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EFFECTS OF ENZYME SUPPLEMENTATION IN PRACTICAL DIET FOR ROHU (*LABEO ROHITA*) FINGERLINGS

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ABSTRACT

The effect of pretreatment of agro-based supplementary feed with a crude fungal xylanase produced by *Aspergillus foetidus* (MTCC 4898) were evaluated on rohu fingerlings. The agro-based supplementary feed (defatted groundnut oil cake (GNOC) and rice bran) was treated with crude xylanase at the rate of 184 U/gm. The pretreatment of feed with xylanase resulted in a significant ($P<0.05$) increase in the reducing sugar content and non-significant decrease in the crude fiber content. The growth performance and chemical composition of fingerlings fed with xylanase treated diet were found to be better than fishes fed on the control diet. At the same time, mean survival of rohu fingerlings was 100%. The results of the present study indicate that xylanase treatment of supplementary feed can be effective for improving the growth of the rohu fingerlings.

Key words*: non-starch polysaccharides (NSPs), Rohu, supplementary feed, xylanase.*

INTRODUCTION

Very high cost of fish meal and soybean meal has encouraged the researchers to use alternative locally available plant protein sources for fish feed. Oilseed byproducts such as rapeseed, cotton seed, sunflower and groundnut meals are being used as partial substitutes for fish meal and soybean meal [1 & 2]. Oilseed meals are rich in protein; and some of them are traditionally used as feed for farm animals. However factors like amino acid imbalance and presence of anti-nutritional factors limit the incorporation of oilcakes in aqua feed [3]. Enhancement of the nutritive value of these ingredients by processing to increase the bioavailability of nutrients and to reduce or remove anti-nutritional factors by the inclusion of appropriate additives could result in oilseed meals being incorporated at higher levels in fish feed [4].

Use of enzymes as additives in plant based feeds has improved feed utilization in terrestrial animals. Commercial feed enzymes are routinely used in poultry and pig feed to improve nutritive value of plant based feed [5]. Cheah *et al.* [6] have reported significant increase in the protein content of palm kernel meal by solid state fermentation with various species of *Trichoderma* fungus. Endoglucanases and endoxylanases hydrolyse β-1, 4-bond in cellulose and xylan polymers respectively and reduce the size of soluble nonstarch polysaccharides (NSPs) within the digestive tract. The action of these enzymes is also supposed to aid in the release of nutrients bound by plant cell walls. In aquaculture, addition of NSP degrading enzymes to canola meal has been shown to improve growth rates of juvenile tiger prawns; and endoglucanase as well as endoxylanase enzymes could play a significant role in the utilization of plant materials in crustacean diets [7].

The present study has been undertaken to investigate the possibility of use of crude fungal xylanase in improving the nutritional quality of practical feed of carp, mainly defalted groundnut oil cake (GNOC) and rice bran, and their influence on the growth and carcass composition of fishes. Limited information is available on the use of feed enzymes in the supplementary feed of Indian major crops (IMCs). The effects of crude fungal xylanase treated practical feed has been checked on the growth rate, survival rate and body composition of *Labeo rohita* fingerlings. The findings suggest that by enhancing the nutritive value of practical diet of IMCs by enzyme treatment, growth rate of the fishes can be enhanced; and this warrants further investigation.

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MATERIALS AND METHODS

Preparation of fish feed

Rice bran and groundnut oil cake (GNOC) were obtained from a local market, ground to fine powder and sieved before being subjected to enzyme treatment. Feed formulation contained rice bran and GNOC (1:1) as well as guar gum as a binder (2% of the feed). After pelleting, pellets were oven dried at 50°C and used as fish feed.

Enzyme production and its treatment to fish feed

Xylanase was produced indigenously by solid state fermentation on corn cobs using *Aspergillus foetidus* MTCC 4898 [8]. Treatment of fish feed with enzyme was carried out by mixing rice bran and GNOC (1:1) with crude fungal xylanase at the rate of 66 ml / 100 g feed (v/w) with 280U per ml of enzymatic activity. This enzyme-feed mixture was incubated at 37°C for 2 h before pelleting.

Experimental procedure

Fingerlings of *Labeo rohita* were obtained from Govt. Fish Rearing Centre Navli (Dist. Anand, Gujarat). The fishes were acclimatized in a 5000-L fiberglass tank for two weeks and fed with GNOC and rice bran (1:1). For the feeding trials, duplicate groups of 25 fingerlings (mean weight 5.30±0.15g) were maintained in 150L glass aquaria. Both experimental and control feed were fed to duplicate groups of experimental and control fishes at the rate of 2% of the body weight per day in equal feedings at 09.00 and 17.00 hours. All the aquaria were cleaned and refilled with fresh water every morning. Continuous aeration in all the aquaria was maintained during experimentation. Physicochemical parameters of water viz. temperature, pH, dissolved oxygen and total organic carbon were measured at every week from all the aquaria during experimentation to maintain water quality and measured as 21.5-31.5 °C, 8.03-8.67, 6.23-8.68 mg/l and 106.7-115.1 ppm respectively [9]. Fishes in each aquarium were weighed once every week and the feeding ration adjusted accordingly. The feeding trial was conducted for 30 days.

Analytical methods

Feed ingredients, experimental diets, fish muscles and fish liver were analysed according to the AOAC [10] as follows: protein (N×6.25), by micro kjeldahl digestion and distillation after acid digestion; ash, by ignition at 600 °C in a muffle furnace until a light gray or white ash was formed; crude fiber, by total dietary fiber assay kit from SIGMA (TDF-100A); lipid, by extracting the residue with 40-60 °C petroleum ether for 8 h in a Soxhlet apparatus; total carbohydrate, by Anthrone method; glycogen, by extracting with 30% KOH and the yield was determined by anthrone-sulphuric acid method. The release of reducing sugar from the xylanase treated feed and untreated feed were measured at 0 h and 2 h incubation period by DNS method [11].

Determination of growth parameters

Fishes were weighed once a every week and from the data of average initial and final weight, specific growth rate (SGR=$\log_e$ final body weight - $\log_e$ initial body weight /number of Exp. Days x 100), feed conversion ratio (FCR=feed given (gm)/fish weight gain (gm)) and survival rate (SR=final no. of fishes/ initial no. of fishes x 100) were calculated.

Statistical Analysis

The data obtained were analyzed statistically by one-way analysis of variance (ANOVA). Differences between means were assessed using Duncan's multiple range test [12]. All differences were regarded as significant at $P < 0.05$.

RESULTS AND DICUSSION

In pond culture, Indian major carps (IMCs) are generally fed on a mixture of rice bran and various varieties of oil cakes. These oil cakes form the major dietary protein source in formulated feed for the carps [13]. In the present experiment to improve the nutritive value of practical feed of rohu, ground nut oil cake (GNOC) and rice bran were treated with crude fungal xylanase. Proximate composition of GNOC and rice bran is shown in the Table - 1.

Table - 1 Chemical composition of the diet (% dry matter basis).

Feed ingredient	Moisture	Crude Protein	Carbo-hydrate	Lipid	Crude Fiber	Ash
Oil cake	11.18	38.37	18.63	10.83	15.8	5.59
Rice bran	9.65	15.06	39.01	14.16	8.85	8.41

Increased use of plant proteins in aquafeed has generated interest in the use of exogenous enzyme additives in aquafeed [14]. Endogluconase and endoxylanases have been reported to play a significant role in the utilization of plant material in crustacean diets [15]. Enzyme treatment to the feed is reported to demonstrate significant improvement in the growth and feed utilization in tilapia [16].

The present study demonstrates that xylanase treatment to the feed seems to be responsible for the better growth performance of rohu fingerlings probably by improving the nutritional quality of feed and by the removal of NSPs from the plant based feed. This is evident from the significant increase in the reducing sugar, from 13.33 mg/gm to 44.16 mg/gm, and decrease in the total fiber from 11.32 % to 9.79 % in the enzyme treated feed by the action of xylanase (Table - 2).

Table - 2 Reducing sugars and crude fibers of raw and xylanase treated practical feed at 0 h and after 2 h incubation.

Parameters	Raw feed		Treated feed	
	0 h	2 h	0 h	2 h
Reducing sugar (mg/gm)*	13.50 ±0.28^a	15.33 ±0.65^a	13.33 ±0.69^a	44.16 ±0.69^b
Crude fiber (%)*	11.16 ±0.92^a	11.10 ±0.80^a	11.32 ±0.95^a	9.76 ±0.87^a

Data represent the mean ± S.E.M. of three replicates. Values on the same line with different superscripts are significantly different ($P<0.05$). *Values reported on wet weight bases

The maximum free reducing sugar was increased in 2h incubation in experimental feed. In untreated feed, increase in free reducing sugar was 13.5% during 2h incubation; whereas the increase was 231% in xylanase treated group. This indicated the effectiveness of presently used xylanase in degrading complex carbohydrates into simple sugars. In 2h incubation, fiber content of the xylanase treated feed was reduced by 14%, while no change was observed in untreated feed. This indicates that the crude fungal xylanase is found to be effective in improving the nutritional state and hydrolyzing some of the complex NSPs like xylan from the plant based feed.

Rohu fingerlings readily accepted the experimental diet and were observed to consume feed aggressively throughout the experimentation. No mortality has been observed. Growth, feed utilization efficiency and survival rate of rohu fed with experimental diet are given in Table 3. Rohu fed with enzyme treated diet exhibited better growth than the fishes fed control diet. Significant improvement in percent weight gain (99 %) and SGR (103 %) were observed in experimental fishes; whereas improvement in FCR was non-significant in same group. Improvement in the growth of the fishes seems to be because of the increase in the level of reducing sugar and probably decrease in the NSPs in the feed with xylanase treatment. These could probably be explained by improving the protein-sparing effect of the dietary carbohydrate during protein synthesis. The protein sparing action of carbohydrate has been demonstrated in many fish species [17, 18]. Halliwell *et al.* [19] reported increase in reducing sugar concentration in palm kernel meal treated with cellulolytic fungus *T. Koningii*. Incorporation of dates containing simple carbohydrate in tilapia feed as a replacement for starch was found to improve the growth of the fishes [20]. Application of exogenous enzyme to the plant based poultry and pig feed significantly improved the growth, feed utilization efficiency and nutrient availability [21]. Nile tilapia and red hybrid tilapia fed diet treated with feed enzymes showed significantly higher weight gain [16, 22]. It has already been reported that plant based feed used in aquaculture could be improved by overcoming antinutritional effects of NSPs associated with plant cell walls [23]. Buchnan *et al.* [7] reported that the addition of NSP degrading enzymes to canola meal diets could result into improved growth rate of juvenile tiger prawns. In *salmo salar*, addition of proteolytic enzymes and carbohydrases to the feed containing plant protein has been used to improve growth performance [24]. In the

present study, 100 % survival rate of fishes with xylanase addition rules out the toxic effect of crude fungal xylanase.

Table - 3 Growth, feed utilization efficiencies and survival rate in *Labeo rohita* fingerling.

Parameters	Control diet	Experimental diet
Initial weight (gm)	5.93±0.09 ^{ns}	5.37±0.04 ^{ns}
Final weight (gm)	6.76±0.07 ^{ns}	6.85±0.08 ^{ns}
Weight gain (%)	14.74±0.08 ^{a}	29.36±0.06 ^{b}
SGR (%/day)	0.32±0.01 ^{a}	0.65±0.06 ^{b}
FCR	1.98±0.06 ^{ns}	1.76±0.21 ^{ns}
SR (%)	100	100

Data represent the mean ± S.E.M. of three replicates. Values on the same line with different superscripts are significantly different ($P<0.05$). ns = Non significant. SGR: Specific Growth Rate. FCR: Feed Conversion Ratio. SR: Survival rate.

Proximate composition of body muscles and liver has indicated that xylanase addition to the feed improved protein content in muscles and liver. It has also improved the deposition of glycogen in muscle (Table 4). Improvement in the deposition of protein in muscle and liver could probably be explained by improving the protein-sparing effect of dietary carbohydrate. Increase in the glycogen content in muscle suggests the role of increased level of reducing sugar in the feed. However lipid content of muscle and liver has not been influenced by the improvement in reducing sugar level of experimental diet. Though, several investigators have reported the higher level of carbohydrate in the diet with increased deposition of lipid in the fishes [20, 25 & 26].

Table - 4 Muscle and Liver composition of *Labeo rohita* after 30 days.

	Protein (mg %)	Glycogen (mg %)	Lipid (mg %)
Muscle composition			
Control group	13.15±0.77	0.10±0.01	1.12±0.05
Experimental group	14.82±0.47	0.21±0.07	0.97±0.04
Liver composition			
Control group	5.60±0.25	4.89±0.15	8.09±0.85
Experimental group	6.03±0.57	4.15±0.14	7.25±0.57

Data represent the mean ± S.E.M. of three replicates.

The results of the present study indicate that rohu fingerlings fed with practical diet treated with crude fungal xylanase shows better growth performance as compared to fishes fed with untreated diet. It has also resulted in higher deposition of protein in muscles and liver. Pretreatment of practical feed of carp, ground nut oil cake and rice bran, with indigenously produced crude fungal xylanase using corn cob has potential to improve nutritional quality of practical feed as well as growth performance of rohu. Further investigations are required in this direction.

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