Comparative Effects of *Chlorella* and *Spirulina* on Growth, Pigmentation, Breeding, and Stress Tolerance in Guppy (*Poecilia reticulata* Peters, 1859)

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ABSTRACT

Fish coloration and growth can be enhanced by supplementing *Spirulina* in feed, though it is relatively expensive. As a cost-effective alternative, *Chlorella* was used in this study to replace *Spirulina*. The experiment was conducted by feeding one-day-old guppy (*Poecilia reticulata*) fry with either a control feed without algal supplementation (C), a diet supplemented with 5% *Spirulina* (5SP), or a diet supplemented with 5% *Chlorella* (5CH). After 30 days, the fry fed 5CH had significantly poorer growth performance ($p<0.05$) compared to 5SP, but not different from the control. Interestingly, there was a trend toward improved final weight and specific growth rate in the 5CH group, though the differences were not statistically significant compared to both 5SP and the control. Similar trends were observed for fry production per female. Additionally, no significant differences were found among treatments in terms of survival (ranged 96–97%), stress tolerance, and most reproductive traits (such as fry size). The colour intensity of fish fed both the 5SP and 5CH diets was significantly enhanced ($p<0.05$) compared to the control group. In conclusion, supplementing guppy feed with 5% *Chlorella* can replace *Spirulina* at the same rate for improving colour intensity, without negative effects on growth, survival, stress, or reproductive performances. However, further studies using higher inclusion levels of *Chlorella* are recommended, given the slight trend toward improved final weight and fry production in fish fed the *Chlorella*-supplemented diets.

Keywords: *Chlorella*, Ornamental fish, *Poecilia reticulata*, *Spirulina*

INTRODUCTION

While growth performance is a key focus in conventional aquaculture, colour intensity determines the demand for ornamental fish, making pigmentation a crucial factor in their value. Although processed astaxanthin is commonly used to enhance fish colouration (Barone *et al*., 2018), ornamental fish feed producers prefer herbal sources due to their added benefits, such as gonad development (Sinha and Asimi, 2007), enhancement of growth and antioxidant capacity (Liu *et al*., 2019), cost-effectiveness (Yesilayer *et al*., 2008), and environmental sustainability (Gupta *et al*., 2007).

Spirulina is a nutrient-rich superfood, approved by the American Food and Drug Administration (FDA) as safe for human consumption (Sotiroudis and Sotiroudis, 2013). It is widely used in pharmaceuticals, cosmetics, and as an aqua

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feed additive, driving up both its demand and price. Studies have consistently shown that *Spirulina* is an effective pigmentation enhancer for ornamental fish (James *et al*., 2006; Ezhil and Narayanan, 2013; Behera *et al*., 2018).

Chlorella, on the other hand, is less frequently used in aquafeeds, and its pigmentation potential is under-researched. However, it has been shown to promote fish growth, breeding, digestion, and health (Radhakrishnan *et al*., 2015; Enyidi, 2017). Additionally, *Chlorella* is more cost-effective, as it can be produced cheaply using modern technology, and its cultivation is simple with short harvest cycles (Li *et al*., 2015, Widayat *et al*., 2018). Therefore, *Chlorella* could be a more economical alternative to *Spirulina* if it proves effective in improving growth, pigmentation, stress tolerance, and other possible parameters. However, there is a lack of comparative research between *Chlorella vulgaris* and *Spirulina*. This experiment was carried out to evaluate the effectiveness of *Chlorella* powder as an alternative additive to *Spirulina* in guppy fry feed.

MATERIALS AND METHODS

The experiment was conducted in an indoor aquarium at the Asian Institute of Technology, Thailand.

Fry production

A total of 600 guppy brooders (450 females + 150 males) of the Golden Tuxedo strain were purchased from an ornamental fish farm. The fish were conditioned for a week before the trial. The brooders were stocked in glass tanks, each with a capacity of 100 m^{-3}, maintaining a male:female ratio of 1:3. Fish were hand-fed ad libitum twice daily with a breeder feed containing 40% crude protein. Water quality parameters, including total ammoniacal nitrogen, pH, and dissolved oxygen, were kept within acceptable ranges. Fry were produced starting from the third week, and one-day-old fry were collected during the fourth week to be used for the experiment, ensuring sufficient numbers for the nursery tanks.

Setting up the glass tanks for the trial

Nine glass aquaria, each with a capacity of 240 m^3 (107.5 cm length × 46.5 cm width × 46.5 cm height), were used to stock the fish. Water was filtered through a dedicated filtration system for each tank, and aeration was provided by a HAILEA ACO-380 air blower (Hailea Group Co. Ltd, Guangdong, China). Air temperature and light intensity were monitored using an AS 803 Smart Sensor lux meter (ARCO, Dong Guan, China), and regulated to 27.0±1.0 °C and 974±95 Lux, respectively. The photoperiod was set to 12 h (light)/12 h (dark) using fluorescent bulbs. The experiment followed a completely randomized design (CRD) with three treatments (control, C; 5% *Spirulina* diet, 5SP; and 5% *Chlorella* diet, 5CH), each replicated three times.

Experimental diets

Iso-caloric and iso-protein (4,468.6±29.7 Cal GE·g^{-1} and 47.5±0.2%, respectively) research feeds (C, 5SP, 5CH) were formulated (Table 1). *Spirulina* (FaYo Natural Health, Thailand) and *Chlorella* (Macrobio World, Thailand) powders were included in 5SP and 5CH diets at 5%, replacing an equivalent amount of crude protein from fishmeal in the control diet.

The ingredients were mixed thoroughly for each diet formula. Distilled water was heated and gradually added to the mixture to form a dough. The dough was shaped into small balls and passed through a VENZ SP 1/2 feed machine (VENZ, Bangkok, Thailand) to produce feed pellets. The feeds were dried at 60 °C for 24 h in a Memmert UF 110 laboratory oven (Memmert GmbH+, Schwabach FRG, Germany), cooled, and then powdered. The feeds were stored in sealed bags and kept refrigerated until use.

Proximate composition analysis

The proximate composition of the experimental feeds was analyzed following the AOAC (1990) protocol. The moisture content was determined using the Air Oven Method. The crude protein was measured by the Kjeldahl Method with

Table 1. Formulation and proximate composition of the research feeds.

Ingredients	C	5SP	5CH
Fish meal	51.1	46.3	46.9
Spirulina powder	0.0	5.0	0.0
Chlorella powder	0.0	0.0	5.0
Soybean	18.8	19.5	19.5
Rice bran	6.0	5.0	4.8
Corn	4.3	5.0	5.0
Egg yolk powder	10.0	10.0	10.0
Vitamin C	0.2	0.2	0.2
Fish oil (Tuna)	4.0	3.4	3.0
Vitamin mixture	2.0	2.0	2.0
Mineral mixture	1.0	1.0	1.0
CMC[1]	2.0	2.0	2.0
DCP[2]	0.5	0.5	0.5
Probiotic	0.1	0.1	0.1
Crude protein (%)	47.6±0.8	47.1±0.1	47.8±1.0
Crude lipid (%)	7.7±0.2	7.0±0.3	7.8±0.1
Moisture (%)	10.1±0.3	10.0±0.1	9.8±0.1
Ash (%)	12.7±0.2	12.0±0.0	11.9±0.0
Fibre (%)	1.5±0.1	1.6±0.0	1.5±0.1
Energy (Cal·g^{-1})	4,456±104	4,474±157	4,481±132

Note: [1]Carboxylmethyl Cellulose; [2]Di Calcium phosphate; Manufacturers: (*Spirulina*) - FaYo Natural Health Co. Ltd., Thailand; (*Chlorella*) - Macrobio World, Thailand

a FOSS Kjeltec 8100 apparatus (FOSS Analytical AB, Hoganas, Sweden). Ash content was determined using a Lab Tech LEF-115S-1 muffle furnace (DAIHAN LABTECH, Namyangju, Republic of Korea), while crude lipid content was analyzed by the Soxhlet Method using a FOSS Soxhtec 2043 apparatus (FOSS ScinoCompany Limited, Suzhou, China). Crude fiber was determined using the Weende Method operating with a FOSS Fibertec 1020 instrument (FOSS Scino Company Limited, Suzhou, China). The Gross energy content was measured with a LECO AC 500 Calorimeter (LECO, Michigan, USA). Amino acid profiles of the feeds, *Spirulina, Chlorella*, and fishmeal were analysed using a Biochrom 30+ amino acid Analyzer (Biochrom, Cambridge, UK), according to AOAC (2005).

Stocking, feeding, and monitoring of growth performance

One-day-old thirty fry of *Poecilia reticulata* (average size of 5.4±0.8 mg) were randomly selected and stocked in each glass aquarium. Feed pellets were shaped into balls, and the fry were fed ad libitum three times daily at 9:00 a.m., 12:00 a.m., and 3:00 p.m.

Survival rate and growth performance were calculated using the following equations.

$$\text{Survival rate} = (\text{Final fish number}/\text{Initial fish number}) \times 100$$

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

Daily weight gain (DWG)
= Weight gain (g)/Number of days

Relative weight gain (RWG)
= [Weight gain (g)/Initial weight (g)]×100

Specific growth rate (SGR)
= [(Ln final weight-Ln initial weight)/number of days]×100

Water quality management

The bottom of each aquarium was siphoned out every morning and evening before feeding and the removed water was replaced by conditioned water up to a 15 cm level. Every five days, two-thirds of the water volume in each aquarium was totally removed and replenished. Dissolved oxygen (DO) levels were monitored daily using the LAQUA DO 220 meter (HORIBA Advanced Techno, Kyoto, Japan), and temperature and pH were recorded with the EUTECH pH 150 meter (Thermo Fisher Scientific Eutech, Singapore). Total ammoniacal nitrogen was tested weekly following the APHA (1998) protocol. The water quality parameters were maintained within optimal ranges (average±SD): pH 7.8±0.4, total ammoniacal nitrogen 0.152±0.051 mg·L^{-1}, DO 6.1±2.4 mg·L^{-1}, and water temperature 25.9±0.7 °C).

Sorting and further rearing

After 30 days of rearing, fish body weight was measured using the OHAUS PIONEER PA 214C digital balance (OHAUS, Parsippany, USA). Fish were then sexed by observing the genital spots, and seven females and seven males from each replicate were isolated for an additional month of rearing. Five males from each replicate were used for pigmentation analysis, and one male and three females from each replicate were used for breeding. Fish were fed ad libitum twice daily with the same experimental feed.

Stress test

On day 30, ten male fish were randomly selected from each treatment for a stress tolerance test, following Lim *et al*. (2003). Fish were immersed in pre-aerated 0.5 L beakers containing 30 ppt salinity. Mortality was recorded every 3 min over a 2-h on period, and the stress tolerance index was calculated based on total and early mortality.

Pigmentation analysis

After one month of additional rearing, five male fish from each replicate were anaesthetized with 125 mg·L^{-1} clove oil (Aroma Hub, Thailand) based on a recommendation by Cunha *et al*. (2015). The caudal fins were photographed on a white background using a Nikon D7500 DSLR camera (Nikon Imaging Inc., Tokyo, Japan) with a YONGNUO YN 60 mm macro lens (YONGNUO, Shenzhen, China), under controlled lighting (753± 65 Lux) at 29.0±1.0 °C. The photography followed the guidelines provided by Stevens *et al*. (2007). The digital images were analysed with MATLAB, converting them to greyscale, where pixel valued ranged from 0 (black) to 255 (white). Lower values indicated higher pigmentation (Uribe *et al*., 2018).

Breeding performance

On day 60, one male and three females from each replicate were stocked separately in breeding tanks for three additional weeks. The water level was maintained at 30 cm, with one-third of the surface covered with banana leaves as a substrates for newborn fry. Fry were collected after delivery and monitored daily for 30 days. The number of fry, weight, and total length were daily recorded.

Statistical analysis

Growth performance, survival, colour intensity, and stress tolerance data were compiled in MS Excel and analyzed using one-way ANOVA followed by mean comparisons using a Tukey's test. Prior to the analyses, normality and homogeneity of the variance were confirmed using Levene test. SPSS software version 22.0 was used for all analyses, and results were considered significant at $p<0.05$, with means expressed as mean ± standard deviation.

RESULTS

Growth performance, survival rate, and stress resistance

The results indicated high survival rates for *Poecilia reticulata* fry across all treatments, with values ranging from 96% to 97%, showing no significant differences between treatments ($p \geq 0.05$).

Although some growth parameters, such as final weight and SGR, showed an increasing trend where the final weight and SGR of the 5CH group were not significantly different from the 5SP group ($p \geq 0.05$; Table 2) most growth-related parameters (WG, RWG, and DWG) were significantly lower ($p<0.05$) in the 5CH group compared to the 5SP group. However, these parameters showed no significant differences when the 5CH group was compared with the control group ($p \geq 0.05$). Additionally, the salinity stress index results were statistically similar across all treatments ($p \geq 0.05$).

Breeding performance

The total fry production per group was statistically similar ($p>0.05$) across all treatments. The number of fry produced per female in the 5SP group was higher ($p>0.05$) than in the control. However, there were no significant difference between the 5SP and 5CH groups, nor between the 5CH and control group. Additionally, the initial average weight and length of the fry produced were statistically insignificant ($p>0.05$; Table 3) across all treatments.

Pigmentation

The colour intensities of fry fed *Spirulina* and *Chlorella*–enriched diets were significantly enhanced ($p<0.05$) compared to the control. However, the colour intensities between the *Spirulina*-fed and *Chlorella*-fed groups (Figure 1) were statistically similar ($p>0.05$; Table 4).

Table 2. Growth performance of *Poecilia reticulata* fry fed diet supplemented with 5% *Spirulina* (5SP), 5% *Chlorella* (5CH), and the control (C, no supplement).

Parameter	C	5SP	5CH
Initial weight (mg)	5.4±0.8^{a}	5.4±0.8^{a}	5.4± 0.8^{a}
Final weight (mg)	56.5±10.5^{a}	91.7±5.3^{b}	62.8± 8.7^{ab}
WG (mg·fish^{-1})	48.8±11.3^{a}	78.6±13.0^{b}	55.5±8.7^{a}
RWG	668.0 ±155^{a}	1,077 ±178^{b}	760.0 ±119.0^{a}
DWG (mg·day^{-1})	1.6±0.4^{a}	2.6±0.4^{b}	1.9±0.3^{a}
SGR	6.7±0.7^{a}	8.2±0.5^{b}	7.2±0.5^{ab}
Survival (%)	96.0±8.0^{a}	97.0±4.0^{a}	96.0 ±4.0^{a}
Stress Index	213.0±151.0^{a}	160.0±49.0^{a}	181.0±34.0^{a}

Note: Mean±SD in each row, superscripted with different lowercase letters, are significantly different ($p<0.05$); WG= Weight Gain; RWG= Relative Weight Gain; SGR= Specific Growth Rate

Table 3. Breeding performance of *Poecilia reticulata* fed diet supplemented with 5% *Spirulina* (5SP), 5% *Chlorella* (5CH), and the control (C, no supplement) during first 30 days after hatching.

Parameter	C	5SP	5CH
Fry production per female	18.7±7.1^{a}	43.0±2.3^{b}	29.7±6.1^{ab}
Initial weight of a fry (mg)	4.0±3.0^{a}	4.0±3.0^{a}	5.0±3.0^{a}
Initial length of a fry (mm)	7.0±1.0^{a}	8.0±1.0^{a}	8.0±0.0^{a}

Note: Mean±SD in each row, superscripted with different lowercase letters, are significantly different ($p<0.05$).

Table 4. MATLAB values related to the color intensities of *Poecilia reticulata* fed diet supplemented with 5% *Spirulina* (5SP), 5% *Chlorella* (5CH), and the control (C, no supplement).

Treatment	MATLAB value
C	111.23±0.90[a]
5SP	85.37±2.23[b]
5CH	95.17±2.86[b]

Note: Mean±SD in each row, superscripted with different lowercase letters, are significantly different (p<0.05).

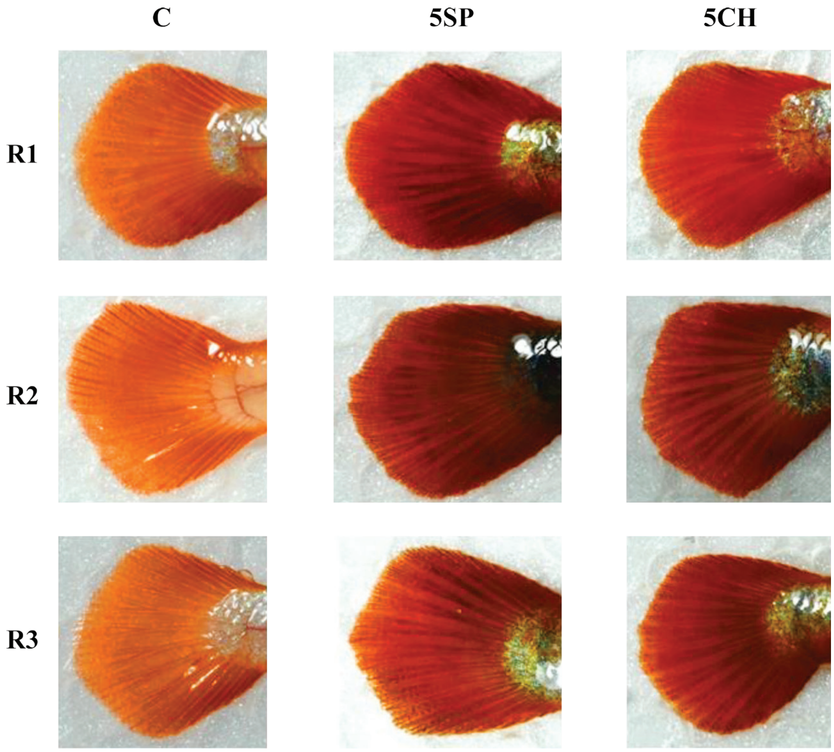

Figure 1. Images of the caudal fins of *Poecilia reticulata* fed diet supplemented with 5% *Spirulina* (5SP), 5% *Chlorella* (5CH), and the control (C, no supplement); (R1, R2, R3 = Replicates related to each treatment).

Amino acid profiles

The laboratory analysis revealed that all essential amino acids (EAAs), except histidine, were lower in *Chlorella* compared to fishmeal and *Spirulina* (p<0.05; Table 5). Histidine content was similar between *Spirulina* and *Chlorella*, though both had lower histidine levels than fishmeal. In contrast, fishmeal had higher (p<0.05) levels of histidine and lysine. *Spirulina* exhibited significantly higher levels of arginine, isoleucine, leucine, threonine, and valine compared to both fishmeal and *Chlorella*. Additionally, *Chlorella* had lower levels of methionine, alanine, and aspartic acid compared to fishmeal and *Spirulina*.

All tested non-essential amino acid in *Chlorella* were significantly lower (p<0.05) compared to fishmeal and *Spirulina*. Furthermore, the EAAs contents of all research diets was statistically equal (p<0.05; Table 6).

Table 5. Amino acid contents of fishmeal, *Spirulina*, and *Chlorella* used in the experiment (mg·100 g^{-1}).

Amino acid	Fish meal	*Spirulina*	*Chlorella*
Arginine	4,116.7±9.1^{b}	4,492.3±27.9^{c}	2,527.8±28.0^{a}
Histidine	757.2±45.8^{b}	545.4±20.6^{a}	534.0±19.6^{a}
Isoleucine	2,520.3±67.2^{b}	3,476.9±22.7^{c}	1,785.0±20.4^{a}
Leucine	4,762.2±89.1^{b}	5,679.6±1.5^{c}	4,202.5±8.7^{a}
Lysine	5,690.8±64.3^{c}	3,443.2±18.0^{b}	3,127.3±17.3^{a}
Methionine	2,105.3±113.2^{b}	1,933.1±44.5^{b}	1,162.9±40.2^{a}
Threonine	2,806.4± 26.8^{b}	3,420.2±51.1^{c}	2,269.9±65.8^{a}
Valine	3,120.0±33.0^{b}	4,154.4±22.4^{c}	2,499.9±18.2^{a}
Alanine	3,470.2±22.5^{b}	5224.7±33.1^{c}	2,923.0±34.6^{a}
Aspartic Acid	6,187.6±80.8^{b}	6,666.3±133.2^{c}	4,433.3±108.8^{a}
Cystine	1,180.0±24.4^{b}	1,299.2±17.2^{c}	944.3±18.3^{a}
Glutamic acid	9,160.6±80.2^{c}	8,448.6±15.9^{b}	5,617.4±17.3^{a}
Glycine	4,099.9±67.3^{c}	3,450.9±15.4^{b}	2,716.8±11.8^{a}
Proline	2,602.5±107.4^{ab}	2,832.2±73.5^{b}	2,369.4±47.0^{a}
Serine	2,784.4±43.9^{b}	3,463.2±18.9^{c}	2,078.6±24.6^{a}

Note: Mean±SD in each row, superscripted with different lowercase letters are significantly different ($p<0.05$); Phenylalanine, Tryptophan, and Tyrosine were not detected.

Table 6. Amino acid profiles of treatment diets used in the experiment (mg·100 g^{-1}).

Amino acid	C	5SP	5CH
Arginine	2,984.0±7.0^{a}	2,801.7±5.3^{a}	2,807.0±48.8^{a}
Histidine	532.3±32.4^{a}	549.9±18.2^{a}	564.3±5.6^{a}
Isoleucine	1,881.4±74.7^{a}	1,904.3±31.3^{a}	1,846.6±3.3^{a}
Leucine	3514.9±71.6^{a}	3440.8±26.0^{a}	3448.2±32.7^{a}
Lysine	3,520.7±110.4^{a}	3,359.7±28.4^{a}	3,395.9±57.8^{a}
Methionine	1,363.4±.78.1^{a}	1,297.8±12.6^{a}	1,287.3±30.0^{a}
Threonine	1,985.0±47.7^{a}	1,989.8±25.7^{a}	1,927.8±23.3^{a}
Valine	2,472.8±66.1^{b}	2,399.2±14.7^{ab}	2,407.4±46.7^{ab}
Alanine	2,976.3±57.9^{a}	2,901.8±22.1^{a}	2,917.0±66.8^{a}
Aspartic Acid	4,796.0±128.9^{a}	4,631.1±30.9^{a}	4,590.0±92.4^{a}
Cystine	1,208.5±144.8^{a}	1,023.5±15.6^{a}	1,188.5±258.8^{a}
Glutamic acid	6,948.8±67.7^{a}	6,559.6±181.5^{a}	6,642.1±62.4^{a}
Glycine	2,778.5±59.5^{a}	2,627.1±7.9^{a}	2,667.7±47.9^{a}
Proline	1,947.0±11.7^{a}	2,080.0±29.7^{a}	2,177.0±87.9^{a}
Serine	2,136.8±65.3^{a}	2,186.0±2.3^{a}	2,139.5±40.6^{a}

Note: Mean±SD in each row, superscripted with different lowercase letters, are significantly different ($p<0.05$); Means without superscripts are non-significantly ($p≥0.05$) different; Phenylalanine, Tryptophan and Tyrosine were not detected.

DISCUSSION

Effects of Chlorella supplement on survival

Nursery stage fish are often unable to digest formulated feeds due to the low enzyme generation and the absence of the stomach (Lauff and Hofer, 1984). However, guppy fry are born with developed organs (Coad, 2017), enabling them to consume formulated feed from birth. In this experiment, all fish, regardless of the diet, had similarly high survival rates (96–97%). *Spirulina* was successfully replaced by *Chlorella* in the fry diets without affecting survival. Although *Chlorella* has a lower essential amino acid (EAA) profile than that of *Spirulina* ($p<0.05$, Table 5), the overall nutrient composition of the feeds was similar (Table 6), which may explain the equal survival rates.

Growth performance of fish fed Chlorella- and Spirulina- supplemented diets

Results indicated that *Spirulina* cannot be fully replaced by *Chlorella* for enhancing growth performance. *Spirulina* has consistently shown superior growth benefits in fish (Abdel-Warith *et al*, 2019; EL-daim *et al*., 2019; Almulhim *et al*., 2023). For example, Khanzadeh *et al*. (2016) found that incorporating 8.1–9.6% *Spirulina* with partial fishmeal replacement improved growth in *Trichopodus tricopterus*. James *et al*. (2006) also obtained maximum growth in *Xiphophorus helleri* with an 8% of *Spirulina* diet. *Spirulina* diets offer higher digestibility for crude protein, lysine, crude fiber, energy, dry matter, carbohydrates, and DHA compared to *Chlorella* diets (Raji *et al*., 2020). Additionally, higher EAA content of *Spirulina*, particularly lysine and methionine, supports better growth and feed efficiency (NRC, 2011).

Poecilia reticulata fry fed *Spirulina*-supplemented diets showed significantly better growth ($p<0.05$) than those fed other diets. These are attributed to superior amino acid profile, higher protein, vitamins, and mineral contents of *Spirulina* (Verdasco-Martin *et al*., 2019). The digestibility of *Spirulina*, due to its cellulose-free cell wall (Habib *et al*., 2008), further enhances growth. Further, phycocyanin in *Spirulina* also increases digestive enzyme activity, boosting growth performance (Biabani *et al*., 2019).

There is limited research comparing the combined use of *Chlorella* and *Spirulina* in aquafeeds. The present study did not show any benefits of supplementing *Chlorella* in the feed for growth, in contrast to some previous reports (Radhakrishnan *et al*., 2015; Enyidi, 2017).

However, *Chlorella* meal is an economical feed ingredient compared with fishmeal and *Spirulina* (Barone *et al*., 2018). Researchers have confirmed that *Chlorella* enhances fish growth mainly because of its growth factor (CGF) and other major components, a short-chain polypeptide protein and nucleic acid complex (Li *et al*., 2015). Therefore, further research with varying inclusion levels of *Chlorella* in the feed is needed to reveal the potential for better growth performance in *P. reticulata*.

Effects of Chlorella supplement on stress

Since ornamental fishes are transported long distances, stress tolerance without mortality is an expected quality of a fish. A method has been developed to test fish stress tolerance, whereby the lower values of the stress tolerance index indicate the higher stress tolerance (Lim *et al*., 2003). In this study, fish fed different diets showed no significant differences in stress tolerance, despite a higher trend was shown for the *Chlorella* and *Spirulina* supplemented diets. Similar methionine and lysine levels in all diets may have contributed to the comparable stress tolerance results, as these EAAs have been linked to improved stress response (Onura *et al*., 2022). Although the stress index values of all treatments tended to be lower than the control group ($p\geq0.05$), they were still higher than previous studies where Vitamin C reduced stress indexes significantly (Lim *et al*., 2002). Enhancing stress tolerance in formulated feeds may require further adjustments, as suggested by Sahin *et al*. (2012).

Effects of Chlorella supplement on breeding performance

The breeding parameters of *P. reticulata* were similar across all treatments, except for the average number of fry per female, which was significantly higher in the *Spirulina*-fed group compared to the control, which the *Chlorella*-fed group was not different from both the control and the *Spirulina*-fed groups. The results suggested a positive trend of *Chlorella* in improving this trait in guppy. The present study is supported by James *et al*. (2006) who found that diets with *Spirulina* significantly improved the breeding performances of *Xiphophorus helleri*, where the fish fed 8% *Spirulina* had four times heavier gonads compared with the group fed 1-3% *Spirulina*-supplemented diet. Therefore, it is surmised that *Chlorella* may have favourable effects on breeding performances of guppy, and thus necessitate further study with higher concentration of *Chlorella* supplementation.

Effects of Chlorella supplement on pigmentation

Fish pigmentation can be enhanced by adding colour enhancers to their diet (Ako *et al*., 2000; Sathyaruban *et al*., 2024; Perera *et al*., 2024), as fish cannot synthesize pigments on their own (NRC, 2011). While *Spirulina* is widely used for this purpose, it is expensive. *Chlorella* is a more affordable alternative (Barone *et al*., 2018). Several studies revealed that novel ingredients, such as microalgae and insect meal, can improve fish coloration (Rout *et al*., 2013; Perera and Bhujel, 2022). While *Spirulina* is more effective than other microalgae in enhancing pigmentation (Sathyaruban *et al*., 2021), some studies suggest that *Chlorella* also has pigmentation benefits (Gouveia *et al*., 2003; Gouveia and Rema, 2005; Ezhil *et al*., 2008). Additionally, *Chlorella* can be enriched with higher levels of pigments and flavonoids, depending on its culture medium (El-fayoumy *et al*., 2019). However, its impact on pigmentation may vary across different fish species due to differences in digestibility and palatability. For example, *Chorella* has been less effective in enhancing muscle pigmentation in carnivore species like rainbow trout (Gouveia *et al*., 1997).

CONCLUSIONS

The study showed that *Chlorella* enhanced the pigmentation of guppy but had no significant effect on growth. Additionally, the inclusion of *Chlorella* did not negatively impact breeding performance or stress tolerance. Therefore, it can be considered a cost-effective alternative to *Spirulina* as a colour enhancer in the diet of *Poecilia reticulata* diet. However, further research is recommended to explore its potential benefits for growth and breeding performance.

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