



Color intensity and growth performance of common clownfish (*Amphiprion ocellaris*) enhanced by dietary *Spirulina* (*Arthrospira platensis*)

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Abstract

In this study, the effect of dietary *Spirulina* (*Arthrospira platensis*) on color intensity, growth performance, total ammonia–nitrogen excretion (TAN), and profitability of common clownfish (*Amphiprion ocellaris*) was investigated. Three isoproteic (46%) and isolipidic (13%) diets were formulated as one control (C) and two different levels (15 and 30%) of *Spirulina* (SP15 and SP30) substituted for fish meal and cornmeal. Ten clownfish (initial body weight of 0.55 ± 0.04 g) per aquarium were distributed to nine glass aquariums (60 L) with three replicates. Fish were hand-fed a day to the satiation thrice. Clownfish were weighed in bulk, and the photographs were taken biweekly during the 12-week experiment. The determined regions of the images were standardized with a raster graphics editor and recorded the color parameters by considering both CMYK (cyan magenta yellow key) and RGB (red green blue) models. After the feeding trial, fish were starved for 3 days, and the total ammonia–nitrogen (TAN) was analyzed with the ammonia salicylate method on the next day for 12 h. At the end of the trial, the final mean weights (FMW) and specific growth rates (SGR) of SP15 (FMW: 1.95 ± 0.18 g, SGR: $1.75 \pm 0.04\%/day$) and SP30 (FMW: 2.05 ± 0.14 g, SGR: $1.81 \pm 0.05\%/day$) were significantly higher than the C (FMW: 1.68 ± 0.12 g, SGR: $1.58 \pm 0.06\%/day$) group ($P < 0.05$). Both color models have proved the enhancement of the skin coloration (significant improvements were recorded in the colors, including red, green, magenta, and key, $P < 0.05$). Also, it was determined that the TAN was decreased with the increase of the *Spirulina* level (29.8, 22.9, and 17.6 mg-N/100 g fish/12 h for C, SP15, and SP30, respectively) in the diet ($P < 0.05$). However, a high level of *Spirulina* in the diet negatively affected profitability. Consequently, common clownfish's growth, color, and TAN improved with the dietary *Spirulina*. Considering that *Spirulina* is an expensive raw material, adding it into the diet at medium levels (15%) is recommended.

Keywords Marine aquarium · Ornamental fish · Microalgae · Fish feeding · Pigmentation

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Introduction

Millions of freshwater and marine fish, invertebrates, and plants are traded worldwide in the aquarium industry. Although freshwater species dominate this industry, marine ornamental species have a higher economic value than freshwater fish (Groover et al. 2020). Marine aquariums have become more popular by increasing marine species considered ornamental fish. In the current perspective, it can be stated that the marine aquarium sector is worth multimillion-dollar and also the ornamental marine fish trade reached 300 million dollars (Palmtag 2017) in recent years. However, about 20–30 million marine aquarium fish are traded in a year worldwide (Wabnitz et al. 2003; Rhyne et al. 2012). But as a disadvantage of this popularity, the number and diversity of marine ornamental species collected from nature are increasing. Therefore, the importance of marine ornamental fish aquaculture is attentional. Stakeholders of the industry have begun a considerable effort to promote these species' culture and minimize the negative impacts on the wild populations of marine aquarium species (Pouil et al. 2020). Among the critical problems encountered in breeding marine ornamental fish are that the fish's nutritional needs and reproductive activities are not known precisely and that these fish do not have the same color content as their counterparts in nature during production.

It is crucial to ensure optimum conditions for the species in ornamental fish culture. Thus, enhancing culture conditions is expected to affect vital parameters such as growth performance or reproductive efficiency and other factors, including coloration. Skin coloration is a visually determining parameter in marketing strategy for the ornamental fish trade (Luo et al. 2021). Thus, it can be said that brilliant and vibrant colors determine the price of ornamental fish in the aquarium industry. However, color fading can be observed in fish for various reasons under the culture conditions. Furthermore, like other animals, fish do not synthesize their color pigments, and their coloration can be improved by administering pigment-enriched feed (Ramamoorthy et al. 2010; Güroy et al. 2012). Many plants and animal-based dietary pigment sources of carotenoids positively affect the skin coloration of fish (Luo et al. 2021). For instance, microalgae have been used widely in recent years because of the coloring of fish and the improving the growth performance of fish (Rosas et al. 2019).

Spirulina (Arthrospira platensis) is a popular microalgae species used in aquafeeds due to its widespread commercial production, rich protein content, essential fatty and amino acids, vitamins, and minerals (Rosas et al. 2019; Zhang et al. 2020). Positive effects of *Spirulina* inclusion in the ornamental fish diet on growth and pigmentation were approved by the previous studies conducted with various freshwater aquarium species (Güroy et al. 2012; Karadal et al. 2017; Biabani Asrami et al. 2019; Erdogan 2019; Kargin and Dikbaş 2020; Lili et al. 2021; Mohammadiazarm et al. 2021). Although there are previous dietary *Spirulina* applications on common clownfish, which is a widespread species of marine aquariums (Marques 2019; Hadijah et al. 2020; Díaz-Jiménez et al. 2021), no study has been conducted on the effects of this microalga on color intensity with both RGB and CMYK models. However, previous studies pointed out that this microalga is used for water quality control and treating effluents in fish farming (Wuang et al. 2016; Nogueira et al. 2018). In addition, *Spirulina* as both a dietary supplement and a live has improve the growth performance of fish and the water quality (Güroy et al. 2012; Karadal et al. 2017; Nogueira et al. 2018; Zhang et al. 2020). In this study, we tested the effect of dietary *Spirulina* on the color intensity, growth performance, total ammonia–nitrogen excretion, and profitability of common clownfish.

Materials and methods

Ethics statement

The experimental protocol was approved by the local ethics committee of Çanakkale Onsekiz Mart University, Çanakkale, Turkey, under protocol number 2021/04–03.

Rearing system and fish

Clownfish (*Amphiprion ocellaris*) were obtained from a commercial facility in İzmir, Turkey (Akvatek Aquaculture Inc.), and transferred to the Marine and Freshwater Fish Unit. Fish were fed a commercial tropical saltwater fish diet (50% protein, 7% lipid, 6.7% fiber, 7.5% ash; Sera Marin Granulat) during the acclimation period for 2 weeks, before the start of the feeding trial. Nine glass aquariums (50×40×30 cm) within 3000-L saltwater (33.85±0.22‰) sump system were used in the study. A partial marine water exchange (15% total system volume) was performed twice, weekly. The water flow rate was 3 L/min and water quality was monitored daily. Water temperature was maintained at 24.8±0.07 °C and dissolved oxygen at 7.82±0.04 mg/L (Handy Polaris, Oxy-Guard International A/S, Birkerød, DK), average pH at 8.04±0.01 (HI 9125 Hanna Instruments Inc., Woonsocket, RI, USA), and 0.05±0.01 mg/L NH₄-N (Hach Lange DR 2800, Hach Lange Loveland, Colorado, USA). The photoperiod was maintained at 12:12 h (light:dark). Clownfish (mean body weight 0.55±0.04 g) were randomly distributed among nine aquariums at the density of 10 fish per aquarium with three replicates for each dietary treatment. Fish were weighed individually at the start and the end of the trial.

Spirulina cultivation

Spirulina (*Arthrospira platensis*) used in this study was obtained from the Algae Culture Unit of Yalova University Armutlu Vocational School, Yalova, Turkey. Each culture was inoculated with an initial Spirulina biomass concentration of 0.237±0.004 OD (optical density in 680 nm wavelength measured by Hach Lange DR 2800 spectrophotometer). The temperature (29.8±0.2 °C) and pH (10.11±0.38) of culture tanks were checked daily. Culture was grown in Schlösser's medium (Schlösser 1982) in the graded volumes of Erlenmeyer flasks (250 mL to 5 L). The growing culture was adapted to glass-tube photobioreactors and then fiberglass raceway tanks. Spirulina was harvested by passing microalgae culture through the 45-µm plankton mesh when the culture's optical density reached 1.8 and above. The biomass was washed with tap water and then was filtered to obtain a dark green slurry. The harvested Spirulina biomass was dried in a tray dryer at 40 °C for 24 h and then was ground to receive the Spirulina powder.

Experimental design and diets

The feeding trial was carried out with three different experimental feeds, including control (C, without Spirulina inclusion) and 15% and 30% Spirulina-added diets (SP15 and SP30) by replacing fish meal and cornmeal (Table 1). Dietary ingredients were mixed with water in a food mixer (IBT-22, Dirmak Food Equipment, İzmir, Turkey)

Table 1 Formulation and chemical composition of the experimental diets

	C	SP15	SP30
Fish meal	43.5	33.5	23.5
Spirulina meal	-	15.0	30.0
Soybean meal	15.0	15.0	15.0
Wheat meal	15.0	15.0	15.0
Corn meal	12.8	7.8	2.8
Wheat gluten	3.0	3.0	3.0
Fish oil	8.0	8.0	8.0
Vitamin premix ^a	0.8	0.8	0.8
Mineral premix ^b	0.9	0.9	0.9
Vitamin C	0.5	0.5	0.5
Mono ammonium phosphate	0.5	0.5	0.5
Chemical composition (%)			
Crude protein	45.69	45.76	45.82
Crude lipid	12.61	12.70	12.80
Crude ash	6.36	5.07	3.79
Crude fiber	1.46	1.43	1.41
Nitrogen free extract (NFE)	33.88	35.04	36.18

^aDSM Nutritional Products, Turkey. Per g mixture: vitamin A: 4000 IU, vitamin D₃: 400 IU, vitamin E: 40 mg, vitamin K₃: 2.4 mg, vitamin B₁: 4 mg; vitamin B₂: 6 mg; calcium d-pantothenate: 10 mg, vitamin B₆: 4 mg, vitamin B₁₂: 0.01 mg, niacin: 40 mg, folic acid: 1.2 mg, biotin: 0.1 mg, vitamin C: 40 mg, inositol: 60 mg

^bDSM Nutritional Products, Turkey. Per g mixture: copper (Cu): 12.5 mg, manganese (Mn): 90 mg, cobalt (Co): 0.4 mg, zinc (Zn): 80 mg, iodine (I): 1.8 mg, selenium (Se): 0.15 mg, iron (Fe): 65 mg

until a soft, slightly moist consistency was achieved. This dough was then extruded cold press (PTM P6, La Monferrina Inc., Rome, Italy) to produce the 1-mm pellets. The moist pellets were then fan-dried and stored at $-18\text{ }^{\circ}\text{C}$ until use. Diets were given three times daily at 09:00, 12:00, and 16:00. Fish were hand-fed to the satiation for 12 weeks. Feed intake (FI) was assessed from the difference between the feed given to the fish and the uneaten feed siphoned from the aquarium 20 min after the feeding period. The FI was recorded to calculate the feed conversion ratio.

Sampling and chemical analysis

Proximate analyses of the raw materials, Spirulina (Table 2), and the diets were performed using standard methods (AOAC 2000). Dry matter was measured by drying at $105\text{ }^{\circ}\text{C}$ until a constant weight was achieved; crude lipid was determined by ether extraction, crude protein by the Kjeldahl method after acid digestion using a Behr system, and crude ash incineration at $525\text{ }^{\circ}\text{C}$ for 12 h in a muffle furnace. The crude fiber was determined by acid-alkali hydrolysis and ignition of the dried sample for 3 h. In Table 1, nitrogen-free extracts (NFE) were calculated as $\text{NFE} = 100 - (\text{crude protein} + \text{crude lipid} + \text{crude ash} + \text{crude fiber})$.

Table 2 Proximate compositions of raw materials

	Dry matter	Crude protein	Crude lipid	Crude ash	Crude fiber
Fish meal	91.65	77.50	9.04	12.82	0.25
Soybean meal	84.95	48.50	1.92	3.00	6.00
Wheat meal	88.11	15.06	1.48	2.00	3.00
Corn meal	88.97	62.96	0.67	1.80	2.50
Wheat gluten	90.00	79.44	5.00	1.00	0.01
Spirulina meal	94.43	52.25	6.71	0.00	0.00

Amino acid analysis

The amino acid compositions of the experimental diets are detailed in Table 3. The sample (60 mg) of diet was hydrolyzed in 10 mL of 6 M HCl in screw-capped tubes. The tubes, which flushed with N, were heated at 110 °C for 24 h. The hydrolysates were rotary-evaporated to dryness under vacuum at 40 °C and re-dissolved in a sodium citrate buffer at pH 2.2. The amino acids were separated by ion-exchange chromatography on a sodium column and detected following post-column derivatization with ninhydrin. Identification and quantification of the detected amino acids were performed using external standards after adjustments by linear regression.

Evaluation of growth performance

Growth performance was monitored after discontinuing feeding for 1 day by weighing the fish in bulk biweekly. All fish were anaesthetized with clove oil (10 µL/L) during the weighing procedure (Pedrazzani and Neto 2016). Growth parameters, including feed conversion ratio (FCR), specific growth rate (SGR), the protein efficiency ratio (PER), and survival rate (SR), were calculated as following equations:

$$FCR = \text{feed intake} / \text{weightgain}$$

Table 3 Amino acid compositions of the experimental diets (%)

	C	SP15	SP30
Methionine	0.95	1.12	1.28
Cystine	0.60	0.59	0.58
Lysine	2.63	2.78	2.92
Tryptophan	0.56	0.71	0.86
Threonine	1.19	1.45	1.71
Isoleucine	1.55	1.85	2.14
Histidine	1.07	1.29	1.52
Valine	1.69	1.95	2.20
Leucine	3.36	3.79	4.21
Arginine	1.67	1.92	2.17
Phenylalanine	3.04	3.04	3.03

$$PER = [(final\ fish\ weight) - (initial\ fish\ weight)] / protein\ intake\ (PI)$$

$$SGR = 100 \times [Ln\ final\ fish\ weight) - (Ln\ initial\ fish\ weight)] / experimental\ days$$

where PI was calculated as $PI = (\text{feed intake} \times \text{protein of ratio feed}) / 100$.

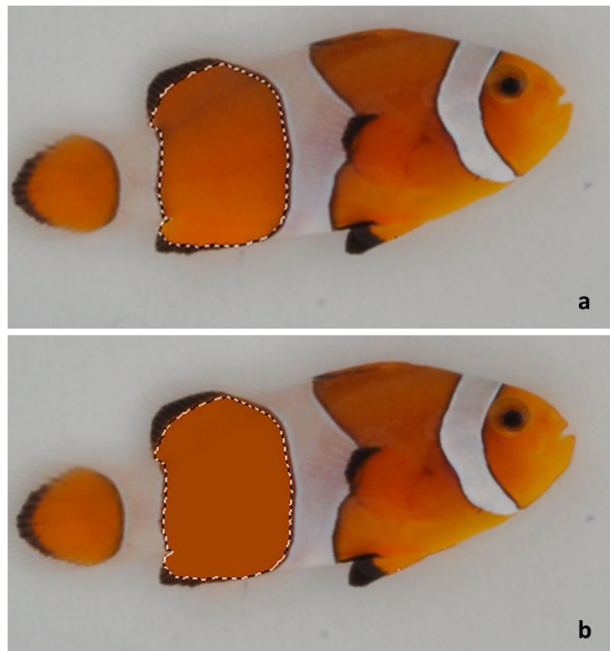
$$SR = 100 \times (\text{total fish count} - \text{dead fish count}) / \text{total fish count}$$

Determination of color intensity

Before weighing, all fish were photographed at the beginning, the end, and biweekly intervals. Fish were placed to the white surface under the two natural white color light sources and a digital camera (Nikon D3100, Tokyo, Japan) was located 25 cm above the fish (Yasir and Qin 2009). The gray, yellow, and red reference cards were used for calibrating and taking the images of the full-body color of clownfish. The body region of clownfish, where it is between the last two white bands (Fig. 1), was photographed and the images were analyzed with the Adobe Photoshop CS6 13.0.1 (Adobe Systems, California, USA). Standardized color values of the experimental groups were determined by the “Average” tool in the Adobe Photoshop (Filter > Blur > Average) biweekly.

The color analyses were considered with the CMYK (cyan magenta yellow key) and RGB (red green blue) models. In RGB space, the maximum value (255) of the red, green, and blue represents the white color, and the absence of light (where the red, green, and blue = 0) indicates the black color. This is a solid black when 100% of C, M, Y, and K. In

Fig. 1 Selection of the body region of common clownfish for using the color intensity technique. **a** Selected area of the real skin part of the fish, **b** standardized color intensity of the selected area of fish was determined by the “Average” tool in the Adobe Photoshop (Filter > Blur > Average)



contrast, the white color is obtained when all colors are set to 0%. Hence, in the CMYK model, different levels of % saturation of cyan, magenta, yellow, and black colors determine the actual color.

Determination of total ammonia–nitrogen (TAN) excretion

After the feeding trial, fish were starved for 3 days to ensure the evacuation of food from the gut. On the morning of the fourth day, aquariums were thoroughly cleaned, and fish in all groups were fed with their experimental diets to apparent satiation. After 30-min postprandial, water flow to each aquarium was discontinued, and uneaten food was removed. After 12-h postprandial, the water samples were taken with an automatic pipette from the aquariums and transferred to the $\text{NH}_4\text{-N}$ reagent cuvettes (Hach Lange LCK 304). Separate (and new) cuvettes were used taking for each aquarium's water samples. TAN concentrates were analyzed with the ammonia salicylate method and measured in a Hach Lange DR 2800 spectrophotometer (Karadal et al. 2017). TAN excretion levels were determined by subtracting the baseline value of each aquarium according to the following formula:

$$\text{TAN}(\text{mg} - \text{N}/100 \text{ fish}/12\text{h}) = [(\text{final ammonia concentration} - \text{initial ammonia concentration}) \times \text{water volume}] / \text{final mean fish weight}/\text{sampling period}$$

Economic analysis

The economic analysis in terms of economic conversion ratio (ECR) and economic profit index (EPI) was developed by Martínez-Llorens et al. (2007), and it was determined using the following formulae:

$$\begin{aligned} \text{ECR}(\text{€} / \text{g}) &= \text{feed conversion ratio} \times \text{feed price}, \\ \text{EPI}(\text{€} / \text{fish}) &= (\text{final mean weight} \times \text{fish salesprice}) \\ &\quad - (\text{economic conversion ration} \times \text{weight gain}) \end{aligned}$$

The cost of feed ingredients was listed by IndexMundi (<http://www.indexmundi.com/commodities>). Clownfish sale price is determined as 5.8 €/g according to commercial ornamental fish importers in Turkey (sale price for 1 g of clownfish is 5.8 €).

Statistical analysis

The Shapiro–Wilk W test and Levene test were examined to verify normality and homogeneity of variance before further analysis was undertaken. All data were subjected to a one-way analysis of variance (ANOVA) after proving the normality and homogeneity of the data. Tukey's multiple range test was used to rank groups when interaction was found different using Statgraphics Centurion XVI (Statpoint Technologies Inc., The Plains, VA) statistical software (Zar 1999). All means were calculated with standard errors (\pm SE). Differences were considered significant at the 95% confidence interval.

Table 4 Growth performance of clownfish fed with experimental diets during the 12 weeks

	C	SP15	SP30
Initial mean weight (g)	0.55 ± 0.03	0.57 ± 0.04	0.58 ± 0.04
Final mean weight (g)	1.68 ± 0.12 ^a	1.95 ± 0.18 ^b	2.05 ± 0.14 ^b
Specific growth rate (%/day)	1.58 ± 0.06 ^a	1.75 ± 0.04 ^b	1.81 ± 0.05 ^b
Feed conversion ratio	2.31 ± 0.09 ^b	1.68 ± 0.14 ^a	1.61 ± 0.12 ^a
Feed intake (g)	2.60 ± 0.23	2.31 ± 0.20	2.37 ± 0.18
Protein efficiency ratio	0.95 ± 0.04 ^a	1.30 ± 0.09 ^b	1.36 ± 0.07 ^b
Survival rate (%)	99.33 ± 0.46	100.00 ± 0.00	100.00 ± 0.00

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups

Results

The growth performance of clownfish fed with different dietary Spirulina levels is given in Table 4. At the end of the 12 weeks, the final mean weight (FMW), specific growth rate (SGR), and protein efficiency ratio (PER) of Spirulina groups (SP15 and SP30) were significantly higher than the control (C) group ($P < 0.05$). The FMW of the SP30 group (2.05 g) was higher than the SP15 group (1.95 g), even if there are no statistical differences between these groups. Feed conversion ratio (FCR) of the C was the highest among the experimental groups ($P < 0.05$). There were no statistical differences between the feed intake (FI) and the survival rates (SR) of the experimental groups ($P > 0.05$).

Initial and final RGB (red green blue) color model data of body and caudal regions of clownfish are presented in Table 5. Final R and G of both body and caudal regions and final B of the caudal part of the C group were significantly higher than Spirulina groups ($P < 0.05$). The final body B of the SP30 was markedly lower than the C and SP15 ($P < 0.05$).

Table 5 Red green blue (RGB) coloration of both body and caudal regions of clownfish fed with diets containing different Spirulina levels

Region	Parameter	C	SP15	SP30
Body	Initial R	187.30 ± 1.84	188.40 ± 1.89	185.10 ± 1.92
	Final R	182.88 ± 4.43 ^b	166.13 ± 2.78 ^a	159.25 ± 3.36 ^a
	Initial G	90.20 ± 3.93	89.40 ± 3.69	87.50 ± 2.46
	Final G	81.38 ± 2.67 ^b	63.00 ± 3.59 ^a	58.13 ± 4.69 ^a
	Initial B	5.30 ± 1.26	7.50 ± 1.38	7.30 ± 1.45
	Final B	2.38 ± 0.89 ^b	2.25 ± 0.73 ^b	0.50 ± 0.27 ^a
Caudal	Initial R	203.20 ± 2.36	203.10 ± 2.97	201.40 ± 2.20
	Final R	193.88 ± 4.40 ^b	173.13 ± 4.83 ^a	171.38 ± 4.99 ^a
	Initial G	103.40 ± 3.38	100.40 ± 3.66	99.10 ± 2.59
	Final G	87.13 ± 2.59 ^b	61.25 ± 3.46 ^a	63.13 ± 3.53 ^a
	Initial B	5.60 ± 1.20	7.40 ± 1.48	7.10 ± 1.78
	Final B	2.13 ± 0.64 ^b	0.75 ± 0.25 ^a	0.38 ± 0.18 ^a

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups

Table 6 Cyan magenta yellow key (CMYK) coloration of body regions of clownfish fed with diets containing different Spirulina levels

Color type	Weeks	C	SP15	SP30
C	0	20.80 ± 1.07	20.50 ± 0.93	20.70 ± 1.24
	2	20.70 ± 1.86	21.10 ± 2.75	20.90 ± 2.23
	4	21.00 ± 0.76	22.30 ± 0.94	22.00 ± 0.71
	6	21.20 ± 0.76	22.50 ± 0.86	22.10 ± 0.50
	8	22.00 ± 0.60	23.90 ± 0.98	22.90 ± 0.46
	10	21.90 ± 0.82 ^a	24.70 ± 0.78 ^b	24.50 ± 0.43 ^b
M	12	21.63 ± 1.21 ^a	26.00 ± 0.71 ^b	26.13 ± 1.54 ^b
	0	62.30 ± 1.35	62.40 ± 1.75	62.50 ± 1.24
	2	62.10 ± 2.84	62.90 ± 3.20	63.20 ± 3.54
	4	62.10 ± 1.15	64.70 ± 1.05	65.50 ± 1.89
	6	64.00 ± 1.94	66.60 ± 1.54	67.40 ± 1.38
	8	67.10 ± 1.37	67.20 ± 1.70	68.90 ± 1.64
Y	10	64.00 ± 1.45 ^a	69.20 ± 0.84 ^b	69.10 ± 1.62 ^b
	12	65.38 ± 2.12 ^a	72.13 ± 1.76 ^b	72.75 ± 1.51 ^b
	0	98.40 ± 0.85	97.50 ± 1.04	98.20 ± 0.70
	2	96.70 ± 2.23	96.80 ± 2.69	97.70 ± 1.58
	4	95.50 ± 3.07	98.60 ± 1.40	98.50 ± 1.50
	6	98.60 ± 1.40	99.50 ± 0.50	99.50 ± 0.50
K	8	98.10 ± 1.29	98.70 ± 0.87	99.80 ± 0.20
	10	99.10 ± 0.90	99.90 ± 0.10	100.00 ± 0.00
	12	98.88 ± 1.13	99.50 ± 0.50	99.75 ± 0.25
	0	7.00 ± 0.67	8.10 ± 0.77	8.20 ± 0.80
	2	7.30 ± 0.79	9.20 ± 1.86	9.10 ± 1.34
	4	7.30 ± 0.63 ^a	11.10 ± 1.47 ^b	11.50 ± 0.57 ^b
K	6	7.90 ± 0.85 ^a	12.10 ± 1.43 ^b	12.30 ± 0.90 ^b
	8	7.80 ± 1.01 ^a	12.20 ± 1.59 ^b	12.50 ± 1.00 ^b
	10	8.60 ± 0.79 ^a	12.50 ± 0.85 ^{ab}	14.40 ± 1.67 ^b
	12	9.13 ± 0.79 ^a	12.63 ± 0.89 ^b	16.13 ± 1.09 ^c

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups

The CMYK (Cyan Magenta Yellow Key) color model values of both body and caudal regions of clownfish fed with experimental diets are detailed week by week (0 to 12) in Tables 6 and 7. At the 10th and 12th weeks (for body region), the cyan color of SP15 and SP30 groups was significantly higher than the C group ($P < 0.05$). The SP15 and SP30 were markedly higher than C at the 10th and 12th weeks for both areas for magenta color ($P < 0.05$). No significant differences were found between the groups for both body and caudal regions for yellow color ($P > 0.05$). The key of C group was the lowest one at the 4th, 6th, 8th, and 12th weeks for the body region and the 8th and 10th weeks for the caudal region ($P < 0.05$). The key of SP30 was higher than C at the 10th and 12th weeks for the body and caudal regions, respectively ($P < 0.05$). The highest key was noted in SP30 at the 12th week for the body region among the experimental groups ($P < 0.05$).

Table 7 Cyan magenta yellow key (CMYK) coloration of caudal regions of clownfish fed with diets containing different *Spirulina* levels

Color type	Weeks	C	SP15	SP30
C	0	17.00 ± 1.35	17.20 ± 1.54	17.00 ± 1.20
	2	17.20 ± 1.06	17.30 ± 1.21	17.40 ± 1.59
	4	17.40 ± 0.40	17.50 ± 0.45	17.88 ± 0.67
	6	18.20 ± 0.77	18.20 ± 0.63	18.50 ± 0.45
	8	18.80 ± 0.89	19.40 ± 0.79	19.70 ± 0.86
	10	19.20 ± 0.71	20.70 ± 0.84	21.50 ± 1.67
M	12	20.13 ± 1.16	23.25 ± 0.96	23.88 ± 2.26
	0	58.20 ± 1.19	58.80 ± 1.85	58.80 ± 2.19
	2	58.30 ± 2.44	59.40 ± 3.07	59.70 ± 3.18
	4	58.10 ± 2.19	60.70 ± 1.65	61.75 ± 2.25
	6	62.30 ± 2.58	66.80 ± 2.04	67.90 ± 2.07
	8	64.20 ± 2.12	67.60 ± 1.38	68.20 ± 1.64
Y	10	64.80 ± 1.36 ^a	69.70 ± 1.59 ^b	70.60 ± 0.70 ^b
	12	64.13 ± 2.97 ^a	73.25 ± 1.50 ^b	72.50 ± 1.79 ^b
	0	98.40 ± 0.85	96.80 ± 1.42	97.90 ± 0.91
	2	97.70 ± 1.58	97.90 ± 1.70	98.00 ± 1.53
K	4	98.70 ± 1.19	99.50 ± 0.50	100.00 ± 0.00
	6	96.80 ± 2.15	99.50 ± 0.40	99.80 ± 0.20
	8	98.30 ± 1.70	99.70 ± 0.30	100.00 ± 0.00
	10	96.90 ± 2.28	97.80 ± 1.48	99.80 ± 0.20
	12	96.38 ± 2.42	100.00 ± 0.00	100.00 ± 0.00
	0	4.60 ± 0.43	4.70 ± 0.45	4.80 ± 0.49
K	2	4.50 ± 0.72	5.20 ± 1.02	5.30 ± 0.93
	4	4.60 ± 0.60	6.40 ± 0.82	6.88 ± 1.67
	6	5.40 ± 0.88	7.00 ± 0.56	7.80 ± 1.23
	8	5.40 ± 0.60 ^a	8.50 ± 0.62 ^b	8.80 ± 1.21 ^b
	10	5.50 ± 0.83 ^a	9.00 ± 1.03 ^b	9.20 ± 1.05 ^b
	12	5.62 ± 0.65 ^a	11.75 ± 1.52 ^{ab}	12.50 ± 2.87 ^b

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups

The total ammonia–nitrogen (TAN) excretion of clownfish fed with *Spirulina* diet reduced compared to fish fed with non-*Spirulina* or fewer amounts of *Spirulina* diets, gradually (Fig. 2). The TAN of the C group was significantly higher than SP30 ($P < 0.05$).

Economic analyses of clownfish fed with *Spirulina*-included and non-*Spirulina*-included diets during the 12 weeks are listed in Table 8. The economic conversion ratio (ECR) of the C and economic profit index (EPI) of the SP30 groups were significantly lowest among the experimental groups ($P < 0.05$).

Discussion

The present study demonstrated the effects of *Spirulina* on growth performance, coloration, and total ammonia–nitrogen excretion of 0.56 g common clownfish (*Amphiprion ocellaris*). Growth performance regarding final mean weight (FMW), specific growth rate (SGR),

Fig. 2 Total ammonia–nitrogen excretion (TAN) of common clownfish fed with experimental diets containing different levels of Spirulina meal as 0 (C), 15% (SP15), and 30% (SP30) after 12 h. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups

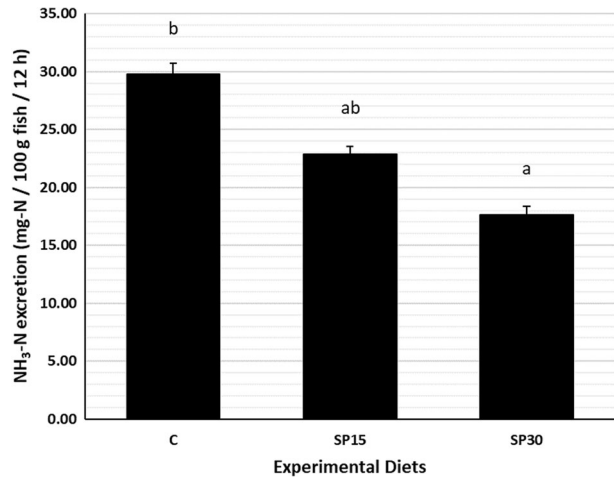


Table 8 Economic analysis of clownfish fed with experimental diets during the 12 weeks

	C	SP15	SP30
Economic conversion ratio (€/g)	0.0115 ± 0.0005 ^a	0.0150 ± 0.0006 ^b	0.0169 ± 0.0001 ^b
Economic profit index (€/fish)	0.30 ± 0.01 ^b	0.30 ± 0.04 ^b	0.17 ± 0.02 ^a

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups

feed conversion ratio (FCR), and protein efficiency ratio (PER) of clownfish enhances with 15% dietary Spirulina inclusion, in comparison to the highest level (30%) which has similar effects as this ratio. Hudaidah et al. (2019) reported comparable results in their study conducted with 0.75 g percula clownfish (*A. percula*) in indoor conditions. They fed the fish with three levels of dietary Spirulina (0.9, 1.2, and 1.5%) and stated that the 1.2% inclusion improved FMW significantly. Güroy et al. (2012) declared that the 2.5, 5, and 10% levels of Spirulina have similar in FMW and SGR. However, inclusion groups were significantly higher than control in 3.75 g yellow tail cichlid (*Pseudotropheus acei*). However, gradual improvements have also resulted in FMW, SGR, and PER of the guppy (*Poecilia reticulata*) and oscar fish (*Astronotus ocellatus*) in previous studies (Biabani Asrami et al. 2019; Mohammadiazarm et al. 2021). Erdogan (2019) assessed the 10% Spirulina for FMW and SGR and 5 and 10% for PER were higher than the 20% group in 3.15 g dolphin cichlid (*Cyrtocara moorii*). The author described that the extra levels of dietary Spirulina had hardened the feed and feed intake (accordingly in growth performance) decrease in fish with difficulty eating. Furthermore, Rosas et al. (2019) detailed that Spirulina is a high-cost feed additive but using small portions in aquafeeds enhances health and growth. In this study, the economic profit index (EPI) of SP15 was higher than SP30. Nevertheless, Nhan et al. (2019) suggested low-cost dietary additives in cultivating clownfish species.

Coloration is a significant factor in the acceptability of an ornamental species in the aquarium market and typically determines the price (Ho et al. 2013; Karadal et al. 2017).

In that case, fish sacrifice in traditional methods is used for skin coloration. Eventually, the colorimeter or visual analysis resulting in minimal stress became more prevalent in recent years (Nhan et al. 2019). This study ranked the color intensity of common clownfish with digital and print-based color models (RGB and CMYK). Overall, these models showed that both body and caudal color intensities were improved with *Spirulina* inclusion, ignoring the microalgae level. The color intensity in fish might be enhanced with ingested and assimilated carotenoids (Ebenezar et al. 2020). Dietary natural carotenoid sources such as carrot, marigold, hibiscus, rose, gutweed, paprika, and sweet potato positively affected the coloration of common clownfish (Ramamoorthy et al. 2010; Nhan et al. 2019; Ebenezar et al. 2020) while Hekimoğlu et al. (2017) and Hudaidah et al. (2019) reported microalgae such as *Nannochloropsis oculata*, *Porphyridium cruentum*, and *Arthrospira platensis* had no significant effects on pigmentation of clownfish species (*A. frenatus* and *A. percula*). Hekimoğlu et al. (2017) commented that this situation emerged by containing the low carotenoid level in microalgae species. It is thought that coloration of common clownfish was positively affected, as a high proportion of dietary *Spirulina* meal was used in our study. Furthermore, dietary *Spirulina* level gradually enhanced the coloration of freshwater ornamental fish species, including yellow tail cichlid (*P. acei*), goldfish (*Carassius auratus*), and oscar fish (*A. ocellatus*) in previous studies (Güroy et al. 2012; Kargın and Dikbaş 2020; Mohammadiarm et al. 2021). Biabani Asrami et al. (2019) recorded phycocyanin extracted from *Spirulina* similarly affected guppy (*Poecilia reticulata*). However, cichlid studies pointed the medium levels of dietary *Spirulina* can improve the color intensity of the ornamental species (Erdogan 2019; Lili et al. 2021) as in this study.

Spirulina assimilates nitrogenous compounds, including ammonia and nitrate, rapidly decreasing their concentrations (Wuang et al. 2016). Dietary *Spirulina* reduced clownfish's total ammonia–nitrogen excretion (TAN) in this study. Similar results were reported in studies conducted with *sebae* clownfish (*Amphiprion sebae*) fed with six different microalgae (class: Cyanophyceae), including *Spirulina* (Gopi et al. 2010). Karadal et al. (2017) also declared that dietary *Spirulina* decreases TAN of 2 g kenya cichlid (*M. lombardoi*).

In aquaculture, microalgae are used as growth enhancers, natural carotenoid sources, metabolism, and immune system regulators. *Spirulina* is a popular microalga with all the aspects listed above in the ornamental fish sector. The results clearly show that the 15% dietary inclusion of *Spirulina* had a significant effect on the growth, coloration, and ammonia excretion of common clownfish while preserving the profitability.

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Data availability All data generated and analyzed during this study are presented in this article.

Code availability Not applicable.

Declarations

Ethics approval The present study was carried out by animal welfare and the ethics of the experiment. This study complied with the Guidelines of the EU Directive 2010/63/EU for animal experiments.

Conflict of interest The authors declare no competing interests.

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