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Effect of papaya (*Carica papaya*) Leaf meal on proximate composition and digestibility of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fingerlings

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Abstract
This experimental study was performed to evaluate the beneficial effect of papaya leaf meal (PLM) on proximate composition and digestibility of Nile tilapia, (*Oreochromis niloticus*) Fingerlings. Dietary supplementation of PML at an inclusion rate of 0% (T_0), 2% (T_1), 4% (T_2), 6% (T_3) and 6% (T_4) were fed to the fish @ 3% body weight. The experimental study duration was conducted for 60 days. Significantly ($p<0.05$) highest digestibility level 74.356±0.1757 and crude protein (15.840±0.0321) T_4 were observed at the 8% (T_4) inclusion level of PML in the fish diet. While the lowest digestibility was 66.141±0.4105 was recorded in 0% (T_0) group, which indicated the better digestion of experimental diet as compared to the control diet. The proximate composition of the experimental diet was significantly different ($p<0.05$). The highest level of crude protein (20.710±0.0536) was reported in 6% (T_3) while lowest (20.440±0.0523) was found in 2% (T_1). The maximum percent of fat (lipid) (8.190±0.0351) was reported in 2% (T_1) and lowest (8.120±0.0251) was reported in 0% (T_0). The highest percentage of carbohydrate (53.570±0.0896) was observed in 6% (T_3) whereas lowest percentage of carbohydrate (53.320±0.1322) was observed in 0% (T_0). The highest level of ash in experimental diet (8.750±0.0611) was noticed in 0% (T_0) and lowest ash (8.410±0.0529) was noticed in 6% (T_3). The highest moisture (9.470±0.0378) in 8% (T_4) and was lowest moisture (9.140±0.0346) in 6% (T_3). The proximate composition of fish carcass has shown a significant difference ($p<0.05$) among all the treatments. These results demonstrated that, PLM at the rate of 8% in the diets of fish showed a beneficial effect on proximate composition and digestibility of Nile tilapia and this level of PLM can be used in the diet of fishes.

Keywords: Nile tilapia, papaya leaf meal (PLM), digestibility, proximate composition

Introduction
In many countries throughout the world, aquaculture is becoming a more attractive and important aspect of national development and poverty reduction initiatives (Prabu and Santhiya 2016) [1]. With a digestibility of over 90%, fish is a high-quality source of animal protein, important fatty acids, in particular long-chain polyunsaturated fatty acids (LCPUFA), as well as minerals (Kijora *et al.*, 2006) [2]. The state of world fisheries and aquaculture is vital for providing millions of people with adequate food, nutrition, and employment. In 2018, there were 179 million tonnes of fish produced worldwide, of which 88% were consumed for human consumption. Due to this output, per capita consumption in 2018 reached a record-high of 20.5kg. Global fish exports rose to USD 164 billion in 2017, with 54% of that amount coming from poorer nations. Production from catch fisheries was 96.4 million tonnes worldwide in 2018. 87.5 and 12.5% of the world's production came from inland and marine fisheries, respectively (FAO, SOFIA, 2018) [3]. The Indian aquaculture sector makes a significant economic contribution. Millions of people are employed and receive precious foreign exchange from it. India produces 6.56% of the world's total fish, making it the second-largest fish producer in the world. There were about 37.27 lakh tonnes of fish produced in the marine sector and 104.37 million tonnes of fish produced in the inland sector (Handbook on Fisheries Statistics, 2020) [4].

Fish is a rich in nutrients and a great source of animal proteins. Artificial feed must be provided so that fish can grow quickly and reach their optimum weight in a short period of time in order to improve fisheries and maximise returns from freshwater resources. Due to its ability to comply with the protein requirements of fish, fish meal is regarded as the best component among commonly utilised feed ingredients (Alam *et al.*, 1996) [12]. Due to the growing price and erratic supply of fish meal, it is important to replace it in fish feed with less

expensive elements of plant origin (Higgs *et al*. 1995) [5]. The World Health Organisation (WHO) encourages adding medicinal plants or herbs to the fish diet as a way to increase fish consumption while reducing the use of chemicals. (Dada, 2015) [6]. In the past, leaves were used to treat a variety of diseases, including jaundice, dengue fever, malaria, and dengue itself. They also had immunomodulatory and antiviral properties. Young leaves are abundant in cynogenetic compounds (benzylglucosinolate), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), alkaloids (carpaine, pseudocarpaine, and dehydrocarpaine I and II), flavonoids (kaempferol and myricetin), and alkaloids (carpaine, pseudocarpaine, and dehydrocarpaine I and II). As opposed to mature leaves, the Carica papaya Linn. fruit and leaf both contain carotenoids, specifically -carotene, lycopene, and anthraquinones glycoside, and as a result, they have medicinal properties such as anti-inflammatory, hypoglycaemic, abortifacient, hepatoprotective, and wound healing. More recently, its antihypertensive and antitumor activities have also been established. Due to their significance in many traditional formulations, leaves are subject to standardisation for a number of factors, including moisture content, extractive values, ash values, swelling index, etc. (Anjum V *et al.*, 2013) [13]. The purpose of the current study was to determine the practical applicability of papaya leaf meal on digestibility and proximate composition of Nile tilapia diet as alternative feedstock.

Materials and Methods

Experimental Fish and Maintenance

The Nile tilapia fingerlings randomly were selected for the experimental study. A total quantity of 200 fingerlings were obtained the Seed Production and Research Unit, MPUAT, Udaipur. For period of 60 days' study (15 July 2022 to 14 September 2022) 20 FRP tanks containing 225-liters capacity were used. In a 500 L capacity FRP rectangular tank with a basic feed, the fish were acclimated for a week. The feeding stopped 24 hours prior to the experiment's start.

After a week fishes were stocked in 5 treatments of experiment with 4 replicates at the rate of 10 Tilapia fingerlings were transferred to each experimental tank of uniform average body weight (29.09 gm). Before introducing fishes all the 20 tanks were disinfected properly and filled with 200 litres filtered underground water. All the experimental tanks were covered with nylon net to prevent jumping out of fish and to prevent external contaminants. During the experiment aeration was provided at least 8 hours per day by air stones diffusers connected to aerator. Fingerlings were fed once daily @ 3% of their body weight.

Basal Diet

The basal diet was prepared by using groundnut oil cake, rice bran and wheat flour (40:40:18) with 2% vitamin and mineral mixture. To determine the digestibility, Cr2 O3 was included in the diets as an additional ingredient.

Table 1: The details of the ingredients used for basal diets (g/kg)

S. No.	Ingredients	Amount (%)
1.	Groundnut oil cake	40
2.	Rice brane	40
3.	Wheat flour	18
4.	Vitamin & mineral mixture	2

Preparation of Experimental Diet

The papaya plant leaf meal was added in the basic diet at four different levels i.e. control–T_0 (without papaya leaf meal) and treatments: T_1 (2%), T_2 (4%), T_3 (6%), T_4 (8%) replacing equal amount of basal diet (Table 1). The plant 'Papaya' (*Carica papaya*) leaf was collected from the Aquaculture Research and seed production unit, Directorate of Research, MPUAT, Udaipur. The leaves were clean and dried in shade at room temperature for 2 weeks. The leaves were grinded in a mechanical grinder after that the meal was sieved by (80 µ diameter) pore size sieve. Then the meal was stored in sealed plastic container at room temperature until it is used. The dry ingredients (consisting of groundnut cake, rice bran, wheat flour & vitamin and mineral mixture) of the basal diets were thoroughly mixed and made dough and placed in autoclave at 15 lbs pressure for 30 minutes. The paste was then extruded through a hand pelletizer. The resulting linguine like diet (2.0 mm diameter) was air dried and stored in air tight containers for further use.

Table 2: The details of experimental diet (%)

S. No.	Treatment	Basal diet (%)	Papaya leaf meal (%)	Total (%)
1.	Control (T_0)	100	0	100
2.	T_1	98	2	100
3.	T_2	96	4	100
4.	T_3	94	6	100
5.	T_4	92	8	100

Water quality analysis

Using APHA's (2005) [7] standard procedures, the water quality parameters of temperature, pH, electrical conductivity, dissolved oxygen, alkalinity, and total hardness were measured.

Proximate composition of experimental diets and fish

The proximate composition of the diet and experimental fishes were estimated by standard protocols (AOAC, 1995) [8]

Estimation of moisture

Loss in weight on drying is determined to calculate % moisture and dry matter. First, a glass container was dried in an oven at 103±2 °C for 30 minutes. After drying, the container was placed in a desiccator for 45-60 minutes. Then it was cooled, the weight of the container was recorded. 5 gm of a fresh sample of powdered fish and feed were placed in different containers and weighed accurately. Containers were placed in an oven and maintained at 103± °C for 6 hrs. After that containers were cooled in a desiccator for 45-60 minutes. Containers were weighted again. The moisture loss as in form of weight loss was reported in % as per the following

$$\text{Formula: } \%\ Moisture = \frac{\text{Fresh weight of the sample} - \text{dry weight of the sample}}{\text{Fresh weight of the sample}} \times 100$$

Estimation of Crude Protein by Micro kjeldahl Method

Organic matter of samples was digested with concentrated H_2SO_4 in the presence of H_2O_2 till it becomes colourless. The intensity of the colour developed by Nessler's reagent in the presence of NaOH and sodium silicate is measured by a Spectrophotometer. The nitrogen contents were calculated by

multiplying with an international protein factor (6.25), the crude protein content is obtained.

Apparatus & equipment's
Kjeldahl flask 100 ml, measuring cylinder, volumetric flask 50 and 100 ml, pipette, beaker, digestion assembly and colorimeter, etc.

Reagents
Sulphuric acid (concentrated), Hydrogen peroxide 30%, Nessler reagent, Sodium silicate 10% solution and Sodium hydroxide 10% solution.

Digestion process
Sample of fish feed and dried fish '0.1' g were taken which was well-grounded and transferred into a 100 ml dry Kjeldahl flask so that it should not stick to the neck. After that 2 ml concentrated H_2SO_4 was added to the Kjeldahl flask, mixed with the catalyst contents of the flask. This assembly was placed on the digestion assembly and heated till the sample is digested properly. Then flasks were cooled and 0.5 ml (approx. 10 drops) of 30% H_2O_2 were added and again contents of flasks were heated. This process was repeated till it became clear and absolutely colourless. Digested contents of the Kjeldahl flask were transferred into a 100 ml volumetric flask by washing it 3-4 times with the help of distilled water and volume was made up to mark.

Colour development
5 ml of digested solution were transferred into a volumetric flask and a few ml of water was added. Then 2 ml of 10% NaOH and 1 ml of 10% sodium silicate solution were added and some water was also added. Contents were mixed thoroughly and 1.6 ml of Nessler's reagent was added to the flask while shaking. After this volume was made up to the mark. First readings of the standard working solution were noted down using a blue filter or by adjusting the Spectrophotometer at a wavelength of 420 nm. The concentration of nitrogen was plotted on X-axis and reading on the Y-axis and a standard curve was prepared. Nitrogen content in the sample was determined with the help of the standard curve. After that crude protein was calculated by multiplying the 6.25 conversion factor (for fish/animal) with the Nitrogen-content.

Estimation of Fat
Samples of fish and fish feed were extracted with diethyl ether in a continuous extractor. The solvent was distilled off. The residues were dried and weighed.

Materials
Anhydrous diethyl ether (60-80 °C), Soxhlet's apparatus and Water bath

Method
3 gm of dried and powdered sample was placed in a filter paper pouch (Whatman No. 40). After preparing a proper pouch of Whatman filter paper, weight was noted. With the help of Soxhlet's apparatus fat of samples were extracted with petroleum ether at 60 °C. The extraction was continued for 12 hours at a condensation rate of 2-3 drops/sec. When the extraction process was completed, samples were dried for 30 minutes at 100 °C. After that weight of the pouches was recorded.

Fat contents mg/ 100g of dried sample = W_i-W_t Where,

W_i = Initial weight of the sample

W_t = final weight of the sample

$$\% \, fat = \frac{\text{Wt.of fat (g)}}{\text{Wt.of sample (g)}} \times 100$$

Estimation of Ash
Materials
Muffle furnace, Silicon crucibles and A tong.

Method
5.0 g of a dried and powdered sample of fish and fish feeds were taken in a silicon crucible and the total weight was recorded. Samples were then incinerated in a furnace which was preheated to 550 °C for 4 hours. Then crucibles were transferred with the help of a pair of tongs to the desiccators. After these samples were cooled and weighed again.

$$\% \, Ash = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$

Estimation of Carbohydrate

Carbohydrate content was calculated by difference method:

$$\% \, Carbohydrate = 100 - (\% \, moisture + \% \, crude \, protein + \% \, total \, lipids + \% ash)$$

Statistical Analysis
The collected data were statistically analysed with the help of statistical package SPSS 16. The significant dissimilarities between the groups were determined using one-way ANOVA and Duncan's multiple range tests. All data is presented as mean± SD, with a statistical significance level of $p<0.05$.

Results and Discussion
The result of the current study revealed that, the digestibility of *Oreochromis niloticus* fingerlings affected throughout 60 days were significantly ($p<0.05$) by different concentration levels of papaya leaf meal.
Analysis of the experimental diet's digestibility and comparison with the control demonstrated that all treatments showed digestibility that were significantly higher than the control. (Table 4) The highest digestibility level 74.356±0.1757 was observed in T_4 followed by 71.121±0.2290 in T_3, 70.084±0.1957 in T_2, 68.624±0.5191 in T_1 and lowest was 66.141±0.4105 in T_0. The apparent protein digestibility has shown a significant difference ($p<0.05$) among all the treatments. The range of digestibility in Nile tilapia fed with guava meal was determined to be (61.49%) by Santos *et al.* (2009) [14], which is similar to the current experimental result. Sharma (2021) [15] reported high protein digestibility with high percentage of fenugreek seed meal on *Cyprinus carpio* which is similar to current findings. Proximate composition of fingerlings of *Oreochromis niloticus* showed that experimental fishes that were fed with 8% inclusion level of papaya leaf meal were found to perform better among all treatment groups in the matter of crude

protein. The highest content of crude protein (15.840±0.0321) was found in T_4 followed by (15.440±0.1422) in T_3 and lowest crude protein (14.630±0.0115) was found in T_2. The highest level of fat (lipid) (3.400±0.0208) was observed in T_1 and lowest (3.260±0.0378) was reported in T_4. The highest carbohydrate (3.480±0.0264) was observed in T_1 whereas lowest (3.030±0.0305) in T_0. The highest ash (3.890±0.0230) was noticed in T_2 and lowest ash (3.123±0.0240) in T_3. The highest moisture (75.060±0.1193) was noticed in T_2 and lowest (72.100±0.1479) in T_4. (Table 5) The proximate composition of fish carcass has shown a significant difference (p<0.05) among all the treatments. Alemu *et al.* (2013) [10] reported crude protein 13.3-15.8 and moisture 79.5-80.9% in fillet of Nile tilapia. Sanvriya (2021) [11] reported crude protein in fish carcass 15.76-16.47, fat 3.2-4.02, ash 3.3-3.6 and moisture 70.40-73.15 in *Labeo rohita* fed with banana peel powder with is correspondence to current findings.

Table 4: Apparent Protein Digestibility of *Oreochromis niloticus* fed with different level of papaya leaf meal in different treatments.

Treatments	Apparent Protein Digestibility				
	0-15 days	16-30 days	31-45 days	46-60 days	0-60 days
T_0 (Control)	66.486^{ab}±0.75011	65.407^{a}±0.7932	65.425^{a}±0.1269	66.418^{a}±0.9808	66.141^{a}±0.4105
T_1	64.019^{a}±2.3926	68.630^{b}±0.1647	69.407^{b}±0.9419	70.736^{b}±0.9410	68.624^{b}±0.5191
T_2	69.556^{bc}±0.2516	69.367^{b}±0.1230	70.408^{b}±0.6402	70.646^{b}±0.2540	70.084^{c}±0.1957
T_3	70.729^{cd}±0.0920	70.707^{c}±0.1754	71.049^{b}±0.4692	71.722^{b}±0.4625	71.121^{c}±0.2290
T_4	73.646^{d}±0.5319	73.936^{d}±0.3335	74.157^{c}±0.2588	75.320^{c}±0.4534	74.356^{d}±0.1757

Data expressed as Mean ± SE (n=3). Mean values in the same column sharing different superscripts are significantly different (p<0.05)

Table 5: Proximate composition of carcass of *Oreochromis niloticus* fed with different level of papaya leaf meal in different treatments.

Treatments	Moisture (%)	Crude protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
T_0 (Control)	74.920^{b}±0.2138	14.960^{b}±0.0435	3.310^{ab}±0.0230	3.780^{c}±0.0378	3.030^{a}±0.0305
T_1	74.840^{b}±0.1250	14.840^{ab}±0.0709	3.400^{c}±0.0208	3.423^{b}±0.0425	3.480^{c}±0.0264
T_2	75.060^{b}±0.1193	14.630^{a}±0.0115	3.350^{bc}±0.0208	3.890^{d}±0.0230	3.090^{ab}±0.0346
T_3	74.940^{b}±0.1135	15.440^{c}±0.1422	3.290^{ab}±0.0173	3.123^{a}±0.0240	3.170^{b}±0.458
T_4	72.100^{a}±0.1479	15.840^{d}±0.0321	3.260^{a}±0.0378	3.690^{c}±0.0230	3.110^{ab}±0.0435

Data expressed as Mean ± SE (n=3). Mean values in the same column sharing different superscripts are significantly different (p<0.05)

Table 6: Proximate composition of experimental diet

Treatments	Moisture (%)	Crude protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
T_0 (Control)	9.330^{b}±0.0404	20.480^{a}±0.0529	8.120^{a}±0.0251	8.750^{b}±0.0611	53.320^{a}±0.1322
T_1	9.380^{bc}±0.0378	20.440^{a}±0.0523	8.190^{a}±0.0351	8.530^{a}±0.0461	53.460^{a}±0.0964
T_2	9.200^{a}±0.0346	20.510^{a}±0.0529	8.150^{a}±0.0208	8.740^{b}±0.0611	53.400^{a}±0.0472
T_3	9.140^{a}±0.0346	20.710^{b}±0.0536	8.170^{a}±0.0251	8.410^{a}±0.0529	53.570^{a}±0.0896
T_4	9.470^{c}±0.0378	20.570^{ab}±0.0529	8.130^{a}±0.0251	8.490^{a}±0.0288	53.340^{a}±0.0435

Data expressed as Mean ± SE (n=3). Mean values in the same column sharing different superscripts are significantly different (p<0.05)

Conclusion

According to the results of the current study, a higher dose of papaya leaf meal at an inclusion level of 8% is considered best for the Digestibility and better feed utilization of *Oreochromis niloticus* fingerlings. Fish showed papaya leaf meal at the rate of 8% to fish diets is good for use in aquaculture to enhance the proximate composition of fish carcasses and feed, and the apparent protein digestibility of Nile tilapia fingerlings.

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