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Effect of *Leucaena leucocephala* Leaf Meal in Diet of *Liza parsia*

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ABSTRACT

To study the effect of *Leucaena leucocephala* leaf meal (LLM) in diet of *Liza parsia* on feed intake, growth and digestibility, feeding trials were conducted in fibre reinforced plastic (FRP) tanks containing 400 litre strained clear brackish water with four groups of *Liza parsia* fry in triplicates having twenty five fishes of average wt. ranging from 1.51 to 1.79 g per replicate. Pellet feeds (iso-proteinous) with three different levels of LLM i.e. 10%(LLM10), 20% (LLM20) and 30% (LLM30) were formulated and prepared using locally available feed ingredients and fed to fishes of group II, III and IV, respectively and compared with fishes of group I fed test diet containing no LLM (LLM0). In every morning leftover feed was siphoned out and 50% of water of rearing tank was exchanged. After 154 days of experiment it was found that average daily weight gain(ADG), feed conversion ratio (FCR), protein efficiency ratio (PER) and specific growth rate (SGR) did not differ significantly among the groups. Nutrient (organic matter, crude protein, ether extract) digestibility did not differ significantly among the groups. Carcass composition of fishes of four groups also did not differ. Histopathological study of liver tissues showed that liver tissue degeneration took place when *Leucaena leucocephala* leaf meal was incorporated beyond 10% level. Hence it is concluded that *Leucaena leucocephala* leaf meal (CP-22.68%) can be incorporated up to 10% in diet of *Liza parsia* without affecting performance of fish.

Key words: *Liza parsia*, Plant protein, *Leucaena leucocephala* leaf meal, Growth, FCR, Digestibility

INTRODUCTION

Protein is the major macronutrient whose requirement is prioritized in nutritional studies, because it represents the highest cost in commercial feeds, as well as plays an important role in the growth of fishes. Lack of adequate level of protein in the diet results in cessation of growth and loss of weight. Of the total protein supplied in the diet, a part of it is used to synthesize new protein and the rest is being converted to energy.

Plant proteins are relatively cheaper per unit of nutrient than animal protein. Among the plant protein sources *Leucaena leucocephala* is a fast-growing and drought resistant tropical leguminous tree, which offers probably the widest assortment of uses. It is also cheaper and afordable by most farmers compared to expensive cereals and oil-seed cake supplements (Rubanza et. al. *,2005)*. Its leaf meal is rich in protein and has been widely used as a protein source in ruminant and poultry feed in tropics. A few studies have been conducted to evaluate the nutritive value of *Leucaena leucocephala* leaf meal (LLM) as protein source in fish feed, but the data obtained are conflicting. *Leucaena* foliage (leaflets plus stems) is a potential source of cheap protein to ruminants due to its good nutritional quality (Jones 1994). Its amino acid pattern is comparable with that of soya bean and fish meal and other animal feed sources available in developing nations (Kale, 1987). *Leucaena* should not be a major portion of the diet in nonruminant animals as *Leucaena* foliage contains low level of mimosine (Norton 1994) and low tannin antinutritive factors (McNeill et al. 1998). Although most of these animals eat the plants with relish, they are less able to tolerate mimosine than are ruminants (Ruskin, 1984). Non-ruminants generally do not tolerate rations that contain more than 5% to 10% *Leucaena* (dry weight). The efficiency of various alternative protein sources as partial or complete dietary replacement for fish meal has been evaluated in fish diet, e.g. poultry by-product meal (Shapawi et al.,2007), poutry offal meal (Hasan and Das, 1993), soybean meal (Langer et al., 2011) and silk worm pupae (Langer et al., 2011). Hossain and Jauncey (1989) evaluated mustard, linseed and sesame oil cakes as dietary protein sources for the

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Table 1. Feed offered with different level of *Leucaena* leaf meal (LLM)

Ingredients (%)	LLM-0	LLM-10	LLM-20	LLM-30
Wheat flour	15	15	15	15
Fish meal	14	18	22	26
Soybean meal	30	20	10	0
Rice bran	37	33	29	25
Mineral mix.	2	2	2	2
Binder	2	2	2	2
Leucaena leaf meal	0	10	20	30
Cost/Kg feed (₹)	20.65	18.95	17.25	15.55

common carp fingerlings. Soybean meal is the most commonly used plant protein and often constitutes approximately 30-40% of the feed of fishes. Therefore, present experiment was conducted to study the effect of LLM by replacing soybean meal in the diet of *Liza parsia*, an important brackishwater species abundantly available in Sunderban area of West Bengal (Chandra and Sagar, 2010).

MATERIALS AND METHODS

Four isoproteinous (crude protein-28%) diet with different levels of *Leucaena* leaf meal (0%,LLM0; 10%, LLM10; 20%, LLM20 and 30%, LLM30) were formulated and prepared using locally available feed ingredients (Table 1) to replace soybean meal at different level (0, 33.33, 66.66 and 100%) with LLM for *Liza parsia*. To study the effect of LLM on feed intake and growth, four groups of *Liza parsia* juveniles were taken with three replicates in each group. Twenty five fishes of average wt. ranging from 1.51 to 1.79 g were stocked per replicate in fibre reinforced plastic (FRP) tank containing 400 litres strained clear brackish water. Fishes of four groups I, II, III and IV were fed LLM0, LLM10, LLM20 and LLM30 feed, respectively. In every morning leftover feed was siphoned out and 50% of water of rearing tank was exchanged. Temperature, dissolved oxygen, pH, salinity and alkalinity of the water of different tanks were measured (APHA, 1998) on weekly interval to maintain the uniformity in water quality and the range was 31.5-33.5°C, 7.2-8.8 ppm, 7.95-7.97, 3.0-6.5 ppt and 96-128 ppm, respectively. For determination of digestibility chromium oxide was included at 0.5% as an inert marker. Experiment was continued for 154 days. A weighed quantity (10% of body weight) of diet was offered twice daily. Leftover feed was recovered, dried and subtracted from the feed offered for computing the feed consumed. Faeces were collected through siphoning with plastic pipe four hour after each feeding for consecutive seven days, washed gently with distilled water, dried in the oven at 60°C and accumulated for analysis for digestibility studies.

The proximate principles of diet and faecal sample were determined following AOAC (1995) method and chromium oxide content of faecal matter was estimated by wet digestion method of Furukawa and Tsukahara (1966) as described for study of digestibility in fish. Other parameters like feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were also calculated after experiment.

At the end of the experiment fishes of each group

Table 2. Chemical Nutrient composition of feed with different level of LLM

Nutrient (% DM)	LLM-0	LLM-10	LLM-20	LLM-30
OM	84.08	84.24	83.92	83.89
CP	28.11	28.07	28.04	28.03
EE	4.72	5.10	5.48	5.86
CF	4.47	4.46	5.10	5.17
Ash	15.92	15.76	16.08	16.11
AIA	4.10	3.96	5.70	3.17
NFE	46.78	46.61	45.30	44.83

Table 3. Performance of *L. parsia* fed different feed containing LLm

Parameter	Group I LLM-0	Group II LLM-10	Group III LLM-20	Group IV LLM-30
Initial body weight (g)	1.79±0.15	1.61±0.05	1.51±0.05	1.71±0.04
Final body wt (g)	6.50±0.55	5.82±0.16	5.30±0.37	5.35±0.17
Total weight gain (g)	4.71±0.66	4.21±0.20	3.79±0.42	3.65±0.12
Av. Daily gain (mg)	30.59±4.26	27.32±1.32	24.61±2.75	23.68±0.81
FCR	7.42±1.20	7.98±0.41	9.02±0.94	9.18±0.33
PER	0.47±0.07	0.42±0.02	0.38±0.04	0.36±0.01
SGR	0.84±0.10	0.83±0.04	0.81±0.07	0.74±0.01
Digestibility (%)				
DM	79.43±0.38	80.71±0.51	79.71±0.62	79.01±0.86
OM	84.39±0.10	84.58±0.60	84.89±0.46	83.44±0.29
CP	93.01±0.52	92.91±0.40	93.31±0.10	92.46±0.48
EE	79.13±3.79	65.75±5.62	71.40±8.09	76.69±2.52
NFE*	88.67^{b}±0.91	88.29^{b}±0.97	86.73^{ab}±0.16	84.89^{a}±0.17

* P<0.05, Figures with superscripts in a row differ significantly (P<0.05)

were sacrificed for analysis of proximate composition. Liver samples were collected in 10% neutral buffered formalin for histopathology. After fixation, standard histological procedure was followed for tissue dehydration and paraffin embedding. The tissue blocks were sectioned at 5µ and stained with haematoxylin and eosin (H & E) according to Stevens and Wilson (1996).

The experimental data were subjected to analysis of variance (ANOVA) using GLM procedure of SPSS (2008) software. The method of least significant difference was applied for comparison between the treatments, following the method of Snedecor and Cochran (1973).

RESULTS AND DISCUSSION

In the present study, pellet feeds (isoproteinous) with three different levels of LLM i.e.,10% (LLM10), 20% (LLM20) and 30% (LLM30) were prepared using locally available feed ingredients and fed to fishes of group II, III and IV, respectively. Fishes of group I (Control) were fed balanced diet without any LLM.

From the nutrient composition of feed (Table 2) it was found that ether extract (EE) was higher in LLM10, LLM20 and LLM30 groups whereas crude fibre (CF) was higher in LLM20 and LLM30 groups which might be due to increase of fish meal percent in said feeds and also due to pigment and wax coming from *Leucaena* leaf meal (LLM). Temperature, dissolved oxygen, pH, salinity and alkalinity of the water of different tanks were measured on weekly interval and the range were 31.5-33.5°C, 7.2-8.8 ppm, 7.95-7.97, 3.0-6.5 ppt and 96-128 ppm, respectively. From the result of experiment it was found that after 154 days of experiment total body weight gain, average daily gain, FCR, PER and SGR though showed decreasing trend with increasing level of *Leucaena leucocephala* but did not differ significantly among the groups (Table 3). Low dietary protein level in

Table 4. Carcass composition of *L. parsia* fed different level of LLM

Body composition (%)	Group I LLM-0	Group II LLM-10	Group III LLM-20	Group IV LLM-30
OM	82.17±0.93	80.25±0.55	80.46±0.31	81.97±0.68
CP	50.88±0.76	51.21±0.56	50.71±0.51	50.76±0.58
EE	23.19±0.85	19.51±0.36	21.65±1.76	22.83±1.41
NFE	8.10±0.77	9.52±0.19	8.12±0.97	8.38±1.00
Ash	17.83±0.93	19.75±0.55	19.54±0.31	18.03±0.68

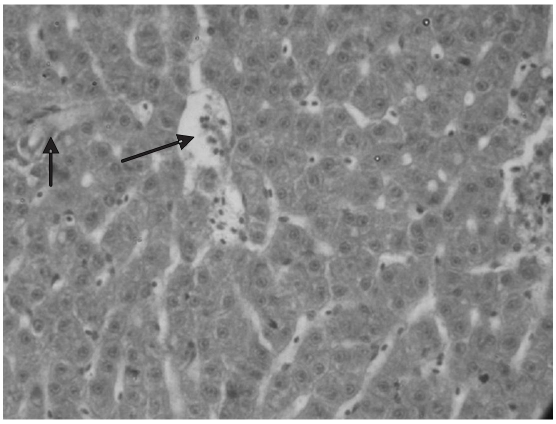

Fig.1. Rupture of blood vessels and escape of erythrocytes in to the interlobular space with connective tissue proliferation and zones of fatty degeneration (40x).

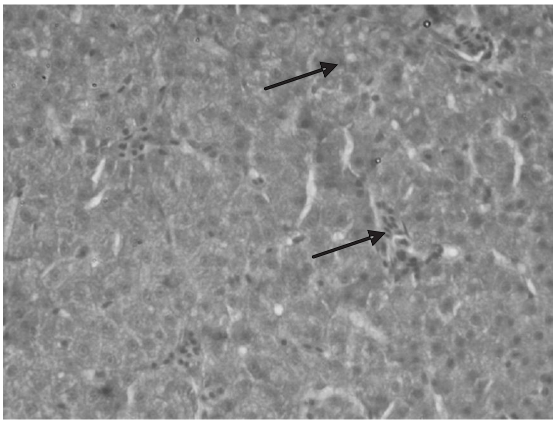

Fig.2. Congestion and hemorrhage with connective tissue proliferation and zones of fatty degeneration (40x).

feed produced higher FCRs, possibly due to intake of inadequate nutrient levels to promote growth (Zhou et al., 2007).

Digestibility of nutrients were also similar (P>0.05) among the groups (Table 3). When soybean meal was totally replaced by LLM apparent digestibility of protein was 92.46% which was higher than that (68.3-72.9%) reported by Hasan et al., (1994). NFE digestibility was lower (P<0.05) in LLM30 group as compared to that of LLM0, LLM10 and LLM20 groups which might be due to higher CF in LLM30. Carcass composition of fishes (Table 4) of four groups also did not differ which is similar with the findings of Hasan et al., (1997). An increasing level of raw *Leucaena* leaf meal was associated with a decrease in the carcass protein content of rohu fingerlings (Bairagi et al., 2004). Improved growth performances in fish fed diets with *Leucaena* as a dietary protein source have been reported for Java tilapia (Ghatnekar et al., 1983), Nile tilapia (Pantastico and Baldia, 1980) and Indian major carp (Ghatnekar et al., 1983). In contrast, reduced performances when fed *Leucaena* leaf meal have been reported for Java tilapia (Wee and Wang, 1987), Nile tilapia (Santiago et al., 1988), Indian major carps (Hasan et al., 1994).

Histopathological study of liver tissues indicated that there were no pathological lesions in liver of fish fed 10% *Leucaena* leaf meal. But when fishes were fed *Leucaena* leaf meal at 20%, fibrous tissue proliferation around the sinusoids with connective tissue proliferation around hepatocytes and when fed at 30% level degeneration of liver tissues as represented by congestion and hemorrhage with connective tissue proliferation and zones of fatty degeneration (Fig 1 & 2) was observed . The result of present study indicated that when soybean meal was replaced with 30% LLM, performance in respect of growth, FCR and nutrient utilization were not affected in *Liza parsia* but beyond 10% inclusion of LLM liver tissues got damaged. Therefore, it may be concluded that *Leucaena leucocephala* leaf meal can be incorporated at 10% level in the diet of *Liza parsia* without any adverse effect and thus cost of feeding can be reduced.

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