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# Impact of Sustainable Feed from Vegetable Waste on Coloration in Black Molly (*Poecilia sphenops*)

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## Abstract

A large amount of vegetable and fruit waste is dumped into landfills and rivers every year, posing an environmental hazard. Many of these wastes, including outer leaves, stems, peels and leftover parts are high in nutrients and can be used in the preparation of feed for livestock. To observe the effect of vegetable waste on the pigment concentration of fish, a three-month experiment was conducted. Black mollies (*Poecilia sphenops*), which are known for their dark skin pigmentation were taken as an experimental species. A total of 18 Black Molly fingerlings (6 per group) were used in the experiment. Survival rate, SGR and FCR was estimated to assess the performance. Pigment concentration data were examined using one-way ANOVA ($p<0.05$) with SPSS (Version 26). An experimental feed ($F_3$) was prepared by combining three types of vegetable wastes: sponge gourd peel, cauliflower stem and cabbage outer leaves. The effect of this feed was compared to control feed ($F_2$) and commercial feed ($F_1$). The pigment concentration in an experimental fish was measured at 450, 475 and 500 nm before starting the experiment and following its completion. Fish fed with the experimental feed ($F_3$) showed a significantly higher concentration of pigments (0.032 µg $g^{-1}$, $p<0.001$) compared to fish fed on $F_1$ and $F_2$. This study concludes that incorporating vegetable waste into fish feed improves pigmentation in fish and offers an environmentally friendly solution to such kind of waste disposal.

**Keywords:** Black molly, Environmental pollution, Fish feed, Vegetable waste

## Introduction

In the Indian diet, vegetables are the main source of nutritional security (Ilakiya *et al.*, 2020). However, a substantial amount of fruit and vegetable waste is generated during the collection, handling, transportation and processing of manufacturing plants. Most of this waste, both in solid and liquid forms, is generated by the processing industry only. All of this waste is biodegradable and is commonly disposed of into the rivers or in the landfills, which can cause the water pollution. Increased water pollution from such waste can lead to eutrophication in the waterbody which results in hypoxia in water body and can cause a negative impact on an aquatic organism.

On the other hand, in aquaculture systems, 60-70% of production costs in aquaculture are attributed to feed inputs, particularly in intensive ornamental fish farming systems (Hasan, 2007). Many commercially available formulated fish feeds which are way more costly due to the inclusion of high-cost ingredients such as fish meal and synthetic pigments. These are key components in supplementary feeding for the normal growth and maintenance of ornamental fishes. However, these ingredients also contribute significantly in high-cost of feed for ornamental fish. Hence, there is a need to reduce feed costs further and promote its use in low-input aquaculture for improved growth and survival rates (Kumar *et al.*, 2017).

The objective of this study was to utilize the vegetable waste as fish feed and by doing it to mitigate the environmental pollution. Vegetable waste like cabbage outer leaves, sponge gourd peel and cauliflower stem are generally not

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usable parts of vegetables and are good source of protein, carbohydrates, lipids, vitamins and minerals. These nutrients enhance their suitability as ingredients for livestock feeds. Feed ingredients have significant impacts on both fish health and the environment (Debbarma *et al.*, 2023) and by using this kind of waste as an ingredient for fish feed, it is possible to reduce the feed cost while simultaneously mitigate the environmental pollution and nutrient loss in the form of such waste.

## Materials and Methods

This research was performed at the Department of Aquatic Biology, Veer Narmad South Gujarat University, Surat (21°09'07.80" N and 72°46'54.80" E) from June 2022 to September 2022, with an aim to investigate the impacts of vegetable waste on the pigments of the common ornamental fish Black Molly (*Poecilia sphenops*). Over the three months of period, fish were maintained in controlled aquarium conditions with consistent water quality parameters. Different groups were fed various diets to assess their impact on pigmentation intensity and overall fish health.

### *1. Feed Preparation*

For the preparation of the experimental feed from vegetable waste, three different types of vegetable waste (sponge gourd peel, cabbage leaves and cauliflower stems) were collected from nearby vegetable markets and after thoroughly washing with tap water, all the raw materials were sun-dried. Other ingredients, such as GOC (groundnut oil cake), wheat flour and rice bran, were also collected and all the ingredients were thoroughly ground and made into the powder form. Powdered materials were then sieved using a small size mesh and all the ingredients were weighed in a fixed ratio (Table 1) to prepare formulated experimental feed or $F_3$. After well mixing and blending of all the raw material pelleting was done by using a pellet extruder and then prepared pellets were sundried in a dust-free condition. The control feed or $F_2$ was prepared using the same ingredients but without the inclusion of vegetable waste and commonly available commercial feed for ornamental fish was used as $F_1$. The control feed ($F_2$) was formulated with basic plant-based ingredients (wheat flour, rice bran and GOC) without any pigment supplementation to evaluate the baseline effects of a non-pigmented diet on fish growth and coloration.

### *2. Selection of the Experimental Species*

To assess the effect of vegetable waste-based feed on the pigments of ornamental fishes, the black molly (*Poecilia sphenops*) was selected as the experimental species. The black molly is one of the popular freshwater ornamental fish. It is generally kept in aquarium due to its attractive and richly pigmented body. It is also a hardy and lab-friendly species which makes it a suitable species for the investigation of pigment changes. Being omnivorous, this species readily accepts various types of feed, including a plant-based diet, which aligns well with the experimental feed prepared from vegetable waste.

### *3. Aquarium Setup and Maintenance*

The aquarium was setup in the Aquarium Room of the Department of Aquatic Biology. Three aquariums with a capacity of 15 litres each were filled to three-quarters of their total volume and tagged the names as Aquarium-A, Aquarium-B and Aquarium-C for the experiment. During the experiment, each aquarium was provided with an individual air pump to ensure proper aeration. Six fingerlings of black molly were stocked in each aquarium. While the sample size was limited to six fish per group due to the infrastructural constraints, this design was maintained consistently on all treatments to allow for comparative analysis. However, larger sample sizes may improve statistical robustness in future studies.

The stocking density (6 fish per 15 L aquarium) was selected based on standard practices for ornamental fish trials (FAO, 1997), ensuring sufficient space and minimizing stress under controlled laboratory conditions.

### *4. Feed Application*

To observe the effect of the experimental diets on the pigments of black mollies, the $F_3$ (experimental feed), $F_2$ (control feed) and $F_1$ (commercial feed) were provided to the fish in the Aquarium-3, Aquarium-2 and Aquarium-1, respectively (Figure 1). The feeds were given twice a day to the fishes @ 3% of their total biomass.

### *5. Observation of water parameters:*

To understand the effect of water quality on fish health, some basic physico-chemical parameters were analysed at 15 days interval. Temperature was measured by using lab thermometer, turbidity was measured by using Digital Turbimeter EQ-811 and pH was measured by using Systronics pH System 362.

### *6. Observation and Calculation of Growth, Pigments and Survival*

Before the start of the feeding trial, baseline measurements for growth with reference to weight, pigments and overall health were recorded to establish initial growth and pigments. Throughout the experimental period, fish were monitored regularly to observe any changes in behaviour, feeding response and mortality rates. After giving the feeds to fish for over three months, final measurements were taken to calculate growth, pigments and survival rate. These data were crucial to assess the effectiveness of each feed formulation on fish development and viability.

*a) Survival Rate*

The survival rate of the experimental fish was calculated using the following formula:

$$\text{Survival rate} = \frac{\text{Number of fish at the end stage of the experiment}}{\text{Number of fish at the initial stage of the experiment}}$$

*b) Growth Parameters*

To know the effect of experimental diet on the growth of black molly, weight measurement of fish was done and the growth was calculated as SGR (Specific Growth Rate) % and FCR by using following formulas.

$$\text{Specific Growth Rate (SGR) (\%/day)} = \frac{\ln W_2 - \ln W_1}{T} \times 100$$

Where, $W_2$ represents the mean final weight of the fish, $W_1$ denotes the mean initial weight of the fish, ln refers to the natural logarithm and T signifies the duration of the experiment.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed given}}{\text{Total biomass harvested}}$$

*c) Pigment Estimation*

Pigments were compared in fish fed on commercial diet, control diet and vegetable waste-based diet by estimating the pigment concentrations before and at the end stage of the trial. Fish samples for pigment estimation were randomly collected from each aquarium and scale and caudal fin samples were taken. Pigment extraction was performed using the spectrophotometric method (Harpaz and Padowicz, 2007). In this method, the collected samples were preserved at -20 °C for 2 days. After freezing, the samples were homogenized and weighed and then pigments were extracted using 80% acetone. After adding 3 ml of acetone, samples were kept in the dark condition for 24 hours. Each sample was centrifuged at 3000 rpm for 10 min. and after that supernatants were taken into the separate test tube. The extracted pigment concentrations were measured in a UV Spectrophotometer by using three different wavelengths at 450, 475 and 500 nm to avoid any chance of mistake.

Pigments were quantified using the following formula:

$$\text{Pigments } (\mu g\ g^{-1}) = \frac{4\times\text{Optical Density Value}\times\text{Total volume of sample taken}}{\text{Weight of sample (mg)}}$$

*d) Statistical Analysis*

Collected data were statistically analysed by using Excel and SPSS (Version 26) software. A significant difference between the pigments in all the fish was determined using a One-way ANOVA at a significance level of $p<0.05$.

## Results and Discussion

*a) Water Quality Observation*

Water quality plays a crucial role in the fish health, with temperature being a significant component that influences the metabolic rates of fish as with rise in temperature, fish metabolism also rises (Clarke and Johnston, 1999). As all the experimental tanks received the same water supply, the water temperature in Aquarium-1, Aquarium-2 and Aquarium-3 were found to be the same; where the lowest water temperature was 26 °C, while the highest temperature was 28 °C. The pH level is the second important water parameter after temperature around which, the water samples taken from Aquarium-1, Aquarium-2 and Aquarium-3 had minimum pH levels of 7.383, 7.392 and 7.329, and the maximum pH levels of 8.207, 8.396 and 8.367, respectively (Table 2). The minimum turbidity levels were 0 NTU, 0 NTU and 2 NTU, and the maximum turbidity levels were 11 NTU, 9 NTU and 8 NTU for Aquarium-1, Aquarium-2 and Aquarium-3 respectively (Table 2). These results suggest that the water quality does not affect the growth of fish.

*b) Growth Observation*

Any increase in the body of fish, particularly in size and weight is considered as a growth, which depends on various internal and external factors like, ability to utilize the feed, age, species, space, competition and water quality where the fish is living (Pauly, 1980; Clarke, 1991). Ornamental fishes require good nutrient rich food for the growth and maintenance of their body and the vegetable waste-based feed provides that nutrients. During the experiment, it was observed that the fish fed on $F_3$ showed positive growth as the SGR % for fish fed on $F_3$ was calculated 0.023% with 2.35±0.20 g weight before the experiment and 2.4±0.32 g weight at the end of the experiment with lowest FCR at 2.38. No positive growth was observed in case of fish fed on $F_1$ and $F_2$ as the SGR % for fish fed on $F_1$ was calculated 0.00% with 2.42±0.18 g initial weight and 1.90±0.20 g final weight with highest FCR at 8.76. The SGR % of fish fed on $F_2$ was also found 0.00% with 2.12±0.20 g initial weight and 1.7±0.01 g weight was found at the end of the experiment with moderate FCR at 5.4 (Table 3).

*c) Survival rate*

The survival rate of fish fed on $F_2$ was found to be very low, at only 16.67% compared to the fish fed on experimental feed which had the highest survival rate of 66.67%. The fish fed on commercial feed also showed a lower survival rate of 33.33% only (Figure 1). This outcome indicates that the dietary vegetable-waste inclusions can stimulate non-specific immunity. Mo *et al.* (2020) and Nassar *et al.* (2024) have also documented a similar trend for Nile tilapia fed fermented *Moringa oleifera* (elevated phagocytic and lysozyme activities) and for grass carp given fruit-vegetable waste pellets (enhanced resistance to *Aeromonas hydrophila*). It shows that the vegetable waste proved to have good nutritional quality as fish fed on them remained disease-free during the entire experiment.

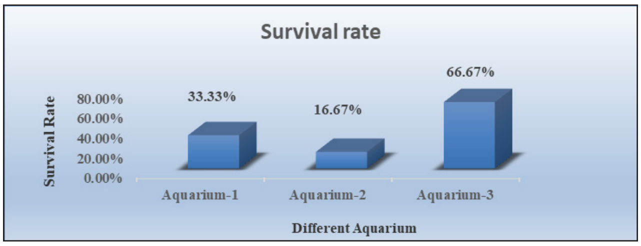


Figure 1: The survival rate of fishes fed on different feeds at the end of the experiment [Note: Aquarium-1 is for fish fed on commercial feed; Aquarium-2 is for fish fed on control feed and Aquarium-3 is for fish fed on the experimental feed]

*d) Pigment Observation*

Ornamental fishes are known to have a wide range of colours and patterns on their body, which generally decides their market values. For the healthy growth and maintenance of their body, ornamental fishes require pigments in their diet as they do not synthesize colour producing carotenoids (Sathyaruban *et al.*, 2021). In nature, this requirement is fulfilled by feeding on a variety of plankton which is available

Table 1: Experimental treatment groups and diet provided during the experiment

| Sl. No. | List of ingredients | Aquarium-1 [Commercial Feed ($F_1$)] | Aquarium-2 [Control feed ($F_2$)] | Aqurium-3 [Vegetable waste-based feed ($F_3$)] |
|---|---|---|---|---|
| 1 | Vegetable waste (Cabbage outer leaves + cauliflower stem + sponge gourd peel) | - | - | 20% |
| 2 | Wheat flour | - | 12.5% | 10% |
| 3 | Rice bran | - | 37.5% | 30% |
| 4 | Groundnut oil cake | - | 50.0% | 40% |

[Note: In vegetable waste, all the three vegetables such as cabbage outer leaves, cauliflower stem and sponge gourd peel were added in equal ratio to make 20% vegetable waste powder; ornamental fish feed available at the market was taken as Commercial feed ($F_1$) and administrated to the fishes in Aquarium-1; control feed and vegetable waste-based feed were administrated to the fishes in Aquarium-2 and Aquarium-3, respectively]

Table 2: Water quality parameters of experimental setup during the experiment

| Parameters | Aquarium-1 | | Aquarium-2 | | Aquarium-3 | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Min. | Max. | Min. | Max. |
| Temperature (°C) | 26 | 28 | 26 | 28 | 26 | 28 |
| pH | 7.383 | 8.207 | 7.392 | 8.396 | 7.329 | 8.367 |
| Turbidity (NTU) | 0 | 11 | 0 | 9 | 2 | 8 |

[Note: Min. is for minimum and Max. is for Maximum water parameter]

Table 3: Growth of fish with reference to SGR % and FCR

| Parameter | Diet $F_1$ | Diet $F_2$ | Diet $F_3$ |
|---|---|---|---|
| Initial weight in g (Mean ± SE) | 2.42±0.18 | 2.12±0.20 | 2.35±0.20 |
| Final weight in g (Mean ± SE) | 1.90±0.20 | 1.7±0.01 | 2.4±0.32 |
| SGR % | 0.00 | 0.00 | 0.023 |
| FCR | 8.7631 | 5.4 | 2.3854 |

[Note: SGR % is for specific growth rate % and FCR is for Feed Conversion Ratio; $F_1$ is for fish fed on commercial feed; $F_2$ is for fish fed on control feed and $F_3$ is for fish fed on experimental feed; Initial weight is for weight of fish before starting the experiment and final weight is for weight of fish at the end of the experiment; Mean ± SE is for Mean ± Standard Error and g is for gram]

in natural waterbody. Although the pigments in fish are genetically determined (Rana *et al*., 2023), ornamental fishes may lose their colouration under controlled conditions due to the absence of planktonic growth. To meet this requirement, the feed supplements should be rich in pigments and other nutrients. It is proven that in case of fish fed on $F_2$ as the feed was prepared from only few basic ingredients without any inclusion of vegetable waste and fishes fed on it showed the lowest pigment concentration at 0.005 (±0.001) μg g$^{-1}$ (Figure 2), 0.006 (±0.001) μg g$^{-1}$ (Figure 3) and 0.005 (±0.001) μg g$^{-1}$ (Figure 4) when analysed at 450, 475 and 500 nm, respectively. This result is not only significantly lower ($p<0.001$) as compared to the two other groups $F_1$ and $F_3$, but it is also significantly lower ($p<0.001$) than the initial pigments of fishes which were taken before the experiments. This suggests that the basic ingredients such as wheat flour, groundnut oil cake and rice bran are not sufficient to provide pigments to the ornamental fish and also indicate the importance of pigments in the diet of an ornamental fish.

An intestine of fish absorb required pigments (Reboul, 2013) from the provided feed and then deposited into the different body parts including scales, skin, muscle, gonads (Grether *et al*., 1999) and moderate amount of pigment was deposited on the body of fish fed on $F_1$ or commercial feed as the pigment concentration when observed at 450 nm, which was consistent with earlier reports that plant-derived carotenoids yield modest coloration relative to high-pigment diets (Nhan *et al.*, 2019). It was found to be significantly ($p<0.001$) lower than the initial pigments as it was 0.016 (±0.001) μg g$^{-1}$ for fish fed on $F_1$ and 0.103 (±0.001) μg g$^{-1}$ before starting the experiment (Figure 2). The pigments in fish that fed on $F_1$ were significantly higher ($p<0.001$) than the initial values, though still lower than those in fish fed on $F_3$. When observed at 475 nm and 500 nm, it was found to be 0.011 (±0.001) μg g$^{-1}$ and 0.005 (±0.001) μg g$^{-1}$, respectively before starting the experiment and 0.015 (±0.001) μg g$^{-1}$ and 0.013 (±0.001) μg g$^{-1}$, respectively in fish fed on $F_1$ (Figure 3 and 4). But the pigment concentration in fish that fed on $F_1$ was significantly lower ($p<0.001$) than the pigment concentration found in fish that fed on $F_3$ when observed at 450, 475 and 500 nm as it was 0.031 (±0.001) µg $g^{-1}$, 0.032 (±0.001) µg $g^{-1}$ and 0.019 (±0.001) µg $g^{-1}$, respectively for fish fed on $F_3$ (Figure 2, 3 and 4). This is evident that vegetable waste provides more pigments to the black molly than the synthetic pigments found in commercial feeds. This may be because plants possess natural pigments, which are accountable for the bright and appealing colour of fruits and vegetables (Ramzan *et al*., 2025) and these findings show that these kinds of pigment-rich vegetables can enhance the colour of fish when they are fed on it.

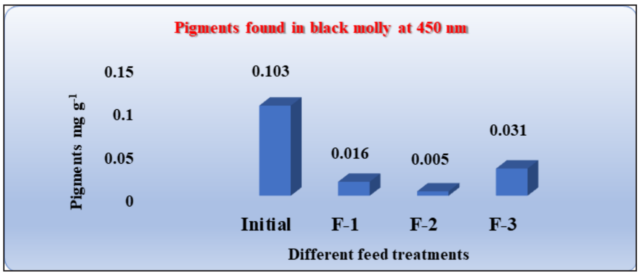


Figure 2: Pigments (µg $g^{-1}$) at 450 nm found in fishes fed on different feeds [Note: Initial is for pigments before starting the experiments, $F_1$ is for fish fed on commercial feed, $F_2$ is for fish fed on control feed and $F_3$ is for fish fed on experimental feed]

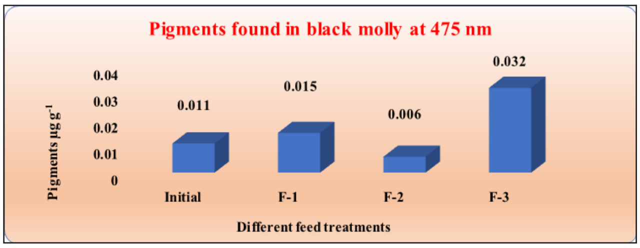


Figure 3: Pigments (µg $g^{-1}$) at 475 nm found in fishes fed on different feeds [Note: Initial is for pigments before starting the experiments, $F_1$ is for fish fed on commercial feed, $F_2$ is for fish fed on control feed and $F_3$ is for fish fed on experimental feed]

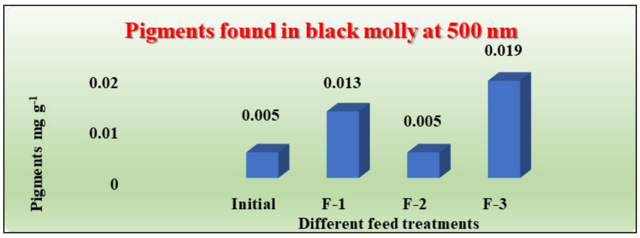


Figure 4: Pigments (µg $g^{-1}$) at 500 nm found in fishes fed on different feeds [Note: Initial is for pigments before starting the experiments, $F_1$ is for fish fed on commercial feed, $F_2$ is for fish fed on control feed and $F_3$ is for fish fed on experimental feed]

Carotenoids and melanin are two major groups of pigments, which are widely studied in an ornamental fish. Carotenoids are responsible for their orange, red and yellow colours while gray, black and brown colours come from melanin (Price *et al*., 2008). Pigment-rich artificial diets for ornamental fish feeds generally contain carotenoids for pigment supplements in which natural compounds, as well as synthetic astaxanthin, are widely used which may lead to higher costs of feeds and also degrade the environmental health. Nowadays, natural- and plant-based pigments are gradually replacing some chemically synthesized pigments (Li *et al*., 2025). Much of the plant wastes, particularly vegetable waste contains plenty of pigments and this is evident by this experiment as chlorophylls in cabbage and sponge gourd peel helped to increase the pigments in fish fed on the experimental feed. The pigment concentration in the fish fed on $F_3$ was significantly higher ($p<0.001$) than the pigments that found in fish before starting the experiment. When observed at 475 and 500 nm, it was found at 0.032 (±0.001) µg $g^{-1}$ and 0.019 (±0.001) µg $g^{-1}$, respectively at the end of the experiment, which was 0.011 (±0.001) µg $g^{-1}$ and 0.005 (±0.001) µg $g^{-1}$ before starting the experiments (Figure 3 and 4). The pigments found in fish fed on the vegetable waste-based feed ($F_3$) were significantly higher ($p<0.001$) than the fish fed on commercial feed and control feed indicating the high nutritional values of vegetable waste used in an experiment.

While findings clearly demonstrate the benefits of experimental feed in enhancing pigment concentration in black mollies, similar results were observed when same experiment was done on gold fish (*Carassius auratus*), but some limitations are also there such as the small sample size used in an experiment. When the sample size is less than 10 fish per treatment, the statistical power goes substantially lower and the risk of Type II error elevates, which could potentially hide the biologically important effects (Searcy-Bernal, 1994; Galkanda-Arachchige *et al*., 2022). It is recommended that a priori power analyses be conducted to determine appropriate replication levels in order to enhance the future findings more reliable. Additionally, the composition of nutrients and concentration of pigment content in vegetable waste can be change season to season and it also depends on source and storage facility for ingredients. The composition of vegetable waste generated by the markets are also vary in each season (Tedesco *et al*., 2021); hence, it was good choice to take the particular vegetable waste (cabbage leaves, cauliflower stem and sponge gourd peel) as the combination for fish feed ingredients.

## Conclusion

The findings demonstrated that the pigment concentration found in the fish fed on vegetable waste-based diet was significantly higher than the fish fed on commercial feed and almost twice than the fish fed on control feed with good survival, highest growth and lowest FCR. The results of this study indicate that the vegetable waste can provide the pigments to the fish and strengthen their immunity for the healthy growth and maintenance of their body. When vegetable waste is used as an ingredient for fish feeding, it has been found that it reduces the cost of feed and helps the environment by reducing pollution. The current study offers promising insights; however, its results should be interpreted with care because of the limited sample size and absence of experimental replication. Further trials that include larger populations, repeated experiments and longer observation periods will be needed to provide strong confirmation.

Additionally, future research could explore the biochemical profiling of pigment deposition, long-term impacts and test the feeding efficiency on a wider range of ornamental fish species. Despite these limitations, the evidence of this study suggests that formulating fish feeds from vegetable wastes is a sustainable, cost-effective and eco-friendly approach instead of conventional feeds. It can also improve the fish colouration thereby promoting the eco-friendly aquaculture practices.

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## References

Clarke, A., 1991. What is cold adaptation and how should we measure it? *American Zoologist* 31(1), 81-92. DOI: https://doi.org/10.1093/icb/31.1.81.

Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* 68(5), 893-905. DOI: https://doi.org/10.1046/j.1365-2656.1999.00337.x.

Debbarma, S., Devi, N.C., Yirang, Y., Yadav, N.K., Lal, J., 2023. Emerging importance of sustainable feed ingredients as a substitution of traditional fish meal in fish feed production: An essential step for sustainable aquaculture. *Research Biotica* 5(1), 11-15. DOI: https://doi.org/10.54083/resbio/5.1.2023/11-15.

FAO, 1997. *Aquaculture Development*. FAO Technical Guidelines for Responsible Fisheries No. 5. FAO, Rome, Italy. p. 40.

Galkanda-Arachchige, H.S.C., Hussain, A.S., Davis, D.A., Improvement in laboratory research: Effects of stocking density, variation and sample size on outcomes of growth studies with shrimp. *Aquaculture Research* 53, 1-13. DOI: https://doi.org/10.1111/are.15620.

Grether, G.F., Hudon, J., Millie, D.F., 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proceedings of the Royal Society B (Biological Sciences)* 266(1426), 1317-1322. DOI: https://doi.org/10.1098/rspb.1999.0781.

Harpaz, S., Padowicz, D., 2007. Colour enhancement in the ornamental dwarf cichlid *Microgeophagus ramirezi* by addition of plant carotenoids to the fish diet. *The Israeli Journal of Aquaculture-Bamidgeh* 59(4), 195-200. DOI: https://doi.org/10.46989/001c.20536.

Hasan, M.R., 2007. *Economics of Aquaculture Feeding Practices in Selected Asian Countries*. FAO Fisheries Technical Paper No. 505. FAO, Rome, Italy. p. 205.

Ilakiya, T., Parameswari, E., Davamani, V., Yazhini, G., 2020. Organic vegetable production. *Research Biotica* 2(2), 50-54. DOI: https://doi.org/10.54083/resbio.2.2.2020.50-54.

Kumar, P., Jain, K.K., MunilKumar, S., Sudhagar, S.A., 2017. Alternate feeding strategies for optimum nutrient utilization and reducing feed cost for semi-intensive practices in aquaculture system - A review. *Agricultural Reviews* 38(2), 145-151. DOI: https://doi.org/10.18805/ag.v38i02.7946.

Li, X., Wang, F., Ta, N., Huang, J., 2025. The compositions, characteristics, health benefits and applications of anthocyanins in *Brassica* crops. *Frontiers in Plant Science* 16, 1544099. DOI: https://doi.org/10.3389/fpls.2025.1544099.

Mo, W.Y., Choi, W.M., Man, K.Y., Wong, M.H., 2020. Food waste-based pellets for feeding grass carp (*Ctenopharyngodon idellus*): Adding baker's yeast and enzymes to enhance growth and immunity. *Science of the Total Environment* 707, 134954. DOI: https://doi.org/10.1016/j.scitotenv.2019.134954.

Nassar, A.A.M.A., Gharib, A.A.E., Abdelgalil, S.Y., AbdAllah, H.M., Elmowalid, G.A., 2024. Immunomodulatory, antioxidant, and growth-promoting activities of dietary fermented *Moringa oleifera* in Nile tilapia (*Oreochromus niloticus*) with *in-vivo* protection against *Aeromonas hydrophila*. *BMC Veterinary Research* 20, 231. DOI: https://doi.org/10.1186/s12917-024-04070-3.

Nhan, H.T., Minh, T.X., Liew, H.J., Hien, T.T.T., Jha, R., 2019. Effects of natural dietary carotenoids on skin coloration of false Clownfish (*Amphiprion ocellaris* Cuvier, 1830). *Aquaculture Nutrition* 25(4), 979-988. DOI: https://doi.org/10.1111/anu.12887.

Pauly, D., 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. *ICES Journal of Marine Science* 39(2), 175-192. DOI: https://doi.org/10.1093/icesjms/39.2.175.

Price, A.C., Weadick, C.J., Shim, J., Rodd, F.H., 2008. Pigments, patterns, and fish behavior. *Zebrafish* 5(4), 297-307. DOI: https://doi.org/10.1089/zeb.2008.0551.

Ramzan, K., Zehra, S.H., Balciunaitiene, A., Viskelis, P., Viskelis, J., 2025. Valorization of fruit and vegetable waste: An approach to focusing on extraction of natural pigments. *Foods* 14(8), 1402. DOI: https://doi.org/10.3390/foods14081402.

Rana, S., Bari, A.A., Shimul, S.A., Mazed, M.A., Nahid, S.A.A., 2023. Enhancement of body coloration of sword-tail fish (*Xiphophorus helleri*): Plant-derived bio-resources could be converted into a potential dietary carotenoid supplement. *Heliyon* 9(4), e15208. DOI: https://doi.org/10.1016/j.heliyon.2023.e15208.

Reboul, E., 2013. Absorption of vitamin A and carotenoids by the enterocyte: Focus on transport proteins. *Nutrients* 5(9), 3563-3581. DOI: https://doi.org/10.3390/nu5093563.

Sathyaruban, S., Uluwaduge, D.I., Yohi, S., Kuganathan, S., 2021. Potential natural carotenoid sources for the colouration of ornamental fish: A review. *Aquaculture International* 29(4), 1507-1528. DOI: https://doi.org/10.1007/s10499-021-00689-3.

Searcy-Bernal, R., 1994. Statistical power and aquacultural research. *Aquaculture* 127(4), 371-388. DOI: https://doi.org/10.1016/0044-8486(94)90239-9.

Tedesco, D.E.A., Scarioni, S., Tava, A., Panseri, S., Zuorro, A., 2021. Fruit and vegetable wholesale market waste: Safety and nutritional characterisation for their potential re-use in livestock nutrition. *Sustainability* 13(16), 9478. DOI: https://doi.org/10.3390/su13169478.