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Effect of *Moringa oleifera* leaves on the growth and enzymatic activities of *Labeo rohita* by replacing with fish meal

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Abstract

Moringa oleifera (*M. oleifera*) is the most common source of plant proteins in fish diet, because it is cheap source of protein and easily availbale.The present study was designed to investigate the effect of (*M. oleifera*) on the growth and enzymatic activities of *Labeo rohita (L. rohita)* supplemented diet of *Moringaoleifera* (*M.oleifera*)leaves, experiment was conducted in four groups i.e. controll group (no *M. oleifera*supplemets) and experimental groups T1 (15%), T2 (30%), T3 (45%). All the fish were reared in the glass aquaria in the replicate of three. After the completion of experimetal trial fish weight were noticed, maximum weight gain (15.23 ± 0.08) was observed in the T2 group compared to other groups. After analyzing the amylase(0.55 ± 0.07) and cellulase activties (0.53 ± 0.09) was high in T2 compared to other experimental groups, which results in efficent digestion. All the water quality paramters were within range. Thus it is concluded from the present study that repacing 30% (*M.oleifera*) with fish meal can increase the growth rate and enzymatic actvities of (*L. rohita*).

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Introduction

Moringa is popular among plants for proteins source and is using in the diet of fish, (Hardy, 2010). *Moringa* is getting attention in the aquaculture because all the parts, i.e. leaves, flower and seeds can be used (Makkar and Becker, 1997).

The leaves of *Moringa* contain high amount of crude protein and approximately 260 g/kg of leaves contain 87% of true proteins. Few essential amino acids are found in the leaf of *Moringa* plant i.e. Cysteine, tryptophan and lysine (Makkar and Becker, 1996). Amino acid comparison of soyabean meal and leaves of *Moringa* show almost similar amount of essential amino acid (Foidl *et al*., 2001). This amino acid are present in abundant amount that it can be used for the preparation of animal feed (Afuang *et al*., 2003). *Moringa* leaves are alternative to proteins source because of its high proteins content, antioxidant enzymes and oils (Ayotunde and Ofem, 2008). *Moringa* contain ascorbic acid carotenoids and minerals. Studies have shown that, Moringa leaves is consider favorable protein source for addition in fish diets at small levels (Ayotunde and Ofem, 2008.).

The Rohu (*L. rohita*) is the most popular fish among fish of Indian subcontinent. Rohuis extremely tasty and esteemed fish species than others among Indian major carps (Ibrar *et al.,* 2017). The use of marketable feed has become unavoidable for the achievement of cyprinid culture under extensive culture conditions predominantly Rohu along with other carps It eats zooplankton and phytoplankton during young stage (Abid *et al*., 2009). *L. rohita* and *Cirrhinus mrigala* are the commonly cultured species of Pakistan, fish meal is expensive source of protein ingredients in the feed of fish, and therefore during the present study, fish meal was replaced with *M. oleifera* as plant protein source, growth performance and enzymatic activities were evaluated after two months of trial.

Material and methods

Maintaining fish

L. rohita fingerlings with average body weight (ABW) of 3.62 ± 0.09 were taken from the nursery ponds of UVAS Ravi Campus Pattoki. All the fish were properly kept under laboratory conditions in the fiber glass aquaria (15″L× 8w″× 8″D) for 15days for acclimatization. All the aquaria were fitted with aerators for oxygen supply and heaters for marinating temperature of the water. Fish were stocked at the stocking density of 2g/L, fish were given 4% feed according to the body weight two time a day.

Experimental design

The present study was aimed to study the effect of *M. oleifera* on the growth enzymatic activities of *L. rohita* by replacing fish meal with protein source of *M. oleifera*. Total four groups were designed Control (C) lacking *M. oleifera* three treatments (T1) 15%, (T2) 30%, (T3) 45% of *M. oleifera* plant proteins source. Experiment was conducted for 60 days.

Processing of Moringa oleifera leaves.

M. oliefera leaves, stem and flowers were taken from a small town Choti Zareen, District Dera Ghazi Khan, Punjab province and were sun dried. After drying properly *M. oliefera* leaves, stem and flower were properly grinded in the grinder separately.

Diet preparation

Four types of diet were prepared i.e. *M. oleifera* 15%, *M. oleifera* 30% and *M. oleifera*45%. In control *M. oleifera* meal was not mixed. In all four diets different types of ingredient were mixed to prepared perfect diet. Their Inclusion Level (IL) is given Table 1.

Chromic oxide was added to check the digestibility of feed by fish. When all these ingredients were mixed properly then to make pellet water was added and mixture of all ingredients were put into the pellet machine. These pellets were dried in the drying oven.

Data collection

After the completion of experimental periods fish were weight with electric balance and length was also measured with ruler, fish were dissected for intestine collection of amylase and cellulase activities, after removal, intestine was put into the nitrogen cylinder, the preserved for further analysis.

Limnological parameters

Water quality parameters were checked daily with multi-parameter (Pentair, Professional plus multipara meter instrument) and all the water quality parameters were with proper range required for carp's culture.

Growth parameters

All the growth parameters were measured by the methods described by (Ibrar *et al.,* 2017).

Enzyme studies

Analysis of enzyme activity

The stored samples were brought to University of Veterinary & Animal Science (UVAS) physiology lab for the analysis of Amylase and cellulase enzyme activity.

Sample preparation

The gut content of fish was collected in a micro-centrifuge tube and homogenized with chilled tris HCl with homogenizer. The homogenate was centrifuged at 6000rpm at -4°C for 15 min and the supernatant was collected and stored at -20°C. This method was described by Bernfeld, (1955).

Spectrophotometer was used to check absorption at 540 nm. Readings were also noted for blank and standard. Samples were taken in caveats tubes and turned the blank to 0. The readings of samples were taken and bands were compared it with standard. And the cellulose was determined by the method adapted by Denison and Koehn (1977).

Statistical analysis

All the data are presented as a Means ± SE, statistical software *Statix-8.1 was used for statistical analysis of the readings.*

Results

After completion of the experimental period fish were analyzed for weight gain, T2 have significantly high ($P<0.05$) weight gain, length and FCE (%) compared to other groups, similar T2 have better feed conversion compared to other fish, Moreover the initial weight of all the fish were non-significant ($P>0.05$).

After analyzing the intestine of (*L. rohita*) the amylase and cellulase activity was maximum in the fish feed 30% *M. oleifera*, while the control fish have low level of enzymatic activities compared to the experimental groups.

Table 1. Showing feed formulation.

Ingredients	Control	*M. oleifera* 15%	*M. oleifera* 30%	*M. oleifera* 45%
Rice polish	51	42	35	30
M. oleifera meal	0	11	21	32
Fish meal	33	25	25	26
Sunflower meal	13	14	13	14
Sunflower oil	2	2	5	4
Mineral mix	1.0	1.00	1.1	1.3
Vitamin mix	1.00	1.003	2	1.00
Chromic oxide	.01	1.00	5	1.0

Discussion

Study conduced on the *L. rohita* show improved results in the weight gain and amylase activities with *M. oleifera* by replacing with fish meal. After completion of 60 days study fish were analyzed for weight gain, T2 have significantly high ($P<0.05$) weight gain (15.23 ± 0.08), length (6.11 ± 0.07) and FCE (%) (76.33 ± 2.3) compared to other groups, similar T2 have better feed conversion compared to other fish. Final weight gain of the Nile Tilapia increased with increasing *Moringa* leaves (El-Nad and Khames *et al*., 2015). The present results support Abo State *et al.* (2008) observation who observed high growth rate with 10% and 12% *Moringa* leaves compared to control diet.

Table 2. Preparation of samples for amylase activities.

Reagents	Sample	Standard	Blank
Starch sol 1%	1 ml	-	1 ml
Phosphate buffer	1 ml	-	-
Prepared sample	1 ml	-	-
Incubate all test tubes at 37˚C for 15 minutes in incubator			
0.1%glucose sol	-	1 ml	-
DNS reagent	1 ml	1 ml	1 ml
Keep tubes in boiling water bath for 1 min and cool it at room temperature			
Water	-	2 ml	2 ml

Table 3. Growth parameters of *L. rohita* after 60 days of experimental trial.

Growth parameters	C	T1	T2	T3
Initial weight (g)	3.62a ± 0.09a	3.67 ± 0.04^{a}	3.59 ± 0.02^{a}	3.63 ± 0.02^{a}
Weight gain(g)	7.2 ± 0.04^{d}	11.25 ± 0.09^{b}	15.23 ± 0.08^{a}	10.12 ± 0.06^{c}
Length(cm)	4.2 ± 0.03^{d}	6.11 ± 0.07^{b}	6.97 ± 0.04^{a}	5.73 ± 0.03^{c}
FCR	2.34 ± 0.02^{a}	1.82 ± 0.04^{c}	1.31 ± 0.03^{d}	2.11 ± 0.09^{b}
FCE (%)	42.72 ± 0.17^{d}	54.94 ± 1.9^{b}	76 .33 ± 2.3^{a}	47.39 ± 1.5^{c}

Data are presented in the form of (Mean ± SE), alphabets show significant difference, C (control), T1 (15%), T2 (30%), T3 (45%) of proteins.

The feed conversion was high in the treatment supplemented with *M. oleifera* leaf (El-Nad and Khames *et al.*, 2015). Replacing moringa leaf, in the diet of fish Nile tilapia (*Oreochromis niloticus*), don't depress the growth activities,(Richter *et al.*, 2003; Afuang *et al.*, 2003), during the present results *Moringa* show better results compared to control and thus supporting the above statement.

According to Puycha *et al.* (2017) Bocourti's catfish (*Pangasius bocourti*) showed better feed conversion, supplemented with *Moringa* leaf, and no mortality was recorded , similarly during the present findings fish supplemented 20% of *Moringa* leaf better feed conversion were reported and all the fish were remain healthy.

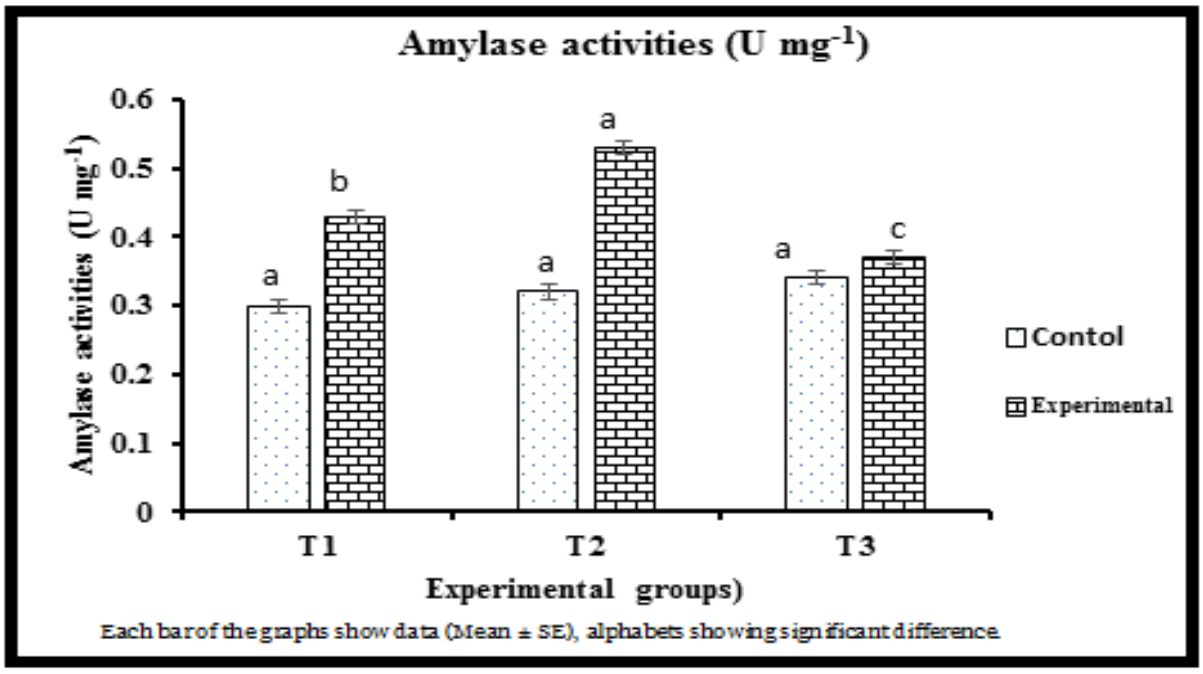

Fig. 1. Showing amylase activities of *L. rohita*.

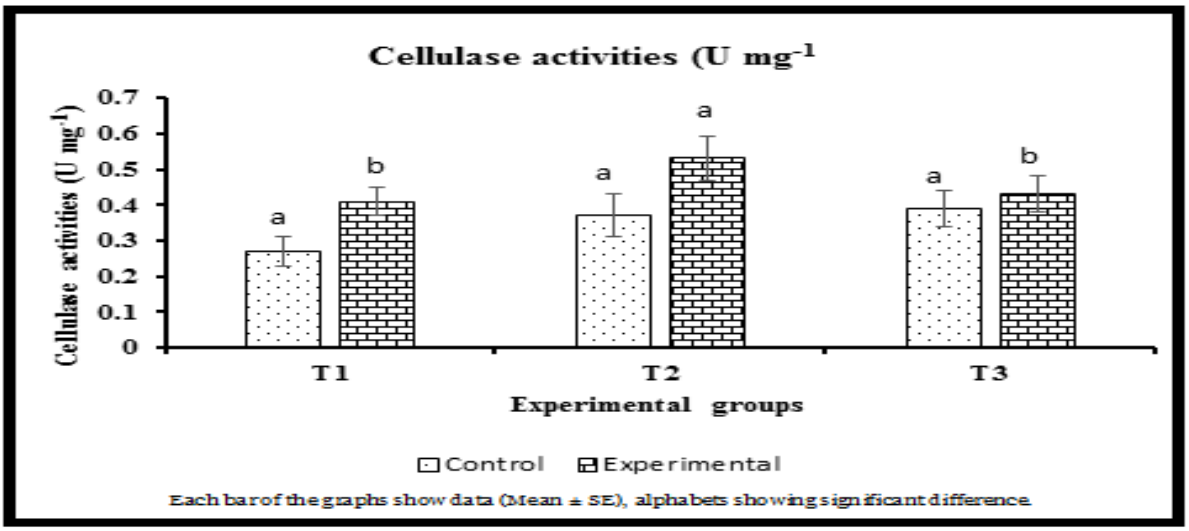

Fig. 2. Showing Cellulase activities of *L. rohita*.

The amylase activity was maximum in the fish feed 30% *M. oleifera* compared to feed lacking *M. oleifera*, 15% and 45% supplementation of *M. oleifera*. The results shows that addition of *M. oleifera*in the fish feed results in beneficial effect on the amylase activity added 30%*M. oleifera*. Cellulase activities was also high in the T2 groups followed by T3 and T1 respectively.

Conclusion

Thus from the present study it is concluded that replacing *M. oleifera* with fish meal enhances growth and enzymatic activities of the *L. rohita* and recommended for feed.

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