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Effect of Henna Leaves (*Lawsonia inermis*) Incorporated Fish Feed on Growth, Survival and Pigmentation of Guppy, *Poecilia reticulata* (Peters, 1859)

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ABSTRACT

The study was conducted during April, 2024–June, 2024 at Aquaculture wet laboratory of Dr. MGR Fisheries College and Research Institute, Thalainayeru. This research sought to examine how incorporating powdered dried henna leaves (*Lawsonia inermis*) into fish food impacts the growth, survival and pigmentation of guppy. For the experimental trial, 120 male guppies were stocked in 8 tanks (30 fishes treatment^{-1} in duplicate). The fish were fed with four isonitrogenous diets (33% Crude Protein) with different concentrations of Henna incorporation: Control (0%), H_1 (1%), H_2 (2%), and H_3 (3%) for 60 days. At the end of the experiment, fish samples were analysed for growth parameters, survival, and pigment accumulation. Compared to the control group, H_3 exhibited significantly higher ($p<0.05$) growth indicators, including specific growth rate, net weight gain, and weight gain percentage. The survival rate was significantly greatest ($p<0.05$) in H_2, followed by H_3, H_1, with lowest survival rate observed in control group. The tissue carotenoid concentration was significantly ($p<0.05$) highest in control group, followed by H_2, H_3 and was significantly ($p<0.05$) lowest in H_1. Colour intensity assessment using image J software® revealed that red colour exhibited significant lower value ($p<0.05$) in H_3 compared to other groups (control, H_1 and H_2), while the green and blue colours exhibited significant greater value ($p<0.05$) in the control compared to all treatment groups (H_1, H_2 and H_3). The present study demonstrated that incorporating 3% dried leaf powder of *Lawsonia inermis* in guppy feed enhanced growth parameters while simultaneously reducing carotenoid content. However, 2% incorporation was found to improve the survival rate.

KEYWORDS: Colouration, feed additives, fish diet, guppy, *Lawsonia inermis*

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1. INTRODUCTION

Ornamental fish keeping has become an important occupation and hobby across the world, transcending national and cultural boundaries. The history of ornamental fish keeping dates back to around 2000 B.C. (as believed) (Mukherjee et al., 2009; Sinha, 2017; Satam et al., 2018). The global ornamental fish industry, comprising over 2,500 species, is dominated by freshwater species like guppies and neon tetras (Dey, 2016). The vibrant and brilliant colours of the ornamental fishes increase consumer willingness towards the buying of fish and determine the market potential of the species (Mukherjee et al., 2009). Among the millions of varieties and strains of ornamental fish, the live bearer guppy (*Poecilia reticulata*), also known as the rainbow or million fish, occupies an indispensable place in the global ornamental fish trade, as it belongs to the family Poecilidae and is known for its ease of breeding, low maintenance, and highly attractive strains (Mokhtar, 2015; Dernekbasi et al., 2010). The scientific literature presents various studies on colour enhancement in ornamental fish using natural and artificial compounds. The different natural and herbal compounds tested include black mulberry in rainbow trout (*Oncorhynchus mykiss*) (Shekarabi et al., 2020), *Lantana camara* in sword tail (*Xiphophorus helleri*) (Zutshi and Madiyappa, 2020a), red pepper (*Capsicum annum*) in yellow tail cichlid (*Pseudotropheus acei*) (Yigit, 2019), *Wolffia arrhiza* and *Spirulina platensis* in Bengal loach (Gogoi et al., 2018), *Chlorella sp.* enriched cladocerans in Siamese fighting fish (*betta splendens*) (Rasdi et al., 2020) and marigold (*Tagetes erecta*) in rosy barb *(Punctius conchonius*) (Jagadeesh et al., 2015), to name a few. These herbal products, besides being safe to use, also reduce feed cost compared to expensive artificial colouring agents when administered orally (Wrolstad and Culver, 2012; Chong et al., 2020; Christaki et al., 2020; Unver and Hamzaçebi, 2020). Among the various pigments used for colour enhancement of fishes, carotenoids are yellowish red coloured lipid soluble pigment that play a crucial role in colour impartment in fish. However, these pigments cannot biosynthesized naturally by fish (Langi et al., 2018; Unver and Hamzaçebi, 2020; Imran et al., 2025). The measurement of colouration was carried out by measuring the carotenoid content of the fish and also by using digital images of the fish (Siegenthaler et al., 2017; Islamy et al., 2025). Lawsone, another red-orange pigment, is a naturally occurring derivative of 1,4-naphthoquinone with molecular formula $C_{10}H_6O_3$ (Kumar et al., 2024). However, the role of lawsone in colour enhancement of most fishes is still unknown. A natural source of lawsone, *Lawsonia inermis,* (also known as hina, the henna tree, the mignonette tree, and the Egyptian privet), is a shrub belonging to the family Lythraceae (Al-Snafi, 2019; Singam et al., 2020). This medicinal herb is commonly used as a dyeing agent for skin, hair and fingernails, and fabrics including silk, wool, and leather (Islam et al., 2024). It exhibits diverse pharmacological attributes, such as antibacterial, antifungal, antiparasitic, anti-oxidant, hepato-protective, analgesic, anti-inflammatory, wound healing, burn healing, anti-diabetic, anti-cancer and immune-modulatory properties due to the presence of phytochemicals like phenolics, flavonoids, saponins, alkaloids, terpenoids, quinones, coumarins, xanthones, resins, and tannins (Al-Snafi, 2019; Adeel et al., 2018). The major pigment in *Lawsonia inermis* is lawsone (2-hydroxy-1,4-naphthoquinone), also known as hennotannic acid (Ranjith et al., 2021). However, studies on the effect of henna (*Lawsonia inermis*) as the feed additive for enhancing growth, survival and colouration of guppy is lacking. Hence, the present study was undertaken to investigate the effect of henna powder on the growth, survival, and colouration of the guppies.

2. MATERIALS AND METHODS

2.1. Procurement, acclimatization, and stocking of fish

The study was conducted during April, 2024 – June, 2024 at Aquaculture wet laboratory of Dr. MGR Fisheries College and Research Institute, Thalainayeru (10° 31’ 48.9828” N Latitude, 79° 45’ 15.3324” E Longitude). A total of 120 male guppy fish were procured for the study. Only male guppies were selected to ensure homogeneous colour distribution and to avoid breeding during the experiment. The fishes were brought to the laboratory, acclimatized for two weeks, and fed formulated feed containing 33% Crude Protein and 5% Crude Lipid at 7% of their average body weight. The experimental setup consisted of 8 plastic tubs (100 l capacity each), with one control and three treatments in duplicate. Each tub was stocked with 15 randomly selected acclimatized guppy fish, following a Completely Randomized Design (CRD).

2.2. Preparation of experimental feed

The experimental feed was prepared with four locally available ingredients: corn flour, wheat flour, fish meal, and groundnut oil cake. Fish meal (42% crude protein) was prepared manually using lesser sardines. The dried henna powder (5% moisture content) was incorporated into the formulated feed as a feed additive. The henna leaves were shadow-dried for 4 days, pulverised, and then sieved through 80-mesh sieves. Four different experimental diets were prepared: Control (0% henna powder), H_1 (1% dried henna powder), H_2 (2% dried henna powder), and H_3 (3% dried henna powder). The pulverised wheat flour, corn flour, fish meal, groundnut oil cake, and dried henna leaf powder were steam cooked, pelletized (2 mm die size) and then sun-dried to a moisture content of 10% for 2 days. All the diets were isonitrogenous (33% CP) (Table 1). The proximate

Table 1: Composition and proximate analysis of experimental Diets (g kg^{-1} on a dry weight basis)

Ingredients		Dietary group			
		Control diet	Henna incorporated diet		
		Control	H1	H2	H3
Fish meal[a] (42% CP)	Ingredients (g)	380	380	380	380
Groundnut oil cake[b] (44% CP)		350	350	345	340
Corn flour[b]		120	120	120	120
Wheat flour[b]		150	140	135	130
Dried henna powder[a]	Additive (g)	0	10	20	30
Proximate composition					
Moisture (%)		2.01±0.15	5.97±0.14	4.97±0.18	4.17±0.09
Crude Protein (%)		33.17±0.1	33.17±0.15	33.14±0.05	33.59±0.14
Lipid (%)		5.13±0.03	5.68±0.11	6.43±0.21	4.50±0.05
Ash (%)		5.22±0.03	4.97±0.14	5.61±0.02	5.46±0.19

[a]Prepared manually at laboratory; [b]Procured from the local markets

composition of the fish feed was analysed according to standard AOAC methods (Anonymous, 2005).

2.3. Feeding experiment

The experiment was carried out in an indoor system. The fishes were fed twice daily (9.00 AM and 5.00 PM) using the prepared experimental diets. Water exchange (30 %) was done twice a week to remove uneaten feed and faecal matter. Water quality was monitored fortnightly, once and the study was carried out over 60 days (Figure 1).

2.4. Growth and water quality analysis

Sampling of the fish was done at the end of the experimental period, and the growth data, including net weight, weight gain %, specific growth rate of the fish, and survival rate, were analysed using the below formulas:

Weight gain of fish (%)=(FW–IW)×100/Initial weight

Net weight gain of fish (g)=FW–IW

Specific growth rate of fish (SGR) (%)=100× [ln (FW) – ln (IW)] /Duration of experiment (days)

Survival rate of fish (%) = (100×TFH)/ TFS

(FW: Final weight; IW: Initial weight; TFH: Total fish harvested; TFS: Total fish stocked)

The water quality parameters, such as dissolved oxygen (DO), alkalinity, ammonia, hardness, and temperature, in the experimental tubes were analysed every 15 days using standard APHA methods (Anonymous, 1985).

2.5. Evaluation of pigmentation in fishes

The accumulation of pigments in fish was measured in three ways: i) Total carotenoid analysis using spectrophotometer, ii) Colour intensity measurement by image J software iii) Visual observation using the Toca colour finder.

2.5.1. Total carotenoid analysis using spectrophotometer

The total carotenoid concentration in fish tissue was determined using a modified version of the method outlined by Olson (1979). Initially, the fish body tissue, excluding the head and alimentary canal, was weighed. A 1 g portion of the tissue was placed in a clean 10 ml glass vial with a screw cap, to which 2.5 g of anhydrous sodium sulphate was added. The mixture was gently mashed with a glass rod, followed by the addition of 5 ml of chloroform. The vial was then stored overnight at 0 °C in a freezer for extraction. From the 1–2 cm supernatant formed, 0.3 ml aliquots were taken and diluted with absolute ethanol to 3 ml. The optical density of the aliquot was measured using a UV-VIS spectrophotometer (Systronic, digital 108, India) at 380, 450, 470 and 500 nm, using a blank. The wavelength showing maximum absorption was used to calculate the optical density.

The total tissue carotenoid concentration was estimated using the following formula:

Total carotenoids concentration=(10×Wavelength at maximum absorption)/[0.25×sample weight (g)]

Where, 10=Dilution factor, 0.25=extinction coefficient

2.5.2. Colour intensity measurement by image j software

The images of the fish samples were captured under the same background light intensity at the same time at the end of the experiment using a modern camera. The fish were digitally cropped point by point with the help of image J software®. The colour of the fish was measured through Image J software® for red, green, and blue (RGB) values.

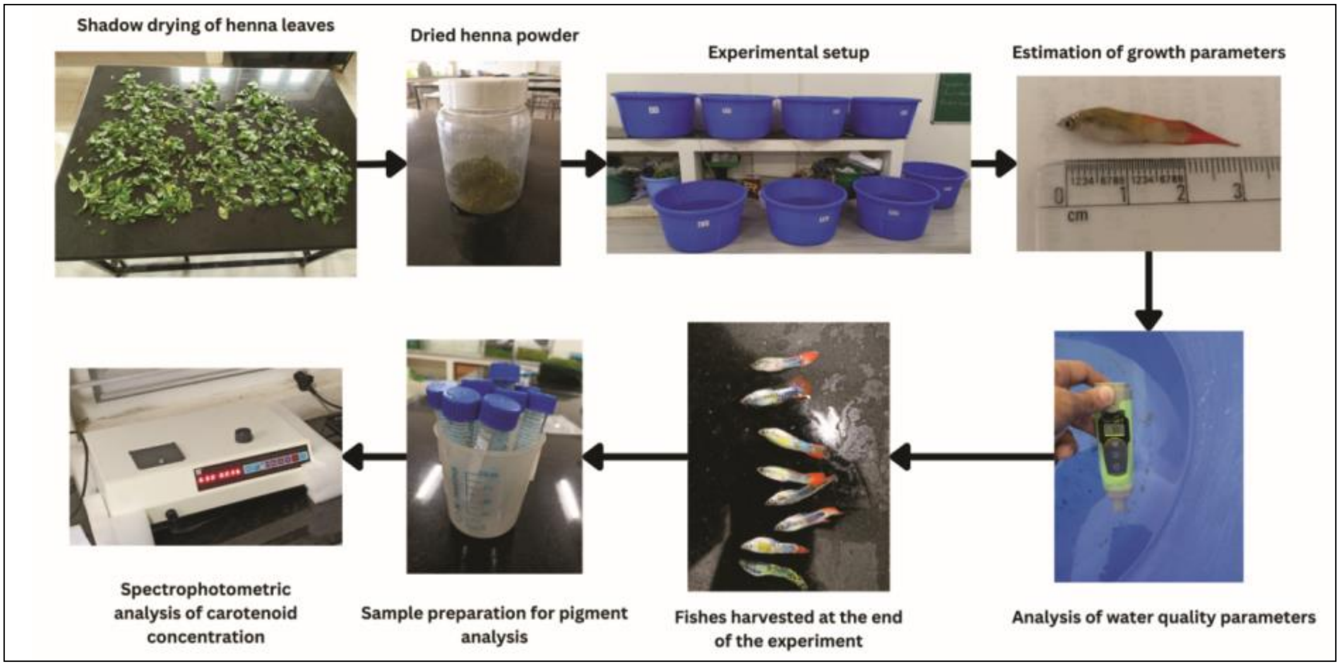

Figure 1: A detailed diagrammatic representation of the henna-incorporated fish feed formulation was provided, illustrating the step-by-step process flow of the experiment, from shadow drying of henna leaves to the spectrophotometric analysis for determining carotenoid concentration

2.5.3. Visual observation by toca colour finder chart

The colour of the fish was also manually analysed in an unbiased manner with the help of the Toca colour finder by a 5-member panel, without any visual impairment. The colour finder had seven scores, with the scores increasing in intensity of colour (scale 1 represents dark colour, and scale 7 represents bright, vibrant colour). The scores were collected individually and recorded for further analysis.

2.6. Statistical analysis

All analyses were done in triplicate. All data were presented in mean±SE using a spreadsheet (Microsoft Excel 2019). A one-way analysis of variance (one-way ANOVA) was conducted to evaluate the significant differences among the treatments for survival, growth, and colour data analysis. Duncan's multiple range test, along with one-way ANOVA, was also performed to compare the means of survival, growth, and colour data using SPSS version 26.

3. RESULTS AND DISCUSSION

3.1. Growth, survival and water quality analysis

The effect of experimental diets on the growth parameters and survival of *Poecilia reticulata* is given in Table 2. The SGR, net weight gain, and weight gain % of H_3 significantly (p<0.05) greater compared to the control group, followed by H_1. However, no statistical difference (p>0.05) was observed among the control and H_2 group. The mean final weight was significantly (p<0.05) higher in H_1 than in the control group, followed by H_3 and H_2, while the mean final length in H_3 was significantly (p<0.05) greater than in the control group. The survival rate was significantly (p<0.05) higher in H_2 than in the control group, followed by H_3 and H_1. Table 3 presented the effect of experimental diets on the water quality parameters of the experimental tubs, and it was observed that there was no notable difference or deterioration in the water quality parameters among the groups. In this study, H_3 exhibited significantly higher (p<0.05) growth indicators, including specific growth rate, net weight gain, and weight gain percentage, compared to the control group. H_1 showed the next highest values for these parameters. Similarly, a 100 µl Ethanolic extract of *Foeniculum vulgare* seed at a 100 µl concentration was found to increase the weight gain compared to the control diet in guppies (Zareen et al., 2023). A diet formulated with *Lantana camara* fed to orange molly (*Poecilia sphenops*) also increased growth (Zutshi and Madiyappa, 2020b). Sathyaruban (2024) reported that the specific growth rate of the guppies increased when fed with a 5% palmyra-enriched fruit pulp incorporated fish feed. In this conducted study, the survival rate was significantly higher in H_2 (2% henna incorporated feed) (91.64 %) than control (54.11%). Cassim and Epa (2023) also observed that male guppy fry fed with 15% *Cucurbita maxima* peel incorporated fish diet showed an increased survival rate than the feed with no incorporation. A comparable observation was made in catfish (*Clarias gariepinus*), where henna leaf extract increased the survival

Table 2: Impact of experimental diets on the growth and survival of *Poecilia reticulata*

Parameter	Control	H_1	H_2	H_3
Mean initial weight of the fish (g)	0.1926±0.01^a	0.1927±0.02^a	0.195±0.01^a	0.1865±0.01^a
Mean initial length of the fish (cm)	2.41±0.06^a	2.49±0.05^a	2.22±0.06^a	2.54±0.04^a
Mean final weight of the fish (g)	0.2184±0.01^a	0.2815±0.02^b	0.2707±0.01^b	0.2786±0.01^b
Mean final length of the fish (cm)	2.971±0.07^a	3.064±0.07^{ab}	2.878±0.13^a	3.307±0.06^b
Net weight gain of the fish (g)	0.026±0.01^a	0.089±0.02^b	0.075±0.01^{ab}	0.091±0.01^b
Weight gain (%)	13.44±7.75^a	46.11±11.5^b	38.84±9.87^{ab}	49.4±7.98^b
Specific Growth Rate	0.15±0.11^a	0.54±0.15^b	0.48±0.12^{ab}	0.64±0.08^b
Survival rate (%)	54.11±0.11^a	75.1±0.1^b	91.64±0.01^c	83.27±0.06^d

Mean values with different superscripts (a, b, c and d) in a row are significantly different (p<0.05). Values are presented as mean±SE, (n=14)

rate and acted as an antimicrobial agent at a 4% extract concentration (Karina et al., 2015). Methanolic extract of *Lawsonia inermis* at 60 and 600 mg.kg^{-1} body weight (intraperitoneally injected) was also found to enhance the survival, non-specific immune response, and disease resistance against *Aeromonas hydrophila* in common carp (Soltanian and Fereidouni, 2016). Similarly, a 0.1 g l^{-1} of garlic extract bath treatment supported the elimination of *Ichthyophthirius multifilis* parasitic infection and enhanced the survival rate in guppies (Sahandi et al., 2023). An aquous garlic extract concentration of 1.5 ml kg^{-1} incorporated into fish feed increased the survival rate in guppy fries (Ahmadniaye et al., 2020). A 20% concentration of *Lawsonia inermis* extract exhibited inhibitive properties against *Ichthyophonus* sp. in blue tilapia (Ameen et al., 2018), and 15% dietary inclusion of moringa leaf powder significantly increased mucosal immunity in guppies (Bisht et al., 2020). The enhanced survival rate confirmed the immune-boosting properties of *Lawsonia inermis,* suggesting its further exploration as a cost-effective and eco-friendly alternative to antibiotics in aquaculture.

Generally, guppies thrive at an optimal temperature of 24–29°C and they need special cooling care during summer (Shah et al., 2017). In this study, the high mortality recorded in the control group was due to elevated temperature (30.5±0.35°C) (Table 3), as the trial was conducted during the summer. The temperature remained high throughout the trial for all treatments. The mortality in H_2 was significantly (p<0.05) lower than in the control group, which may be attributed to henna, as it could have reduced the environmental stress experienced by the fish. However, this hypothesis needed further testing and validation to confirm the effect of henna on stress resistance and immunological parameters of guppy.

3.2. Analysis of pigmentation

3.2.1. Total carotenoid analysis using spectrophotometer

The carotenoid content in the skin and flesh of guppies was observed during initial and final day of the experiment (Table 4). The carotenoid concentration was significantly highest (p<0.05) in control group (9.212±0.22 µg g^{-1}), followed by H_2 (7.779±0.07 µg g^{-1}) and H_3 (7.737±0.05 µg g^{-1}), with significantly (p<0.05) lowest value observed in H_1 (4.937±0.05 µg g^{-1}). All groups showed significantly higher carotenoid levels (p<0.05) compared to the initial carotenoid level (1.650±0.02 µg g^{-1}) measured before the start of the experiment (0 day). No significant (p>0.05) difference was observed between the H_2 (7.779±0.07 µg g^{-1}) and H_3 (7.737±0.05 µg g^{-1}) groups. A significant increase (p<0.05) in carotenoid content was observed in the H_1 group

Table 3: Analysis of water quality parameters in the experimental tanks

Parameter	Control	H_1	H_2	H_3
Temperature (°C)	30.5±0.2	30.43±0.08	30.43±0.14	30.33±0.08
Dissolved oxygen (mg l^{-1})	6.4±0.05	6.6±0.12	6.43±0.14	6.96±0.12
Alkality (mg l^{-1})	155.66±3.75	161.66±3.84	149±2.64	165±2.3
Hardness (mg l^{-1})	112.43±1.41	130.81±0.59	122.3±6.16	144.04±12.45
Ammonia (mg l^{-1})	0.062±0.006	0.069±0.007	0.098±0.02	0.069±0.009
pH	8.6±0.12	8.5±0.17	8.3±0.15	8.4±0.1

The values were expressed as mean ± SE, with a sample size of n=3

Table 4: Variation in the concentration of carotenoid pigments between treatments

Treatments	Initial concentration	Final concentration			
		Control	H_1	H_2	H_3
Total carotenoid content (µg g^{-1})	1.650 ±0.009[a]	9.212 ±0.12[b]	4.937 ±0.02[c]	7.779 ±0.04[d]	7.737 ±0.03[d]

Values with distinct superscripts (a, b, c and d) in a row indicate a significant difference (p<0.05). Data are presented as mean±SE, with n=3

compared to the initial carotenoid level, but a significant decrement was observed in contrast to the control group (p<0.05). Both H_2 and H_3 groups showed a significant (p<0.05) increase in carotenoid content compared to the initial carotenoid value (1.650±0.02 µg g^{-1}), but their levels were significantly lower (p<0.05) than those in the control group (9.212±0.22 µg g^{-1}). The initial carotenoid value was significantly the lowest (p<0.05) of all groups. The most important parameter considered for successful rearing of guppies are pigmentation and growth. Herbal products are commonly used in the ornamental fish industry to enhance growth, colour, and therapeutic properties (Christaki et al., 2020; Chong et al., 2020). In a 60 day trial, garlic and sweet pepper-incorporated fish feed was found to increase growth, colouration and immunity in male fantail guppies (Mousavi et al., 2023). The colouration was significantly increased in Red Eyed Orange Molly (*Poecilia sphenops*) when fed with *Lantana camara* incorporated feed compared to a commercial diet (Zutshi and Madiyappa, 2020b). The colour of the guppies was improved when fed with a 1% palmyra-enriched fruit pulp incorporated fish diet, and higher inclusion percentages were found to enhance immunity (Sathyaruban, 2024). In this study, the carotenoid level in both H_2 (7.779±0.04 µg g^{-1}) and H_3 (7.737±0.03 µg g^{-1}) groups were notably greater (p<0.05) than the initial carotenoid value (1.650±0.02 µg g^{-1}). A similar finding was reported by Unver and Hamzacebi (2020), where a 5.92% of henna-incorporated fish diet increased carotenoid content from 1.82 µg g^{-1} to 11.37 µg g^{-1} on the 50th day in red zebra cichlid (*Maylandia estherae*). In a 90 day experiment, Bisht et al. (2023) reported that guppies fed with a 15% *Moringa oleifera* leaf-incorporated diet showed increased pigmentation. Govindharajan et al. (2024) found that 4% inclusion of the marine green algae *Chaetomorpha aerea* improved skin mucosal immune parameters and colouration in guppies over a 30 day trial. Similarly, 15% inclusion of *Nannochloropsis* sp. in fish feed also increased pigmentation in guppies (Sultana et al., 2022). Carotenoid content and growth were enhanced in male guppy fries fed a 15% *Cucurbita maxima* peel-incorporated fish diet (Cassim and Epa, 2023). In a 6 weeks trial, two different formulated fish feeds containing *Ipomoea batatas* leaf meal (10%) and *Artocarpus heterophyllus* seed meal (3%) increased carotenoid concentration in guppy fries (Nishshanka et al., 2021). Guppies fed a 15% spirulina (*Arthrospira platensis*) incorporated fish diet exhibited increased growth and pigmentation compared to the control and lower inclusion groups, and it also acted as an alternate protein source to fish meal (Bisht et al., 2022). However, this observation does not apply to Henna powder as it was deficient in protein. Similarly, the moisture content of the control feed (2%) was lower compared to moisture content of henna-incorporated fish feed (5%). This might be due to the hygroscopic nature of henna powder incorporated in treatment feed which needed to be evaluated by further studies.

The significant increase in carotenoid content in the control group compared to the initial value might be due to the presence of carotenoid-containing fish meal used as an ingredient in the feed formulation. The significant decrease in carotenoid content was observed in henna-incorporated fish feed (H_1, H_2 and H_3) when compared to henna-free fish feed (control group) might be postulated to the compounds present in henna such as tannins, saponins, lawsone, and other phenolic compounds, that reduce the gut absorption and bio-availability of carotenoids (Al-Snafi, 2019). Hence, additional research was required to investigate the digestibility of henna and its effect in gut absorption. At present, there was no bio-chemical method for estimating lawsone in fish. Therefore, upcoming research should focus on the formulation and subsequent standardization of techniques for estimating lawsone and investigating its roles in growth, metabolic activities, disease resistance, and

Table 5: Estimation of pigment impartment in *Poecilia reticulata* using RGB Image J software and Toca colour finder

Parameter	Control	H_1	H_2	H_3
Red	106.26 ±3.88[a]	101.96 ±7.88[ab]	100.79 ±5.07[ab]	90.02 ±1.81[c]
Green	101.43 ±5.11[a]	82.65 ±4.06[b]	89.09 ±3.9[b]	85.34 ±2.09[b]
Blue	77.24 ±5.44[a]	63.54 ±3.64[b]	69.52 ±3.49[ab]	63.09 ±3.22[b]
Average rank obtained (using Toca colour finder)	4.8	4	3.8	3

Significant differences (p<0.05) were indicated by different superscripts (a, b, c) in a row. The data were shown as mean±SE, with n=8 immune response in fish.

3.2.2. Colour intensity measurement by image j software and the toca colour finder

The redness, greenness and blueness of the fish image were observed using image J software® at the end of the experiment (60 days). The red colour was significantly (p<0.05) lower in H_3 compared to the other groups (control, H_1 and H_2), with redness value showing a decreasing trend as the concentration of henna incorporation increased (Table 5). The green and blue colours were significantly higher in the control group than in all treatment groups (H_1, H_2 and H_3). The green and blue colour in the H_3 group were significantly (p<0.05) lower than in all other groups (Figure 1). Visual observation using the Toca colour finder chart were done at the end of the experiment. The average ranks given by panel members showed that the colour intensity was highest in the control group, followed by H_1, H_2 and H_3 (Table 5). In another study using Adobe photoshop and Image J software for RGB analysis, 10% of cochineal powder (*Dactylopius coccus*) incorporated fish feed was found to increase the red value of *Betta splendens* (Islamy et al., 2025). However, the current study shows that the red, green and blue (RGB) values were notably reduced (p<0.05) in henna-incorporated fish feed than control group. RGB analysis in ImageJ software reflected the values based on the brightness of the red, green and blue colours of the experimental fish. The decrease in RGB values with an increase in the percentage of henna incorporation might be due to the lawsone pigment, which imparted darkness to the

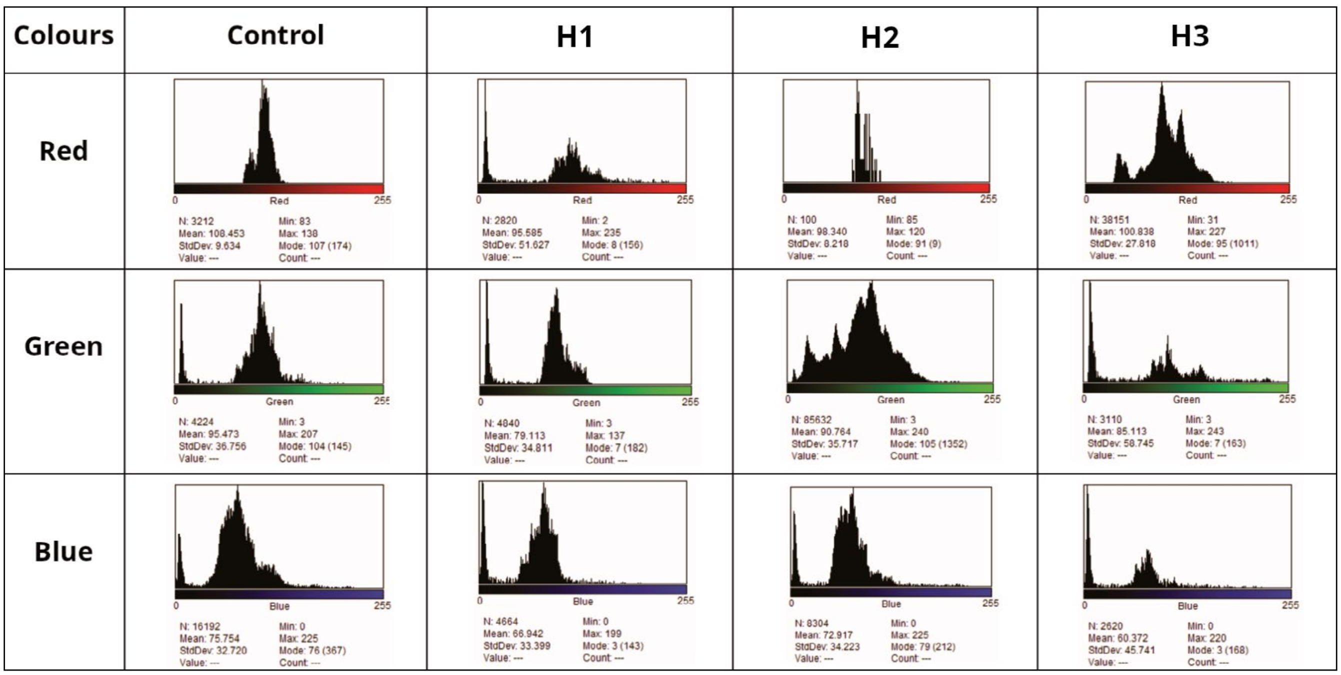

Figure 2: Histograms of red, green, and blue (RGB) colour analysis of guppy photo samples analysed using Image J software. The mean values in this graph illustrated the colour variations among the treatments resulting from different concentrations of henna incorporation in the formulated fish diet

fish (Figure 2). The study, which also included a ranking of colour intensity by panel members using the Toca Colour Finder chart, further supported this observation.

4. CONCLUSION

Incorporating 3% dried leaf powder of *Lawsonia inermis* in the diets of *Poecilia reticulata* increased growth parameters, while 2% incorporation improved survival rates. Moreover, the results also showed that higher levels of henna incorporation reduced tissue carotenoid content, while simultaneously increasing the darkness of the fish, which might be attributed to lawsone. This study paved the way for further research on the roles of lawsone in growth, metabolic activities, disease resistance, and immune response in fish.

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