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Effect of dietary supplementation of papaya leaf on growth and feeding bioenergetics of Indian major carp, *Labeo rohita*

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Abstract
There has been success with the use of plant ingredients in aquaculture as they are cheaper, biodegradable, biocompatible and growth promoters. Therefore, the aim of this study was to determine the effects of adding *Carica papaya* leaf to the diet of *Labeo rohita* on growth and feeding parameters. Feeding the fishes with different concentrations of *C. papaya* leaf diet had an impact on the growth rate of the fish. Incorporation of 10% of papaya leaf in the feed enhances the assimilation and conversion efficiency of *Labeo rohita*. This finding confirmed *C. papaya* leaf as a growth promoter.

Keywords: *Labeo rohita*, *Carica papaya*, bioenergetics, growth promoter, feed

1. Introduction
Aquaculture, which is a traditional method of fish farming that has been in existence for about 2000 years (Balon, 1995; Dunham *et al*., 2001) [2, 9], has become a widespread practice worldwide due to its ability to meet the increasing demand for fish products. In India, the freshwater aquaculture system predominantly relies on the composite carp culture, which involves a combination of three to six species. The three major carp species in India, including catla, rohu, and mrigal, are extensively farmed and contribute significantly to the Indian aquaculture production. In addition, exotic species such as silver carp, grass carp, and common carp are also farmed in the country (Jayasankar *et al*., 2018) [12]. Among the Indian major carps, *Labeo rohita* (Rohu) is a highly preferred species for culture and contributes more than 80% of the major carp culture of India (Dhanapal *et al*., 2013) [8]. It is mainly due to its high commercial value, the taste and good looking white colour of the meat. It is a bottom feeder, omnivore fish which grows fast.

The importance of nutrition in the growth and reproduction of cultured fish cannot be overstated. However, the high cost and limited availability of fish feed have prompted research into potential substitutes. Feed additives are edible substances added to fish feed in small amounts to improve its quality, leading to better growth performance and decreased mortality rates. Feeding rate and food conversion efficiency are key factors used to assess the quality of fish food. The goal of fish feed production is to create an affordable feed with a good amino acid profile, high protein content, low fiber and carbohydrate content, minimal indigestible anti-nutrients, high digestibility, and excellent palatability, often using plant-based protein sources. Plant products have been found to have several beneficial properties, including antistress, appetizer, antimicrobial, immuno-stimulation, and growth enhancement, with no adverse effects observed (Farag *et al*., 1989; Citarasu *et al*., 2002; 2003; Sagdic and Ozcan, 2003; Chakrabarti *et al*., 2012) [10, 6-7, 18, 4].

Sunitha *et al*. (2017) [21] discovered that including 1% to 2% *Phyllanthus niruri* in fish feed increased consumption rate, assimilation and conversion efficiency, protein, carbohydrate, and lipid content in muscle, whole body, and serum of *Cyprinus carpio* fish. Bhosale *et al*. (2010) [3] found soybean to be a promising cost-effective alternative to fish meal as a source of nutrition.

Carica papaya, a plant known for its antiviral, antibacterial and antifungal properties due to papain and chymopapain enzymes, can also increase white blood cells and platelets, normalize clotting, and repair the liver (Aravind *et al*., 2013) [1]. Based on this information, a study was conducted to determine the effect of *Carica papaya* leaf-supplemented diet on *Labeo rohita* fish's growth and bioenergetics with the following objectives.

- To assess the impact of the feed supplementation on the growth of the fish.
- To analyse the effect of feed supplementation on the energy budget of the fish.

2. Materials and Methods

2.1 Fish Feed Preparation

The components used for the preparation of fish feed were wheat bran, groundnut cake, beetroot, carrot, egg, spleen amaranthus, tapioca powder, fish oil, vitamin C tablet, mineral mix and papaya at different concentrations. The quantity of each ingredient of the fish feed is shown in Table 1. Wheat bran, groundnut cake, beetroot, carrot, egg, spleen amaranthus, and papaya were well dried, steam boiled and ground finely. The food components, excluding papaya leaf, were thoroughly mixed and kneaded with enough water to form a dough, and the appropriate amount of vitamins and minerals were added. Following the preparation of the dough, it was divided into four parts and papaya leaf powder (Carica papaya) was added at varying concentrations to each part. The first part of the food without papaya served as control feed (0%). To the 2nd, 3rd and 4th parts papaya leaf powder was added in proper dose to get 10%, 20% and 30% papaya incorporated in experimental feed 1, 2 and 3 respectively. The feed was mixed well and extruded in the form of noodles using a mechanical extruder. This was then dried and used to feed the fish throughout the study period.

Table 1: Ingredients of fish feed

Ingredients	Weight in grams
Wheat Bran	250
Groundnut cake	200
Beetroot	100
Carrot	100
Egg	4.5
Spleen amaranthus (Arai keerai)	100
Tapioca powder	200
Fish oil	2
Vitamin C	2.6603
Mineral mix	
Calcium chloride	0.3
NaCl	0.0315
Zinc sulphate	0.003
Potassium iodide	0.0015
Copper sulphate	0.0037
Total	1000 g

2.2 Collection and Maintenance of experimental fish

The fingerlings of Rohu were procured from Chittar Dam Fisheries Department and acclimatized to laboratory condition. The water was renewed to maintain sufficient oxygen and the fishes were fed with control food. During the acclimatization period, the water in the tank was changed every 24 hours, and the fish were provided with ad libitum feeding of control feed.

2.3 Experimental Setup

Four groups (control, experimental 1, experimental 2, and experimental 3) of 14 fishes each were formed. To maintain replicates, each group was further divided into two sub-groups of 7 fishes each. The initial length and weight of the fishes were recorded, and they were then placed in plastic tubs of similar shape and size (diameter – 42 cm, height - 16 cm) filled with tap water. The tubs were covered with nets to avoid the jumping out of fishes. The fish were given a daily morning feeding of measured amounts of the different types of feed: the first group with control food (food without papaya leaf), second, third and fourth group of fishes were fed with 10%, 20% and 30% of the experimental feed (with papaya leaf) respectively. After four hours in the noon, any uneaten feed was removed from each container, and the remaining feed was collected in separate dishes and allowed to dry. Fecal pellets were also collected from each trough before changing the water and then dried. The experiment was conducted for 45 days at room temperature. The fishes were weighed (wet weight) in alive condition and their body length was measured on the 45th day). Also the amount of food eaten and the amount of fecal matter were measured. All these measurements were taken to assess the growth of the fishes.

2.4 Parameters assessed

The present study followed the IBP formula of Petrusewicz and Macfadyen (1970) [16] for the scheme of energy balance which is usually represented as.

$$C = P + R + F + U$$

Where,
C = Food consumed i.e., food given- unfed.
P = Production i.e. difference between the initial wet weight of the fish at the beginning of the experiment and the final wet weight of the fish at the end of the experiment.
R = Respiratory loss
F = Faeces
U = Nitrogenous excretory products.

2.5 Growth

Growth in terms of wet body weight (weight gain) = Final weight - Initial weight
Growth in terms of body length (length gain) = Final length - Initial length

$$\text{Specific growth rate (SGR) \%} = \frac{\textit{Final weight} - \textit{Initial weight}}{\textit{Duration (days)}} \times 100$$

2.6 Consumption rate (Cr) or feeding rate

Feeding rate is a term used to describe the amount of food that a fish consumes per unit of its live body weight over a specific period of time (mg/g.wt/day).
Consumption (C) = Food given – unfed

$$\text{Consumption rate} = \frac{\textit{Food consumed}}{\textit{Initial live weight} \times \textit{Experimental duration}} \times 1000$$

2.7 Assimilation rate (Ar)

Assimilation rate refers to the proportion of food consumed by fish that is absorbed and utilized per unit weight of fish per unit time (mg/g.wt/day).

Assimilation (A) = Consumption – feces

$$\text{Assimilated rate} = \frac{\text{Food assimilated}}{\text{Initial live weight} \times \text{Experimental duration}} \times 1000$$

2.8 Conversion or Production rate (Pr)

Production rate is also known as growth rate or conversion rate. It represents the amount of food converted into body mass per unit weight of the fish per unit time (mg/g.wt/day)
Conversion or Production (P) = Final wet weight - Initial wet weight

$$\text{Conversion Rate} = \frac{\text{Conversion}}{\text{Initial live weight} \times \text{Experimental duration}} \times 1000$$

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Food consumed}}{\text{weight gain (wet weight)}}$$

2.9 Metabolic rate (Mr)

It represents the amount of food metabolized per unit weight of the fish per unit time (mg/ g. wt. / day).
Food metabolized (M) = Food assimilated - Food converted

$$\text{Metabolic rate} = \frac{\text{Food metabolised}}{\text{Initial live weight} \times \text{Experimental duration}} \times 1000$$

2.10 Assimilation Efficiency (%)

The term assimilation efficiency is preferred over the term "approximate digestibility" used by Waldbauer (1968) because not all digested food is necessarily absorbed and assimilated (Pandian, 1967) [14]. Assimilation is defined as the percentage of food energy, absorbed in relation to the food energy consumed.

$$\text{Assimilation efficiency (\%)} = \frac{\text{Food assimilated}}{\text{food consumed}} \times 100$$

2.11 Conversion efficiency (%)

Production efficiency or growth efficiency is another term used to describe the percentage of food energy converted in relation to food consumed. Gross conversion efficiency refers to the percentage of food energy converted in relation to the total food consumed, while net conversion efficiency refers to the percentage of food energy converted in relation to the assimilated food.

$$\text{Gross conversion efficiency, } K_1 (\%) = \frac{\text{Food converted}}{\text{food consumed}} \times 100$$

$$\text{Net conversion efficiency, } K_2 (\%) = \frac{\text{Food converted}}{\text{food assimilated}} \times 100$$

3. Results

The study conducted showed the impact of a diet supplemented with *Carica papaya* leaf on the growth and feeding energetics of the fish, *Labeo rohita.*

3.1 Growth in terms of body weight

Table 2 depicts the effect of *C. papaya* diet on the growth in terms of body length and wet weight and the results showed maximum growth of the fish in experimental 1 group.

Table 2: Growth in terms of body length, wet weight and SGR of the fish, *L. rohita* fed on *C. papaya* leaf supplemented diet for a period of 45 days

Fish Group	Length (cm) (Mean ± SD)	Weight (gm) (Mean ± SD)	SGR %
Control	0.65±0.30	0.38±0.39	0.85
Experimental 1	0.82±0.52	0.49±0.31	1.38
Experimental 2	0.58±0.38	0.32±0.29	0.64
Experimental 3	0.64 + 0.36	0.36±0.25	1.01

3.2 Food Consumption, Food Assimilation and Metabolization

Consumption was higher in the experimental 3 group. The values obtained for assimilation were high in experimental 1 group. Similarly the value for metabolization was also high in experimental 1 group (Figure -1).

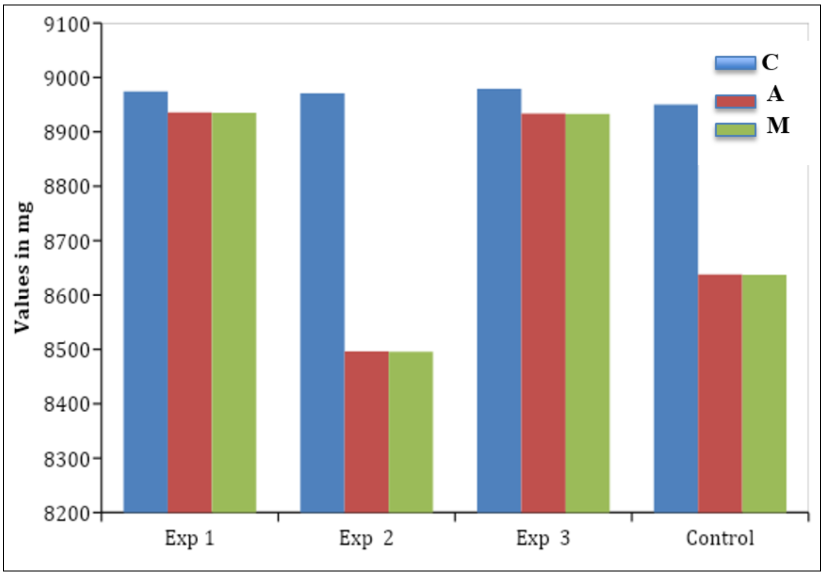

Fig 1: Food consumed (C), assimilated (A) and metabolized (M) by *L. rohita* fed on *C. papaya* leaf supplemented diet for a period of 45 days

3.3 Rate of consumption (Cr), assimilation (Ar), metabolism (Mr), conversion (Pr) and food conversion ratio (FCR)

The consumption rate was higher in the control group. The assimilation rate was higher in experimental 3 groups. Similarly the value for metabolic rate was also high in fishes fed with C. *papaya* leaf incorporated diet with 30% (Table 3). The feed conversion ratio of control group was 23308.09 and experimental groups were 14404.73, 31039.75 and 19604.51 respectively.

Table 3: Influence of different concentrations of *C. papaya* leaf in the feed on the rate of consumption (Cr), assimilation (Ar), metabolism (Mr), conversion (Pr) and food conversion ratio (FCR) of the fish, *L. rohita* fed for a period of 45 days (Rates are expressed in mg / g body weight / day)

Group	Cr	Ar	Mr	Pr	FCR
Control	55.35	53.4227	53.4188	0.0023	23308.09
Experimental 1	41.99	41.8129	41.8105	0.0029	14404.73
Experimental 2	46.95	44.4769	44.4747	0.001	31039.75
Experimental 3	55.22	54.9462	54.9438	0.0028	19604.51

3.4 Efficiencies of assimilation, gross conversion (K_1) and net conversion (K_2)

Assimilation efficiency was observed high in fishes fed with the diet containing 10% papaya when compared to the fishes fed with the diet containing 20% and 30% of papaya. The experimental results indicated that the fish fed with the experimental 1 diet exhibited higher gross and net conversion efficiencies compared to the other diets (Table 4).

Table 4: Efficiencies of assimilation, gross conversion (K_1) and net conversion (K_2) shown by *L. rohita* fed on *C. papaya* leaf incorporated feed for a period of 45 days (values are expressed in %)

Group	Assimilation Efficiency	K_1	K_2
Control	96.50	0.0042	0.004
Experimental 1	99.57	0.0069	0.006
Experimental 2	94.71	0.0032	0.002
Experimental 3	99.49	0.0051	0.005

4. Discussion

In this study, the potential impact of Carica papaya leaf supplementation on the growth performance and feeding energetics of freshwater fish *Labeo rohita* was investigated. The dietary supplementation of *Carica papaya* leaf had an effect on the growth parameters: consumption (C), assimilation (A), food conversion (P), metabolism (M), consumption rate (Cr), assimilation rate (Ar), conversion rate (Pr), metabolism rate (Mr), SGR, FCR, assimilation efficiency, gross conversion (K1), and net conversion (K2) of *Labeo rohita*.

The specific growth rate is a metric used to assess the health and growth of fish in both natural and experimental settings (Sivagami and Ronald, 2018) [20]. The rate of growth of fish fingerlings was different, with different experimental feeds. The body weight was found to be maximum (0.49 g) in 10% feed. The group of fish fed with the experimental diet showed a higher level of feed consumption. The study's findings demonstrated that incorporating papaya leaf meal into the diet of *Labeo rohita* (rohu) fingerlings resulted in improved growth performance and nutrient utilization. The observed increase in growth performance could potentially be attributed to the enhanced feed intake and improved digestibility of nutrients resulting from the inclusion of papaya leaf meal in the diet. *Carica papaya* (Papaya plant) produces a proteolytic enzyme called papain which is known to have various health benefits. The increased fish appetite, improved feed intake, and nutrient digestibility observed in this study could be attributed to the presence of papain in papaya leaf meal, which in turn enhanced the growth of the experimental fish. However, further research is needed to fully explore the potential benefits of papaya leaf supplementation in fish diets.

The results of this investigation were in agreement with those obtained by Hamid (2022) [13], who found that the inclusion of papaya in fish diets led to an improvement in the growth performance of Tilapia. Metabolism refers to the overall rate at which living organisms transform substances. It encompasses catabolism and maintenance functions as well as anabolism, which leads to growth. Assimilation efficiency was observed high in fishes fed with the diet containing 10% papaya (99.57) as compared to the fishes fed with the diet containing 20% and 30% of papaya. Hence, feed with 10% papaya was found to be the best. Results of the present study substantiate the fact that *papaya* leaf incorporated diets have direct growth promoting effects on fingerlings. Grishma Tewari *et al*. (2018) [11] demonstrated that the growth of Cyprinus carpio was promoted by the extract of papaya leaves. Further, Hamid (2022) [13] found significant improvements in weight gain, SGR, and feed efficiency of the red hybrid tilapia with this inclusion level and recommended inclusion level of papaya leaf extract as a feed additive to promote red hybrid tilapia fry growth is between 1% and 2%.

As per the results of this study experimental 1 group has attained high bodyweight and experimental 2 group has high standard length. This shows that papaya leaf meal helps the fishes in gaining their body weight to a certain extent as the quality of the food significantly affects conversion efficiency. The increase in growth rate observed in the study could be attributed to the increase in gross food conversion efficiency (Padian and Raghuraman, 1972) [15], as well as the active principles present in papaya leaf that may induce the secretion of digestive enzymes that might stimulate the appetite and increase the food consumption (Citarasu, 2010; Rinna Hamlin *et al*., 2013; Safrida *et al*. 2020) [5, 17, 19].

5. Conclusion

Based on this study, it could be suggested that *C. papaya* leaf is a promising medicinal plant in aquaculture as it enhances growth of the fish. The freshwater fish cultivators can be benefited economically by using *C. papaya* leaf in the fish feed. Therefore, it can be inferred that incorporating papaya leaf as a supplement in fish feed has the potential to enhance productivity in aquaculture.

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