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Effects of dietary *Spirulina subsalsa* supplementation on growth performance and pigmentation in *Betta splendens*

Efectos de la suplementación dietética con *Spirulina subsalsa* sobre el crecimiento y la pigmentación en *Betta splendens*

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ABSTRACT | Cyanobacteria of the genus *Spirulina* contain high levels of protein, carotenoids, and phycocyanin, compounds associated with improved growth and pigmentation in ornamental fish. This study evaluated the effect of dietary inclusion of *Spirulina subsalsa* on the growth performance and pigmentation of *Betta splendens*. A completely randomized experimental design was used with five treatments: a control diet without cyanobacteria and diets containing *Spirulina subsalsa* at 5, 10, 15, and 18%. Each treatment was evaluated in triplicate using 2 L experimental units over a five-week period. Fish length and weight were measured weekly, and weight gain (WG), specific growth rate (SGR), and condition factor (K) were calculated. Fish showed growth in all treatments; however, no significant differences were detected among treatments in length, weight, WG, SGR, or K ($P > 0.05$). The highest final weight was observed in the 18% inclusion treatment (0.49 g), whereas the greatest mean lengths were recorded in the 10% (3.38 cm) and 18% (3.37 cm) treatments, with no significant differences compared with the control. No changes in fish pigmentation were observed among treatments. These results indicate that *Spirulina subsalsa* may serve as a dietary supplement but not as a complete diet for *Betta splendens*, and that pigmentation was primarily associated with genetic factors.

Palabras clave
cianobacterias
dieta
peces ornamentales
pigmentación
rendimiento de crecimiento

RESUMEN | Las cianobacterias del género *Spirulina* contienen altos niveles de proteína, carotenoides y ficocianina, compuestos asociados con un mejor crecimiento y pigmentación en peces ornamentales. Este estudio evaluó el efecto de la inclusión dietética de *Spirulina subsalsa* en el rendimiento de crecimiento y pigmentación de *Betta splendens*. Se utilizó un diseño experimental completamente aleatorio con cinco tratamientos: una dieta de control sin cianobacterias y dietas que contenían *Spirulina subsalsa* al 5, 10, 15 y 18%. Cada tratamiento se evaluó por triplicado utilizando unidades experimentales de 2 L durante un período de cinco semanas. La longitud y el peso de los peces se midieron semanalmente, y se calculó la ganancia de peso (GP), la tasa de crecimiento específica (TCE) y el factor de condición (K). Los peces mostraron crecimiento en todos los tratamientos; sin embargo, no se detectaron diferencias significativas entre los tratamientos en longitud, peso, GP, TCE o K ($P > 0,05$). El mayor peso final se observó en el tratamiento con un 18 % de inclusión (0,49 g), mientras que las mayores longitudes medias se registraron en los tratamientos con un 10 % (3,38 cm) y un 18 % (3,37 cm), sin diferencias significativas con respecto al control. No se observaron cambios en la pigmentación de los peces entre los tratamientos. Estos resultados indican que *Spirulina subsalsa* puede servir como suplemento alimenticio, pero no como dieta completa para *Betta splendens*, y que la pigmentación estuvo asociada principalmente a factores genéticos.

INTRODUCTION

Spirulina subsalsa Oersted ex Gomont 1892 is a freshwater cyanobacterium characterized by compact helical filaments and the presence of diverse photosynthetic and accessory pigments, including chlorophyll *a*, carotenoids, and C-phycocyanin. These pigments confer its characteristic blue-green coloration; however, marine species exhibiting violet-reddish pigmentation have also been reported (Torres-Ariño *et al.* 2021).

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Photosynthetic pigments such as carotenoids, chlorophylls, and phycobiliproteins play a fundamental role in fish physiology and pigmentation, by supporting antioxidant activity, immune response, and cellular protection against oxidative stress. Additionally, these compounds contribute to metabolic processes associated with growth and survival in aquatic organisms (Díaz-Jiménez *et al.* 2019).

One of the most commercially important freshwater species in the international ornamental fish trade is the Siamese fighting fish, *Betta splendens* Regan, 1910, which ranks among the 20 most widely traded ornamental fish species worldwide (Borrego-Enríquez and Muñoz 2013). This species exhibits high tolerance to fluctuations in physicochemical water parameters, facilitating its maintenance and culture under a wide range of environmental conditions. In addition, it possesses an accessory respiratory structure, the labyrinth organ, which enables aerial respiration and allows survival in environments with low dissolved oxygen concentrations, thereby reducing the need for intensive aeration systems during culture (Vu and Iwasaki 2020).

As in other teleost fish, pigmentation in *Betta splendens* is determined by both genetic and exogenous factors, primarily associated with diet (Borrego-Enriquez and Muñoz 2013). One strategy to enhance coloration in ornamental fish is the dietary inclusion of natural pigments, such as carotenoids, chlorophylls, and phycobiliproteins. These compounds can be absorbed and deposited in skin chromatophores, thereby enhancing body coloration (Ezquerra-Brauer and Chan-Higuera 2021).

The efficiency of pigment deposition in fish is strongly influenced by the chemical structure and bioavailability of dietary pigments. For instance, differences in polarity and molecular structure can affect intestinal absorption and transport efficiency, as well as the incorporation of these compounds into lipoproteins and their subsequent deposition in tissues (Díaz-Jiménez *et al.* 2021).

Among the phycobiliproteins, phycocyanin is one of the most important blue pigments in aquaculture due to its intense coloration and its antioxidant, anti-inflammatory, antimutagenic, and antimicrobial properties, which may confer physiological benefits to aquatic organisms such as fish and crustaceans (Kumar *et al.* 2014, Maza *et al.* 2018).

The inclusion of *Spirulina subsalsa* as a dietary supplement for ornamental fish may represent an alternative to enhance growth and pigmentation in *Betta splendens*. However, information regarding the influence of this cyanobacterium on the growth and coloration of ornamental fish remains limited. Therefore, the objective of the present study was to evaluate the effect of dietary inclusion of *Spirulina subsalsa*on the growth and pigmentation of Siamese fighting fish (*Betta splendens*).

MATERIALS AND METHODS

Culture of *Spirulina subsalsa*

The cyanobacterium *Spirulina subsalsa* was obtained from the Cyanobacteria and Microalgae Collection of Universidad del Mar and cultured in ME medium for 30 days following Cisneros-Morales (2019). Cultures were maintained in 20 L glass containers at a white light intensity of 550 lux and continuous aeration via air bubbling to maintain the cells in suspension. Additionally, the cultures were gently agitated manually to prevent sedimentation and promote homogeneous growth.
Biomass was harvested by filtration using a 30 µm mesh and subsequently dried in a forced-air oven (Felisa®, Mexico) at 59 ± 4 °C for 24 h. The dried biomass was ground to a fine powder using a grain mill and stored in vacuum-sealed bags protected from light at room temperature (23 ± 1 °C) until use in the experiment.

Pigment analysis

A total of 0.05 g of dry biomass of *Spirulina subsalsa* was used. Chlorophyll *a* and carotenoids were extracted using organic solvents (90% acetone), and the resulting extract was analyzed using a UV-Vis spectrophotometer at different wavelengths such as 665 nm (chlorophyll *a*), 480 and 510 nm (carotenoids) (Jeffrey and Humphrey 1975, Humphrey 1979, Torres-Ariño 2019). Furthermore, due to their water-soluble nature, the phycobiliproteins were extracted using phosphate buffer at pH 7 (Bennet and Bogorad 1973, Torres-Ariño 2019). The resulting extract was analyzed by UV-Vis spectrophotometry, with absorbance measured at 615 nm (C-phycocyanin), 652 nm (allophycocyanin), and 562 nm (B-phycoerythrin).

Pigmentation evaluation was performed through photographic records of Betta fish (*Betta splendens*) positioned with their fins extended on a color intensity chart. The images were subsequently used to compare coloration patterns in the skin and fins. Each color on the chart was assigned a numerical value, with 0 considered the reference point, corresponding to the initial white coloration of the fish. Color analysis was performed using the image analysis software CAPCUT®, based on the red-green-blue (RGB) color model, by comparing the white background of the image with variations in fin color intensity (Hancz *et al.* 2003).

Experimental organisms and rearing conditions

Specimens of *Betta splendens* were obtained from the same cohort, with an initial mean length of 1.74 ± 0.11 cm and a mean weight of 0.077 ± 0.015 g. Fish were randomly assigned to five experimental treatments, with three replicate aquaria per treatment and five fish per aquarium, with a total of 15 fish per treatment. Each glass aquarium (2 L) was considered an experimental unit.

Treatments consisted of diets with different inclusion levels of dried *Spirulina subsalsa* biomass (0, 5, 10, 15, and 18%), each evaluated in triplicate. Throughout the experiment, physicochemical water parameters were maintained within the following ranges: dissolved oxygen 3.5 - 4.5 mg L^{-1}, temperature 28 ± 1 °C, and pH 6.5 - 7.5. These parameters were monitored periodically throughout the experimental period.

Preparation of experimental diets

A commercial ornamental fish feed (AZOO®, Taiwan) was used as the basal diet. Experimental diets were prepared by incorporating dried *Spirulina subsalsa* biomass at proportions of 0, 2.5, 5, 7.5, and 9.0 g per 50 g of commercial feed, corresponding to dietary inclusion levels of 0, 5, 10, 15, and 18%, respectively. Thus, five experimental treatments were established, including a control diet without *Spirulina subsalsa* supplementation.
According to the manufacturer, the commercial feed had the following proximate composition: 5% moisture, 42% crude protein, 5% crude lipid, 5% crude fiber, 8% ash, and 35% nitrogen-free extract (NFE). The inclusion of *Spirulina subsalsa* enabled the formulation of diets with varying pigment concentrations, particularly C-phycocyanin.

Feeding regime and growth measurements

Fish were fed at a daily feeding rate equivalent to 9% of the total biomass per treatment over a five-week experimental period. Feeding was carried out three times daily at 09:30, 12:30, and 19:00 h. Weekly growth measurements were conducted to assess fish performance. Total length (cm) was measured using a digital caliper (precision ± 0.01 cm), while body weight (g) was recorded using a precision digital balance (precision ± 0.01 g).

Growth performance parameters

Fish growth was evaluated using growth performance and feed utilization indices derived from weekly weight and length measurements. In addition, temporal trends in fish weight and length were analyzed throughout the experimental period. Weight gain (WG), specific growth rate (SGR), and condition factor (K) were calculated as follows:

$$\mathrm{WG\ (g)} = \mathrm{W}_f - \mathrm{W}_i$$

$$SGR\ (\%\ day^{-1}) = \frac{\ln(\mathrm{W}_f) - \ln(\mathrm{W}_i)}{\mathrm{t}}(100)$$

$$K = \frac{\mathrm{W}}{\mathrm{L}^3}(100)$$

Where W_f is final weight (g), W_i is initial weight (g), and t is time (days). W is body weight (g), and L is total length (cm).

Statistical analysis

Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. When these assumptions were met, differences in weight and length among treatments were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's *post hoc* multiple comparison test was applied to identify differences among treatment means. For specific growth rate (SGR), weight gain (WG), and condition factor (K), data did not meet the assumptions of normality and/or homogeneity of variances; therefore, differences among treatments were analyzed using the Kruskal–Wallis non-parametric test, followed by Dunn's *post hoc* test for multiple comparisons, using a significance level of $\alpha = 0.05$ for all analyses. All statistical analyses were performed using Python 3.14.

RESULTS

Pigment concentration

Spectrophotometric analysis revealed significant differences in absorbance among pigments (Kruskal-Wallis, $H(3) = 9.46$, $p = 0.02$; Fig. 1). The highest absorbance was recorded for chlorophyll a (0.041 ± 0.012 mg mL^{-1}), followed by carotenoids (0.011 ± 0.0025 mg mL^{-1}), whereas C-phycocyanin (0.0037 ± 0.0012 mg mL^{-1}) and allophycocyanin (0.0036 ± 0.0011 mg mL^{-1}) exhibited lower values. *Post hoc* pairwise comparisons indicated that chlorophyll *a* absorbance was significantly higher than that of both allophycocyanin ($p < 0.01$) and C-phycocyanin ($p = 0.011$).

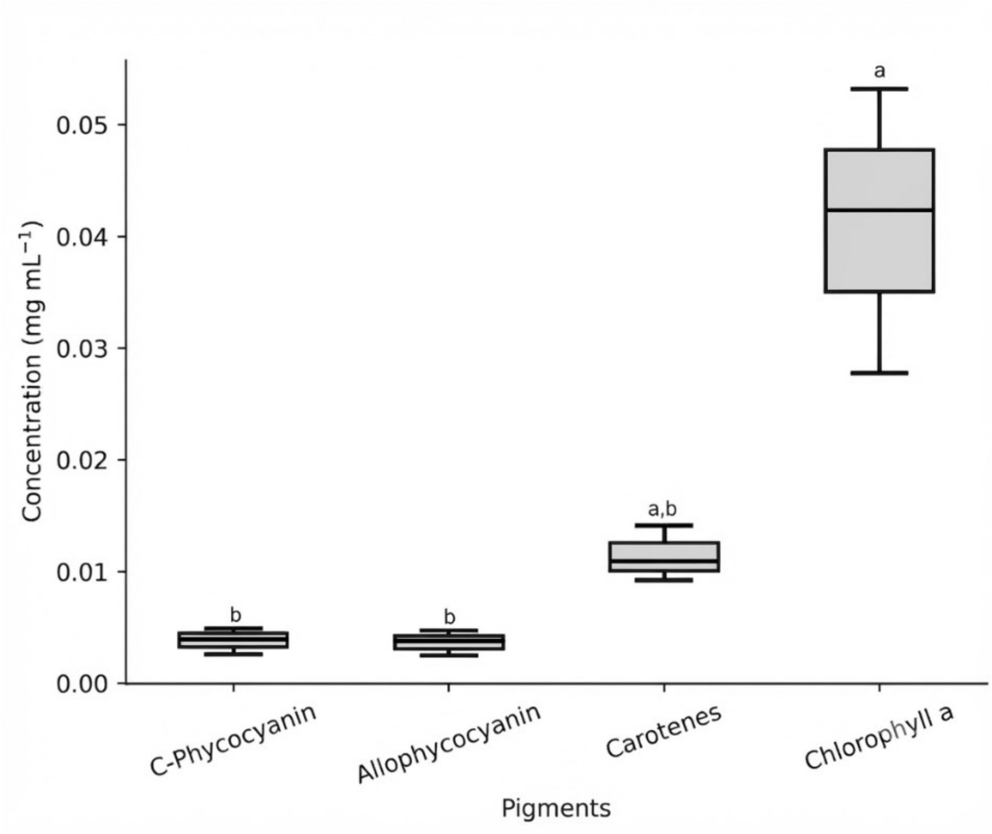

Figure 1. Absorbance of major pigments in *Spirulina subsalsa* biomass. Values are presented as median and interquartile range (IQR). Different letters indicate significant differences among pigments.
Figura 1. Absorbancia de los principales pigmentos en la biomasa de *Spirulina subsalsa*. Los valores se presentan como mediana e intervalo intercuartílico (IQR). Letras diferentes indican diferencias significativas entre pigmentos.

In the experiment using *Spirulina subsalsa* to pigment or increase the color of *Betta splendens* fish, no treatment showed a change in coloration, so they started white and ended without any change.

Growth performance

Total length differed significantly among treatments (ANOVA, $F_{4,65} = 3.37$, $p = 0.01$). The highest total length was observed in the 18% treatment (3.24 ± 0.64 cm), followed by the 5% (3.04 ± 0.24 cm), 10% (3.01 ± 0.40 cm), and 15% (2.94 ± 0.25 cm) treatments, whereas the lowest value was recorded in the control group (2.69 ± 0.33 cm). Tukey's test indicated that only the

18% treatment differed significantly from the control group ($p = 0.007$).

Weight did not differ significantly among treatments (ANOVA, $F_{4,65} = 2.33$, $p = 0.065$). The highest weight (Fig. 2) was observed in the 18% treatment (0.42 ± 0.16 g), followed by the 10% (0.37 ± 0.10 g), 5% (0.35 ± 0.08 g), and 15% (0.35 ± 0.06 g) treatments, whereas the lowest value was recorded in the control group (0.29 ± 0.08 g). Tukey's test indicated no significant differences among treatments ($p > 0.05$), although the comparison between the control and 18% treatments showed a tendency toward significance ($p = 0.06$).

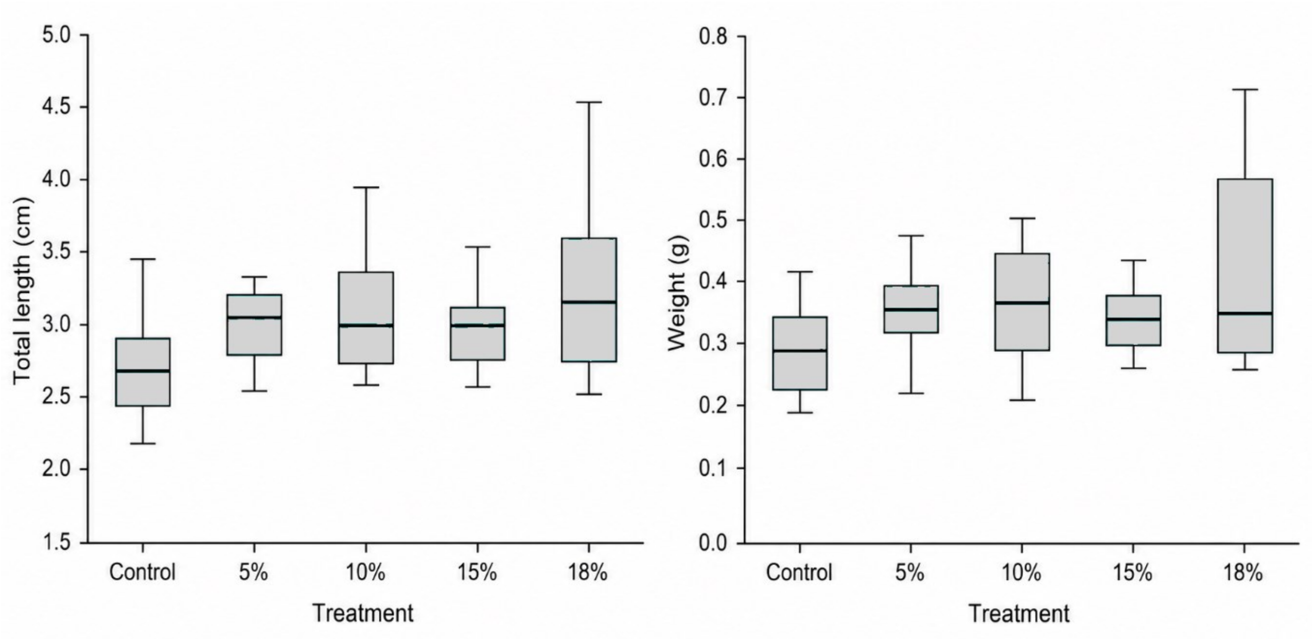

Figure 2. Final body weight (g) and total length (cm) of *Betta splendens* fed diets with different inclusion levels of *Spirulina subsalsa*.
Figura 2. Peso corporal final (g) y longitud total (cm) de *Betta splendens* alimentado con dietas con diferentes niveles de inclusión de *Spirulina subsalsa*.

Total length increased progressively throughout the experimental period in all treatments (Fig. 3). At the beginning of the trial (week 0), fish showed similar mean lengths across treatments, ranging from 1.67 ± 0.16 cm (18%) to 1.79 ± 0.08 cm (control). By week 1, length had increased in all groups, with the highest mean observed in the 18% treatment (2.06 ± 0.19 cm), followed by the 10% (1.98 ± 0.07 cm) and 5% (1.96 ± 0.02 cm) treatments. During week 2, growth continued steadily, with the 10% treatment reaching 2.38 ± 0.07 cm, slightly exceeding the other groups.

A clearer divergence among treatments became evident by week 3, when fish receiving 18% *Spirulina subsalsa* exhibited the greatest length (2.95 ± 0.14 cm) compared with the remaining treatments (2.47–2.67 cm). This pattern persisted through week 4, with the 18% treatment reaching 3.29 ± 0.21 cm, the highest value recorded during the experiment. At the end of the trial (week 5), fish fed 18% *Spirulina subsalsa* maintained the greatest mean length (3.31 ± 0.08 cm), followed by 10% (3.05 ± 0.30 cm) and 5% (3.02 ± 0.03 cm) treatments, whereas the control group showed the lowest value (2.70 ± 0.09 cm).

Body weight increased progressively throughout the experimental period in all treatments (Fig. 3). At the beginning of the trial (week 0) mean weight ranged from 0.07 ± 0.01 g in control and 5% treatments to 0.08 ± 0.01 g in the 10% and 15% treatments, whereas the 18% treatment showed a similar value (0.07 ± 0.02 g). By week 1, weight increased across all groups, with the highest mean observed in the 15% treatment (0.16 ± 0.06 g), followed by the 18% (0.14 ± 0.03 g), and the 10% (0.13 ± 0.01 g) treatments. During week 2, fish in the 5% treatment reached the highest mean weight (0.23 ± 0.02 g), slightly higher than those in 10% (0.22 ± 0.01 g) and control (0.21 ± 0.04 g) treatments. By week 3, differences in growth among treatments became more evident, with the 18% treatment exhibiting the highest weight (0.37 ± 0.02 g) compared with the other treatments (0.24–0.33 g). This pattern continued at week 4, when the 18% treatment reached 0.43 ± 0.04 g, representing the highest value recorded during the experiment. At the end of the trial (week 5), fish fed 18% *Spirulina subsalsa* maintained the greatest mean weight (0.43 ± 0.07 g), followed by 10% (0.37 ± 0.04 g) and 5% (0.35 ± 0.03 g) treatments, whereas the control group showed the lowest value (0.29 ± 0.03 g).

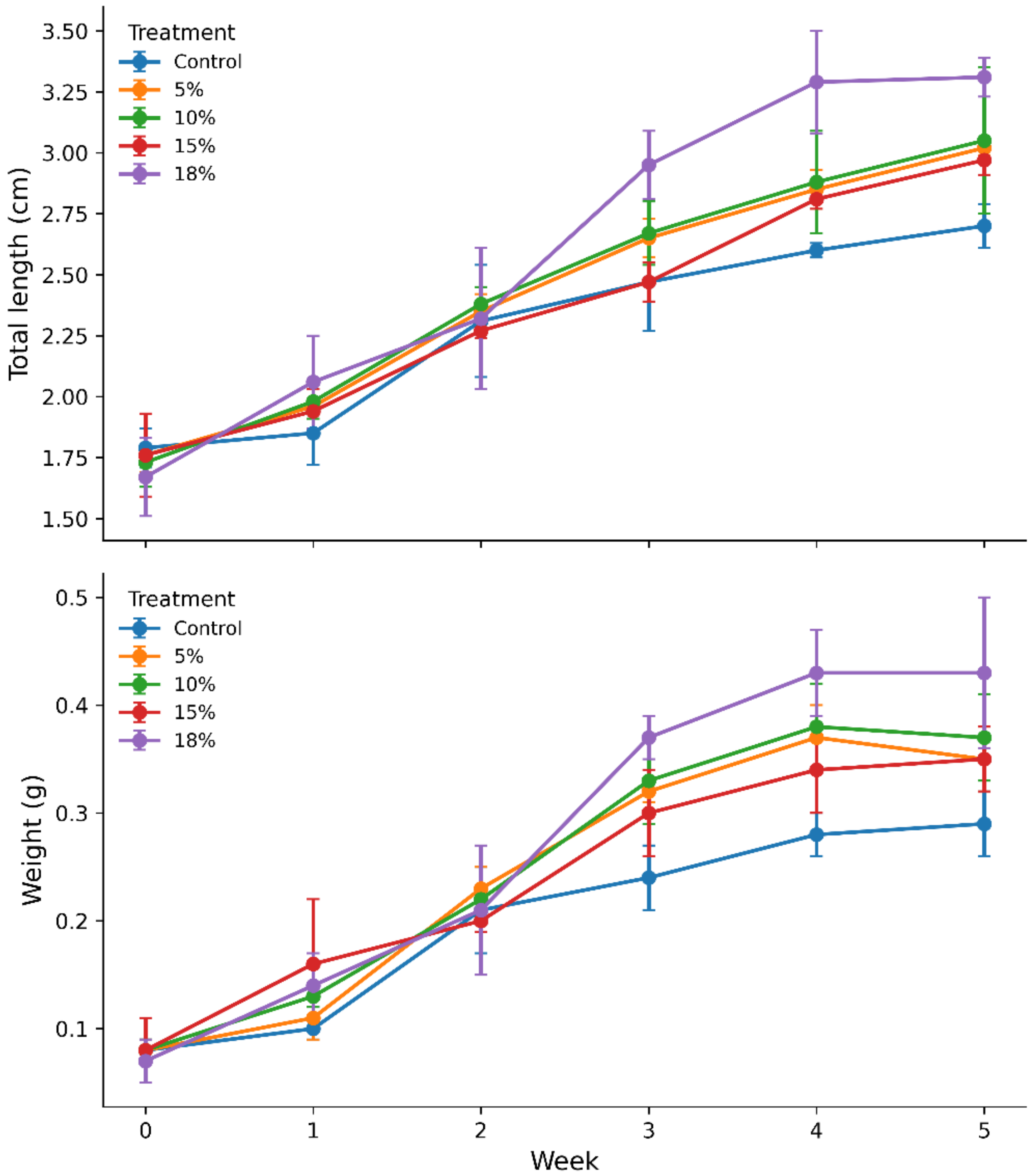

Figure 3. Growth trends in total length (cm) and body weight (g) of *Betta splendens* during the experimental period under different dietary inclusion levels of *Spirulina subsalsa*.

Figura 3. Tendencias de crecimiento en longitud total (cm) y peso corporal (g) de *Betta splendens* durante el periodo experimental bajo diferentes niveles de inclusión dietaria de *Spirulina subsalsa*.

Growth performance and condition indices

Weight gain (WG) did not differ significantly among treatments (Kruskal-Wallis, $H_4 = 8.07$, $p = 0.089$; Fig. 4). The highest WG was observed in the 18% treatment (0.36 ± 0.06 g), followed by the 10% (0.29 ± 0.03 g), 5% (0.28 ± 0.04 g), and 15% (0.27 ± 0.05 g) treatments, whereas the lowest value was recorded in the control group (0.21 ± 0.04 g).

Specific growth rate (SGR) did not differ significantly among treatments (Kruskal-Wallis, $H_4 = 6$, $p = 0.164$; Fig. 4). The highest SGR was observed in the 18% treatment (5.27 ± 0.88 % day^{-1}), followed by the 5% (4.38 ± 0.60 % day^{-1}), 10% (4.29 ± 0.16 % day^{-1}), and 15% (4.28 ± 1.07 % day^{-1}) treatments, whereas the lowest value was recorded in the control group (3.81 ± 0.68 % day^{-1}).

The condition factor (K) did not differ significantly among treatments (Kruskal-Wallis, $H_4 = 2.5$, $p = 0.79$; Fig. 4). Mean K values ranged from 1.43 ± 0.27 in the 18% treatment to 1.69 ± 0.43 in the 15% treatment, whereas intermediate values were observed in control (1.56 ± 0.25), 5% (1.54 ± 0.20), and 10% (1.60 ± 0.21) treatments.

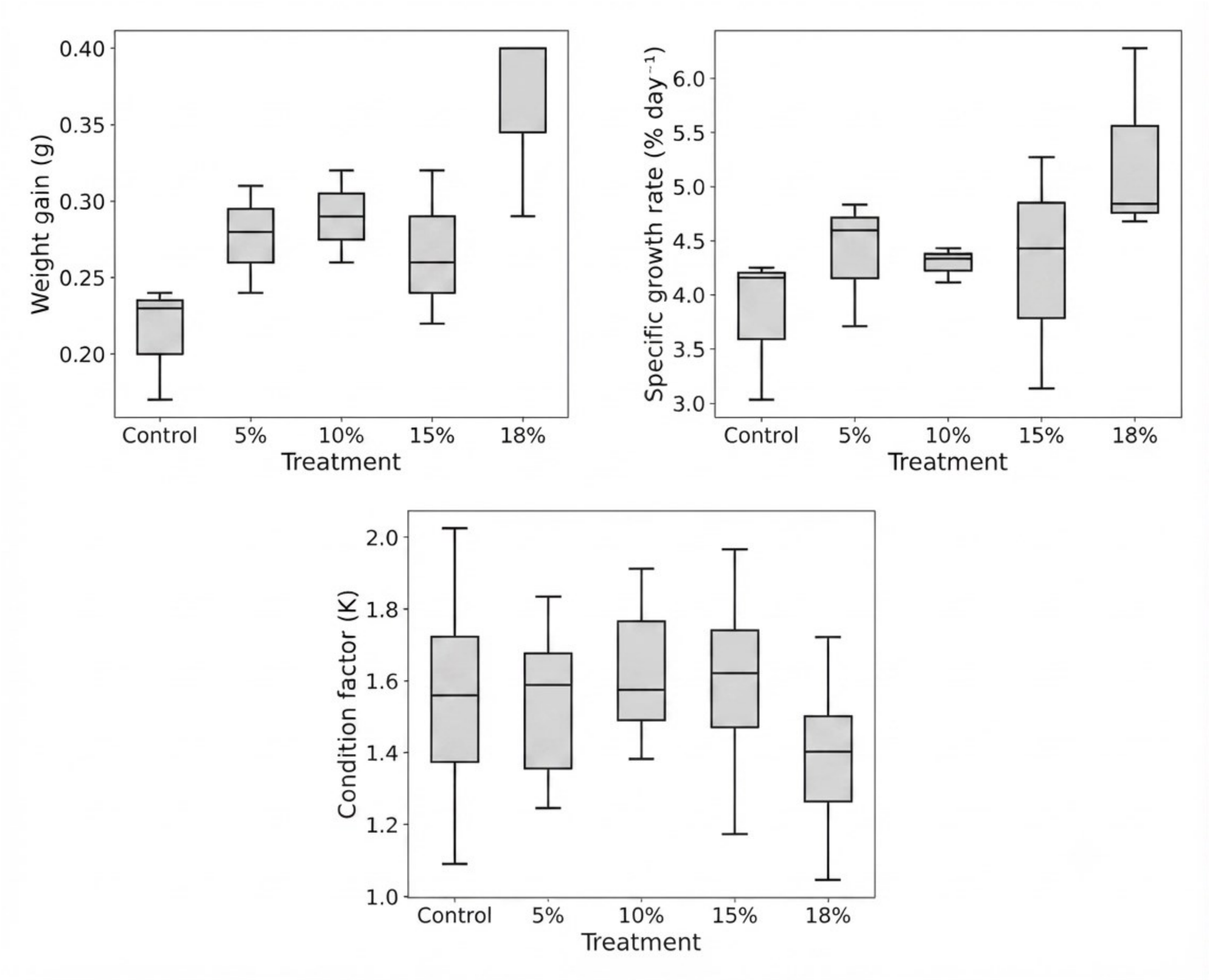

Figure 4. Growth performance parameters of *Betta splendens* fed diets with different inclusion levels of *Spirulina subsalsa*: weight gain (WG), specific growth rate (SGR), and condition factor (K).
Figura 4. Parámetros de desempeño del crecimiento de *Betta splendens* alimentado con dietas con diferentes niveles de inclusión de *Spirulina subsalsa*: ganancia de peso (GP), tasa de crecimiento específica (TCE) y factor de condición (K).

Pigmentation of *Betta splendens*

At the end of the experiment, pigmentation evaluation using photographic records showed no visible changes in the coloration of the skin or fins of *Betta splendens* (Fig. 5). According to the colorimetric scale used, all organisms maintained the initial white coloration, corresponding to a value of 0. Likewise, comparison with the chart's white background confirmed the absence of increased chromatic intensity across all evaluated treatments.

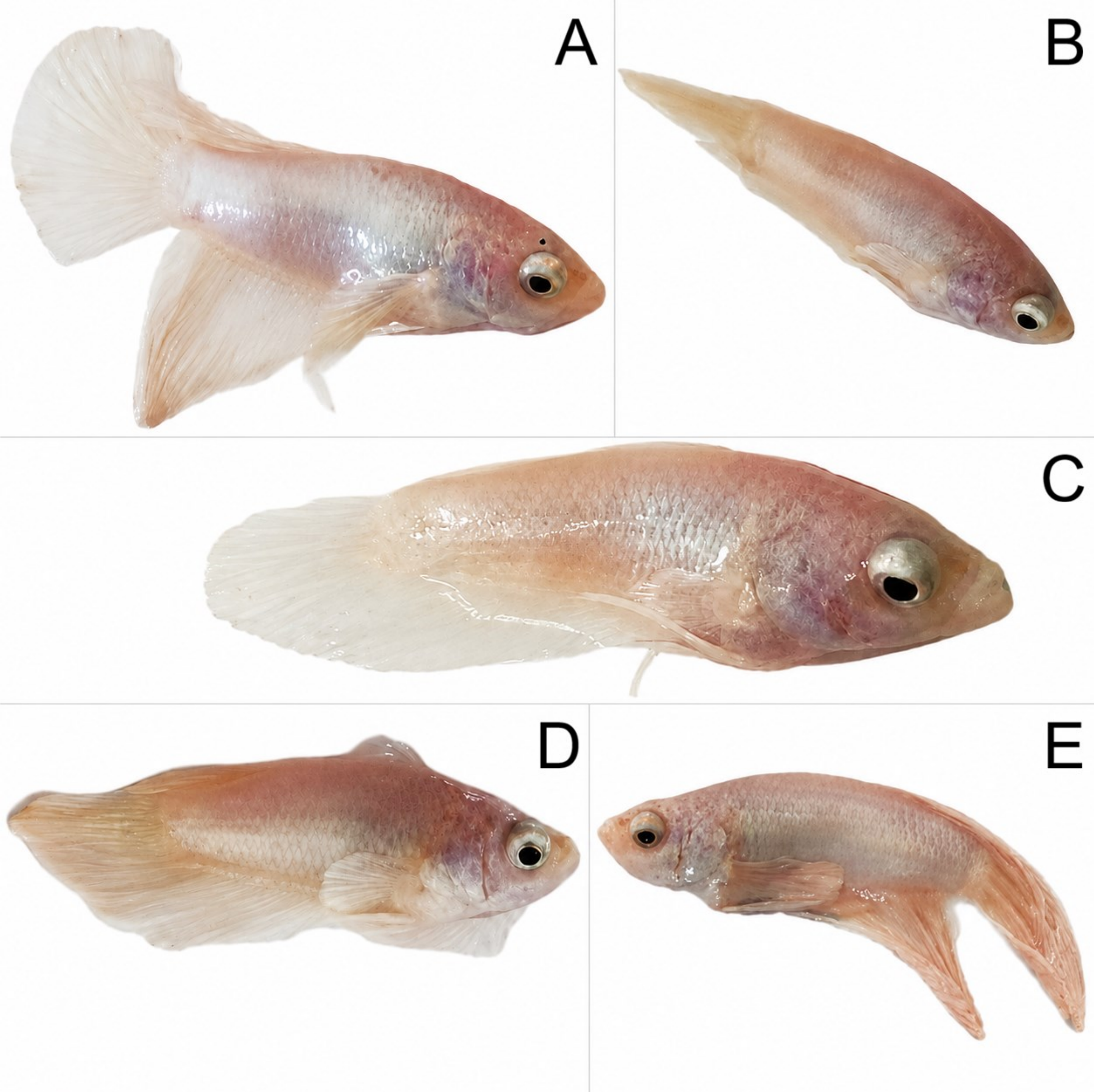

Figure 5. Visual evaluation of pigmentation in *Betta splendens* at the end of the experiment. A) Control, B) 5%, C) 10%, D) 15%, and E) 18% inclusion of *Spirulina subsalsa* in the diet.
Figura 5. Evaluación visual de la pigmentación en *Betta splendens* al final del experimento. A) Control, B) 5 %, C) 10 %, D) 15 % y E) 18 % de inclusión de *Spirulina subsalsa* en la dieta.

DISCUSSION

The pigment concentration in the biomass used for diet formulation differed from expected values, showing a higher proportion of chlorophyll *a,* carotenoids, and a lower yield of C-phycocyanin. This result contrasts with previous studies. For instance, Susanti *et al.* (2019) reported a C-phycocyanin concentration of 0.794 mg mL^{-1} (4.24 of absorbance) in *Spirulina platensis*, whereas Sadat-Ghoreyshi (2025) reported maximum values of 1.41 mg mL^{-1} (7.53 of absorbance) of C-phycocyanin, 0.034 mg mL^{-1} (0.881 of absorbance) of carotenoids, and 0.118 mg mL^{-1} (9.96 of absorbance) of chlorophyll *a* under controlled light conditions. Although pigment composition is influenced by culture conditions, light quality, and storage time, no changes in pigment concentration were detected after seven months of storage, suggesting that the low C-phycocyanin content was associated with the initial biomass yield.

During diet preparation, the pigment incorporation method proved effective, as the nutritional composition remained unchanged after the inclusion of *Spirulina subsals a*extract. Proximate analysis confirmed that nutrient composition was maintained regardless of pigment concentration, likely due to minimal thermal exposure during processing. This approach is consistent with that reported by Vivaldo-Patraca (2019) for the use of this cyanobacterium in feed formulations.

The results of the present study showed that dietary inclusion of *Spirulina subsalsa* in *Betta splendens* did not produce significant differences in growth performance parameters (WG, SGR, and K). However, numerically higher weight and length values were observed at highest inclusion level (18%). After five weeks, fish reached a mean total length of 2.98 cm (an increase of 1.31 cm) and a mean final weight of 0.35 g (a weight gain of 0.28 g). Similarly, Komilus *et al.* (2023) reported increases in body weight following the inclusion of crab shell waste, with no significant effects on other growth performance parameters or coloration.

These results contrast with studies reporting significant improvements in growth associated with microalgal supplementation. Patria *et al.* (2024) documented significant increases in growth and relative growth rate in *Betta splendens* with the inclusion of *Arthrospira maxima*, whereas Norazmi-Lokman *et al.* (2020) reported improvements in SGR and feed utilization efficiency as a function of feeding frequency. Similarly, Rincón *et al.* (2012) reported that supplementation with *Arthrospira maxima* did not significantly affect most growth performance parameters, although FCR (1.87) was lower than that observed in the present study (2.61). In the study by Saekhow (2022), under controlled conditions and over a longer experimental period (8 weeks), higher gains in weight (0.58 g) and length (1.94 cm) were reported, corresponding to approximately 0.36 g and 1.21 cm over five weeks, values slightly higher than those obtained in the present study.

The treatment with the highest inclusion level of *Spirulina subsalsa* exhibited the highest growth values, although no significant differences were detected among treatments. This pattern is consistent with Dernekbasi *et al.* (2010), who reported that increasing dietary inclusion of *S. platensis* in *Poecilia reticulata* led to greater weight gain, although differences among treatments were not statistically significant. Similarly, previous studies have shown that the effects of *Spirulina* spp. on fish growth are species-specific and dependent on inclusion levels (James *et al.* 2009, Dernekbasi *et al.* 2010, Vasudhevan and James 2011, Rahimnejad 2013, Dar *et al.* 2014, Kim *et al.* 2015, Adel *et al.* 2016). In the present study, no significant differences were detected ($P > 0.05$), indicating that supplementation did not adversely affect growth or survival, consistent with findings reported by Rojas-Villalta *et al.* (2024) in *Oreochromis niloticus*.

No changes in coloration were observed in the fish, in contrast to studies reporting significant improvements in pigmentation following dietary carotenoid supplementation. Kiswara *et al.* (2020) reported an increase in red color intensity in *Betta splendens* fed *Artemia salina* enriched with *Tagetes erecta*, whereas Yuslan *et al.* (2021) observed significant differences in the coloration of *Betta splendens* when the fish were fed copepods previously enriched with carrot and spinach. Similarly, Patria *et al.* (2024) documented increased color intensity associated with the inclusion of *Arthrospira maxima*.

Furthermore, both insufficient and excessive levels of dietary pigments may limit their effective utilization, as imbalances in pigment concentration can reduce assimilation efficiency or increase metabolic costs associated with their processing and excretion (Díaz-Jiménez *et al.* 2019).

The absence of pigmentation changes observed in the present study is consistent with Borrego-Enríquez and Muñoz (2013), who described coloration in ornamental fish as a multifactorial trait in which diet represents only one of several contributing factors. The selection of *Spirulina subsalsa* was based on its content of pigments such as chlorophyll a, carotenoids, and phycobiliproteins, which have been associated with physiological and antioxidant effects (Walia 2014, Maza *et al.* 2018, Patria *et al*. 2024). However, under the experimental conditions evaluated, these compounds did not result in observable changes in coloration.

The efficiency of pigment deposition in fish depends not only on dietary availability but also on the bioavailability and chemical nature of the compounds, as well as the capacity of the species to assimilate and transport them. Differences in polarity and molecular structure among pigments, including carotenoids and phycobiliproteins, may influence their absorption and subsequent deposition in chromatophores (Díaz-Jiménez *et al.* 2021).

Pigment deposition is also influenced by species-specific metabolic pathways, which determine the efficiency of assimilation and transformation of dietary pigments. As a result, some compounds are more readily incorporated into tissues than others, leading to variable pigmentation responses among species and experimental conditions (Díaz-Jiménez *et al.* 2019).

Finally, the five-week experimental duration and the method used to evaluate coloration differ from those reported in other studies, which may partly explain the lack of pigmentation response. For example, Vasudhevan and James (2011) reported pigmentation after 90 days of supplementation combined with vitamin C.

Despite advances in the understanding of pigment metabolism in aquatic species, information on the use of mixed pigment sources, such as cyanobacteria, in ornamental fish remains limited, particularly regarding species-specific responses and optimal dietary inclusion levels (Díaz-Jiménez *et al.* 2019).

CONCLUSIONS

Dietary inclusion of *Spirulina subsalsa* did not significantly affect growth, survival, or body condition in *Betta splendens*, indicating that its incorporation at the evaluated levels did not limit the species' productive performance. Likewise, no visible changes in pigmentation were observed, suggesting that under the evaluated conditions, supplementation with *Spirulina subsalsa* was insufficient to elicit evident phenotypic or physiological responses.

The absence of effects on coloration may be attributed to the low concentration of accessory pigments and bioactive compounds in the biomass used. In addition, using organisms from the same cohort reduced initial variability in length, weight, and coloration, allowing a more accurate interpretation of the results. In this regard, coloration in *Betta splendens* was mainly determined by genetic factors rather than dietary supplementation. Therefore, future studies should evaluate longer experimental periods, higher inclusion levels, and biomass with greater pigment concentrations.

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Declarations

The authors declare no conflict of interest.

Statement on ethical use of living organisms

This study was conducted in accordance with the regulations of the Secretaría de Agricultura y Desarrollo Rural (SADER, Mexico). All fish-handling procedures followed the Mexican Official Standard NOM-062-ZOO-1999.

Authorship contribution statement (CrediT)

C.G. Renero-Mondragón: Conceptualization, methodology, formal analysis, visualization, writing – original draft, writing – review and editing. **M.A. Hernández-de Dios:** Methodology, validation, formal analysis, visualization, supervision, writing – original draft, writing – review and editing. **A. Gallardo-Collí:** Supervision, writing – review and editing. **A. Torres-Ariño:** Conceptualization, methodology, investigation, validation, formal analysis, funding acquisition, writing – original draft, writing – review and editing.

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