Acta Aquatica Turcica

E-ISSN: 2651-5474
Home Page: https://dergipark.org.tr/actaquatr 21(1): 042-050, 2025 DOI: 10.22392/actaquatr.1494714

Research Article Araştırma Makalesi

Evaluating Schizochytrium Supplementation on Growth Performance and Skin Colouration in Electric Yellow Cichlids (*Labidochromis caeruleus*)

Sarı Prenses Çiklitlerinde (*Labidochromis caeruleus*) Büyüme Performansı ve Cilt Renklenmesi Üzerinde Schizochytrium Takviyesinin Değerlendirilmesi

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Received: 03.06.2024 **Accepted:** 01.10.2024 **Published:** 01.03.2025

How to Cite: Karataş, B., & Kayan, D. (2025). Evaluating Schizochytrium supplementation on growth performance and skin colouration in Electric Yellow Cichlids (*Labidochromis caeruleus*). *Acta Aquatica Turcica, 21*(1), 042-050. https://doi.org/10.22392/actaquatr.1494714

Abstract: In this study, the effects of *Schizochytrium* sp. supplementation (SCH) to feed on growth and skin coloration in electric yellow cichlid (*Labidochromis caeruleus*) were investigated. Fish (1.93 ± 0.23 g initial weight) were fed with commercial fish feed as the control group (C) and commercial fish feed supplemented with 0.5% (SCH05) and 1% (SCH1) SCH for 60 days. The SCH05 and SCH1 groups displayed the highest final weight (FW), weight gain (WG), and specific growth rate (SGR) values, with similarly favorable feed conversion ratio (FCR) levels compared to the C group ($p<0.05$). The SCH1 group exhibited statistically significant differences from the C and SCH05 groups concerning lightness (*L**) and hue values ($p<0.05$). The redness (*a**) values in the SCH05 and SCH1 groups showed a statistically significant difference compared to the C group ($p<0.05$). However, there were no statistically significant differences in the yellowness (b*) and Ch values among all groups. To conclude, the inclusion of SCH microalga as a dietary supplement markedly promoted growth performance and facilitated the attainment of marketable size in electric yellow cichlids. Additionally, although the enhancement in skin coloration resulting from the addition of 0.5% SCH to the feed did not reach a significant difference, it hints at a promising advantage that could enhance the aesthetic allure and market value of the fish.

Keywords
- Microalgae
- Additive
- Growth
- Ornamental fish
- Skin color

Özet: Bu çalışmada, sarı prenses çiklitlerinde (*Labidochromis caeruleus*) *Schizochytrium* sp. takviyesinin (SCH) büyüme ve deri renklenmesi üzerindeki etkileri araştırıldı. Balıklar (başlangıç ortalama ağırlığı 1.93 ± 0.23 g) 60 gün boyunca kontrol grubu olarak ticari balık yemi (C) ve %0.5 (SCH05) ve %1 (SCH1) SCH takviyeli ticari balık yemi ile beslendi. SCH05 ve SCH1 grupları, C grubuna kıyasla en yüksek son ağırlık (FW), ağırlık kazanımı (WG) ve spesifik büyüme oranı (SGR) değerlerini ve benzer şekilde olumlu yem değerlendirme oranı (FCR) seviyelerini gösterdi. SCH1 grubu, parlaklık (*L**) ve hue değerleri açısından C ve SCH05 gruplarından istatistiksel olarak anlamlı farklılıklar gösterdi ($p<0.05$). SCH05 ve SCH1 gruplarındaki kırmızılık (*a**) değeri, C grubuna kıyasla istatistiksel olarak anlamlı bir fark gösterdi. Ancak, tüm gruplar arasında sarılık (*b**) ve kroma (Ch) değerlerinde istatistiksel olarak anlamlı bir fark bulunamadı. Sonuç olarak, SCH mikroalginin diyet takviyesi olarak dahil edilmesi, büyüme performansını belirgin şekilde teşvik etti ve sarı prenses çiklit balığının pazarlanabilir boyuta daha hızlı ulaşmasını sağladı. Ayrıca, deri renklenmesinde istatistiksel olarak anlamlı bir artış sağlamamasına rağmen, yemlere %0.5 SCH eklenmesi balıkların estetik çekiciliğini ve piyasa değerini artırabilecek umut verici bir avantaj sunmaktadır.

Anahtar kelimeler
- Mikroalg
- Katkı maddesi
- Büyüme
- Süs balığı
- Deri rengi

1. INTRODUCTION

The aquarium sector continues to grow as keeping ornamental fish is a hobby of worldwide interest (Karadal et al., 2017; Sathyaruban et al., 2021). In recent years, significant progress has been achieved in the aquarium industry, leading to establishment of ornamental fish production as a prominent sector within aquaculture (Qaranjıkı, 2017; Mutlu, 2019). The industry encompasses the trade of approximately 5,300 freshwater and 1,802 marine fish species, with an estimated global trade value ranging from 15 to 30 billion dollars (Qaranjıkı, 2017; Yanar et al., 2019; Polat & Yağcilar, 2021). Therefore, ornamental fish production has commercial value within the aquaculture industry in developed and developing countries (Hekimoğlu, 2006). One of the most critical factors contributing to the growth of ornamental fish production is the production and marketing of the most popular species. Cichlids are among the preferred and demanded species in today's market (Yalçın, 2014). Due to their attractive colors, *Labidochromis caeruleus*, commonly known as the electric yellow cichlid, is a highly demanded cichlid species. The electric yellow cichlid fish originates from Lake Malawi located in Africa. Renowned for tranquil disposition in aquariums, electric yellow cichlids (*Labidochromis caeruleus*) can grow up to 10-12 cm in length as adults. These cichlids are well-suited to coexist with other cichlid species in the same aquarium. The vibrant colors and peaceful nature of electric yellow cichlids have recently made it a popular aquarium fish (Cavdar et al., 2020; Yeşilayer et al., 2020).

In aquaculture, prioritizing economic efficiency and attaining optimal growth through utilizing species-specific functional feeds are major objectives. Nutrition significantly impacts fish growth, reproduction, and pigmentation (Pezeshk et al., 2019; Cavdar et al., 2020; Yeşilayer et al., 2020; Hekimoğlu & Sönmez, 2023). Furthermore, high-quality feeds are critical in sustaining fish health throughout the culture process while reducing environmental waste. These issues are pointed out not only for consumable fish culture but also for ornamental fish production (Cavdar et al., 2020). In ornamental fish production, the key determinants of growth, reproduction, and coloration predominantly hinge upon the availability of feed ingredients and additives in suitable proportions (Şahin et al., 2022). Consequently, investigations into ornamental fish diets have underscored the incorporation of natural ingredients (Yeşilayer et al., 2011) and additives (Karsli et al., 2018; Yiğit et al., 2019; Yeşilayer et al., 2020) to enhance reproductive and coloration parameters, bolster survival rates, and fortify disease resistance, thereby ensuring efficacious culture practices.

Microalgae are becoming increasingly important as a feed and supplement in aquaculture and animal feed due to their balanced nutritional content. They have the potential to replace fish meal and other traditional ingredients (Ansari et al., 2021). In recent years, studies in aquaculture have focused on incorporating microalgae as feed additives primarily to maintain the health of cultured organisms, reduce costs, and increase the quantity and quality of the products obtained or produced. Previous studies have confirmed the effectiveness of microalgae as feed supplements for cultured species such as serpae tetra (*Hyphessobrycon eques*) (Berchielli-Morais et al., 2016), guppy (*Poecilia reticulata*) (Biabani Asrami et al., 2019) and koi (*Cyprinus carpio* var. koi) (Prabhath et al., 2019).

Among the algae used for aquafeed, the microalga *Schizochytrium* sp. (SCH) is particularly noteworthy (Souza et al., 2020). This alga has a rich content of docosahexaenoic acid (DHA) (Lewis et al., 1999). Studies have shown that the inclusion of SCH in the diet has positive effects on culture parameters in Nile tilapia, *Oreochromis niloticus* (Sarker et al., 2016; Dos Santos et al., 2019), channel catfish, *Ictalurus punctatus* (Li et al., 2009) and rainbow trout, *Oncorhynchus mykiss* (Lyons et al., 2017). Despite the potential use as a feed additive mentioned above, to our knowledge, no studies investigating the effects of SCH on electric yellow cichlids (*L. caeruleus*) have yet been conducted. Considering the known beneficial properties of SCH, we hypothesize that its inclusion as an additive in fish feed could offer advantages to electric yellow cichlids. Moreover, such utilization could yield valuable insights into the advantageous effects of SCH. Given the economic importance of the electric yellow cichlid in the aquarium industry, along with the numerous benefits of SCH as a feed supplement, this research was conducted for the first time to evaluate the dietary effect of SCH on the growth performance and skin coloration of the electric yellow cichlid (*L. caeruleus*).

2. MATERIALS AND METHODS

2.1. Experimental design and feeding trial

The research took place at the Aquatic Organisms Experimental Unit (SUCAN) within the Faculty of Fisheries at Van Yüzüncü Yıl University, located in Van, Türkiye. A total of 135 electric yellow cichlids obtained from the Aquatic Organisms Experimental Unit, averaging 1.93 ± 0.23 g in weight, were employed for the study. The microalgae was obtained from a commercial company (Marinbio, Aydın/Turkey).

The fish were fed with control feed for two weeks for acclimatization and then distributed in the 9 glass aquariums of 100 L in triplicate. Three different groups were formed with 0 (C), 0.5% (SCH05) and 1% (SCH1) doses of SCH in the feed (Table 1). SCH powder was blended with the commercial feed via spraying, as outlined by Safari et al. (2022), and stored at the 4 ºC until use. During the 60-day study, the fish were fed near satiation with the experimental feeds twice daily, at 09:00 and 16:00. Continuous aeration of the aquariums was ensured using a central air pump (RESUN GF-250 Vortex Blower). Daily water changes of 40-50% were conducted to sustain water quality, and any uneaten food or feces were removed by siphoning to uphold a clean environment. Throughout the research, a 12:12 h (light:dark) photoperiod was maintained. Water quality parameters were assessed weekly using a multiparameter (AZ Instruments/ AZ86031), yielding the following results: temperature: 26.3 ± 0.39 °C; dissolved oxygen: 6.72 ± 0.14 mg L^{-1} and pH: 8.34 ± 0.06.

2.2. Growth performance analysis

All fish were weighed individually at both the start and the end of the study. Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) were computed using the following formulas:

WG (g/fish): Final weight (g) − Initial weight (g),
SGR (% / day): (100 × ((Ln final fish weight) – (Ln initial fish weight)/days)),

FCR: Total feed given (g)/Weight gain (g),

SR (%): (Final number of the fish/initial number of fish) × 100.

2.3. Color analysis

Fish skin color was assessed using a Konica Minolta CR 400 device, with six specimens per group. This involved determining the lightness value (L^*; -100, +100 white), the redness value (a^*; -100 green, +100 red), and the yellowness value (b^*; -100 blue, +100 yellow) of each group. Skin color parameters L^*, a^*, and b^* were measured from areas near the lateral line and dorsal part of the fish. Hue (H°ab) and chroma (Ch) values were derived from the a^* and b^* values. Chroma (Ch) refers to the intensity and vividness of color and is calculated using the formula $Ch = (a^{*2} + b^{*2})^{½}$. Hue, on the other hand, represents the balance between the red and yellow tones in the fillet. If a* is greater than 0, the hue angle is determined by the equation $Hab° = \tan{-1}(b^*/a^*)$. If a^* is less than 0, it is calculated using $H°ab = 180 + \tan{-1}(b^*/a^*)$ (Hunt, 1977). Regarding hue (H°ab), a^* value of 0° corresponds to a red hue, 90° to yellow, 180° to green, and 270° to blue (Hunt, 1977; Yeşilayer et al., 2020). All measurements adhered to the methods outlined by CIE (1976) and were performed using the Konica Minolta CR 400 device.

2.4. Statistical analysis

Data were analyzed using the SPSS 20 for Windows software package (SPSS Inc., Chicago, IL, USA). Before performing the analysis (ANOVA, Duncan test), the underlying assumptions were thoroughly examined. Skewness and kurtosis statistics were first evaluated for each group to ensure compliance with normality assumptions, and the Levene test was applied to assess the homogeneity of variances. Results were expressed as mean ± standard error. Statistical significance was determined at a threshold of $p < 0.05$.

3. RESULTS

3.1. Growth Performance

As shown in Table 2, the SCH05 and SCH1 groups demonstrated the highest FW, WG and SGR values ($p<0.05$). Similarly, the SCH05 and SCH1 groups showed the best FCR levels compared to the C group ($p<0.05$). Among the groups fed with SCH feeds, there were no statistically significant differences in SR ($p>0.05$).

Table 1. Composition of the commercial diet utilized in the investigation.

Ingredient	Quantity	Ingredient	Quantity
Crude protein (%)	47.5	Manganese (Mg/kg)	67
Crude fat (%)	6.5	Zinc (Mg/kg)	40
Crude fibre (%)	2	Iron (Mg/kg)	26
Moisture content (%)	6	Vitamin D (IU/kg)	1860

3.2. Skin coloration parameters

At the end of the 60-day feeding trial, as summarized in Table 3, the SCH1 group was found to be statistically different from the C and SCH05 groups in terms of *L** and Hue values ($p<0.05$). The *a** value in the SCH05 and SCH1 groups showed a statistically significant difference compared to the C group. There were no statistically significant variations in the *b** and Ch values among all groups.

Table 2. Effects of dietary *Schizochytrium* sp. on growth parameters in electric yellow cichlid.

	C	SCH05	SCH1
IW	1.93 ± 0.02	1.93 ± 0.1	1.93 ± 0.1
FW	5.24 ± 0.07[b]	5.77 ± 0.05[a]	5.76 ± 0.06[a]
WG	3.30 ± 0.05[b]	3.84 ± 0.06[a]	3.82 ± 0.04[a]
SGR	1.66 ± 0.00[b]	1.83 ± 0.02[a]	1.82 ± 0.01[a]
FCR	2.14 ± 0.04[b]	1.87 ± 0.03[a]	1.86 ± 0.01[a]
SR (%)	100	100	100

Data were shown as means ± SE. Different lowercase letters on each line indicate significant variations between groups ($P < 0.05$). SCH05, a diet supplemented with 0.5% *Schizochytrium* sp.; SCH1, a diet supplemented with 1% *Schizochytrium* sp; IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate.

Table 3. Effects of dietary *Schizochytrium* sp. on skin coloration parameters in electric yellow cichlid.

	C	SCH05	SCH1
L*	69.80 ± 5.01[a]	65.40 ± 2.42[a]	58.14 ± 1.47[b]
a*	2.11 ± 1.17[b]	4.14 ± 1.02[a]	5.87 ± 0.38[a]
b*	21.32 ± 2.95	22.58 ± 2.77	21.01 ± 4.96
Ch	21.52 ± 2.97	23.03 ± 2.93	21.91 ± 4.79
H°ab	84.65 ± 3.05[a]	79.23 ± 1.05[a]	72.61 ± 3.67[b]

Data were shown as means ± SE. Different lowercase letters on each line indicate significant variations between groups ($P < 0.05$). SCH05, a diet supplemented with 0.5% *Schizochytrium* sp.; SCH1, a diet supplemented with 1% *Schizochytrium* sp; *L**, (+) brightness, (−) darkness; *a**, (+) redness, (−) greenness; *b**, (+) yellowness, (−) blueness; Ch, Chroma; H°ab, Hue.

4. DISCUSSION AND CONCLUSION

Optimizing growth performance and feed conversion ratio (FCR) in ornamental fish farming is necessary for efficient production (Hoseinifar et al., 2023). This study shows that incorporating SCH into the feed significantly improves the growth performance of electric yellow cichlids. Compared to the control (C) group, growth indices such as weight gain (WG) and specific growth rate (SGR) were positively affected in fish subjected to diets containing 0.5% and 1% SCH. Moreover, the groups receiving SCH feeds exhibited marked improvements in FCR values. The observed improvements in growth performance may be attributed to the presence of various bioactive compounds in the algae, including polysaccharides, fatty acids, pigments (especially carotenoids) and minerals. (Chen et al., 2021; Idenyi et al., 2022; Siddik et al., 2024). This might have led to increased consumption of the feeds by enhancing palatability (Xie et al., 2019; Li et al., 2023). Moreover, docosahexaenoic acid, found in high concentrations in SCH, is known to have beneficial effects on growth and development in aquatic organisms by promoting efficient nutrient absorption, enhancing metabolic efficiency, and supporting tissue growth and repair (Raghukumar, 2008; Sarker et al., 2016; Osmond et al., 2021). Additionally, bioactive compounds found in algae can improve the secretion of digestive enzymes, thereby enhancing the digestibility of feed and nutrient absorption (Abdelhamid et al., 2021). Similar to our study, Li et al. (2009) reported that supplementation with 1.0% and 1.5% SCH positively affected the growth performance of channel catfish (*Ictalurus punctatus*). In another study, the dietary inclusion of 3% SCH was reported to enhance the growth performance of golden pompano (*Trachinotus ovatus*) by increasing feed intake and feed utilization (Xie et al., 2019). Additionally, supplementation with SCH was reported to positively affect the growth performance of Nile tilapia (*O. niloticus*) (Dos Santos et al., 2019) and silver pomfret (*Pampus argenteus*) (Li et al., 2023). However, Jorge et al. (2022) found no significant variations regarding the growth performance of Nile tilapia (*O. niloticus*) fed diets SCH (3%) compared to the control group. While there is no information available on the role of SCH as a growth promoter in electric yellow cichlids (*Labidochromis caeruleus*), the impacts of different algae on the growth of electric yellow cichlids have been investigated in various studies. Similarly, to our study, Pezeshk et al. (2018) stated that added to the diet of electric yellow cichlids (*L. caeruleus*) with extracts derived from brown macroalgae (*Sargassum boveanum*), red macroalgae (*Gracilaria persica*), and green macroalgae (*Entromorpha intestinalis*) led to improved growth performance. However, some studies have stated no statistically significant variation in the growth performance of fish when algae were added to the diet as a supplement (Peyghan, 2016; Belbasi et al., 2019).

One of the most attractive features of ornamental fish is their vividly colored appearance (Kop & Durmaz, 2008; Yeşilayer et al., 2020). Hence, improving the vibrant coloration of ornamental fish stands as a crucial quality criterion in the aquarium fish industry (Sathyaruban et al., 2021). In this study, although there was no statistical difference, the yellow (+*b**) coloration increased in the SCH05 group compared to the C group. Additionally, the chroma (Ch) value, indicating color intensity and brightness, increased in the fish in the SCH05 group. However, the electric yellow cichlid is characterized by its yellow coloration, and in this study, it lost its natural coloration due to the increased redness (+*a**) when SCH was added to the feed. This phenomenon could be attributed to the elevated levels of micronutrient content in SCH, including the carotenoid astaxanthin and other bioactive compounds (Xie et al., 2019). Similarly, the addition of SCH to the diet has shown positive effects on fillet coloration in Atlantic salmon, *Salmo salar* (Katerina et al., 2020) and Nile tilapia, *O. niloticus* (Jorge et al., 2022). To date, there is no information available on the effects of SCH on skin coloration in electric yellow cichlids (*L. caeruleus*), but the effects of different algae on skin coloration in electric yellow cichlids have been investigated in various studies. Pezeshk et al. (2018) suggested that extracts obtained from *S. boveanum*, *G. persica*, and *E. intestinalis* algae could be used as feed additives to increase coloration in this species. Similarly, Peyghan (2016) reported a significant increase in skin coloration in electric yellow cichlids fed with *Sargassum angustifolium* and *Laurencia snyderia* algae. However, a study by Belbasi et al. (2019), stated no statistically significant variation in skin coloration compared to the control group in electric yellow cichlids fed with *Spirulina*-supplemented feeds.

In conclusion, the incorporation of SCH as a dietary supplement significantly enhanced the growth performance and expedited the attainment of marketable size in electric yellow cichlids (*L. caeruleus*). Moreover, although the improvement in skin coloration observed by adding 0.5% SCH to the feed was not statistically significant, it indicates a potential benefit that could be crucial for ornamental fish's aesthetic value and marketability. Considering the findings obtained; it is thought that the addition of SCH to feeds may be effective in increasing growth and pigmentation in Electric Yellow Cichlids (*Labidochromis caeruleus*), but further studies are required to prove this.

FUNDING

No financial support was received for the present study.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: BK, DK; Literature: BK, DK; Methodology: BK, DK; Performing the experiment: BK, DK; Data analysis: BK, DK; Manuscript writing: BK, DK, Supervision: BK, DK. All authors approved the final draft.

ETHICAL STATEMENTS

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University (protocol no: 2023/14-12).

DATA AVAILABILITY STATEMENT

The data used in the present study are available upon request from the corresponding author. Data is not available to the public due to privacy or ethical restrictions.

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