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Effects of dietary supplementation with astaxanthin from *Haematococcus pluvialis* on coloration, zootechnical performance, and antioxidant activity of the ornamental fish *Cryptocentrus cinctus*

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ABSTRACT

This study evaluated the effects of astaxanthin from *Haematococcus pluvialis* on coloration, antioxidant activity, and growth performance in *Cryptocentrus cinctus*. Three dietary treatments were formulated with increasing astaxanthin concentrations: A0 (0%—control), A10 (0.01% of the total dry diet), and A15 (0.015% of the total dry diet). Astaxanthin supplementation significantly enhanced skin pigmentation, with A15 fish exhibiting a distinct yellow coloration (R = 144.75; G = 122.9; B = 48.5). Catalase activity was also the highest in the A15 group (25 UCAT mg^{-1}), suggesting improved antioxidant capacity. Zootechnical performance improved with astaxanthin inclusion, as A15 fish demonstrated greater weight gain (0.44 g), length gain (7.93 mm), and a lower feed conversion ratio (1.10). Overall, dietary supplementation with 0.015% astaxanthin from *H. pluvialis* enhanced pigmentation, antioxidant response, and growth efficiency in *C. cinctus*, representing the first report of carotenoid supplementation for this species.

Keywords: Aquaculture; Aquafeed; Gobies; Microalgae; Carotenoid.

Efeitos da suplementação dietética com astaxantina de *Haematococcus pluvialis* na coloração, no desempenho zootécnico e na atividade antioxidante do peixe ornamental *Cryptocentrus cinctus*

RESUMO

Este estudo avaliou os efeitos da astaxantina de *Haematococcus pluvialis* sobre a coloração, a atividade antioxidante e o desempenho de crescimento de *Cryptocentrus cinctus*. Foram formulados três tratamentos dietéticos com concentrações crescentes de astaxantina: A0 (0% — controle), A10 (0,01% da dieta seca total) e A15 (0,015% da dieta seca total). A suplementação com astaxantina aumentou significativamente a pigmentação da pele, com os peixes do grupo A15 apresentando uma coloração amarela distinta (R = 144,75; G = 122,9; B = 48,5). A atividade de catalase também foi maior no grupo A15 (25 UCAT mg^{-1}), sugerindo uma capacidade antioxidante aprimorada. O desempenho zootécnico melhorou com a inclusão de astaxantina, já que os peixes do grupo A15 apresentaram maior ganho de peso (0,44 ± 0,07 g), maior ganho de comprimento (7,93 mm) e menor taxa de conversão alimentar (1,10). De modo geral, a suplementação dietética com 0,015% de astaxantina de *H. pluvialis* aprimorou a pigmentação, a resposta antioxidante e a eficiência de crescimento em *C. cinctus*, representando o primeiro relato de suplementação de carotenoides para esta espécie.

Palavras-chave: Aquicultura; Ração para aquicultura; Gobídeos; Microalgas; Carotenoide.

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INTRODUCTION

Ornamental aquaculture has emerged as a promising commercial activity due to its rapid economic return, smaller space requirements compared to traditional finfish farming, and the high market value of its products. This sector has experienced global expansion, driving growth not only in ornamental fish farming but also in the production and trade of related goods (Ladisa et al., 2017; Ribeiro et al., 2010). In 2017, the global ornamental fish market was valued at approximately US$ 15 billion. Between 2000 and 2011, exports nearly doubled—from US$ 181 million to US$ 372 million—, highlighting the sector's increasing global importance (Ladisa et al., 2017). Brazil has also seen significant progress, ranking eighth among the world's leading exporters with sales surpassing US$ 18.5 million in 2014 (Dey, 2016). At the same time, the country has registered a notable increase in the import of high value-added ornamental species, reflecting a shift toward more specialized and valuable products (Cardoso et al., 2021).

A wide variety of ornamental fish species are currently cultivated commercially. The selection of suitable species for aquaculture depends on multiple factors, including production costs, technological feasibility, market demand, commercial value, and both zootechnical and aesthetic traits such as body shape and coloration. Among marine species, the genera *Chrysiptera*, *Dascyllus*, *Chromis*, *Amphiprion*, *Premnas*, and *Cryptocentrus* are widely farmed (Groover et al., 2020). Within this group, *Cryptocentrus* spp., a member of the Gobiidae family, the most diverse marine fish family, stands out as one of the four most traded groups globally, with several species already under cultivation, including *Cryptocentrus cinctus* (Dimaggio et al., 2020).

Cryptocentrus cinctus, commonly known as the yellow watchman goby, is native to sandy environments in the Western Pacific and forms mutualistic relationships with shrimp. It reaches reproductive maturity at around 75 mm in length and reproduces readily in captivity (Groover et al., 2020). Despite being a commercially attractive species and one of the most farmed, its retail price remains relatively low, particularly for individuals exhibiting a gray coloration, which limits its economic potential (Dimaggio et al., 2020). This limitation, in turn, has generated growing interest in pigmentation enhancement as a strategy to improve its market value.

Recognized as a key phenotypic trait, coloration in ornamental fish has been a longstanding focus of the industry, stimulating advances in diet formulation and technological approaches to enhance color expression (Das & Biswas, 2016; Ribeiro et al., 2010). In response, the market for species-specific feeds designed to improve pigmentation, animal welfare, and growth performance has expanded significantly in recent years (Sipaúba-Tavares et al., 2019). These feeds vary in composition—grain types and sizes, and nutritional content including proteins, lipids, carbohydrates, vitamins, and minerals—, with carotenoids playing a central role in pigmentation (Zuanon et al., 2011).

Species like *C. cinctus* rely on dietary intake of carotenoids, such as lutein, β-carotene, zeaxanthin, canthaxanthin, and astaxanthin, for pigmentation, as they cannot synthesize these compounds (Das & Biswas, 2016). Beyond their visual effect, carotenoids like astaxanthin have been shown to reduce stress, support immune function, and promote growth and reproductive performance (Ebeneezar et al., 2020).

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a carotenoid with strong antioxidant, anti-inflammatory, antitumor, and immunomodulatory properties (Ding et al., 2018). It mitigates oxidative stress by scavenging free radicals and enhancing antioxidant enzyme activity, such as catalase, which plays a key role in immune function (Sandamalika et al., 2021; Zhao et al., 2023). In aquaculture, astaxanthin is widely used not only for its pigmentation effects but also for improving the health and zootechnical performance of fish and shrimp (Jiang et al., 2019; Xie et al., 2018; Zhao et al., 2023). Dietary astaxanthin has also been reported to enhance growth performance, feed utilization efficiency, stress resistance, and reproductive success in several aquatic species (Qiang et al., 2022; Xie et al., 2018; Zhao et al., 2022). It can stimulate immune-related gene expression, modulate inflammatory pathways, and improve mucosal and humoral defense responses, thereby reducing susceptibility to pathogens (Eldessouki et al., 2022; Zhao et al., 2023). Moreover, astaxanthin supplementation has been associated with improved flesh quality and lipid stability, contributing to better product shelf life and consumer appeal (Hart & Colombo, 2022). These multiple benefits make astaxanthin a valuable functional additive in aquafeeds beyond its role as a pigment.

Although synthetic astaxanthin is the carotenoid most widely used in aquafeeds, it presents significant limitations. Its antioxidant activity is estimated to be 20 to 50 times lower than that of natural astaxanthin, and it exhibits reduced bioavailability, along with potential adverse effects (Capelli et al., 2013; Ebeneezar et al., 2020). Consequently, there is growing interest in identifying natural sources of astaxanthin. Among these, the microalga *Haematococcus pluvialis* (Chlorophyta) stands out due to its high astaxanthin content, which can reach up to 5% of its dry biomass (Kim et al., 2018). Astaxanthin accumulation in *H. pluvialis* is closely associated with environmental stress conditions, which induce morphological, physiological,

and biochemical changes, particularly high light intensity and variations in nutrient availability in the culture medium (Christian et al., 2018; Doria et al., 2018).

The objective of this study was to evaluate how diets supplemented with *H. pluvialis* biomass rich in astaxanthin influence coloration, antioxidant activity, and zootechnical performance in *C. cinctus*, to enhance its commercial value.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the Laboratório de Produção de Alimento Vivo of the Universidade Federal Rural de Pernambuco (UFRPE), following a completely randomized design. Inert diets supplemented with *H. pluvialis* biomass rich in astaxanthin were tested across three treatments: A0 (0%, control), A10 (0.01% of the total dry diet), and A15 (0.015% of the total dry diet), each with four replicates, totaling 12 experimental units. Fish were fed their respective diets, and water quality, growth, skin coloration, and catalase activity were evaluated. The study protocol was approved by the Ethics Committee on Animal Use of the UFRPE (protocol no. 2,044,191,021).

Specimen acquisition

Specimens of *C. cinctus* were obtained from a commercial ornamental fish facility and acclimated under laboratory conditions at 27°C. Prior to the trial, fish were fed the control diet twice daily (8 a.m. and 4 p.m.) for four days. Following acclimation, individuals with an initial weight of 1.43 ± 0.31 g and total length of 52.8 ± 1.07 mm were randomly assigned to individual 8-L aquaria (20 × 20 × 20 cm), with a working volume of 5 L. Aeration (0.8 L·min^{-1}) was supplied by an air blower connected to silicone tubing and porous diffusers (air stones), ensuring continuous and uniform oxygenation of the water.

Growing, harvesting, and chemical analyses of *Haematococcus pluvialis* biomass

Haematococcus pluvialis was obtained from the laboratory's culture collection and cultivated in 5-L plastic bottles at 22 ± 1°C under a 12 h light/12 h dark photoperiod and 40 µmol photons·m^{-2}·s^{-1}, provided by 36 W LED lamps, using modified Bold's Basal Medium (Table 1) (Moraes et al., 2023). Astaxanthin accumulation (at the cystic phase) was induced by increasing light intensity to 100 µmol photons·m^{-2}·s^{-1} and by supplementing the medium with 1.98 g·L^{-1} sodium acetate (Moraes et al., 2023).

At the end of cultivation, biomass was harvested by centrifugation (3,500 g, 10 min), frozen at -80°C for 24 h, and freeze-dried for 48 h (Alpha 1-4 LD Plus).

Table 1. Modified Bold's Basal Medium culture medium used in *Haematococcus pluvialis* culture.

Compound	Concentration (mg·L^{-1})
NH_4NO_3	127.3
$CaCl_2.2H_2O$	25.0
NaCl	25.0
KOH	31.0
EDTA $Na.2H_2O$	50.0
K_2HPO_4	75.0
KH_2PO_4	175.0
$FeSO_4.7H_2O$	4.98
$MgSO_4.7H_2O$	75.0
H_3BO_3	11.42
$ZnSO_4.7H_2O$	1.412
$MnCl_2.4H_2O$	0.232
$CuSO_4.5H_2O$	0.252
$Na_2MoO_4.2H_2O$	0.192
$Co(NO_3)_2.6H_2O$	0.08

Dry weight was determined using an analytical balance (± 0.001 g). Dried biomass was analyzed for protein (AOAC, 2012), using the micro-Kjeldahl method (conversion factor 6.25), lipids (Bligh & Dyer, 1959), total carotenoids, and astaxanthin content (Cheng et al., 2016). The *H. pluvialis* biomass in the red (cyst) phase exhibited protein and lipid contents of 18.11 ± 3.2% and 7.46 ± 0.9% of dry weight, respectively. Astaxanthin concentration was 10 ± 0.4 mg·g^{-1}, accounting for approximately 90% of the total carotenoids (11 ± 0.3 mg·g^{-1}). Based on these values, two levels of dietary astaxanthin supplementation were established: A10 (10 mg·g^{-1}) and A15 (15 mg·g^{-1}).

Formulation of experimental diets

The experimental diets were formulated based on the protocol described by Jiang et al. (2019) for *Pseudochromis fridmani*, with *H. pluvialis* biomass replacing an equivalent amount of carboxymethyl cellulose in treatments A10 and A15. Biomass inclusion was adjusted to provide 10 (0.01%) and 15 mg (0.015%) of astaxanthin per 100 g of feed (Table 2). Ingredients were sequentially mixed until homogenized. Fish oil was added to the dry mix, followed by water (25% of total weight). The resulting mixture was extruded into 1-mm pellets, dried at 50°C for 24 h, and stored in light-proof containers at 4°C until use.

Proximate composition (moisture, ash, protein, and lipid) was determined following the Association of Official Agricultural Chemists's (AOAC, 2012) recommendations. The carbohydrate

content was estimated by difference, subtracting the measured protein, lipid, moisture, and ash from 100% of the dry biomass. The proximate composition of the experimental diets is presented in Table 2. The diets showed similar proximate composition ($p > 0.05$), averaging 48.5% protein, 15% lipid, 6% carbohydrate, 15% ash, and 15% moisture.

Table 2 Composition of experimental diets formulated for *Cryptocentrus cinctus*.

	A0	A10	A15
Ingredient	**%**	**%**	**%**
Fishmeal[1]	67.0	67.0	67.0
Wheat flour[2]	7.8	7.8	7.8
Wheat gluten[2]	7.0	7.0	7.0
Fish oil[1]	6.0	6.0	6.0
Cornstarch[2]	5.0	5.0	5.0
Soybean oil[3]	4.0	4.0	4.0
Dicalcium phosphate[4]	0.5	0.5	0.5
Carboxymethylcellulose[5]	2.1	1.1	0.6
Haematococcus pluvialis biomass	0	1.0	1.5
Vitamin and mineral premix[6]	0.6	0.6	0.6
Proximate composition*			
Protein	47.04 ± 2.14	47.92 ± 0.30	50.45 ± 2.46
Lipid	14.30 ± 4.12	15.87 ± 1.44	15.61 ± 1.57
Carbohydrate	8.98 ± 6.03	6.01 ± 1.47	4.17 ± 2.86
Ash	15.15 ± 1.46	14.83 ± 0.23	15.16 ± 0.14
Moisture	14.51 ± 0.11	15.35 ± 0.15	14.60 ± 0.77

*Values represent mean ± standard deviation (n = 3). No significant differences were observed among treatments (Tukey's test, $p > 0.05$); [1]Bio Food Products Ltda., São Paulo, SP, Brazil; [2]Cerealista Express Ltda., São Paulo, SP, Brazil; [3]Okamoto Ltda., Goiânia, GO, Brazil; [4]N-Max, Brazil; [5]*H. pluvialis* biomass replaced an equivalent amount of carboxymethyl cellulose (Quimisul SC Ltda., Joinville, SC, Brazil) used in the control diet (A0); [6]Premix (DSM, Wagga Wagga, Australia) containing, per kg: zinc sulfate monohydrate (125 g), silicon dioxide (200 g), magnesium oxide (50 g), copper sulfate pentahydrate (3.8 g), calcium pantothenate (D form, 12 g), thiamin nitrate (vitamin B1, 4 g), nicotinic acid (22.5 g), pyridoxine hydrochloride (vitamin B6, 4 g), folic acid (vitamin B9, 1 g), manganese oxide (125 g), silicon dioxide (amorphous, 50 g) and retinyl acetate (vitamin A, 2,500,000 IU).

Feed stability

Feed stability was evaluated following Obaldo et al. (2002). In triplicate, 2 g of each feed was placed in 500 mL bottles containing 100 mL seawater (salinity 30) and maintained at 27°C with aeration for 4 h. Samples were then filtered (20 µm), dried at 105°C for 24 h, and weighed on a semi-analytical balance. Pellet stability was calculated as the percentage of dry matter retained post-leaching relative to the original dry matter. Feed stability, measured as dry matter retention, was 96 ± 2.6% (A0), 88 ± 0.5% (A10), and 87 ± 2.7% (A15).

Rearing conditions and monitoring

Each fish was maintained individually in an 8-L aquarium, with a working volume of 5 L (n = 4 per treatment). Feeding occurred twice daily (8 a.m. and 4 p.m.) at 2% body weight for 60 days. Aquaria were maintained with 5-L seawater (salinity 30) and constant aeration (0.8 L·min^{-1}). Weekly, temperature, pH (Kkmoon pH/EC-983), dissolved oxygen and salinity (MultiProbe System 5565 YSI), ammonia, and nitrite (APHA, 2012) were monitored. Units were siphoned weekly to remove waste, with a 20% water exchange. Biometric data (weight, length, height) were recorded at the start and end of the trial using a precision scale (0.1 mg) and digital calipers (Mitutoyo 530-114BR).

Zootechnical performance

The zootechnical parameters evaluated were weight gain, length gain, feed conversion ratio, specific growth rate, length-specific growth rate, and survival rate, calculated according to Eqs. 1–6:

$$\text{Weight gain (g)} = \text{Final weight} - \text{Initial weight} \quad (1)$$

$$\text{Length gain (mm)} = \text{Final length} - \text{Initial length} \quad (2)$$

$$\text{Feed conversion ratio} = \text{Dry matter intake} / \text{Biomass gain} \quad (3)$$

$$\text{Specific growth rate (\% day}^{-1}\text{)} = 100 \times (\ln \text{final weight} - \ln \text{initial weight}) / \text{Time} \quad (4)$$

$$\text{Length-specific growth rate (\% day}^{-1}\text{)} = 100 \times (\ln \text{final mean length} - \ln \text{initial mean length}) / \text{Time} \quad (5)$$

$$\text{Survival rate (\%)} = (\text{Final number of individuals} / \text{Initial number of individuals}) \times 100 \quad (6)$$

Coloration analysis

Coloration was assessed on day 0 and day 60. Images were taken using a Sony DSC-H400 camera under constant LED illumination inside a standardized photo box, with the camera positioned at 20 cm from the sample and a shooting angle of 90°. Fish were positioned on Petri dishes and anesthetized with 100 mg·L^{-1} eugenol to reduce handling stress. RGB color values were measured in Adobe Photoshop CC 2018 at three body points (head, dorsal region, caudal fin), with three replicates per point; mean values were used for analysis (Stevens et al., 2007). In addition, color parameters (L*, a*, b*) were calculated from

the same digital images according to the procedure described by Yam and Papadakis (2004), allowing for standardized color evaluation in the CIELAB color space.

Catalase antioxidant activity

Catalase (CAT) activity was determined from crude liver extracts homogenized in 20 mM potassium phosphate buffer (pH 7.4) with 0.1% Triton X-100 and 150 mM NaCl (1:20 dilution). Samples were centrifuged at 10,000 g for 10 min at 4°C. The assay mixture consisted of 2-mL potassium phosphate buffer (50 mM, pH 7), 0.05-mL H_2O_2 (0.3 M), and 0.05-mL homogenate. The decomposition of H_2O_2 was monitored at 240 nm for 60 s. CAT activity was expressed as U·mg^{-1} protein (μmol H_2O_2 min^{-1}) (Soares et al., 2021).

Statistical analysis

Data normality and homoscedasticity were verified using the Shapiro-Wilk and Bartlett's tests, respectively. Analysis of variance ($p < 0.05$) was applied to normally distributed data, followed by Tukey's post hoc test. For non-parametric data, the Kruskal-Wallis' test was used. Pearson correlation analysis was performed between variables ($p < 0.05$). Analyses were conducted using R software (R Core Team, 2022).

RESULTS

Zootechnical performance, antioxidant activity, and water quality

Dietary astaxanthin supplementation significantly affected fish performance (Table 3). The A15 diet led to the highest weight gain and length gain, along with the lowest feed conversion ratio. No significant differences were observed for survival or specific growth rates.

Catalase activity in liver tissue differed significantly among treatments ($p < 0.05$). The A15 group showed the highest activity (25 U CAT·mg^{-1}), while A0 had the lowest (12 U CAT·mg^{-1}) (Fig. 1). Water quality parameters remained within the recommended range for Gobiidae culture: dissolved oxygen 5.14 ± 0.02 mg·L^{-1}, salinity 30.6 ± 1.01 g·L^{-1}, pH 7.93–8.18, NH_4^+ < 0.50 mg·L^{-1}, NO_2^- < 0.05 mg·L^{-1}, and temperature 27.7–28.3°C.

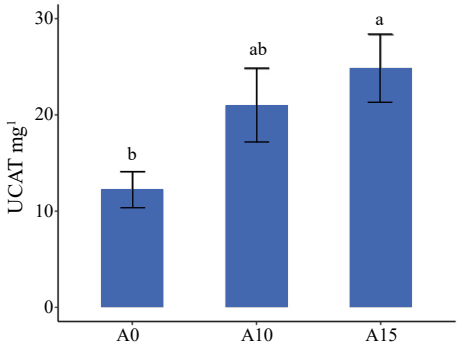


Figure 1. Catalase activity (UCAT·mg^{-1}) in *Cryptocentrus cinctus* fed diets supplemented with astaxanthin from *Haematococcus pluvialis* (A0: 0%, A10: 0.01%, A15: 0.015%). Columns represent mean values, with vertical bars indicating standard deviations for four replicates.

Coloration of *Cryptocentrus cinctus*

Fish pigmentation was significantly affected by astaxanthin supplementation. At the end of the trial, both A10 and A15 treatments showed increases in red and green values compared to the control (A0), with blue values decreasing only in A0 ($p < 0.05$). Differences between initial and final pigmentation values were significant for red, green, and blue in A10 and A15 ($p < 0.05$), while the measurement location (head, dorsal, or caudal fin) had no significant effect ($p > 0.05$). Representative images confirm the visual enhancement in pigmentation (Figs. 2 and 3).

Table 3. Zootechnical performance of *Cryptocentrus cinctus* fed with inert diets with different concentrations of astaxanthin from *Haematococcus pluvialis**.

	WG (g)	LG (mm)	FCR	SGR (%·day^{-1})	LGR (%·day^{-1})	SR (%)
A0	0.33 ± 0.10^{ab}	5.49 ± 1.12^{b}	2.10 ± 0.57^{b}	0.36 ± 0.10	0.17 ± 0.03	100
A10	0.25 ± 0.03^{b}	5.01 ± 0.56^{b}	2.00 ± 0.58^{ab}	0.43 ± 0.10	0.17 ± 0.03	100
A15	0.44 ± 0.07^{a}	7.93 ± 1.31^{a}	1.10 ± 0.16^{a}	0.49 ± 0.14	0.19 ± 0.08	100

*Values represent mean ± standard deviation (n = 4). Different letters within the same column indicate significant differences (Tukey's test, $p < 0.05$); WG: weight gain; LG: length gain; FCR: feed conversion ratio; SGR: specific growth rate; LGR: length growth rate; SR: survival rate; A0: 0% (control); A10: 0.01%; A15: 0.015%.

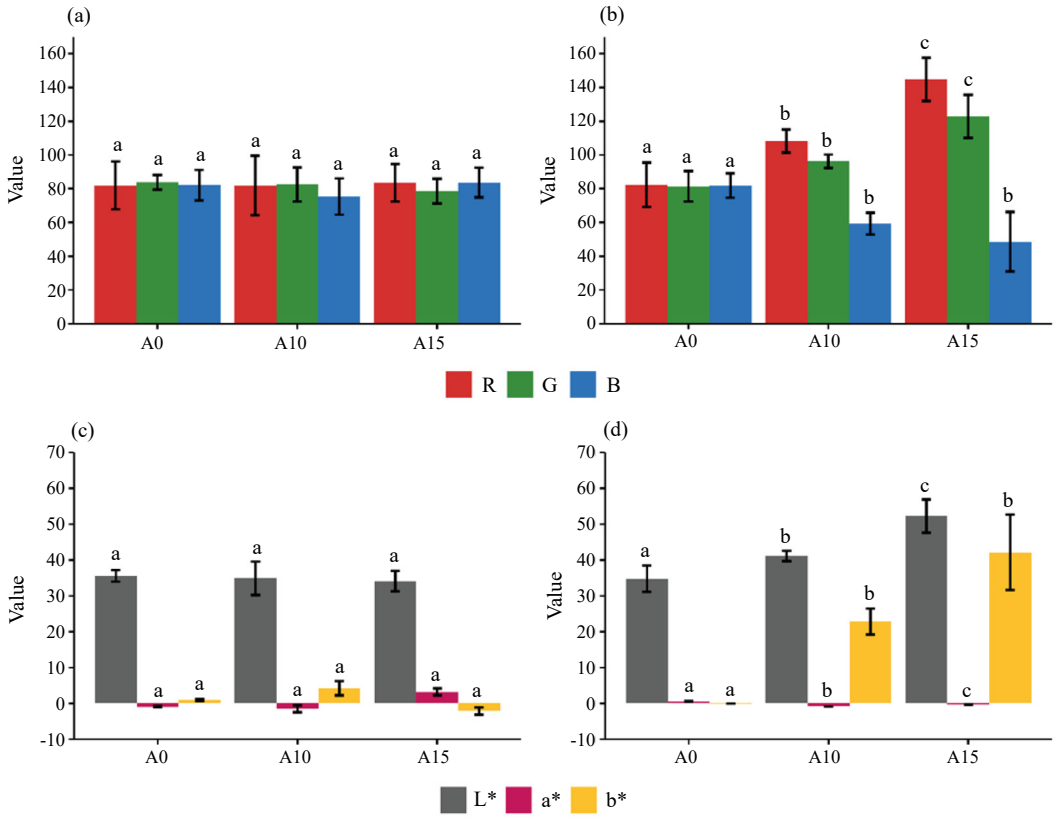


Figure 2. Mean coloration (RGB values) and (L*a*b* values) of *Cryptocentrus cinctus* fish fed inert diets containing different concentrations of astaxanthin from *Haematococcus pluvialis* (A0: 0%, A10: 0.01% of the total dry diet, A15: 0.015% of the total dry diet) at (a and c) the beginning and (b and d) the end of the experimental period. Different letters indicate significant differences between treatments by Tukey's test ($p < 0.05$). Columns represent mean values, with vertical bars indicating standard deviations for four replicates.

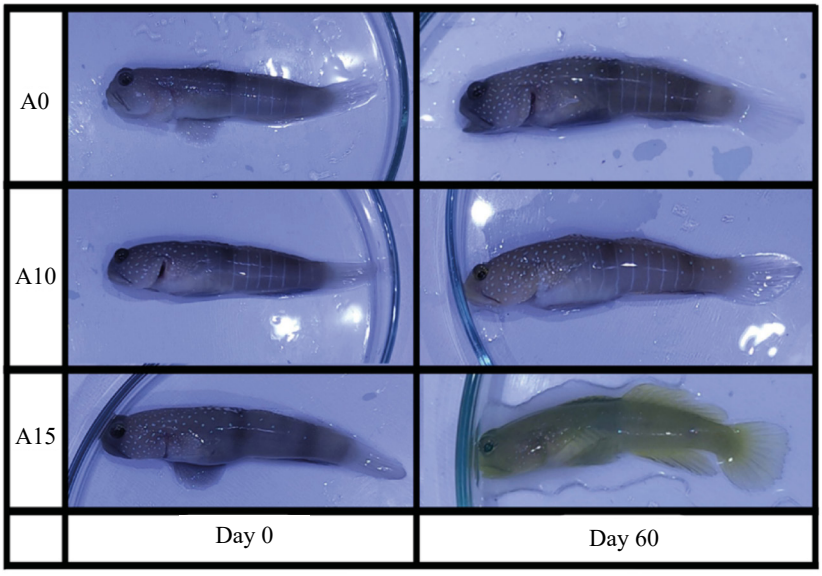
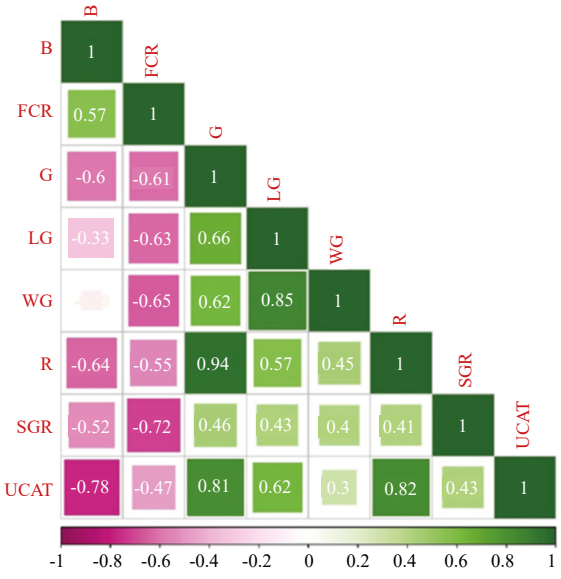


Figure 3. Effects of dietary concentration of astaxanthin-rich *Haematococcus pluvialis* (A0: 0%, A10: 0.01%, A15: 0.015%) and supplementation time (0 and 60 days) on the skin coloration of *Cryptocentrus cinctus*.

Correlation analysis

Pearson's correlation analysis ($p < 0.05$) revealed significant relationships between pigmentation, performance, and antioxidant activity (Fig. 4). RGB color channels were interrelated: red and green were positively correlated (r = 0.94), while blue was negatively correlated with both red (r = -0.64) and green (r = -0.60). Green values were also positively correlated with length gain (r = 0.66), weight gain (r = 0.62), and CAT activity (r = 0.81), and negatively with feed conversion ratio (r = -0.61). Feed conversion ratio showed strong inverse correlations with length gain (r = -0.63), weight gain (r = -0.65), and length growth rate (r = -0.72). CAT activity was positively associated with red (r = 0.82), length gain (r = 0.62), and negatively with blue (r = -0.78).



R: red; G: green; B: blue; FCR: feed conversion ratio; LG: length gain; WG: weight gain; SGR: specific growth rate.

Figure 4. Pearson correlation matrix ($p < 0.05$) among color parameters (R, G, B), zootechnical indices (FCR, WG, LG, SGR), and catalase activity (U CAT mg^{-1}). Positive correlations are shown in green, and negative correlations in pink.

DISCUSSION

Characterization of *Haematococcus pluvialis* biomass and the formulated inert diets

The reduced protein and lipid levels observed in this study are consistent with the metabolic transition of *H. pluvialis* from the vegetative (green) phase to the cystic (red) phase, during which protein content typically declines (15–23%) and lipid accumulation increases (up to 37%) (Shah et al., 2016). This substantial lipid accumulation enhances the nutritional value of *H. pluvialis* for aquafeeds, particularly due to its favorable fatty acid profile, which includes high levels of palmitic (C16:0), oleic (C18:1n9c), linoleic (C18:2n6c and C18:2n6t), and α-linolenic (C18:3n3) acids (Moraes et al., 2022).

Growing demand for sustainable and natural alternatives to conventional ingredients in aquafeeds has led to increasing research on the application of microalgae, either as feed additives or direct nutritional sources. Prominent examples include *Navicula* sp. (Abreu et al., 2019), *Nannochloropsis oculata*, *Schizochytrium* sp. (Sarker et al., 2020), *Scenedesmus obliquus* (Silva et al., 2022), and *H. pluvialis* (Zhao et al., 2022). This trend aligns with the objective of reducing the environmental impacts of fishmeal and fish oil obtained from wild fisheries, while promoting animal health and product quality (Sarker et al., 2018). Microalgae serve as a valuable source of essential nutrients, including lipids, fatty acids, sterols, proteins, amino acids, phycobiliproteins, and carotenoids (Moreno-Garcia et al., 2017; Nagappan et al., 2021).

Among these, astaxanthin from *H. pluvialis* is considered the most commercially valuable compound due to its potent antioxidant, anti-inflammatory, and pigmenting properties, with applications across the nutraceutical, pharmaceutical, and animal feed sectors (Mota et al., 2022). In terms of pellet stability, diets supplemented with *H. pluvialis* showed lower dry matter retention compared to the control (A0), likely due to the reduced content of carboxymethylcellulose, a binding agent replaced by microalgal biomass. Nonetheless, feed consumption remained unaffected, as fish typically consumed the diet shortly after feeding. Comparable findings were reported by Obaldo et al. (2002), who observed high dry matter retention (≥ 88%) after 6 hours of immersion in water. Therefore, the physical stability of the formulated diets was considered suitable for aquaculture application.

Effects of dietary astaxanthin supplementation on coloration, antioxidant activity, and zootechnical performance of *Cryptocentrus cinctus*

Coloration represents a key attribute in the ornamental fish industry, reflecting not only aesthetic value but also physiological condition, including immune function and reproductive status (Eaton et al., 2016). Since fish are incapable of endogenously synthesizing carotenoids, pigmentation is predominantly influenced by dietary intake (Jiang et al., 2019). In aquaculture, astaxanthin is frequently utilized to intensify pigmentation and enhance immune responses (Lu et al., 2021).

In this study, dietary supplementation with natural astaxanthin significantly enhanced skin pigmentation in *C. cinctus*, with the highest color intensity observed in fish fed the A15 diet (15 $mg \cdot g^{-1}$ of astaxanthin). The rise in red and green channel values in the RGB model indicates enhanced carotenoid deposition in the skin, reflecting pigment accumulation within specific chromatophore types. This effect is mainly associated with increased activity and pigment deposition in xanthophores (orange and yellow color) and erythrophores (orange and red color) (Lau et al., 2023). These findings suggest that astaxanthin was efficiently absorbed, metabolized, and incorporated into pigment cells, resulting in a visible enhancement of coloration. Similar responses have been reported in *Cichlasoma citrinellum* (Pan & Chien, 2009) and *Xiphophorus hellerii* (Putra et al., 2020), in which dietary supplementation with 16–20 $mg \cdot g^{-1}$ astaxanthin from *H. pluvialis* resulted in increased pigment deposition and more vivid coloration.

From a commercial standpoint, such enhancement of coloration directly increases the aesthetic appeal and market value of *C. cinctus*, as color intensity and uniformity are key determinants of consumer preference and price in the ornamental fish trade. Therefore, dietary supplementation with natural astaxanthin represents a practical, sustainable, and biologically effective approach to improving both the visual quality and economic potential of this species, highlighting the close relationship between nutrition, cellular physiology, and phenotypic color expression (Lau et al., 2023).

Astaxanthin also influenced antioxidant defense, as evidenced by elevated CAT activity in the A15 treatment. This carotenoid has been shown to enhance CAT function, which is essential for neutralizing reactive oxygen species like hydrogen peroxide (H_2O_2), thereby reducing oxidative stress (Sandamalika et al., 2021; Zhao et al., 2023). In aquatic environments, fish are frequently exposed to stressors that promote reactive oxygen species formation, leading to cellular damage (Elvitigala et al., 2013). Enhancing endogenous antioxidant enzymes is thus critical for maintaining fish health and supporting growth performance. Dietary supplementation with astaxanthin from *H. pluvialis* not only impacted CAT activity but also affected the zootechnical aspects of *C. cinctus*.

In addition to physiological benefits, dietary astaxanthin improved growth metrics. Fish receiving the A15 diet exhibited significantly greater weight and length gains, alongside a reduced feed conversion ratio. These results are consistent with previous studies reporting improved growth following astaxanthin supplementation (Rahman et al., 2016; Zhao et al., 2022), though other reports present contrasting findings (Micah et al., 2022; Nogueira et al., 2021). Such variability likely stems from differences in species, developmental stage, environmental conditions, carotenoid source, and feeding duration (Lim et al., 2018). Notably, *H. pluvialis*-derived astaxanthin has been shown to outperform both synthetic and yeast-derived sources in promoting intestinal health and nutrient assimilation in *Oncorhynchus mykiss* (Zhao et al., 2022). Importantly, environmental parameters during the trial remained within optimal ranges for goby cultivation (Dimaggio et al., 2020), ensuring that water quality did not interfere with the observed effects.

Pearson correlation analysis revealed significant relationships among pigmentation, antioxidant activity, and zootechnical performance. RGB color components were interdependent, with blue values inversely correlated with red and green, while red and green exhibited a strong positive correlation. As yellow hues in *C. cinctus* are achieved through a combination of high red and green and low blue values (Sun et al., 2012), the enhanced pigmentation observed in A15 suggests effective deposition and utilization of astaxanthin.

Furthermore, the significant correlation between feed conversion ratio and the green component of the RGB color space indicates that improved feed efficiency (*i.e.*, lower feed conversion ratio) may enhance astaxanthin assimilation, contributing to more intense yellow pigmentation in *C. cinctus*. The activity of the CAT enzyme may also influence this color expression, given its key role in oxidative stress mitigation and cellular detoxification (Zhao et al., 2023). Enhanced pigmentation is frequently associated with improved metabolic performance and nutrient utilization, serving as a visual indicator of overall physiological health (Ebeneezar et al., 2020).

Thus, this work helped to better assess the possible effects of adding the microalga *H. pluvialis* biomass in its red phase, in which there is a higher concentration of the carotenoid astaxanthin, on skin color, growth, and CAT activity from *C. cinctus*. To our knowledge, this is the first report evaluating carotenoid supplementation in the diet of *C. cinctus*, providing valuable baseline information for future studies aiming to optimize pigmentation, feed formulation, and production value in ornamental aquaculture.

CONCLUSIONS

This study demonstrated that supplementation of inert diets with 0.015% astaxanthin derived from *H. pluvialis* biomass enhanced skin pigmentation in *C. cinctus*, promoting more intense yellow coloration. Additionally, the A15 treatment improved antioxidant status, as indicated by increased CAT

activity, and supported better growth performance and feed efficiency. These findings highlight the potential of *H. pluvialis* biomass as a functional ingredient in ornamental fish diets. Its incorporation can improve visual appeal and physiological condition, contributing to the commercial value and sustainability of ornamental aquaculture.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

AUTHORS' CONTRIBUTION

Conceptualization: Mota, G.C.P., Moraes, L.B.S.; **Methodology:** Mota, G.C.P., Moraes, L.B.S., Ludke, M.C.M.M.; **Formal analysis:** Mota, G.C.P., Moraes, L.B.S., Campos, C.V.F.S., Ludke, M.C.M.M.; **Investigation:** Mota, G.C.P., Moraes, L.B.S., Oliveira, D.W.S., Ludke, M.C.M.M.; **Data curation:** Mota, G.C.P., Moraes, L.B.S., Oliveira, D.W.S., Campos, C.V.F.S.; **Writing — original draft:** Mota, G.C.P., Moraes, L.B.S.; **Writing — review and editing:** Campos, C.V.F.S., Oliveira, C.Y.B., Gálvez, A.O., Bezerra, R.S.; **Resources:** Ludke, M.C.M.M., Gálvez, A.O., Bezerra, R.S.; **Project administration:** Gálvez, A.O.; **Funding acquisition:** Gálvez, A.O., Bezerra, R.S.; **Supervision:** Bezerra, R.S.; **Final approval:** Moraes, L.B.S.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

We declare that no artificial intelligence tools were used in the preparation of this manuscript.

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