level, and experimental conditions, highlighting the need to evaluate its effects at defined dietary levels.

Prebiotics, particularly mannan-oligosaccharides (MOS), are also used as functional feed ingredients because of their potential to modulate intestinal microbial populations and support gut health (Abdel-Latif *et al*., 2025; Jouga *et al*., 2026). Although both *A. platensis* and commercial prebiotics have attracted attention in aquaculture nutrition, their effects should be evaluated using complementary indicators, including growth performance, survival, intestinal microbial populations, digestive enzyme activities, and blood parameters. Furthermore, changes in bacterial populations alone are insufficient to establish a specific prebiotic mechanism. Comparative evaluation of these dietary supplements under the same experimental conditions may therefore provide useful information about their effects on rainbow trout.

Therefore, the present study aimed to investigate and compare the effects of dietary *A. platensis* supplementation at 1, 1.5, and 2 g/kg and a commercial MOS-based prebiotic at 1 g/kg on the growth performance, survival, intestinal microbial populations, digestive enzyme activities, hematological parameters, and serum biochemical indicators of rainbow trout (*O. mykiss*).

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Material and methods

Cyanobacterial cultivation and proximate composition

The biomass of *A. platensis* was cultivated at the Academic Center for Education, Culture, and Research (ACECR) in Sari, Mazandaran, Iran. Briefly, the cyanobacterium was grown in modified Zarrouk's medium (Zarrouk, 1966) in a 12 m² open raceway pond equipped with paddlewheels to provide continuous mixing. The culture was maintained at a mean temperature of 29 ± 1 °C and an average light intensity of 4 klux. Biomass was harvested approximately 14 days after inoculation, corresponding to the late exponential growth phase. The harvested biomass was washed, freeze-dried, and ground into a fine powder. Proximate composition, including crude protein, crude lipid, ash, and moisture contents, was determined in triplicate using the applicable official methods of AOAC International (AOAC, 2016).

Experimental fish, diets, and feed management

A total of 400 healthy rainbow trout (*O. mykiss*) with an initial average weight of 10.0±0.2 g were obtained from a local hatchery. Prior to the trial, the fish were acclimated to the experimental conditions for 14 days and fed a basal diet without additives. Following acclimation, they were randomly distributed into 20 fiberglass tanks representing 5 dietary treatments with 4 replicates. Experimental diets included a control diet (without additive), three levels of *A. platensis* (1, 1.5, and 2 g/kg), and one level of prebiotic (1 g/kg of diet, *AgriMOS*®, a mannan-oligosaccharide) as a positive control. Diets were formulated using UFFDA software. The ingredients and chemical composition of the basal diets is shown in Table 1. The fish were hand – fed twice daily at a rate of 2% of their wet body weight for 56 days. Throughout the experiment, water temperature (14.5±0.8 ºC), dissolved oxygen (7.2±0.5 mg/L), pH (7.4±0.3), and total ammonia nitrogen (0 – 0.12 mg/L) were strictly maintained within optimal limits via daily 30% water exchanges.

Table 1: Ingredients and nutrient composition of the basal experimental diets.

Ingredient	Amount (%)
Fishmeal	35.00
Soybean meal	15.00
Meat meal	22.50
Wheat flour	2.00
Corn flour	15.82
Fish oil	5.00
Beet molasses	2.00
DL-Methionine	0.84
Lysine	0.77
Mineral and vitamin premix*	1.00
Salt	0.07
Calculated composition	
Gross energy (kcal/kg)	3614
Dry matter (%)	93.80
Crude protein (%)	43.00
Ash (%)	13.00
Crude fat (%)	11.00

* Vitamin and mineral mixture (g/kg): ρ-Aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; Ca-pantothenate, 1.0; pyridoxine-HCl, 0.17; riboflavin, 0.73; thiamine-HCl, 0.22; menadione, 0.17; α-tocopherol, 1.45; cyanocobalamine, 0.0003; calciferol, 0.03, L-ascorbyl-2-phosphate-Mg, 0.25; folic acid, 0.05; choline chloride, 29.65, NaCl, 1.838; $MgSO_4 \cdot 7H_2O$, 6.85; $NaH_2PO_4 \cdot 2H_2O$, 4.36; KH_2PO_4, 11.99; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 6.79; Fe-citrate, 1.48; Ca-lactate, 16.35; $AlCl_3 \cdot 6H_2O$, 0.009; $ZnSO_4 \cdot 7H_2O$, 0.17; $CuCl_2$, 0.0005; $MnSO_4 \cdot 4H_2O$, 0.04; KI, 0.008 and $CoCl_2$, 0.05.

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Growth performance and survival

At the conclusion of the 56–day feeding period, fish were fasted for 24 h and anesthetized with benzocaine (50 mg/L) before final weight measurements. The performance parameters were calculated using standard formulas:

Weight gain (g/fish) = Mean final body weight (g) – Mean initial body weight (g)

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed fed (g)}}{\text{Total weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final numver of fish}}{\text{Initial number of fish}} \times 100$$

Hematological and biochemical analyses

Blood was withdrawn from the caudal vessel into a syringe containing 10% EDTA solution. Red blood cell (RBC) and white blood cell (WBC) counts were evaluated using a Neubauer hemocytometer. Hematocrit (HCT) values were assessed via the microhematocrit capillary technique (Goldenfarb *et al.*, 1971), and hemoglobin (Hb) concentration was quantified spectrophotometrically utilizing the cyanmethemoglobin method (Drabkin and Austin, 1935). Serum was separated via centrifugation at 3000 × g for 15 min at 4ºC and stored at - 80º C. Serum glucose was determined colorimetrically using a diagnostic kit. Serum malondialdehyde (MDA) concentration, representing lipid peroxidation, was evaluated using the thiobarbituric acid reaction method (Esterbauer and Cheeseman, 1990).

Digestive enzyme assays

At the end of the trial, amylase, lipase, and protease activities in the intestinal contents of the fish were evaluated in triplicate. Amylase activity was determined using the 3,5-dinitrosalicylic acid (DNS) method (Bernfeld, 1951). Lipase activity was determined utilizing *p*-nitrophenyl palmitate (pNPP) dissolved in isopropanol as the substrate (Markweg-Hanke *et al.*, 1995). Total protease activity was analyzed by measuring tyrosine release from casein digestion (Tsuchida *et al.*, 1986).

Intestinal microbiota profiling

The ileums were excised, and their contents were collected by gently pressing the intestinal wall to extrude the content into sterile tubes at the end of the 56-day feeding trial. Samples within each replicate were pooled, kept on ice, and immediately transported to the laboratory for the enumeration of microbial populations. One gram of ileal content was homogenized in 9 mL sterile peptone water (0.1%) and serially diluted (10-fold). Using these diluted sub-samples, *Bacillus subtilis* was enumerated on Nutrient Agar after incubation at 37 °C in an aerobic chamber for 48 h (Guban *et al.*, 2006). Additionally, coliforms and *E. coli* were counted on CHROMagar ECC (Paris France) after incubation at 37 °C in an aerobic chamber for 48 h (Sallam, 2007). Results were expressed as Log_{10} CFU/g of intestinal content.

Statistical analysis

Data were analyzed by analysis of variance using GLM procedures of SAS software

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(SAS Institute Inc, 2023). Means were compared using Duncan's new multiple ranges test. The level of significance was reported at $P < 0.05$. The statistical model was as bellow:

Yij= μ + Ti+ eij,

Where, Yij is the value of each observation, μ is observations mean, Ti is the effect of treatment, and eij is the random error.

Results

Composition of Arthrospira platensis

The proximate composition of the dried *A. platensis* biomass is presented in Table 2. Crude protein was 74.0±0.27%, whereas crude fat was 1.73±0.14%. The biomass contained 1.03±0.07% calcium, 1.43±0.08% phosphorus, and 12.53±0.04% ash.

Table 2: Proximate composition of the *Arthrospira platensis* dry biomass (Mean ± SD)

Crude Protein (%)	Crude Fat (%)	Calcium (%)	Phosphorus (%)	Ash (%)
74.0±0.27	1.73±0.14	1.03±0.07	1.43±0.08	12.53±0.04

Growth performance and survival

Dietary inclusion of both 2.0 g/kg *A. platensis* (AP-2.0) and the commercial prebiotic significantly increased final body weights and significantly lowered FCRs compared to the control group in both the early (1-4 weeks) and late (5-8 weeks) rearing periods. Survival rates were significantly higher in the AP-1.5, AP-2.0 and prebiotic groups than in the control group (p<0.05; Table 3).

Table 3: Growth performance and survival of rainbow trout fed diets with different levels of *A. platensis* and prebiotic additive (56 days)

Parameter	Control	AP- 1.0	AP- 1.5	AP- 2.0	Prebiotic	SEM	*p*-Value
1-4 Weeks							
Body weight (g)	17.9^{c}	19.24^{b}	19.80^{b}	20.18^{a}	20.02^{a}	2.11	<0.003
FCR	0.63^{a}	0.58^{b}	0.57^{b}	0.53^{c}	0.54^{c}	0.05	<0.0001
5-8 Weeks							
Body weight (g)	32.72^{c}	43.25^{b}	47.68^{b}	56.02^{a}	55.82^{a}	10.01	<0.002
FCR	1.61^{a}	1.46^{ab}	1.41^{b}	1.35^{c}	1.36^{c}	0.25	<0.002
Survival rate (%)	90.57^{c}	95.0^{bc}	97.02^{b}	99.0^{a}	99.12^{a}	2.0	<0.015

Values in the same row with different superscripts differ significantly (p<0.05). FCR: feed conversion ratio.

Hematological and serum biochemical profile

Serum MDA was significantly lowered in fish fed diets supplemented with 1.5 g/kg (AP-1.5) and 2 g/kg (AP-2.0) of *A. platensis* relative to the control and prebiotic groups (p<0.05; Table 4). However, other parameters, including glucose, RBC, WBC, hematocrit, and hemoglobin, showed no significant differences among dietary treatments (p>0.05).

Digestive enzyme activity

Dietary supplementation with 2.0 g/kg of *A. platensis* (AP-2.0) significantly increased intestinal protease activity (0.412 U/mg protein) compared to both the control (0.300 U/mg protein) and prebiotic groups (0.349 U/mg protein) (p<0.05; Table 5). Conversely, no statistically significant modifications were registered in either amylase or lipase activities (p>0.05).

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Table 4: Hematological and biochemical parameters of rainbow trout fed experimental diets (at 56 days).

Parameter	Control	AP-1.0	AP-1.5	AP-2.0	Prebiotic	SEM	*p*-Value
MDA (µmol/dL)	60.53^{a}	54.11^{ab}	42.86^{b}	23.71^{c}	53.98^{ab}	2.49	0.011
Glucose (mg/dL)	100.32	99.99	100.11	100.44	100.08	5.05	0.320
RBC ($\times 10^{6}$ /µL)	3.55	3.56	3.49	3.50	3.57	0.03	0.357
WBC ($\times 10^{3}$ /µL)	16.77	16.69	16.74	16.80	16.75	0.91	0.285
Hematocrit (%)	37.74	37.72	36.99	37.70	37.70	0.56	0.666
Hemoglobin (g/dL)	6.10	6.09	6.00	6.12	6.02	0.66	0.210

Values in the same row with different superscripts differ significantly (p<0.05). MD: malondialdehyde; RBC: red blood cell count; WBC: white blood cell count

Table 5: Intestinal digestive enzyme activities (U/mg protein) of rainbow trout (at 56 days).

Parameter	Control	AP-1.0	AP-1.5	AP-2.0	Prebiotic	SEM	*p*-Value
Protease	0.300^{c}	0.334^{bc}	0.358 ^{ab}	0.412^{a}	0.349^{b}	0.04	0.002
Amylase	15.31	15.36	15.33	15.33	15.34	1.44	0.242
Lipase	0.024	0.023	0.023	0.020	0.022	0.008	0.113

Values in the same row with different superscripts differ significantly (p<0.05).

Intestinal microflora

Evaluation of the intestinal microflora at day 56 revealed that supplementing diets with 1.5 and 2.0 g/kg of *A. platensis* significantly increased intestinal counts of *Bacillus subtilis* compared with the control group. Coliform counts were significantly lower in the AP-1.5, AP-2.0, and prebiotic groups than in the control group (p<0.05, Table 6).

Table 6: Intestinal microflora population (Log_{10} CFU/ g) of rainbow trout (at 56 days).

Microflora	Control	AP-1.0	AP-1.5	AP-2.0	Prebiotic	SEM	*p*-Value
Bacillus subtilis	4.23^{c}	4.26^{c}	4.77^{b}	5.10^{ab}	5.98^{a}	0.44	0.003
Coliforms	3.91^{a}	3.85^{a}	3.50^{b}	2.55^{bc}	1.31^{c}	0.20	0.001

Values in the same row with different superscripts differ significantly (p<0.05).

Discussion

Modern aquaculture systems increasingly prioritize non-chemotherapeutic alternatives, such as prebiotics and cyanobacterial feed ingredients, to alleviate pathogen pressure, improve fish health, and support production efficiency (Abdel-Latif *et al*., 2025; Jouga *et al*., 2026). In this study, dietary supplementation with 2.0 g/kg *A. platensis* (AP-2.0) significantly improved final body weight and decreased the feed conversion ratio (FCR) of rainbow trout (*O. mykiss*), with no significant difference in growth performance between AP-2.0 and the positive-control prebiotic group. This aligns with recent findings demonstrating that dietary *Spirulina* can improve fish growth, although the magnitude of the response varies among fish species and experimental conditions (Trevi *et al.*, 2023; McLean *et al*., 2025; Mohammadian *et al*., 2025). Similarly, improved growth performance has been reported in largemouth bass fed diets containing *A. platensis* powder (Zhang *et al.*, 2024). However, the effects of *Spirulina* depend on its inclusion strategy. In contrast to the beneficial response observed in the present study, complete replacement of fishmeal with *A. platensis* reduced final body weight and worsened FCR in rainbow trout (Rosenau *et al.*,

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2022). This difference may be related to changes in dietary composition and nutrient utilization associated with complete fishmeal replacement, compared with the low-dose supplementation strategy used in the present study. The nutritional value of *A. platensis*, including its protein and bioactive constituents, may contribute to its potential as a functional feed ingredient (McLean *et al*., 2025; Spínola *et al*., 2024; Hajati *et al.*, 2024). The improved growth performance may be partly attributed to the high nutritional value and digestibility of *A. platensis*, which can support efficient nutrient utilization and growth in fish.

Furthermore, intestinal protease activity was significantly higher in the AP-2.0 group, whereas amylase and lipase activities were not significantly affected by dietary treatment. In contrast, dietary *A. platensis* supplementation significantly increased the activities of all three digestive enzymes in *Nile tilapia* and *thinlip mullet* (Eissa *et al.*, 2024; Abdel-Latif *et al.*, 2022). Similarly, a recent study in common carp reported increased protease and lipase activities following dietary inclusion of *Spirulina* (Harmantepe and Yılmaz, 2025). These differences suggest that digestive responses to *A. platensis* are not necessarily uniform across fish species and experimental conditions. Protease activity is directly involved in protein digestion, and its increase in the AP-2.0 group may have contributed to the improved growth performance and feed conversion observed in the present study. By contrast, the absence of significant changes in amylase and lipase indicates that supplementation at the levels tested did not produce a detectable effect on the measured activities of the enzymes involved in carbohydrate and lipid digestion. Differences in fish species, dietary formulation, and inclusion levels may help explain the contrasting digestive enzyme responses reported across studies.

A key finding of the present study was the significant reduction in serum malondialdehyde (MDA) levels in the AP-1.5 and AP-2.0 groups. MDA is widely used as a biomarker of lipid peroxidation and oxidative damage in fish (Rizzo, 2024; Lopes *et al.*, 2025). Consistent with these findings, dietary supplementation with 5–10% *A. platensis* reduced lipid peroxidation in rainbow trout and was accompanied by changes in total antioxidant capacity and antioxidant-related gene expression (Teimouri *et al.*, 2019). The antioxidant potential of *A. platensis* may be partly associated with bioactive constituents such as C-phycocyanin, carotenoids, and other antioxidant compounds (Spínola *et al*., 2024; Nazmi *et al.*, 2026). However, antioxidant responses to dietary algal ingredients are not uniform across studies, as they may depend on fish species, inclusion level, dietary formulation, and experimental conditions; some studies report changes in antioxidant capacity or related responses without consistent improvements across all oxidative-stress indicators (Mohammady *et al.*, 2026). In the present study, MDA levels in AP-2.0 were also significantly lower than those in the positive-control prebiotic group, suggesting a stronger response to the highest *A. platensis* inclusion level for this specific indicator of lipid peroxidation. Overall, these findings support the potential of dietary *A. platensis* to improve oxidative

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status in rainbow trout, although the magnitude of its effects may vary with dietary and experimental conditions.

The intestinal tract plays a critical role in nutrient absorption, metabolic function, and immune defense in fish (Siddik *et al*., 2024; Abdel-Latif *et al*., 2025). In the present study, dietary supplementation with *A. platensis* at 1.5 and 2.0 g/kg significantly increased intestinal *Bacillus subtilis* counts while reducing coliform counts, suggesting a prebiotic-like effect on the intestinal microbiota. The complex carbohydrates, extracellular polysaccharides, and other structural components of *A. platensis* may contribute to these microbial shifts by influencing the availability of substrates for intestinal bacteria (Abdo *et al*., 2024; Spínola *et al*., 2024). Potential mechanisms include the production of short-chain fatty acids (SCFAs), antimicrobial compounds, and changes in intestinal pH, which may favor beneficial bacteria and restrict coliform populations (Jouga *et al*., 2026; Neissi *et al.*, 2024). Although these mechanisms were not directly assessed in the present study, the observed changes in bacterial counts suggest that *A. platensis* supplementation may help promote a more favorable intestinal microbial balance. Consistent with this interpretation, recent studies have linked dietary *Arthrospira* supplementation to improvements in intestinal morphology and selected immune responses in fish (Sherif *et al.*, 2024). However, responses may vary with fish species, inclusion level, and biomass processing, as changes in intestinal microbial composition do not necessarily coincide with improvements in all immune parameters (Peralta-Sánchez *et al.*, 2025). Taken together, these findings support the potential of *A. platensis* to promote a favorable intestinal environment, although the extent to which such microbial changes translate into improved intestinal barrier function and mucosal immunity may depend on the dietary and biological context.

Conclusion

This study demonstrates that dietary supplementation with *Arthrospira platensis*, particularly at 2.0 g/kg, can improve growth performance, feed utilization, survival, and selected physiological responses in rainbow trout (*Oncorhynchus mykiss*). At the end of the 56-day trial, fish fed AP-2.0 achieved a final body weight of 56.02 g, representing a 71.3% increase compared with the control group (32.72 g), while the feed conversion ratio decreased by 16.1% (from 1.61 to 1.35). Survival increased from 90.57% in the control group to 99.0% in AP-2.0, an improvement of 8.43 percentage points. Moreover, intestinal protease activity increased by 37.3% relative to the control, whereas serum malondialdehyde (MDA), an indicator of lipid peroxidation, decreased by 60.8%. Dietary supplementation at 2.0 g/kg was also associated with a 0.87-log-unit increase in intestinal *Bacillus subtilis* counts and a 1.36-log-unit decrease in coliform counts relative to the control.

Notably, although growth performance and survival were comparable between the AP-2.0 and commercial-prebiotic groups, AP-2.0 resulted in significantly higher intestinal protease activity (18.1% higher) and lower serum MDA levels (56.1%

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lower). These findings suggest that *A. platensis* may offer specific advantages in digestive enzyme activity and the modulation of lipid peroxidation.

Overall, *A. platensis* at 2.0 g/kg appears to be a promising cyanobacterial feed ingredient for rainbow trout, combining growth performance comparable to that achieved with the commercial prebiotic with favorable effects on selected antioxidant and digestive parameters. Further studies are warranted to evaluate its cost-effectiveness, long-term effects, and suitability as an alternative or complementary ingredient to commercial prebiotics under different aquaculture conditions.

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Conflicts of Interest

The authors declare no conflicts of interest.

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