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Evaluation of jute leaf as substitute of fish meal in the diet of mrigal (*Cirrhinus cirrhosus*) fingerlings

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ABSTRACT

The study was conducted to identify jute leaf powder as an alternate to fish meal in diets of juvenile mrigal (*Cirrhinus cirrhosus*) for 60 days. Tossa jute (*Corchorus olitorius*) leaf was selected to utilize this unexplored nutritious resource rather leaving under water for potential pollution. Three isonitrogenous test diets were prepared and applied as treatments (T) in triplicates (R). In control (T_0) dietary inclusion rate of fish meal was 30%, of which 10% was substituted with jute leaf powder in T_{10} and in T_{20} replacement was 20%. Mrigal fingerlings (9.38±0.13 cm and 7.94 ±0.26 g) were stocked in nine plastic half drums (0.26 m^2 each) at 10 fish per drum and fed test diets. Although, growth parameters among the treatments were statistically similar, the highest mean length gain, weight gain, SGR and production were 1.51 (±0.18) cm, 2.96 (±0.13) g, 0.53 (±0.03) %/day and 4084.00 (±50.67) kg ha^{-1}, respectively in T_{10}. However, significantly higher ($P<0.05$) survival was found in T_{10} (93.33%) and T_{20} (90.00%) compared to T_0 (83.33%). Juveniles in T_{10} and T_{20} showed better tolerance to low pH stress than T_0. Water quality parameters were within acceptable range in all the treatments. Moreover, carcass composition of fish was statistically similar among the treatments. Importantly, feed formulation cost was reduced by 3.7% and 20.4% in T_{10} and $T_{20,}$ respectively compared to T_0. Therefore, the results signify that jute leaf powder could be a promising substitute of fishmeal in mrigal diet without hampering growth along with improved survival and low feed cost.

Keywords: Fish meal, Isonitrogenous, Jute leaf, Mrigal.

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Introduction

Aquaculture is one of the promising and fastest food producing sectors in Bangladesh, which has brought the country to the top 5th position in global context (FAO, 2018). In order to keep pace with the increasing demand, this sector has been intensified greatly. Therefore, the demand for fishmeal, the prime source of dietary protein in aqua-feed (Katheline *et al.*, 2019; Hardy, 1999), is increasing dramatically to support the intensification. It has been estimated that aquaculture feed industry used 372.4 million tons of fish meal and 83.5 million tons of fish oil in 2006, which was equivalent to 16.6 million tons of small pelagic forage fishes (Tacon and Metian, 2008; FAO, 2012). Hence, fish meal based feeding practices in aquaculture is a threat to the conservation of wild fish population, which has already been under reckless fishing pressure. Besides high price, adulteration and uncertain availability of fish meal adversely affect profitability of fish farming (Alceste, 2000). Therefore, the quest for possible cost effective alternate protein sources to replace (complete or partial) fish meal in the aqua-feed has become paramount (Magouz *et al.*, 2008). As animal protein sources are mostly expensive and scarce, locally available plant sources are considered to be one of the cheaper alternatives to lessen feed cost without compromising the quality (Munguti *et al.*, 2006; Francis *et al.*, 2012). Addressing the issue, the present venture has been designed with jute (*Corchorus olitorius*) leaf powder to attain a more economically sustainable fish production by utilizing non-conventional protein sources as a substitution of fishmeal in the diet.

C. olitorius, commonly known as tossa/ traditional jute (also known as long-fruited jute, jute mallow and jew's mallow), is an erect, annual herb belonging to flowering plant of the family

Tiliaceae (Singh *et al.*, 2016). It is one of the abundantly cultured jute verities in Bangladesh and West Bengal (India). In Bangladesh, a large area of around 8.0 - 8.2 lakh hectares has been under jute and kenaf cultivation (85% tossa jute, 8% white jute and 7% kenaf of the area) with annual production of 85-90 lakh bales (Saha, 2011; Al-Mamun *et al.*, 2017). The leaves of jute, which are demulcent, diuretic, febrifuge and also serve as tonic (Singh *et al.*, 2016), are very popular as a leafy vegetable in many Asian, African and European countries (Furumuto *et al.*, 2002; Zeghichi *et al.*, 2003). Food and medical industries have shown increasing interest to Jute leaves because of the nutritional value (Oyedele *et al.*, 2006; Dewanjee *et al.*, 2013). In Bangladesh, jute leaves are available at cheap rate to offer popular vegetable dish. Although jute leaf as fish dietary protein source is not yet well established in the literature, the study of Singh *et al.* (2016) has shown some potentiality in carp diets. Therefore, the present study was conducted to evaluate jute leaf powder as feed ingredient for juvenile mrigal by progressively replacing fishmeal in their diet.

Materials and Methods

Experimental design

The study was carried out for 60 days in the "BAU Aquaponics Oasis" at the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. The experimental design comprised of nine well aerated fish holding tanks (each of 0.26 m^2), labeled and placed in two rows following complete randomized block design for experimental congeniality. The tanks were prepared by cutting, washing, drying, setting with aerators, filling up with water and covered with net frame to prevent the fish jumping out or predatory animals attack. Mrigal fingerlings (average size: 9.38±0.13 cm and 7.94 ±0.26 g) were stocked at a density of 10 fingerlings/ 0.26 m^2 tank after proper acclimatization.

Collection and preparation jute leaf powder

The jute leaves were collected from local market. After collection, the leaves were separated from stems, washed with tap water, aerated with electric fan in room condition to evaporate residual moisture and then dried in a homemade dryer at 40°C. When jute leaves became crunchy they were finely ground using blender and then sieved. However, prior to formulating feed, prepared jute leaf powder was analyzed for nutritional profile (Table 1).

Ingredients selection and fish feed formulation

The following ingredients were selected for feed formulation based on their availability, nutrients content and market price (Table 2).

Table 1. Proximate composition of collected jute (*Corchorus olitorius*) leaf powder on wet matter basis (%).

Nutrients	Composition (%)
1. Moisture	10.06±0.84
2. Crude lipid	1.53±0.43
3. Crude protein	28.83±1.25
4. Crude fiber	7.36±0.74
5. Ash	12.01±0.95
6. NFE (Nitrogen Free Extract)	40.21±2.29

Table 2. The price of ingredients used in feed formulation for mrigal fingerlings.

Name of ingredients	Price (BDT Kg^{-1})
1. Fish meal	80
2. Jute leaf powder	30
3. Mustard oil cake	35
4. Rice bran	35
5. Soya bean meal	42
6. Wheat flour	25
7. Soya bean oil and	80
8. Minerals and vitamin premix.	100

Feeds (test diets) were formulated by emphasizing progressive replacement of fishmeal with jute leaf powder. Three different test diets were formulated by replacing 0, 10 and 20 % of fish meal with jute leaf powder, which were designated as T_0, T_{10} and T_{20}, respectively. In the control (T_0), dietary inclusion of fishmeal was 30% (but no jute leaf powder), which was substituted with jute leaf powder and subsequently reduced to 27% and 24% in T_{10} and T_{20}, respectively. Inclusion rates of different feed ingredients were determined following Pearson's technique to prepare isonitrogenous (around 30% protein) test diets (Table 3).

Table 3. Dietary inclusion rate (g) of different ingredients used in formulating 100 g of the test diets for mrigal fingerlings.

Feed ingredients	T_0 (0% replacement of fish meal with jute leaf powder)	T_{10} (10% replacement of fish meal with jute leaf powder)	T_{20} (20% replacement of fish meal with jute leaf powder)
Jute leaf	0	3	6
Fish meal	30	27	24
Mustard oilcake	15	13	13
Rice bran	25	25	25
Soya bean	10	15	21
Wheat flour	15	12	6
Soya oil	3	3	3
Vitamin mineral premix	2	2	2
Grand Total	100	100	100

Sinking dry pellet feed (1.5 mm diameter) was prepared with extruded feed pellet machine and sun dried. Prepared test diets were stored in air tight polythene bags at 4°C in refrigerator before feeding the fish. The proximate composition of the prepared test diets was also determined that has been shown in Table 4 (AOAC, 1990). Some precautionary measures were also taken in preparing the ingredients for feed formulation such as measured mustard oil cake was soaked overnight and soybean meal was pre-boiled to minimize their glucocyanate effects.

Table 4. Proximate composition (%) of different test diets.

Treatments	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	NFE
T_0	12.88	5.65	30	12.54	4.34	33.49
T_{10}	12.83	6.54	29.95	11.83	5.14	33.61
T_{20}	13.68	5.45	30.01	11.60	5.79	33.27

Feeding of fish, sampling and data analysis

The mrigal fingerlings were fed with the experimental diets at the rate of 10% of their body weight twice daily. The uneaten feed and faces were removed from the tanks with 25% daily water exchange through siphoning and the entire water changed fortnightly. Moreover, fish were sampled fortnightly throughout the study period in order to observe their growth response to the test diets by calculating the growth parameters such as length gain (cm), weight gain (g), percent weight gain, specific growth rate (SGR, % day^{-1}), food conversion ratio (FCR), survival rate (%) and fish production (kg ha^{-1}). Fish carcass profile was also determined following the standard procedure of AOAC (1990). Besides, water quality parameters such as dissolved oxygen (mg L^{-1}), water temperature (°C), pH, ammonia and nitrite contents were measured using portable DO meter, thermometer, pH meter and ammonia testing kits, respectively. However, collected data were subjected to one-way ANOVA for statistical analysis (Snedecor and Cochran, 1994). The least significant difference was used for comparison of the mean values ascertained from different treatments.

Low pH stress test

Tolerance of the test fish against low pH stressor (pH 3) was also observed to determine the effect (if any) of the test diets on fish fitness. Therefore, after the feeding trial, two fish from each rearing tank (6 fish per treatment) were randomly selected and transferred to a 20 L bucket containing water having pH 3. Deep tube-well water was strongly aerated for 24 h and gradually mixed with nitric acid (HNO_3) to avail this low pH water (pH 3). The tanks for stress test were equipped with continuous aeration and kept under ambient temperature. The passing of time to reach 50% mortality was calculated as median lethal time (LT_{50}).

Results and Discussion

Throughout the experiment, the test diets were well accepted by the juvenile mrigal as there was almost no feed left over after twenty minutes of feed delivery. Therefore, inclusion of jute leaf as a substitute of fishmeal presumably did not hamper the palatability of the test diets. Singh *et al.* (2016) similarly experimented with jute leaf powder in the diet of rohu (*Labeo rohita*) fingerlings and reported its suitability in carp diet. Regarding the survival rate, juveniles treated with jute leaf based diets in T_{10} and T_{20}

experienced better survivals (93.33% and 90.00%, respectively) that were significantly superior (P<0.05) to those in T_0 with survival rate of 83.33%. The higher survival rates in T_{10} and T_{20} might be attributed to the rich nutritional profile of jute leaves containing appreciable amount of minerals (viz. potassium, magnesium, iron, copper, and manganese) and vitamins (viz. A, C, E) as well as lipid, protein, and carbohydrates (Steyn *et al.*, 2001; Dansi *et al.*, 2008).

The observed mean length gain and weight gain of mrigal fingerlings in the treatments were as follows: T_0: 1.0±0.57 cm and 2.61± 0.06 g, T_{10}: 1.51±0.18 cm and 2.96±0.13 g and T_{20}: 1.35±0.27 cm and 2.52±0.57 g, respectively (Table 5). Although both the parameters were statistically non-significant (P>0.05) among the treatments but T_{10} gave comparatively better fish increment. Correspondingly, the highest SGR (% day^{-1}) and fish production (kg ha^{-1}) were experienced in T_{10} (0.53±0.03 % day^{-1} and 4084.0±50.67 kg ha^{-1}) compared to T_{20} (0.45±0.11 % day^{-1} and 4030.1±42.42 kg ha^{-1}) and T_0 (0.47±0.05 % day^{-1} and 3979.8±45.04 kg ha^{-1}). However, the FCR (Food Conversion Ratio) was the lowest in T_{10} (2.81±0.71) followed by T_0 (3.19±0.16) and T_{20} (3.30±3.15).

Giving insight to the growth performance of mrigal juveniles, all the parameters were statistically similar except the survival rate where jute leaf presumably performed the key role. Therefore, the results suggest that replacement of fishmeal with jute leaf in mrigal diet could be feasible, without hampering fish growth, which is in agreement with the results of Singh *et al.* (2016).

Table 5. Overall growth performance of mrigal fingerlings fed test diets.

Parameters	T_0 (0% replacement of fish meal with jute leaf powder)	T_{10} (10% replacement of fish meal with jute leaf powder)	T_{20} (20% replacement of fish meal with jute leaf powder)	F value	P - value	Significance
Mean initial length (cm)	9.30 (±0.07)	9.27 (±0.16)	9.56 (±0.15)	4.01	0.07	NS
Mean final length (cm)	10.30 (±0.61)	10.78 (±0.06)	10.91 (±0.11)	2.34	0.17	NS
Mean length gain (cm)	1.00 (±0.57)	1.51 (±0.18)	1.35 (±0.27)	0.47	0.64	NS
% length gain	10.72 (±6.16)	16.34 (±2.27)	15.39 (±3.33)	0.01	0.29	NS
Mean initial weight (g)	7.88 (±0.05)	7.81 (±0.20)	8.11 (±0.52)	0.68	0.54	NS
Mean final weight (g)	10.50 (±0.11)	10.78 (±0.13)	10.63 (±0.11)	3.81	0.54	NS
Mean weight gain	2.61 (±0.06)	2.96 (±0.13)	2.52 (±0.57)	1.36	0.32	NS
% weight gain	33.17 (±0.60)	37.96 (±2.67)	31.73 (±9.01)	1.07	0.39	NS
FCR	3.19 (±0.16)	2.81 (±0.71)	3.30 (±3.15)	0.73	0.73	NS
SGR (% day^{-1})	0.47 (±0.05)	0.53 (±0.03)	0.45 (±0.11)	1.04	0.40	NS
Fish production (kg ha^{-1} 60 days^{-1})	3979.80 (±45.04)	4084.00 (±50.67)	4030.10 (±42.42)	3.81	0.08	NS
Survival rate (%)	83.33^a	93.33^b	90.00^b	4.28	0.04	*

*Note: Values are mean ±Standard deviation from triplicate group. Values in a row having similar letters (s) or without letters do not differ significantly whereas values bearing the dissimilar letter (s) differ significantly as per DMRT (Duncan's New Multiple Range Test). * Significant at P≤ 0.05; NS non-significant at P> 0.05.*

Besides growth performance, the tolerance of the experimental fish to low pH stressor was also assessed (after the final harvest) where they were exposed to water pH 3.0. Water pH is an important regulator of cultured fish and tolerance to adverse pH mostly depends on the fish wellbeing. The recommended pH range for fish culture is 6.8-9.0 whereas, pH 4.0 or below is considered as the acid death point where most fish would die (Swingle, 1967). In this experiment, fish in T_0 showed the least tolerance to low pH stressor (LT_{50} = 8 minutes). In comparison, 50% of the fish of T_{10} died within 16 minutes and of T_{20} died within 17 minutes (LT_{50} = 16 and 17 minutes, respectively) after low pH exposure (Fig. 1). Such result signifies the incorporation of jute leaf powder in fish diet to make them more resilient.

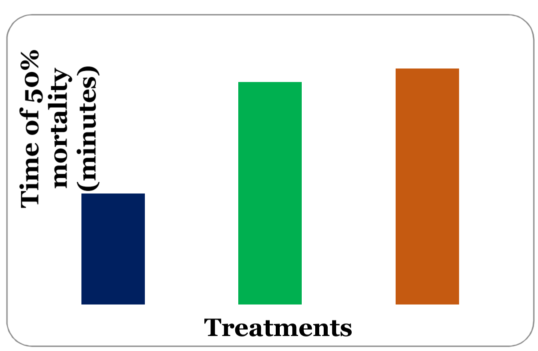

Fig. 1. Low pH stress test.

Regarding the carcass compositions of the experimental juvenile mrigal, all the parameters (moisture, crude protein, crude lipid, crude fiber, ash and carbohydrate contents) were statistically similar (P>0.05) among the treatments (Table 6). Therefore, the results imply that the dietary replacement of fishmeal with jute leaf did not affect the nutrient profile of the fish. Although the observed carcass composition of mrigal juvenile were slightly higher than the findings of Singh *et al.* (2016), who applied jute leaf based diet in rohu (*L. rohita*) fingerlings. The possible reasons might be the presence of fishmeal in the test diets and different fish species subjected in the present experiment. However, further research with higher dietary inclusion of jute leaf in mrigal diets is necessary to visualize the amplified effect of jute leaf in the fish carcass profile. Notably, the incorporation of plant based diets has been reported to boost up carcass protein and fat levels in Indian major carps (Nandeesha *et al.*, 1995).

Table 6. Proximate compositions of mrigal fingerlings (% moisture basis) with different experimental diets.

Parameters	T_0 (0% replacement of fish meal with jute leaf powder)	T10 (10% replacement of fish meal with jute leaf powder)	T_{20} (20% replacement of fish meal with jute leaf powder)	F value	P -value	Significance
Moisture	75.06 (±1.04)	75.81 (±0.82)	75.61 (±0.90)	0.43	0.67	NS
Crude protein	14.07 (±0.64)	14.10 (±0.21)	13.91 (±0.12)	0.20	0.82	NS
Crude lipid	4.93 (±0.41)	5.13 (±0.32)	4.49 (±0.16)	3.25	0.11	NS
Crude fiber	1.13 (±0.03)	1.24 (±0.11)	1.03 (±0.23)	1.50	0.29	NS
Ash	3.71 (±0.46)	3.79 (±0.52)	3.56 (±0.08)	0.25	0.78	NS
Carbohydrate	0.47 (±0.26)	0.82 (±0.28)	0.62 (±0.31)	1.14	0.37	NS

*Note: Values are mean ±Standard deviation from triplicate group. Values in a row having similar letters (s) or without letters do not differ significantly whereas values bearing the dissimilar letter (s) differ significantly as per DMRT (Duncan's New Multiple Range Test). * Significant at $P \leq 0.05$; NS non-significant at $P > 0.05$.*

Considering the feed cost, it was found that the expense of preparing the test diets increased with the inclusion level of fishmeal (Fig. 2). Correspondingly, the highest feed formulation cost was observed in T_0 (50.35 BDT Kg^{-1}) which was 3.7% higher than T_{10} (48.50 BDT Kg^{-1}) and 20.4% higher than T_{20} (40.10 BDT Kg^{-1}). Importantly, feed cost in aquaculture accounts for 70 to 75% of the total cost of fish production (Gadzama and Ndudim, 2019; Katheline *et al.*, 2019). Therefore, substitution of fishmeal (animal protein) with jute leaf powder could considerably lessen the feed cost in aquaculture without affecting fish growth (Singh *et al.*, 2018).

However, the water quality parameters viz. pH, dissolved oxygen (DO), ammonia and nitrite contents in all the treatments (Table 7) were within the acceptable range for fish culture (Swingle, 1967; Rothius and Nhan, 1998; Paul *et al.*, 2014). Therefore, suggesting that the experimental fish were not under stressed condition and water in the tanks did not play any decisive role in the feeding trials.

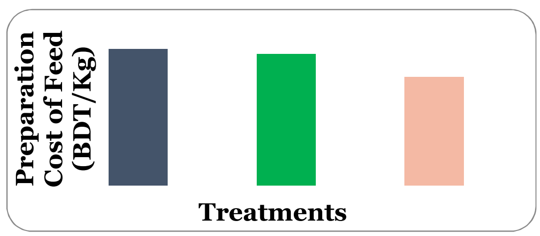

Fig. 2. Formulation cost of different test diets (BDT Kg^{-1}).

Table 7. Water quality parameters of different treatments during the experimental period.

Parameters	T_0 (0% replacement of fish meal with jute leaf powder)	T_{10} (10% replacement of fish meal with jute leaf powder)	T_{20} (20% replacement of fish meal with jute leaf powder)
pH	7.45 (±0.18)	7.50 (±0.12)	7.69 (±0.10)
DO (mg L^{-1})	5.56 (±0.31)	5.05 (±0.64)	5.90 (±0.10)
Temperature (°C)	26.43 (±0.47)	25.86 (±0.75)	25.30 (±0.67)
Ammonia (mg L^{-1})	0.08±0.02	0.07±0.02	0.07±0.01
Nitrite (mg L^{-1}))	0.62±0.07	0.57±0.04	0.61±0.04

Conclusion

Reducing feed cost has always been a crucial issue in aquaculture. The current venture promotes the dietary inclusion of jute leaf powder in order to cost effectively replace fish meal in the diets of mrigal fingerlings without adversely affecting fish growth and carcass composition at improved survival.

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