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Research Article

Application of agricultural phytohormones in carotenoid biosynthesis in *Arthrospira platensis*: effects on productivity and hematological health of *Oncorhynchus mykiss*

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ABSTRACT. *Arthrospira platensis* is a cyanobacterium of high nutraceutical value, notable for its rich protein, polyunsaturated fatty acids, essential minerals, vitamins, and bioactive pigments, including β-carotene, phycocyanin, and chlorophylls. This study evaluated the effect of agricultural hormones (AH)-indole-3-acetic acid (IAA), gibberellins (GA), and abscisic acid (ABA)-on β-carotenoid production. IAA was tested at 5 and 10 ppm. Under stable conditions, AH treatments enhanced growth, with IAA at 5 ppm achieving the highest density (1.2×10^6 filaments mL^{-1}), significantly outperforming ABA and the controls ($P < 0.05$). Pigment responses varied: chlorophyll-*a* and *b* peaked at 10 ppm, while β-carotenoids were maximized at 5 ppm. Higher concentrations (>10 ppm) inhibited growth. Biomass from AH-treated cultures was incorporated into trout fry diets and tested over 50 days. Fish fed treated *Arthrospira* showed marked improvements, with body weight increasing from 2.70 ± 0.13 to 32.70 ± 4.11 g and length from 6.23 ± 0.09 to 13.84 ± 0.575 cm ($P < 0.05$). Diets containing IAA and ABA yielded the highest daily growth rates (463.4 and 406.39 mg d^{-1}, respectively) compared to controls (323-334 mg d^{-1}). Feed efficiency, food conversion rate, and condition factor were enhanced, with survival highest in the ABA group (98.3 *vs.* 85%). Under stress conditions, treated fish exhibited enhanced hematological and biochemical responses, including elevated white blood cell counts, glucose levels, and globulin levels. Superoxide dismutase activity in blood plasma was significantly higher ($P < 0.05$), ranging from 4.76 to 6.32 U mL^{-1}. Overall, AH supplementation increased pigment yield in *A. platensis* and significantly enhanced growth, metabolism, antioxidant defense, and stress resilience in rainbow trout (*Oncorhynchus mykiss*) during early culture stages.

Keywords: *Arthrospira platensis*; rainbow trout fry; agricultural phytohormones; hematological profile; superoxide dismutase; carotenoid biosynthesis; aquafeed supplementation; fish growth and health

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INTRODUCTION

The rapid expansion of global aquaculture, driven by increasing demand for aquatic protein, has led to intensified production systems that place considerable physiological stress on farmed species and elevate the risk of infectious diseases (Banaee et al. 2022, FAO 2022). In the Andean regions of South America, rainbow trout (*Oncorhynchus mykiss*) farming is a growing industry, yet it remains vulnerable to recurrent outbreaks of both chronic and acute diseases. Notable pathogens include *Renibacterium salmoninarum* (causative agent of bacterial kidney disease), *Saprolegnia* spp., *Flavobacterium columnare*, and infectious pancreatic necrosis virus (IPNV), which have been associated with fry mortality rates as high as 80% (Ulloa-Stanojlovic et al. 2022, Romero et al. 2023). These outbreaks are often exacerbated by suboptimal management practices, including over-crowding, nutritional imbalances, and poor water quality, which frequently result in elevated stress, immunosuppression, and cumulative mortality rates of up to 50% (Banaee et al. 2022).

In response, the aquaculture sector has increasingly turned to functional feed ingredients to enhance fish health and reduce reliance on antibiotics. Microalgae, such as *Chlorella* spp. and *Arthrospira platensis*, are particularly promising due to their high protein content, balanced amino acid profile, and abundance of bioactive compounds (Hemaiswarya et al. 2011). In trout and other freshwater species, microalgal supplementation has been shown to improve growth performance, antioxidant capacity, and immune response (Kiron 2012). Key metabolites include carotenoids such as β-carotene, lutein, and zeaxanthin, as well as tocopherols, and enzymes such as superoxide dismutase (SOD), all of which contribute to physiological resilience under stress (Becker 2007, Yaakob et al. 2014, Charoonnart & Saksmerprome 2018).

Despite this potential, the widespread use of *A. platensis* in aquafeeds is constrained by its relatively low productivity and limited carotenoid yield under conventional cultivation conditions (Norsker et al. 2011, Castillo-Cruz et al. 2022). Carotenoids are essential not only for their photoprotective and photosynthetic roles in microalgae but also for their immunomodulatory, antiviral, and anti-inflammatory effects in animals, which are incapable of synthesizing these compounds endogenously (Hegazy et al. 2020). Therefore, enhancing the carotenoid content of microalgal biomass represents a strategic opportunity to develop high-performance, health-promoting aquafeeds.

In plants, carotenoid biosynthesis is regulated through the isoprenoid pathway, with key enzymes such as phytoene synthase (PSY) playing central roles. Phytohormones, including indole-3-acetic acid (IAA), gibberellins (GA), and abscisic acid (ABA), have been shown to upregulate carotenoid biosynthetic pathways, promote pigment accumulation, and improve photosynthetic efficiency (Shewmaker et al. 1999, Bajguz & Piotrowska-Niczyporuk 2013). However, their application to microalgal systems remains underexplored, particularly for producing enriched biomass for aquaculture.

Although previous studies have demonstrated the benefits of *A. platensis* as a dietary supplement in aquaculture, there is a notable lack of research on the use of phytohormone-treated *A. platensis* to enhance carotenoid biosynthesis and its subsequent application in the diets of rainbow trout fry, especially under the high-altitude, stress-prone conditions typical of the Ecuadorian Andes.

This study aimed to evaluate the effects of *A. platensis* enriched with IAA, GA, and ABA on carotenoid productivity and to assess its impact on growth performance and hematological health in *O. mykiss* during early developmental stages. By optimising the nutritional profile of microalgae through phytohormonal stimulation, this research offers a novel, eco-friendly strategy for improving fry health and survival in Andean aquaculture systems.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Aquaculture Laboratory of the Universidad de las Fuerzas Armadas-ESPE, within the Pailones Fish Farming Project, located in San Fernando, Rumiñahui Canton, Pichincha Province, Ecuador. The site is situated at Hacienda "El Prado" (0°23’20”S, 78°24’44”W), at an elevation of 2,940 m above sea level. This region is classified as a montane humid forest zone, with an average temperature of 13 ± 0.89°C, annual precipitation of 1,285 mm, and relative humidity of 69.03%. Water for the aquaculture system was sourced from the Pita River.

Experimental trial on biological material and mass cultivation of *Arthrospira platensis*

This study used the microalga *A. platensis*, strain 001ESPE24, obtained from the Microbiology Laboratory of the Biotechnology Program at Universidad de las Fuerzas Armadas ESPE. Strains were selected from cultures exhibiting optimal purity

and healthy filaments. Reactivation of the strains began by inoculating 1 mL of microalgal medium containing 2×10^4 filaments mL^{-1} into 3 mL of maintenance medium (IASA n1®) in pre-autoclaved test tubes, and exposing them to white LED light for 15 days. To preserve the *A. platensis* cultures, 12 additional replicates were prepared to replace tubes compromised by contamination or cellular mortality, bringing the total to 24. After 15 days of incubation, all cultures were transferred to 500-mL Erlenmeyer flasks containing 100 mL of maintenance medium (IASA n1®) (Benalcázar 2022).

To prevent contamination, the flasks were sealed with cotton stoppers, and aeration was withheld until the culture reached 300 mL, at which point aeration commenced. Refeeding was performed every four days until a final volume of 500 mL was reached. Once the culture reached 500 mL, it was transferred to 4 L bottles containing 1 L of mass-cultivation medium (IASA 2®) and exposed to white LED light under an 18:6 photoperiod. Refeeding continued every four days until the cultures reached 4 L (Benalcázar 2022).

Absorbance measurements determined microalgal status and cellular concentration, which served as the basis for mass cultivation and the establishment of experimental treatments (absorbance: 1.4-1.6; concentration: 7.9×10^5 ± 4,546 filaments mL^{-1}). Cellular density and harvest timing were assessed by UV spectrophotometry (GENESYSTM 10S) at 540 nm (Sandoval 2017). The cultivation period lasted 34 days.

Experimental design

The experiment used a completely randomized design (CRD) with four treatments: three phytohormones and a control. The hormonal treatments applied to each experimental unit (bottle with microalgal culture) were IAA, GA, ABA, and a control. Each hormone was tested at two concentrations (5 and 10 ppm), with the control group at zero ppm. Each hormone-dose combination had three replicates, for a total of 21 experimental units. The initial culture volume was set at 1 L, with an absorbance of 0.3 (75,000 filaments mL^{-1}).

Phytohormone inoculation and spirulina harvest

Stock solutions of IAA, GA, and ABA (Sigma®) were prepared at 1 g L^{-1} in 99% ethanol, homogenized, and filtered through a 0.22 µm membrane. Two concentrations (5 and 10 ppm) were incorporated during the exponential growth phase at an absorbance of 0.7 nm. The *v/v* ratio remained below 1% to prevent cellular damage (Benalcázar 2022).

For harvesting and drying, a 30-µm nylon mesh was used, followed by freezing at -20ºC and subsequent lyophilization at -65ºC using the BIOBASE® system for 48 h. The dried material was stored in sterile containers at 4°C in complete darkness for further analysis (Benalcázar 2022).

Quantified variables

Microalgal cellular density was determined following homogenization and dilution (1:10), using a 10-µL sample placed on a slide and applying the formula:

$$DCinoculum = N \times 10^3/10 \times DF$$

where DC represents the inoculum's cellular density (filaments mL^{-1}), N is the average number of cells per filament count, 10^3 is the conversion factor for 10 µL to 1 mL, and DF is the dilution factor (Arredondo & Voltolina 2007).

$$\text{Growth rate } (\mu) = \text{Ln } x_1\text{-Ln } x_0)/ (t_1\text{-}t_0)$$

where μ represents the growth rate (filaments d^{-1}), t_1 and t_0 are the final and initial times, x_1 and x_0 are the final and initial cellular densities in the logarithmic phase (Arredondo & Voltolina 2007).

$$\text{Doubling time } (Td) = \text{Ln}(2) / \mu$$

where Td is the doubling time, Ln(2) is the natural logarithm of 2, and µ is the growth rate (cells d^{-1}) (Arredondo & Voltolina 2007).

Pigment extraction and analysis

To detect pigments in lyophilized spirulina samples, 100 mg of biomass was homogenized with stainless steel beads at 2,833 g for 1 min. The sample was rehydrated with type 1 water under refrigerated conditions, then 500 µL methanol and 500 µL chloroform were added, and the mixture was centrifuged at 5,000 *g*. The supernatant was processed multiple times until it became transparent. The final extract was diluted in methanol-acetone (8:2, *v/v*), refrigerated at -20°C until analysis (Strickland & Parsons 1972).

The analytical method used was HPLC-DAD. Following filtration through 0.45 µm PTFE membranes, an 8 µL aliquot was injected into a Poroshell 120 EC-C18 column (4.6 × 50 mm, 2.7 µm) operated at a flow rate of 1.2 mL min^{-1} and a column temperature of 38°C. The mobile phase consisted of 100% HPLC-grade methanol, and detection was conducted at 450-500 nm with a runtime of 12 min. This analysis was performed at the laboratories of the Bolívar State University (UEB, by its Spanish acronym).

Administration of spirulina-enriched diets in rainbow trout fry - biological material

In this stage, a total of 300 Spanish-line rainbow trout fry were reared in this study. The fry were evenly distributed into separate compartments within PVC tanks. Throughout the experimental period, key water quality parameters were carefully monitored and maintained as follows: average water temperature of 13 ± 0.5°C, dissolved oxygen levels above 80% saturation, pH at 8.18 ± 0.15, conductivity at 192.71 ± 6.39 µS cm^{-1}, and total dissolved solids at 94.93 ± 3.95 ppm.

Experimental design

A total of 300 rainbow trout fry were randomly distributed across 15 subdivisions (1 m^3 each) within four rectangular tanks. Each subdivision served as an independent experimental unit. Five dietary treatments were randomly assigned to these units, with three replicates per treatment (15 experimental units in total). All diets were formulated to be isoproteic and isocaloric, containing 50% crude protein specifically tailored to fry nutritional requirements. Feed rations were calculated based on the fish's initial body weight and adjusted according to the ambient water temperature. The total daily feed amount was divided into eight equal portions, administered at regular intervals throughout the day to ensure consistent and efficient nutrient delivery.

The dietary treatments consisted of:

- Basal diet (BD): standard balanced feed;
- BD with synthetic pigment Carophyll® (BD-CP);
- BD with *A. platensis* treated with indole-3-acetic acid (IAA) (BD-IAA);
- BD with *A. platensis* treated with gibberellic acid (GA) (BD-GA);
- BD with *A. platensis* treated with abscisic acid (ABA) (BD-ABA).

Hormone-treated spirulina was incorporated at 10 g kg^{-1} of the basal feed. The feeding period lasted 50 days, during which the fry reached an average weight of 32.70 g. Water quality parameters, including temperature, pH, dissolved oxygen, ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), were monitored daily. Additionally, fish were sampled every 10 days from each experimental unit for further analysis.

Physical stress test

To analyze hematological variables in the fish, after 50 days, four fish per dietary group were sampled from tanks and transferred to two rectangular tanks with a useful volume of 0.6 m^3. Each new tank housed 20 fish representing the five dietary groups. Fish in one tank were subjected to water stress (stressed), while those in the other tank remained in normal flow conditions (non-stressed).

A standardized system was developed to regulate water levels at specific intervals, simulating acute fluctuations in water height. The mechanism was based on the gravity siphon principle, utilizing a suction system characterized by the inverse relationship between pressure and fluid velocity within a closed conduit (Einarsdóttir & Nilssen 1996). Before initiating the stress protocol, the tanks were cleaned, and a pipeline with a narrow section and base-reaching cap was installed. Additionally, a vacuum chamber was created for suction and drainage.

The water exchange rate was 50.91% per hour with a flow of 0.92 m^3 h^{-1}. Water level reduction in the stressed tank occurred gradually in cycles of 8 min of filling and 4 min of drainage, lasting 12 min per cycle, for a total of 48 h.

Quantified variables

To measure morphometric and productive variables, 15 fish per experimental unit were sampled throughout the experiment.

The morphometric variables included weight (g) and length (cm); productive variables included specific growth rate (SGR, % d^{-1}), mortality (%), weight gain (mg d^{-1}), feed conversion ratio (FCR), condition factor (CF), and feed efficiency (%).

For hematological variables, fish in the stressed groups were anesthetized with eugenol (10 ppm) for blood extraction from 15 organisms per treatment. Blood samples were collected using 1-mL syringes with sodium heparin (5,000 UI mL^{-1}) to prevent coagulation. Samples were labeled and stored at 4°C for erythrocyte and leukocyte counts, hematocrit, albumin, total protein, glucose, and globulins using standard protocols. Blood plasma was stored at -20°C for subsequent SOD analysis using the Sigma-Aldrich® kit.

Statistical analysis

In the first stage, cellular productivity variables in *A. platensis* cultures were analyzed using a generalized linear mixed model (GLMM) to assess the influence of factors. Subsequently, nonparametric statistical tests (Kruskal-Wallis) were used to evaluate significant differences, followed by Dunn's test to confirm intergroup variations.

In the second stage, morphometric, productive, and hematological variables of the fish were analyzed using ANOVA. Assumptions were tested for compliance, and when violated, an Aligned Rank Transform (ART) approach was applied, allowing factorial models with interactions to be fit to nonparametric data. When significant differences were detected, post hoc tests, such as Tukey's multiple comparisons test or other nonparametric methods, were employed as necessary. Additionally, descriptive statistical measures, including means and standard deviations, were reported to characterize experimental behavior. All statistical analyses were performed using R software (v4.5.0).

RESULTS

Large-scale cultivation of spirulina: cultivation conditions

Environmental conditions were consistently regulated across all experimental units to support optimal culture performance. Average values recorded were: temperature at 19.89 ± 1.11°C, pH at 10.26 ± 0.21, salinity at 1.61 ± 0.15, and light intensity at 16.9 µmol quantum m^{-2} s^{-1}.

Cell growth and absorbance

Significant differences ($P < 0.05$) were observed over the 34-day cultivation period in filament density and absorbance. The variations were evident between the initial and final stages, confirming the influence of treatments on spirulina proliferation. The highest filament concentration was observed in the IAA treatment, reaching 1.2×10^6 filaments mL^{-1}, a substantial increase compared to other treatments. In contrast, the ABA treatment exhibited the lowest filament density, with a recorded value of 491,100 filaments mL^{-1}.

These findings are illustrated in the growth trends (Fig. 1a), where spirulina exhibited greater proliferation in the presence of stimulatory phytohormones such as IAA compared to ABA, which had a less pronounced effect on biomass production. Absorbance followed the same pattern as cell growth, with the IAA group showing the highest absorbance values relative to other treatments (Fig. 1b). Furthermore, a statistically significant correlation ($P < 0.05$) was observed between cell counts and absorbance, with 92% association.

Growth kinetics and doubling time

Productivity parameters, such as SGR and population doubling time (DT days), were affected across all treatments at phytohormone concentrations of 5 and 10 ppm. The treatment that exhibited the highest SGR and shortest DT was spirulina treated with GA, with values ranging from 0.076 ± 0.05% and 9.09 ± 0.60 filaments d^{-1}, respectively. Conversely, the application of ABA at 10 ppm resulted in lower growth kinetics and longer doubling times (0.064 ± 0.007% and 10.83 ± 1.16 days, respectively) (Table 1).

Biomass and photosynthetic pigments

The harvest of wet biomass yielded values ranging between 7.33 and 7.78 g of spirulina L^{-1}. Compared with the control group, significant differences were observed ($P < 0.05$). After lyophilization, dry matter values ranged from 0.43 to 0.61 g L^{-1}.

Phytohormones at different concentrations differentially promoted pigment synthesis in spirulina cultures. For chlorophyll-*a*, the best treatments were 5 and 10 ppm of IAA, with concentrations of 11.83 and 9.33 mg g^{-1}, respectively, whereas the control group maintained a concentration of 8.76 mg g^{-1}. Chlorophyll-*b* exhibited a similar trend, with IAA-treated groups showing concentrations of 0.72 and 0.86 mg g^{-1} of sample. Significant differences were detected between treatments and the control ($P < 0.05$).

The biosynthesis of β-carotene was influenced by IAA at both 5 and 10 ppm concentrations, with production levels of 8.4 and 6.64 mg g^{-1} of sample, respectively, significantly differing from the control group, which exhibited a concentration of 4.82 mg g^{-1} of sample ($P < 0.05$) (Fig. 2).

Experimental diets for rainbow trout feeding

The experiment began with an initial weight and length of 2.70 ± 0.13 g and 6.23 ± 0.09 cm, reaching a final weight and length of 32.70 ± 4.11 g (Fig. 3a) and 13.84 ± 0.575 cm (Fig. 3b). Under these conditions, significant differences ($P < 0.05$) were detected between treatments using phytohormone-enriched diets versus both the balanced feed and carotenoid control groups. The best-performing treatments were those that included IAA and ABA, with final body weights of 38.75 ± 0.25 and 34.42 ± 0.80 g, respectively.

Based on morphometric parameters, key productive indicators were estimated, including final biomass, FCR, feed efficiency, SGR, and daily weight gain, all of which exhibited statistically significant differences compared to the balanced feed control ($P < 0.05$). Daily growth rates in the IAA and ABA treatments ranged from 463.4 to 406.39 mg d^{-1}, whereas the control group maintained values between 323 and 334 mg d^{-1}. Notably, all treatments improved the CF, increasing from 1.12 to 1.21 (Table 2).

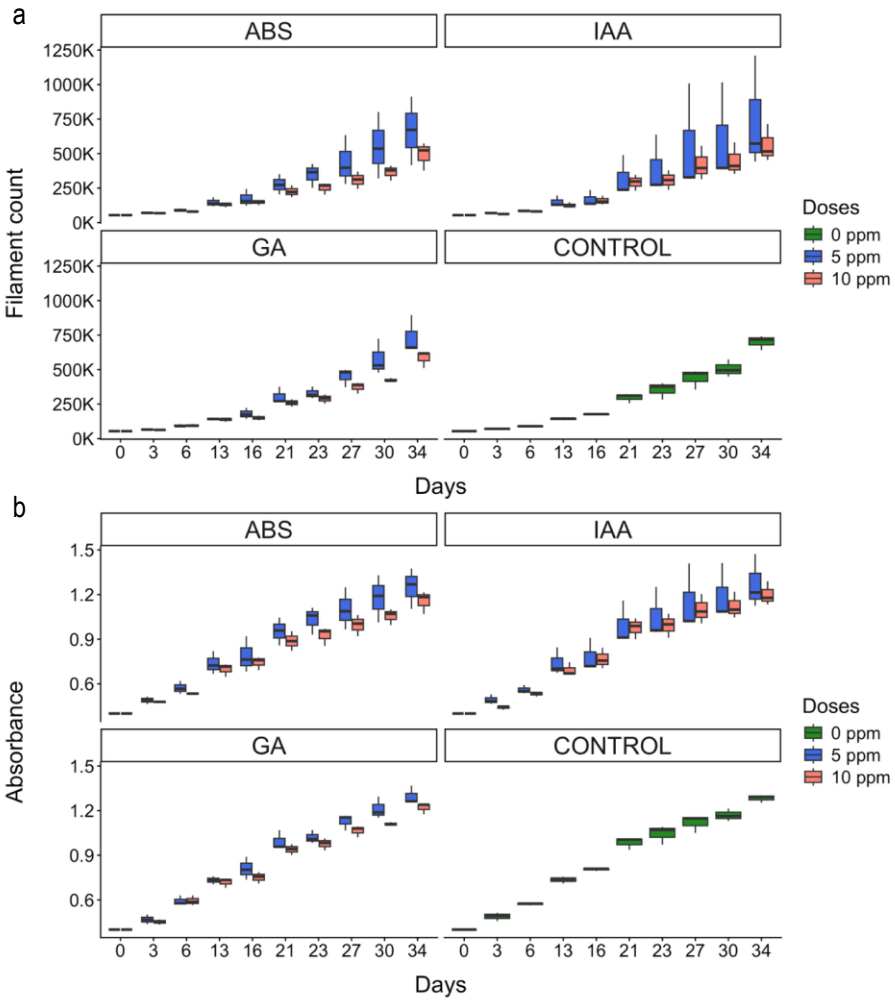

Figure 1. a) Cell growth and b) absorbance of *Arthrospira platensis* over the 34-day cultivation period, according to treatment and dosage. Values represent observed trends over time. A significant effect of time and treatment on growth was detected in the corresponding statistical analysis ($P < 0.05$). ABS: abscisic acid, GA: gibberellins, IAA: indole-3-acetic acid.

Table 1. Doubling time and specific growth rate with the addition of different types of phytohormones. IAA: indole-3-acetic acid, GA: gibberellins, and ABA: abscisic acid.

Concentration	5 ppm		10 ppm	
Phytohormones	Specific growth rate (μ)	Doubling time (days)	Specific growth rate (μ)	Doubling time (days)
IAA	0.074 ± 0.01	9.59 ± 1.80	0.068 ± 0.007	10.22 ± 0.99
GA	0.076 ± 0.05	9.09 ± 0.60	0.070 ± 0.003	9.94 ± 0.47
ABA	0.072 ± 0.01	9.74 ± 1.60	0.064 ± 0.007	10.83 ± 1.16
Control	0.075 ± 0.01	9.21 ± 0.29		

Mortality rates during the experiment varied across phytohormone treatments ($P < 0.05$). The lowest mortality rate was observed in fish treated with ABA (1.67%), while the control group fed balanced feed had a mortality rate of 15% (Table 2).

Physical stress: biochemistry variables

Significant differences ($P < 0.05$) were observed in both the hematological profile and blood chemistry under physical stress conditions. Hematocrit levels in trout fry and juveniles typically range between 40 and

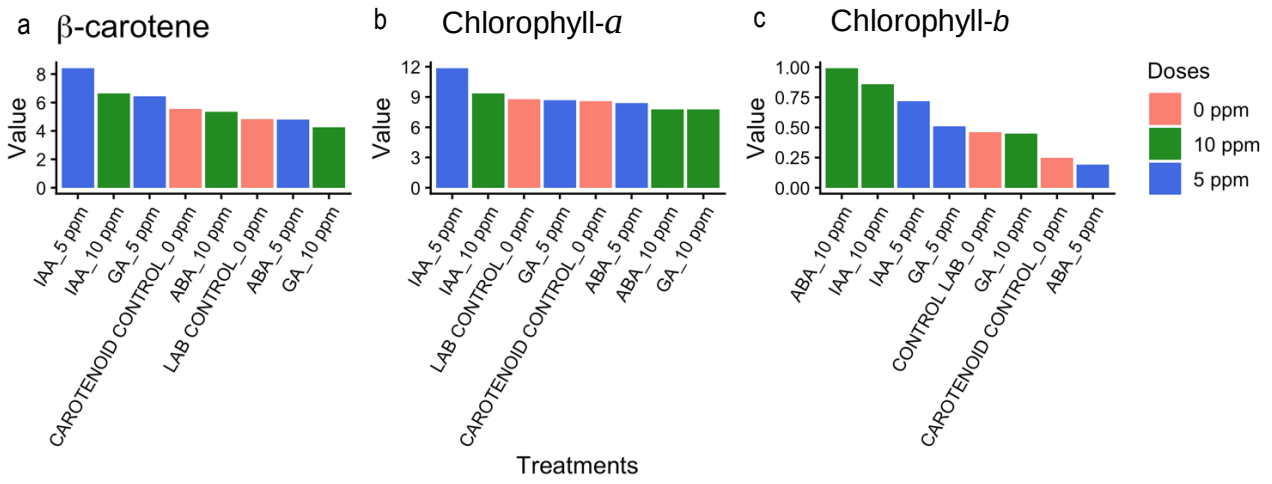

Figure 2. β-carotene, chlorophyll-*a*, and chlorophyll-*b* content (mg g^{-1}) in spirulina stimulated with phytohormones. HPLC analysis performed by UEB on lyophilized samples in the Aquaculture Laboratory of ESPE. Bars illustrate observed trends in pigment accumulation across treatments. Significant differences ($P < 0.05$) correspond to the statistical analysis described in the text. ABA: abscisic acid, GA: gibberellins, and IAA: indole-3-acetic acid.

50%, with red and white blood cell counts varying from 800,000 to 1,500,000 and 8,000 to 14,000 cells mL^{-1}, respectively.

Hematocrit values in phytohormone-modified diets under stress conditions differed significantly from those of the control groups ($P < 0.05$). Red blood cell concentrations remained similar and did not differ significantly from the control in either stressed or non-stressed conditions ($P > 0.05$). In contrast, white blood cell counts increased considerably under stress conditions and showed significant differences from the control ($P < 0.05$) (Fig. 4).

The behavior of white blood cells, such as basophils, differs from that of the balanced control group, with a tendency toward lower percentages ($P < 0.05$). Under stress conditions, eosinophilic cells are detected, albeit in low percentages. Monocyte presence increases under stress across all treatments, whereas lymphocyte levels remain unchanged, showing no significant differences ($P > 0.05$) in concentration (Table 3).

Glucose levels range from 60 to 120 mg dL^{-1}, protein from 3.5 to 5.5 g dL^{-1}, albumin from 0.8 to 2.5 g dL^{-1}, and globulins from 1.5 to 4.5 g dL^{-1}. A significant increase in glucose levels was observed in stressed fish, with statistical differences between the ABA and IAA treatments compared with the balanced feed group, with the increase reaching up to 3.16 times the normal concentration ($P < 0.05$). No significant changes were detected in protein and albumin levels ($P > 0.05$), whereas globulin concentrations in the IAA treatment differed significantly from the control group ($P < 0.05$) (Fig. 5).

SOD levels, expressed in units per milliliters, varied in blood plasma, increasing under physical stress conditions with values ranging from 4.76 to 6.32 in FA treatments. Under normal cultivation conditions, SOD levels ranged from 4.46 to 4.85 units mL^{-1}. The highest SOD levels were observed in the IAA and synthetic carotenoid treatments, showing significant differences compared to the balanced feed ($P < 0.05$) (Fig. 6).

DISCUSSION

The application of agricultural phytohormones stimulates cellular growth and biomass accumulation over short periods, enhancing spirulina cultivation productivity, which is evidenced by the cell density achieved through IAA inoculation over 34 days, reaching 741,400 filaments mL^{-1} with an absorbance of 1.57. Similar studies report filament densities ranging from 700,600 to 723,000 filaments mL^{-1}, with absorbance values between 1.41 and 1.43 over 39 to 45 days of cultivation (Sandoval 2017, Benalcazar 2022, Curipallo 2023).

The growth rates of *A. platensis* under controlled conditions in Andean regions are comparable to those observed in traditional cultivation systems, ranging from 0.0005 to 0.022%. However, these rates are significantly lower than those reported for optimized

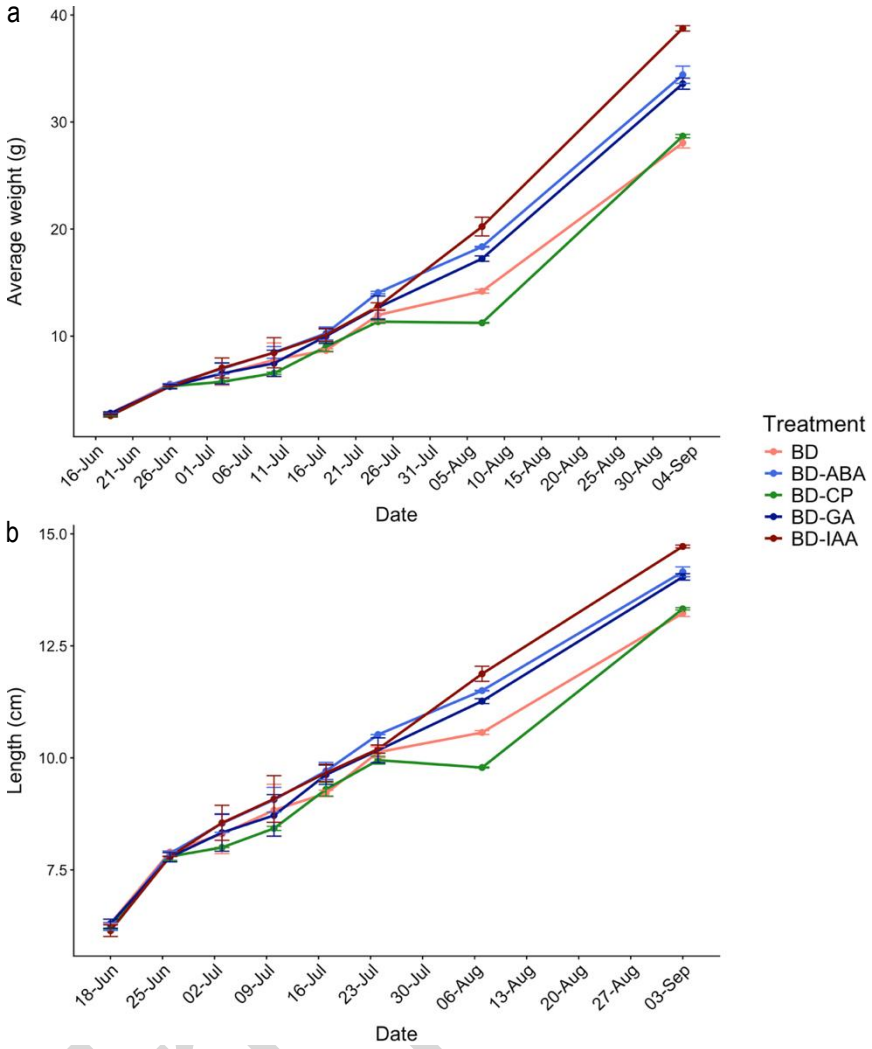

Figure 3. a) Body mass and b) total length of fish fed spirulina-based diets supplemented with different types of phytohormones. Pailones Fish Farming Project (ESPE). Statistically significant differences for both variables among treatments were observed on June 18 and 26; July 3, 10, 17, and 24; August 7; and September 3 ($P < 0.05$). ABA: abscisic acid; GA: gibberellins, IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA.

tubular bioreactor systems, which achieve growth rates of 0.182-0.24% (Ravelonandro et al. 2011, Rojas & Sáez 2022). It is important to note that cellular activity is not solely dependent on cellular promoters but is also influenced by environmental factors such as light intensity and quality, temperature, and pH, all of which regulate cellular activity and impact population doubling times, ranging from 5.43 to 8.61 d^{-1} (Sandoval 2017, Benalcazar 2022, Curipallo 2023).

Photosynthetic pigment production is affected by FA application, with results comparable to those reported by Sandoval (2017), where chlorophyll-*a* concentrations ranged from 10.86 to 11.41 mg g^{-1}. However, chlorophyll-*b* levels in the present study differed: control concentrations were 0.46 mg g^{-1}, and the IAA treatment showed an increased concentration of 0.99 mg g^{-1}, whereas Sandoval (2017) reported values between 3.26 and 3.29 mg g^{-1}. These findings align with previous studies indicating that light and phytohormone signals influence photosynthetic regulation and pigment accumulation in microalgae (García-González et al. 2005, Averina et al. 2018, Han et al. 2018).

Table 2. Productive parameters of rainbow trout (*Oncorhynchus mykiss*) farming from June to September 2024. Pailones Fish Farming Project with experimental spirulina-based diets stimulated with phytohormones. FCR: feed conversion ratio, SGR: specific growth rate, FE: feed efficiency. Statistical difference ($P < 0.05$). ABA: abscisic acid; GA: gibberellins; IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA.

Treatment	Initial biomass (g)	Final biomass (g)	FCR	FE (%)	SGR (% d^{-1})	Daily weight gain (mg d^{-1})	Condition factor (initial/final)		Mortality (%)
BD	56.10 ± 0.41	476.53 ± 20.01	2.27 ± 0.06	44.02 ± 1.16	2.95 ± 0.02	323.65 ± 5.93	1.12	1.21	15.00
BD-CP	51.27 ± 0.61	487.62 ± 2.73	2.09 ± 0.03	47.78 ± 0.62	3.10 ± 0.02	334.87 ± 2.33	1.08	1.21	15.00
BD-ABA	54.36 ± 2.27	677.17 ± 35.31	1.87 ± 0.06	53.55 ± 1.61	3.26 ± 0.07	406.39 ± 10.93	1.12	1.21	1.67
BD -GA	56.04 ± 2.67	638.33 ± 41.04	1.85 ± 0.02	54.14 ± 0.56	3.19 ± 0.07	394.63 ± 7.26	1.12	1.21	5.00
BD-IAA	51.98 ± 3.24	736.25 ± 4.75	1.77 ± 0.05	56.59 ± 1.53	3.47 ± 0.08	463.47 ± 3.30	1.12	1.22	5.00

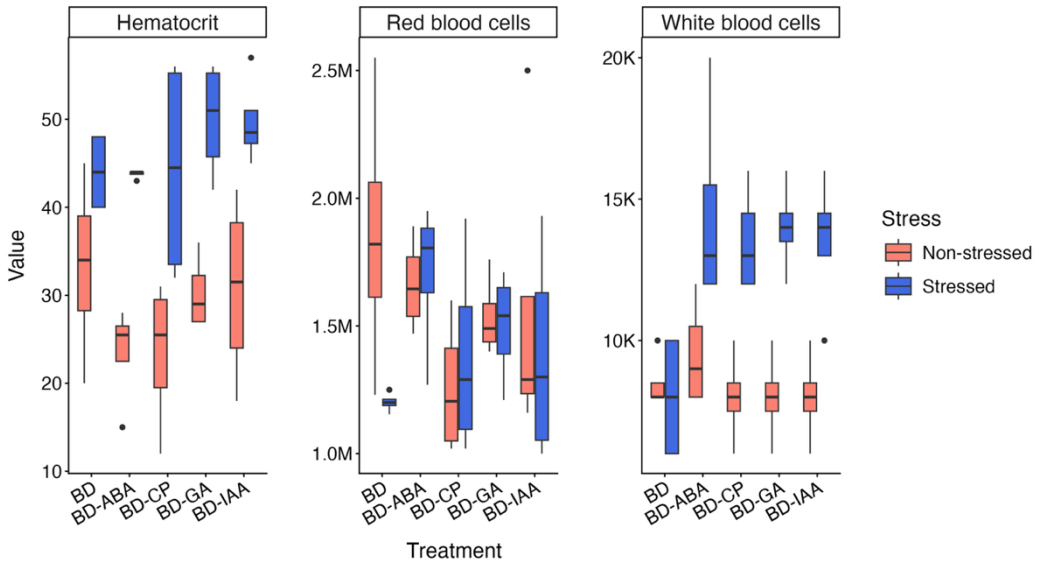

Figure 4. Hematological analysis of rainbow trout (*Oncorhynchus mykiss*) under stressed and non-stressed conditions, showing hematocrit content (%), red blood cell count (cells mL^{-1}), and white blood cell count (cells mL^{-1}). Pailones Fish Farming Project, 2024-2025. Significant effects of stress were observed for hematocrit ($P < 0.05$). White blood cell counts exhibited differences by stress and diet ($P < 0.05$). No significant differences were detected for red blood cell counts. ABA: abscisic acid; GA: gibberellins; IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA.

Chlorophyll-*a* concentrations in terrestrial plants typically range from 0.87 to 15.92 mg g^{-1}, while microalgae show comparable levels, averaging 4.18 mg g^{-1} (Liu et al. 2016, Jacob-Lopes et al. 2020). Chlorophyll-*b* in plants ranges from 0.32 to 6.42 mg g^{-1}, with a mean of 1.72 mg g^{-1}, similar to patterns observed in algal species. Moreover, chlorophyll-*f*, a pigment identified more recently, improves light absorption within the 700-800 nm wavelength range, enabling cyanobacteria to adapt their photosynthesis to environments enriched with far-red and near-infrared light (Averina et al. 2018, Jacob-Lopes et al. 2020). This process, termed far-red light photoacclimation (FaRLiP), permits the substitution of approximately 8% of chlorophyll-*a* by chlorophyll-*f*, thereby broadening photon capture and enhancing photosynthetic efficiency by 33% relative to organisms lacking this pigment (Chen & Blankenship 2011, Kurashov et al. 2019, Jacob-Lopes et al. 2020).

Table 3. Leukogram of rainbow trout (*Oncorhynchus mykiss*) under stressed and non-stressed conditions, showing the content of lymphocytes, monocytes, basophils, neutrophils, and eosinophils (%). ABA: abscisic acid; GA: gibberellins; IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA.

	Basophils (%)		Eosinophils (%)		Lymphocytes (%)		Monocytes (%)		Neutrophils (%)	
Stress	+	-	+	-	+	-	+	-	+	-
BD-ABA	10.42 ± 8.24	15.36 ± 7.24	1.39	-	62.49 ± 10.36	60.50 ± 7.62	25.70 ± 15.78	22.76 ± 2.16	-	1.39
BD-GA	10.70 ± 5.82	14.44 ± 4.81	1.47	1.56	54.25 ± 10.64	64.64 ± 7.78	32.19 ± 6.68	16.22 ± 6.53	1.39	3.14
BD-IAA	7.75 ± 6.47	9.76 ± 3.93	1.04	1.56	67.11 ± 9.27	66.38 ± 5.30	23.05 ± 7.49	20.29 ± 3.17	1.04	2
BD-CP	9.37 ± 2.32	10.99 ± 4.45	1.32	-	62.54 ± 9.29	66.72 ± 10.35	24.15 ± 6.10	21.04 ± 6.80	2.63	1.25
BD	9.37 ± 2.32	7.04 ± 2.32	1.32	-	62.54 ± 9.29	72.50 ± 9.96	24.15 ± 6.10	20.46 ± 8.15	2.63	-

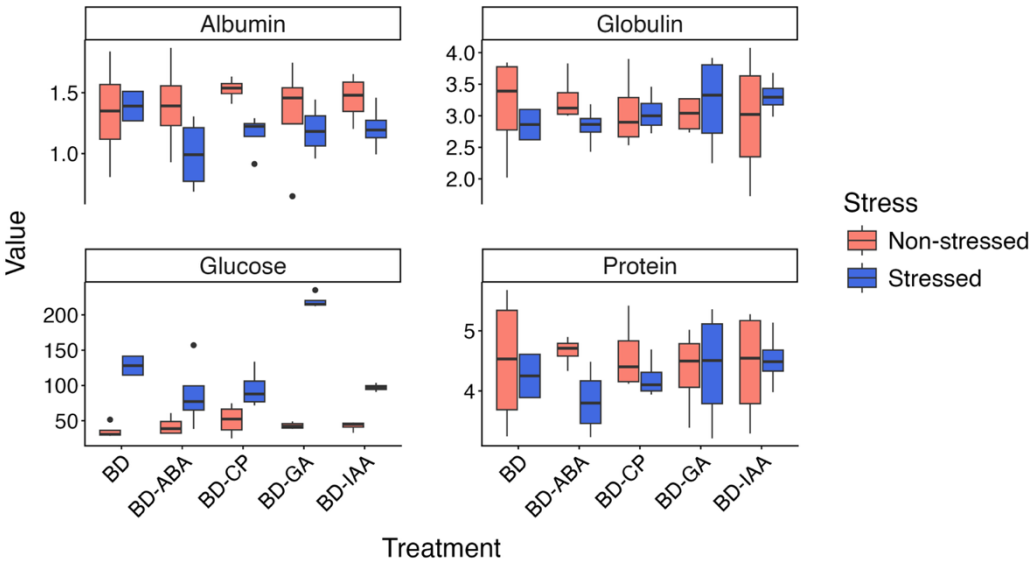

Figure 5. Blood biochemistry of rainbow trout under stressed and non-stressed conditions, showing albumin (g dL^{-1}), globulin (g dL^{-1}), glucose (mg dL^{-1}), and protein (g dL^{-1}) content. ABA: abscisic acid; GA: gibberellins; IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA. Significant increases in glucose were observed under stress, particularly in the BD-ABA and BD-IAA treatments compared with the BD group ($P < 0.05$). Globulin levels were also higher in the BD-IAA group ($P < 0.05$), whereas protein and albumin showed no significant differences ($P > 0.05$).

The production of β-carotene in spirulina showed significant variation among treatments ($P < 0.05$), indicating a positive effect of phytohormones on the carotenogenesis of *A. platensis*. β-carotene levels ranged from 4.81 to 5.35 mg g^{-1} in cultures treated with ABA, 4.27 to 6.44 mg g^{-1} with GA, and 6.64 to 8.40 mg g^{-1} with IAA, while the control group exhibited an average concentration of 4.82 mg g^{-1}.

Previous research has indicated that carotenoid content in microalgae ranges between 2 and 3% of dry weight, supporting the effectiveness of hormonal treatments in enhancing these metabolites (Alsenani et al. 2019). IAA had a significant regulatory impact on β-carotene biosynthesis in *A. platensis*, resulting in increases of 1.37-1.74 times relative to the control. These results underscore its potential use in optimizing spirulina cultivation, with significant applications in biotechnology and nutrition, particularly for developing functional supplements and biofortified foods. Studies on *Haematococcus pluvialis* and *Chlorella*

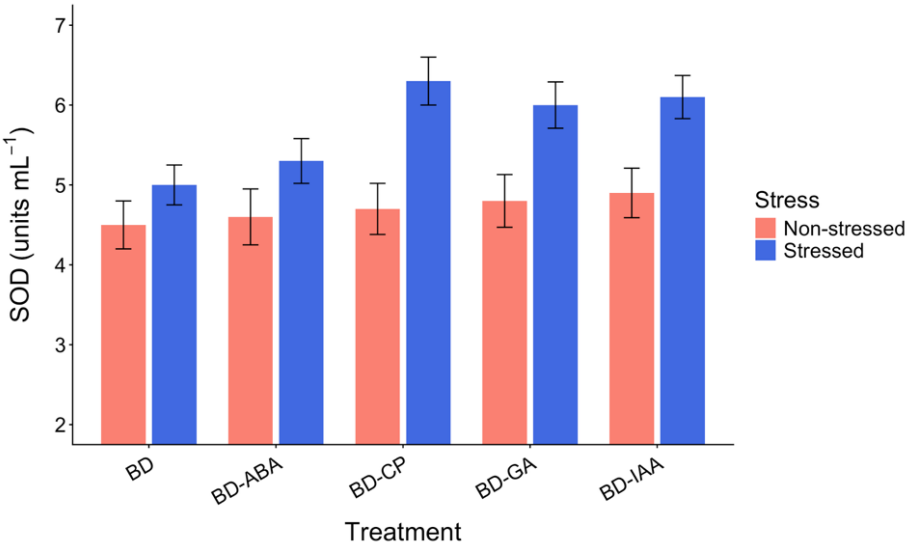

Figure 6. Expression levels of superoxide dismutase (SOD, units mL^{-1}) in experimental treatments of rainbow trout (*Oncorhynchus mykiss*) under stressed and non-stressed conditions. ABA: abscisic acid; GA: gibberellins; IAA: indole-3-acetic acid; BD: basal diet, standard balanced feed; BD-CP: BD with synthetic pigment Carophyll®; BD-ABA: BD with *A. platensis* treated with ABA; BD-GA: BD with *A. platensis* treated with GA; BD-IAA: BD with *A. platensis* treated with IAA.

vulgaris have identified phytoene synthase (PSY) expression as a critical regulatory step in carotenoid biosynthesis, showing comparable responses to hormonal induction (Fraser et al. 2007, Toledo et al. 2010, Cui et al. 2025). In green algae such as *Dunaliella*, *Haematococcus*, and *Chlamydomonas*, *psy* gene expression is positively regulated by light exposure, leading to increased carotenoid synthesis under high irradiance (Steinbrenner & Linden 2003, Pizarro & Stange 2009, Collins et al. 2011, Han et al. 2018, Sathasivam et al. 2021).

Furthermore, overexpression of *psy* in tomato plants has been demonstrated to influence gibberellin synthesis, thereby affecting structural development (Fraser et al. 2007). The findings of this study support the alternate hypothesis that phytohormone treatments enhance spirulina productivity under standard conditions, resulting in increased levels of photosynthetic pigments such as chlorophyll-*a* and β-carotene. This improvement reinforces the potential of *A. platensis* as a functional nutritional supplement for aquaculture diets within sustainable production systems.

Spirulina (*A. platensis*) is widely acknowledged as a valuable nutritional supplement in aquaculture, especially in the farming of rainbow trout (*O. mykiss*). Its biochemical composition is characterized by a high protein content, reaching up to 60% of dry weight, and by richness in polyunsaturated fatty acids, B-complex vitamins, essential minerals such as zinc, manganese, magnesium, and selenium, pigments including phycocyanin and carotenoids, and tocopherols. These features position spirulina as a crucial component for enhancing fish nutrition in intensive aquaculture systems (Becker 2007, Habib et al. 2008).

The spirulina produced in this study exhibited a significant increase in β-carotenoid and chlorophyll-*a* content, which corresponded with a measurable positive effect on trout growth. These findings align with previous research demonstrating benefits such as increased daily weight gain, improved feed conversion ratio, and reduced mortality in rainbow trout, indicating an important role in enhancing fish metabolic efficiency (Teimouri et al. 2013). Furthermore, the inclusion of these pigments has been reported to improve dermal and muscle pigmentation in rainbow trout, thereby enhancing the organoleptic properties of the product for human consumption and elevating its commercial value (Ahmadi et al. 2006, Pulcini et al. 2021).

Compared to traditional fishmeal-based diets, spirulina offers a sustainable alternative by lowering the environmental footprint of aquaculture and decreasing reliance on marine-derived protein sources (Habib et al. 2008). Research indicates that incorporating up to 5% spirulina in fish diets does not adversely affect the SGR or feed conversion FCR in rainbow trout

(Plaza et al. 2018). In contrast, optimal inclusion levels have been shown to promote biomass production, enhance morphometric traits, and improve the nutrient assimilation (Rosenau et al. 2022).

The functional potential of *A. platensis* to enhance fish immune defenses has been extensively documented. Its dietary inclusion in aquaculture has been shown to upregulate the expression of genes encoding pathogen recognition proteins in epithelial tissues, including the gills, intestines, and skin (Youssef et al. 2023). Additionally, the sulfated polysaccharides present in spirulina serve as redox modulators, thereby optimizing cellular immune responses against prevalent bacterial infections in aquaculture environments (Youssef et al. 2023). These biochemical effects contribute to reduced reliance on antibiotics in production systems, thereby reducing ecological risks associated with antimicrobial use in aquatic farming (Dawood et al. 2018, Plaza et al. 2018).

Carotenoids found in microalgae, particularly β-carotenes, are essential for the nutrition and physiological development of rainbow trout. These bioactive compounds exhibit potent antioxidant properties, protecting cells from oxidative damage caused by metabolic processes and environmental stressors (Zambrano & Landines 2011). Research has shown that diets supplemented with *Haematococcus pluvialis*, a microalga rich in astaxanthin and β-carotene, significantly enhance the immune response in rainbow trout by increasing leukocyte and erythrocyte counts in the bloodstream (Youssef et al. 2023). Similarly, supplementation with *A. platensis* has been associated with elevated activities of antioxidant enzymes, including SOD and catalase (CAT), thereby improving resistance to infections and reducing cellular inflammation (Kaulmann & Bohn 2014). These effects were also reflected in increased SOD levels and white blood cell production observed in the current study.

The positive impacts of carotenoids on rainbow trout extend beyond immune function to include blood biochemistry. Supplementation with natural pigments has been shown to elevate plasma protein levels, hemoglobin concentration, and hematocrit values, indicating improved oxygen transport and cardiovascular health in fish (Crupi et al. 2023). Furthermore, carotenoids have been reported to regulate the expression of immune-related genes, enhancing cytokine production and increasing resistance to bacterial infections across various aquatic species (Kaulmann & Bohn 2014, Galasso et al. 2017). Carotenoids increased leukocyte counts, especially lymphocytes and monocytes, and enhanced serum complement and plasma immunoglobulin levels in trout (Amar et al. 2000). Elevated thrombocyte levels additionally suggest improved coagulation capacity and strengthened defense mechanisms against pathogens (Zambrano & Landines 2011, Plaza et al. 2018, Rosenau et al. 2022, Youssef et al. 2023).

Blood biochemistry encompasses key metabolic indicators, such as glucose, plasma proteins (albumin and globulins), and liver enzymes, all critical for energy metabolism and hepatic function, for which spirulina may exert beneficial effects (Dawood et al. 2018). Research has shown that these biochemical parameters are modulated by environmental conditions, physiological stress, and, importantly, dietary composition (Teimouri et al. 2013).

From a nutritional standpoint, spirulina provides polyunsaturated omega-3 and omega-6 fatty acids that help regulate elevated low-density lipoprotein (LDL) cholesterol levels while enhancing high-density lipoprotein (HDL) concentrations, thereby promoting cardiovascular health (Crupi et al. 2023). Moreover, the carotenoids within this cyanobacterium affect glucose homeostasis and lipid metabolism, leading to decreased expression of inflammatory markers (Kaulmann & Bohn 2014).

In the context of oxidative homeostasis, research on trout emphasizes the catalytic function of SOD as a critical enzyme maintaining cellular balance. This enzyme actively facilitates the removal of oxygen-derived free radicals, thereby preserving the structural integrity of various tissues under stressors such as physical strain, high stocking density, and environmental variability (Zambrano & Landines 2011, Banaee et al. 2022). The current study reveals significant variation in SOD activity, with levels reaching up to 6.3 units mL^{-1} in the treated groups, compared with the control group, which exhibited values ranging from 4.7 to 4.8 units mL^{-1}.

Numerous studies have shown that oxidative lipid profiles can induce allosteric modulation of SOD, thereby enhancing enzymatic activity in digestive tissues. (Zambrano & Landines 2011). This physiological adaptation is associated with compensatory mechanisms involving other antioxidant enzymes, such as CAT, which form molecular protection networks to prevent degradation of cellular membranes (Dawood et al. 2018).

The biofortification of aquaculture feeds with natural pigments extracted from algae is gaining recognition as an innovative approach in preventive

nutrition. Pigments such as β-carotene, phycocyanin, astaxanthin, and chlorophyll a have been shown to activate antioxidant signaling pathways, thereby improving immunohematological parameters and decreasing markers of systemic inflammation (Rosenau et al. 2022, Crupi et al. 2023). Notably, these biomolecules not only enhance the lipoprotein profile within muscle tissues but also serve as metabolic precursors for the synthesis of immunomodulatory compounds, thereby reinforcing both innate and adaptive immune defenses in fish against pathogenic challenges (Galasso et al. 2017).

This study provides valuable insights into the dietary use of microalgae, emphasizing the synergistic role of antioxidant-rich species in the development of functional feeds for ecological aquaculture. The inclusion of *A. platensis*, notably rich in carotenoids, demonstrates promising effects on the health, growth, and overall performance of rainbow trout during early developmental stages, particularly under high-altitude aquaculture conditions in the Andean region of Ecuador. The bioavailability and stability of key pigments, such as β-carotene and phycocyanin in spirulina, are highly dependent on environmental and nutritional factors, including water temperature and nutrient inputs. Precise regulation of these variables is critical for optimizing pigment synthesis and preserving the nutritional integrity of fish, with an emphasis on advancing sustainable aquaculture systems.

Credit author contribution

J. Ortiz, F. Verdezoto and J. Galarza: conceptualized the study, validated the results, developed the methodology, performed formal analysis and drafted the original manuscript; D. Muñoz: acquired funding, managed the project, provided supervision and carried out review and editing; J. Ortiz, E. Placencia, J. Vizcarra and M. Calva: contributed to methodology development, validation, supervision and manuscript review and editing; J. Ortiz and M. Rivera: handled methodology implementation, data curation, formal analysis and review and editing; and M. Bangeppagari: reviewed and edited the English version. All authors have read and approved the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval and consent to participate

The use of microalgae in fish diets was conducted under authorization from the Ecuadorian Ministry of Environment, Water, and Ecological Transition (MAATE-DBI-CM-2022-0264). Good Aquaculture Practices (GAP) and stringent animal welfare protocols for *Oncorhynchus mykiss* were rigorously applied throughout the study by the Aquaculture Laboratory at the University of the Armed Forces (ESPE). All fish remained alive during the experimental period and were used solely for non-lethal blood sampling.

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