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PIGMENT STUDY OF GOLDFISH (*Carassius auratus*) FED ON VEGETABLE WASTE WITH SPIRULINA

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Abstract:

Aquafeed generally takes 60-70% part in the input cost of aquaculture. In which, fish meal is generally used as one of the costliest ingredients as a protein source, which makes the feed more costly. In ornamental fish feed, pigment supplements are another cost-increasing ingredient. The experimental feed was prepared by replacing the fish meal and an artificial pigment with the nutrients of three vegetable waste and nutrient-rich blue-green microalgae Spirulina. Prepared feed (F-3) was given to the experimental fishes along with the control (F-2) feed and the commercially available ornamental feed (F-1). Goldfishes were used as an experimental fish and pigments were estimated at the initial phase of the experiment and compared with the pigments found at the end of the experiments at 450, 475 and 500 nm to know the effect of the given feed on the pigments of the fishes. As a result, higher pigments were found in fish fed on experimental feed (F-3) as it was 0.156 ±0.001 µg/g with compare to fishes fed on commercial (F-1) and control (F-2) feed as it was 0.057 ±0.001 and 0.040 ±0.001 µg/g at 475 nm. There was a high significant difference ($P<0.05$) was observed between the pigmentation of fishes from F-3 and F-1. The survival rate was found 100 % in all the fishes with active and healthy fishes in the fishes fed on the (F-3).

IndexTerms: Spirulina, Vegetable waste, Goldfish, Pigments

I. INTRODUCTION

Fisheries and aquaculture have a very important role in the development of the country as India is the 2nd leading country in fish production with an annual fish production of about 9.06 million Metric Tonnes. In aquaculture, fish feed comprises most of the input cost, approximately 60-70 % cost is coming from the fish feed and feeding management **(Kumar *et. al.*, 2017; Coloso, 2014)** due to its high-cost ingredients like fish meal, which is rich source of protein as it contains 50-70% of protein in case of food fish but in case of ornamental fish feed, the pigment supplement is also occupy the big part of cost of feed. Mainly three sources are used for the pigments are plants, animals and synthetic sources. For plant materials marigolds and other flower petals, red paper, carrots, beetroot and *Spirulina* are used as a source of pigments in the feed of an ornamental fish. On the other side, a large amount of vegetable waste and fruit waste is generated from the processing plants and vegetable and fruit markets. Nutrient values are also very high for many of these vegetable wastes and many of these wastes are generally available at any season in India. So, utilization of these kinds of waste can reduce environmental pollution and also be beneficial in reducing the cost of ornamental fish feed.

II. RESEARCH METHODOLOGY

2.1. Selection of ingredients:

Generally, fish feeds contain high-cost ingredients like fish meal and pigments; but the experimental feed was replaced by *Spirulina* powder and to reduce the cost of feed vegetable wastes were incorporated into the feed. We selected the main three vegetable wastes due to their high availability in India at any season and also due to their high nutritional value. The cabbage outer leaves, cauliflower stems and sponge gourd peel were used as vegetable waste. *Spirulina* is a blue-green alga, rich in protein (58-60%), lipid (PUFA, EPA and DHA), carbohydrates (13.6%), Vitamins (Vitamin-B complex, vitamin-C, D and E) and minerals (Potassium, Calcium, Chromium, Copper, Iron, Magnesium, Manganese, Phosphorus, Sodium and zinc), etc.

These both ingredients were incorporated with other traditional ingredients like Groundnut oil cake, Wheat flour, Rice bran, Jaggery was used in fish feed to prepare the experimental feed.

2.2 Feed formulation:

In the diet of an ornamental fish, every nutrient such as protein, carbohydrates, lipids, vitamins, minerals and pigments should be in sufficient amounts in feed and that's why feed formulation is a crucial part of the preparation of fish feed for the nutritionally balanced diet for the growth and maintenance of the growing fish. To fulfil these requirements, the formulation was prepared by using highly nutritious ingredients.

Table-1: Formulation of fish feed pellets prepared from vegetable waste incorporated with *Spirulina*.

Sr. no.	Ingredient	Feed-1 (Commercial feed) %	Feed-2 (Control feed) %	Feed-3 (veg. waste+ *Spirulina* feed) %
1	Vegetable waste (Cauliflower powder + Cabbage powder + Sponge gourd powder)	-	-	24 (8+8+8)
2	*Spirulina* powder	-	-	24
3	Wheat flour	-	12.5	14
4	Rice bran	-	37.5	14
5	Groundnut Oil Cake	-	50	14
6	Jaggery	-	-	10

2.3. Feed preparation:

To fulfil the requirements of feed formula, vegetable wastes were collected from the different vegetable markets of nearby places. After collection, the vegetable waste was washed with clean water and it was partially dried under the roof fan, and then dried in sunlight to make it a powder form. For the preparation of feed in pelleted form, all the ingredients were firstly weighted in a fixed ratio according to the prepared formula. After weighing it, all the ingredients were mixed and blended together to prepare the dough. Sufficient water was added for the required consistency of dough. After that the dough was stem cooked in a stem cooker for 25-30 minutes and the pelleting was done by using a hand pelletizer and it was sundried in a dust-free condition to meet the required quality of dry pellets.

2.4. Proximate analysis of feed and ingredients:

Analysis for the proximate composition of feed ingredients and prepared feed was done for the crude protein by the micro-kjeldhal method IS: 7219 (2015), lipid by Folch *et al* (1957) method; sugar by the Method of sampling and analysis for sugar confectionery IS:6287 (2015), ash and moisture were estimated by gravimetric method AOAC (2000). List of ingredients and the proximate composition of ingredients and the experimental feed are described in table 2.

Table-2: proximate report for the experimental feed, control feed and commercial feed and ingredients of the experimental feed.

Sr.no.	Ingredients	Protein (%)	Sugar (%)	Lipid (%)	Moisture (%)	Ash (%)
1	Rice bran	12.84%	1.59%	0.007 %	3.32%	10.67%
2	Groundnut Oil Cake	35.29%	3.49%	0.003 %	3.86%	9.20%
3	Wheat flour	12.09%	2.02%	0.002%	2.16%	1.86%
4	Cabbage waste leaves	13.83%	4.02%	0.003 %	3.83%	9.26%
5	Cauliflower steam	15.93%	6.76%	0.004 %	3.28%	18.58%
6	Sponge gourd peel powder	3.68%	2.87%	0.004%	2.52%	8.27%
7	*Spirulina* powder	63.00%	0.00%	0.007%	6.06 %	7.76%
8	Commercial feed (F-1)	30.00%	0.00%	0.001%	7.31%	11.96%
9	Control feed (F-2)	22.08%	4.07%	0.013%	6.01%	3.95%
10	Experimental feed (F-3)	26.25%	7.13%	0.003%	5.38%	6.33%

Note: F-1 is for fish feed 1, F-2 is for Feed-2 and F-3 is for Feed-3.

2.5. Aquarium set-up, stocking density and an experimental fish:

To check the impact of feed on an ornamental feed, the aquarium set-up was done by filling up three aquaria with the same capacity by filling it with water. Aquarium were covered with a net to avoid the jumping out of fish and to avoid entry of any kind of insects. Fishes were stocked with four fish per aquarium. Common goldfish (*Carassius auratus*) was selected as an experimental fish. The fishes were acclimatized by giving them a regular feed for a week and then the experimental feed was given to the fishes.

2.6. Application of feed and maintenance of experimental set-up:

Prepared pellets were given to the fish @ 5% of their body weight two times a day, at morning 10 A.m. and at evening 5 P.m. for 3 months. Netting was done in each aquarium on a regular basis to avoid the growth of plankton. Water exchange was done on a weekly basis and the temperature was maintained at 25-30^{0}C and pH was maintained at 7-8. Daily excreta removal was done by siphoning.

2.7. Observation of the impact of feed on an ornamental fish:

The observation was done as a pigment estimation of fish skin before starting the experiment and at the end of the experiment. Pigment estimation was done by pigment extraction method **(Hapaz and Padowicz, 2007).** For that, a random sample of fish was collected from each aquarium and then it was freeze-dried at -20^{0}C in a deep freezer for two days and after two days the fishes were dissected out and the sample was crushed by using a pestle and mortar and 5 ml of chilled 90% acetone was added. The solution was transferred to the test tube and wrapped with carbon paper and kept in a dark condition overnight at room temperature, centrifuged at 2000 rpm for 10 minutes. Pigments were measured at 450, 475, and 475 nm by using a UV Spectrophotometer. And the pigments were calculated by using the following formula.

- **Pigments µg/g** = $\frac{\text{4 X Optical Density Value X total vol. of sample taken}}{\text{Weight of sample (mg)}}$

- **Survival rate:**

The survival rate of fish fed on commercial feed, control feed and experimental feed was calculated by following the formula.

Survival rate = $\frac{\text{Number of fishes at the end stage of the experiment}}{\text{Number of fishes at the initial stage of the experiment}} \text{ X 100}$

2.8. Statistical analysis:

A statistical analysis was done by using Excel and SPSS version 26, One-way ANOVA at $p < 0.05$ was done to test significant differences between the pigmentation of fish fed on commercial feed, control feed and the experimental feed.

III. RESULTS AND DISCUSSION:

Impact of feed on fish:

3.1. Survival:

A 100 % survival rate was found in the fishes fed on the commercial, control and experimental feed.

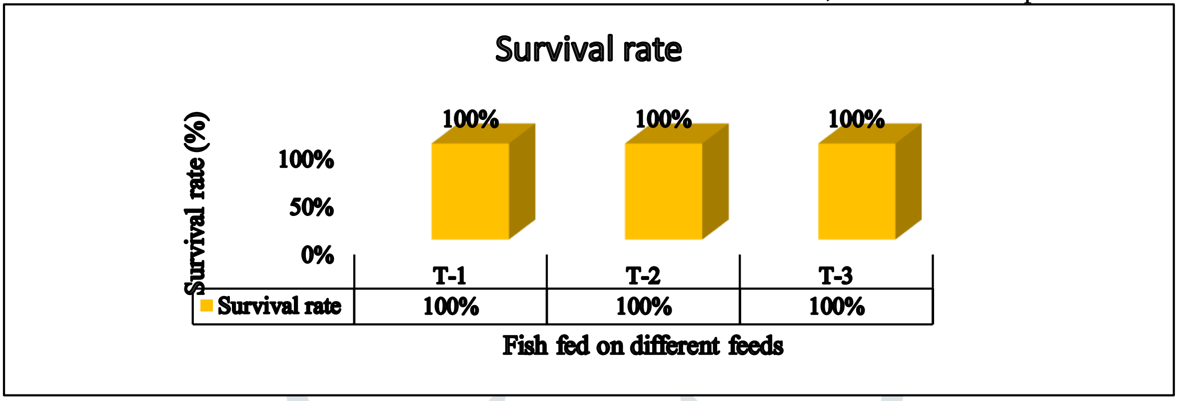

Figure-1: The survival rate of fish fed on different feeds

3.2. Activeness

➢ Experimental fishes were found highly active than the fish fed on commercially available feed and the control feed.

3.3. Disease free

➢ Experimental fishes were found disease free throughout the experiment.

3.4. Visual observation:

Figure-2: visual observation of pigments in fishes before experiments and the pigments in fish fed on the experimental feed at the end of the experiments.

The colors in fishes were started to get bright within 15 days after starting the experiments and at the end of the experiment, it was found to have bright orange color on their skin.

3.5. Pigmentation in the fishes at different wavelengths:

Table-3: Pigmentation in fishes at 450, 475 and 500 nm in fishes fed on different feeds.

Pigments	Initial*	F-1*	F-2*	F-3*
at 450 nm	0.052 ±0.001	0.055 ±0.002	0.036 ±0.004	0.152 ±0.001
at 475 nm	0.053 ±0.000	0.057 ±0.001	0.040 ±0.001	0.156 ±0.001
at 500 nm	0.050 ±0.001	0.054 ±0.002	0.038 ±0.001	0.108 ±0.001

Note: Initial- pigmentation in fish before starting the experiment, F 1*- pigmentation in fish fed on commercially available feed at the end of the experiment, F-2*- pigmentation in fish fed on control feed at the end of the experiment, F-3*- pigmentation in fish fed on experimental feed at the end of the experiments.

3.5.1. Pigmentation in the fishes at 450 nm:

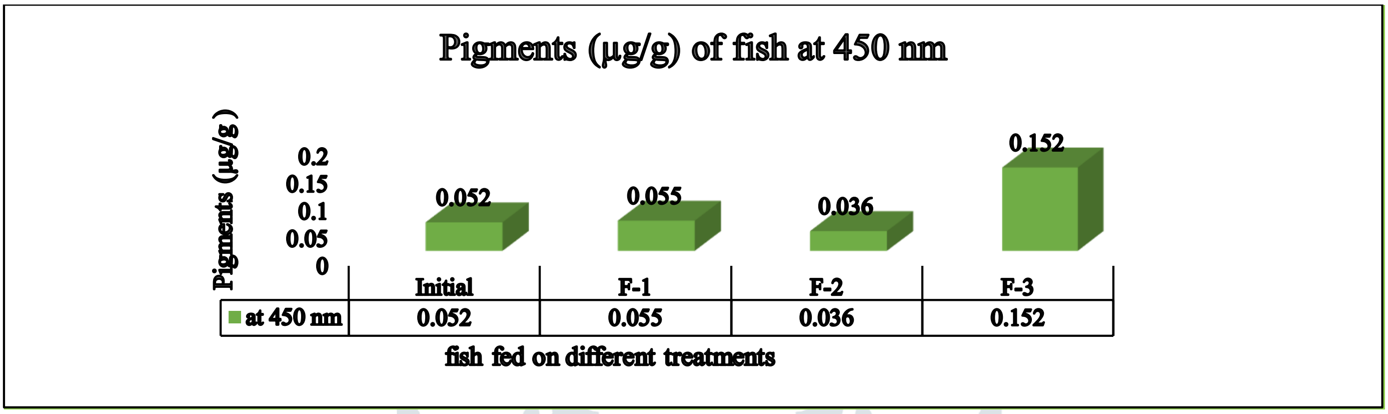

Figure-3: Pigments (µg/g) estimated at 450 nm in fishes fed on different feeds

Initial pigments of fishes at 450 nm were 0.052 ±0.001 µg/g which was nearly similar to the pigmentation of fishes fed on F-1 feed at the end of the experiment as it was 0.055 ±0.002 µg/g. Lower pigmentation was found in the fish fed on the F-2 feed as it was 0.036 ±0.004 µg/g. The highest pigments were found at 450 nm in the fishes fed on the F-3 feed as it was 0.152 ±0.001 µg/g. There was no significant difference (0.769) observed between the pigments in the fish before starting the experiments and the pigment observed in the fish fed on F-1 feed at the end of the experiments. Pigments found in fish fed on control feed (F-2) were significantly low (0.004) than the concentration of the pigments in the fishes before the experiments. Pigments found in fish fed on experimental feed (F-3) were significantly high (0.000) than the pigment concentration in the fishes before the experiments, fishes fed on commercial feed (F-1) and fish fed on control feed (F-2).

Pigmentation in the fishes at 475 nm:

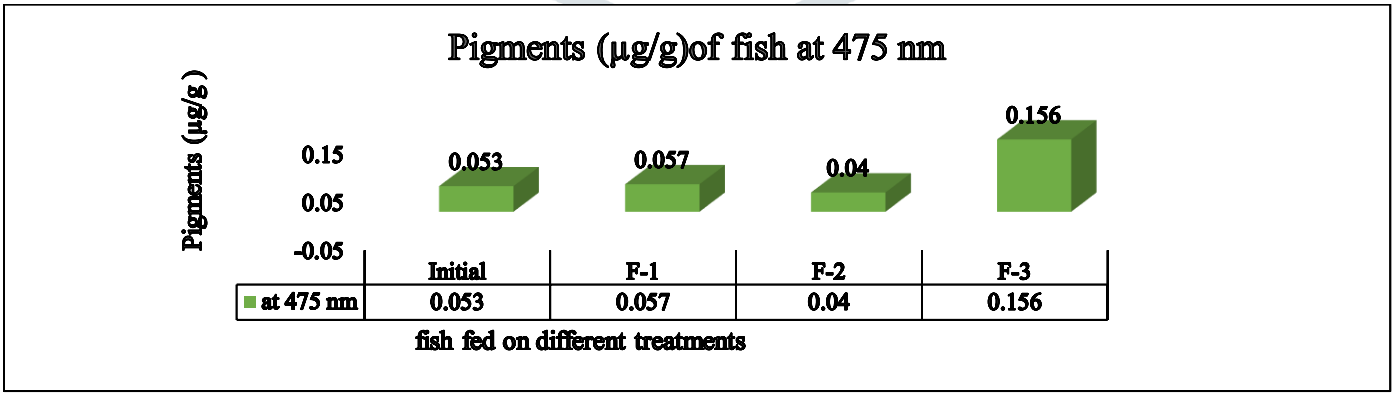

Figure-4: Pigments (µg/g) estimated at 475 nm in fishes fed on different feeds

Pigments at an initial phase of experiments for 475 nm were found 0.053 ±0.000 µg/g similar to Pigments found in fishes fed on F-1 feed as it was 0.057 ±0.001 µg/g. The lowest pigments found at 475 nm were in the fish fed on the F-2 feed as it was 0.040 ±0.001 µg/g. and the highest pigments were found in the fishes fed on the F-3 feed as it was 0.156 ±0.001 µg/g. Pigments found in fish fed on commercial feed were significantly high (0.021) than in the fishes before starting the experiments. The pigment concentration in fish fed on control feed (F-2) and experimental feed (F-3) was significantly high (0.000) than the pigments in fishes before the experiments. Pigments found in fish fed on experimental feed (F-3) were significantly high (0.000) than the pigments in fish fed on commercial feed and control feed (F-2).

3.5.2. Pigmentation in the fishes at 500 nm:

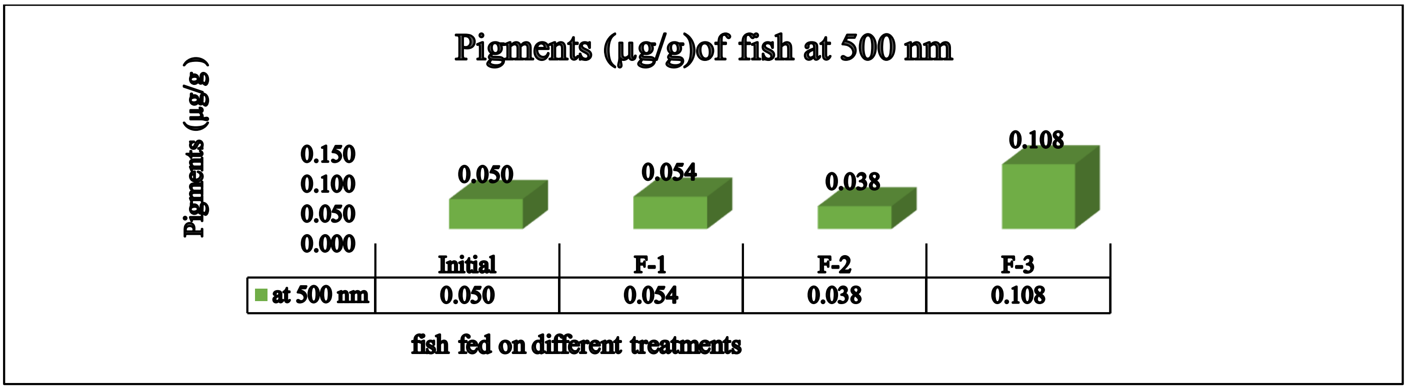

Figure-5: Pigments (µg/g) estimated at 500 nm in fishes fed on different feeds

Pigments at an initial phase of experiments for 500 nm were found 0.050 ±0.001 µg/g value near to Pigments found in fishes fed on F-1 feed as it was 0.054 ±0.002 µg/g. The lowest pigments found at 500 nm were in the fish fed on the F-2 feed as it was 0.038 ±0.001 µg/g. and the highest pigments were found in the fishes fed on the F-3 feed as it was 0.108 ±0.001 µg/g. There was no significant difference (0.089) observed between the pigments in the fish before starting the experiments and the pigment observed in the fish fed on commercial feed (F-1) at the end of the experiments. The pigment concentration in the fish fed on control feed (F-2) and Fish fed on experimental feed (F-3) was significantly high (0.000) than the pigments in fish before starting the experiments. The pigment concentration in fish fed on experimental feed (F-3) was significantly high (0.000) than the pigments found in fishes fed on commercial feed (F-1) and control feed (F-2).

Pigments are crucial nutrients for ornamental fishes for their attractive and bright colors **(Anitha *et. al.*, 2019)** but the fishes are not able to synthesize pigments in their body and ornamental fishes mostly remain in confined areas are lacking of natural pigments due to a lack of pigments **(Arulrasu *et. al.*, 2013)** in the form of plants and planktons in their surrounding nature. Pigment also improves the reproductive system, act as an antioxidant and strengthens immunity. A pigment supplement is important in the formulation of feed for an ornamental feed and the low range of pigments in the fish fed on the control feed proves right because, there were no pigment was incorporated in the control feed. *Spirulina* is a rich source of pigments as it is having carotenoids, C-phycocyanin and chlorophyll-b, etc, which improved the immunity and activeness of fish fed on experimental feed and also enhanced the colors in the fishes as the pigment found in the experimental fishes was way higher than the fishes fed on the commercial feed and the control feed. 100 % survival rate in all the fishes shows the good maintenance and good environment of an experimental set-up.

IV. CONCLUSION:

By using this kind of combination of Vegetable waste with the *Spirulina* as a feed can enhance the quality of pigments in the ready to marketing fishes which may usually increase the market value of fishes with low-cost feed. By utilizing vegetable waste in this manner, the pollution load on the environment also can be reduced.

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