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Effect of Different Levels of A-Cellulose on Growth and Survival of Rohu (*Labeo Rohita*) Fingerlings

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ABSTRACT

Four isonitrogenous and isocaloric diets, with different α-cellulose inclusion levels, were formulated. Diet with 4% α-cellulose in it served as control. Fish ranging from 2.6-3.4 gm each were housed in glass aquaria @10 fish per aquarium. Aquaria were provided with 14L/10D fluorescent light. Trial was conducted for 60 days. No mortality was observed in any group. All diets performed equally well. However, the diet with 12% α-cellulose showed superiority over the rest of the treatments in growth and nutrient digestibility. There was no variation in digestibility of carbohydrates and dry matter contents. Lowest digestibility of fats and protein was observed in 16% α-cellulose containing diet the group which also displayed the lowest growth. The studies have suggested that *Labeo* fingerlings can tolerate complex carbohydrates up to 16% if included in the diet but there is a gradual reduction in performance if inclusion level of this carbohydrate exceeds 12% of the diet.

Keywords: *Labeo* rohita , α-cellulose, Fish growth, Digestibility of nutrients, FCR.

INTRODUCTION

Labeo rohita is the most important fish contributing 35% of the total stocking on the average and 23% of the total aquaculture production in the region (personal observation). It is present in freshwaters of Pakistan, Bangladesh and Burma and has been transplanted in many other countries (Agrawal, 1994). Fish farmers like to stock this fish as their main aquaculture species because it has high consumer preference and fetches high price when marketed. It comes next to *Catla* in growth amongst the Indian major carps. It feeds on phytoplankton, zooplankton, detritus, and vegetable debris. It matures in two years from spawning and breeds from June to July by hypophysation.

Semi-intensive poly fish culture is commonly practiced in Pakistan. Fish heavily depend on natural food produced in pond by frequent applications of organic and inorganic fertilizers. There is no established formula which can totally support the growth and health of this fish.

There are several reasons for non development of effective feed; cost of feed ingredients and their selective digestibility are the major ones. Fish prefer to have animal feed sources which are never cost effective and have drastic implications on the economics of fish farm. Sustainability of fish culture therefore rests with utilization of alternate cheaper protein sources in aqua feeds (Francis *et al*., 2001; Zhou *et al*., 2004) which is really a challenge for scientists.

Lot of efforts has been exhausted to search alternative protein sources and plant protein sources are always at the top. But they have their own limitations. High fiber contents and poor digestibility due to lack of well developed stomach in fish are the major ones (Robinson *et al*., 1981) which is basically cellulose and does not have any nutritional value for fish and need to be restricted to less than 7% of fish diets. McGoogan and Reigh (1996) reported higher digestibility of protein by red drum *Sciaenops ocellatus* for ingredients with less than 2% of fiber but when level exceeded this limit, protein digestibility reduced drastically.

The aim of this research was to assess the effects of increasing dietary doses of αcellulose on growth and feed digestibility juvenile *Labeo rohita*.

MATERIALS AND METHODS

Experimental Fish and its husbandry

The experiment was conducted at the Fisheries and Aquaculture Research Centre (University of Veterinary and Animal Sciences Ravi Campus Pattoki). Fingerlings of *Labeo rohita* ranging from 2.6-3.4 gm each were procured local Fish Seed Production facility and were acclimatized in concrete tanks. A control diet (30% protein) was fed for two weeks prior to the experiment to acclimate the fish to their environment. The same diet was later on divided into four groups to prepare different dietary treatments. Ambient water temperature was maintained by uninterrupted supply of fresh tube well water and the photoperiod of 14 h light: 10 h dark was set by providing artificial lights hanging on aquaria. . Environmental and water quality indicators were closely monitored over the course of the experiment. No major health issues were encountered over the course of this experiment. There were no disease outbreaks, no problems with feed acceptance and there was no mortality.

Experimental design

Trial was designed as Completely Randomized Design (CRD) with three treatments and a control with reportedly the most acceptable fiber level for fish. There were three treatments and a control. Every treatment including control had three replicates. Studies were executed in glass aquaria with water holding capacity of 70 liter each. Aquaria were installed to maintain a flow-through-system with water exchange rate of 5 L min^{-1} $aquarium^{-1}$. Five fish were randomly taken from the e main stock, weighed and measured for baseline data and for comparison of future growth increments in fish. Then three aquaria were randomly allotted to each treatment to avoid possibility of systematic error. After preliminary preparations 10 fish were randomly collected from the main stock and carefully transferred to each properly labeled aquarium.

Experimental diets

Four isonitrogenous and isocaloric diets with different levels of α-cellulose, were formulated. All ingredients were individually finely ground and then mixed in an appropriate ratio to achieve desired protein level (30% protein). One percent Cr_2O_3 was added as marker in dry feed and mixed thoroughly to determine digestibility of different macronutrients. Homogenously mixed feed ingredients were moistened with boiled water (1:0.6 W/V) and pelleted in a laboratory type pellet machine and then dried in oven at 60 ^{0}C to constant weight and then stored in zip lock bags in refrigerator at 4 ^{0}C. Feed mixture was dry pelletized using molasses as binder. Pellets were oven dried and crumbled to size proportionate to the mouth size of fish. Prepared diet was divided into 4 groups. Diet with 4% α-cellulose in it served as control. Treatment 1 contained 8, treatment 2, 12 and treatment 3 contained 16% α-cellulose (Table 1).

Table 1- Initial and final weight of *Labeo rohita* fingerlings fed on formulated feeds for 57 days

Treatment	Initial Weight	Final Weight
4% Cellulose diet	2.579±2.128E-01[a]	5.0±3.8[a]
8% Cellulose diet	2.779±1.768E-01[a]	3.8±2.0[a]
12% Cellulose diet	2.805±1.860E-01[a]	6.1±4.3[a]
16% Cellulose diet	3.373±3.288E-01[a]	5.2±3.7[a]

Data are presented as X±SE

Note: Values in the columns followed by the same superscript letters are not significantly different from each other at $p>0.05$.

Experimental protocol and set-up

Fish were fed @ 5% of its body weight daily at 9.00 A.M. and 2.30 P.M. regularly. Fluorescent lights hanged above the aquaria provided 14L/10D photoperiod for maximum feeding. Water quality was checked every day at noon and at night. Normally measured parameters were; temperature, pH, oxygen content and NH_3 which remained within appropriate ranges for fish growth during the experimental period. Feces were collected 4 hours after feeding by sucking with plastic pipe, blotted and then dried in oven at 80 ^{0}C up to constant weight then stored in refrigerator for proximate analysis. On the termination of the feeding trial, water was totally drained off; all the fish were harvested, weighed and measured for growth data analysis.

Chemical analysis of feed and fecal matter

Individual feed ingredients and freeze-dried feces, collected from triplicate groups of corresponding treatment were ground in a coffee grinder and thoroughly homogenized to collect representative sub-samples. All chemical analyses were done in triplicate and made on dry weight basis. Feed and feces were analyzed for dry matter by drying samples to a constant weight (ISO 1983; ISO 1998). Crude protein (N × 6.25) was determined by Kjeldahl method after acid digestion (ISO, 1979). Total lipids were extracted by petroleum ether in a Soxhlet fat extraction apparatus (ISO/DIS 1996).

Determination of apparent digestibility coefficients (ADC %)

ADC (%) of different nutrients was performed by indirect method using Cr_2O_3 as inert marker (De Silva *et al*., 1997).

$ADC_{DM} = 100[1-(Cr_2O_3 \text{ in diet}/Cr_2O_3 \text{ in feces})]$

$ADC_{N} = 100[1-(Cr_2O_3 \text{ in diet}/Cr_2O_3 \text{ in feces})(\text{nutrient in feces}/\text{nutrient in diet}]$

Statistical Analysis

Each aquarium served as an experimental unit. Data collected analyzed as a completely randomized design using the General Linear Model procedure of IBM SPSS Statistics (Version 19.0.0, SPSS Inc., Chicago, IL, USA). Tukey's Multiple Range Test was applied to determine the significance level among treatments. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Water quality parameters

Water quality parameters remained within appropriate ranges during the course of experiment. DO levels remained at 4 ppm or higher.

Growth

No mortality was observed in any group. All diets performed equally well. However, the diet with 12% α-cellulose showed superiority over the rest of the treatments in growth (Table 1 and 2). Lowest growth was observed on 16% α-cellulose containing diet though initial fish size was quite bigger in treatment 4 than control group and other two treatments. As other growth parameters like, Feed Conversion Ratio (FCR), specific growth rate are tagged with gain in weight hence they followed the same trend as it was observed in weight increments.

Table 2- Absolute growth rate (increase in weight per day in gm), relative growth rate (%increase in weight over the whole time period), specific growth rate (%increase in weight per day) and feed conversion ratio of fish fed on formulated feeds with different α-cellulose levels

Treatment	AGR	RGR	SGR	FCR
4% cellulose diet	$0.025{\pm}0.001^{a}$	$55{\pm}10.5^{a}$	$0.91{\pm}0.2^{a}$	$1.97{\pm}0.3$
8% cellulose diet	$0.02{\pm}0.001^{a}$	$49.4{\pm}9.8^{a}$	$0.76{\pm}0.2^{a}$	$2.45{\pm}0.7^{a}$
12% cellulose diet	$0.03{\pm}0.002^{b}$	$67.6{\pm}7.2^{b}$	$0.77{\pm}0.3^{a}$	$1.8{\pm}0.3^{b}$
16% cellulose diet	$0.01{\pm}0.001^{c}$	$20.2{\pm}4.2^{c}$	$0.33{\pm}0.1^{b}$	$4.67{\pm}1.1^{c}$

AGR= Absolute Growth Rate, RGR= Relative Growth Rate, SGR= Specific Growth Rate and FCR= Feed Conversion Ratio

Data figures with different superscript letters are different from each other at $p<0.01$

The lowest values were observed in treatment 2 indicating acceptable cellulose requirement of this stage of this fish. Dioundick and Stom (1990) and Shia *et al*., (1988) and Liang (1994) observed growth depression and reduction in feed intake in Tilapia when fed on diet containing 10% cellulose. Similarly inclusion of alginate and guar gum (2.5-10%) in rainbow trout diets lowered protein and lipid digestibility (Storebakken, 1985). Situation was little different in current studies. When *Labeo rohita* was fed on diets containing varying percentages of α-cellulose, fish growth increased up to 12% cellulose inclusion level but declined when cellulose level exceeded 12%. Morita *et al*., (1982) have observed improvements in growth and feed efficiency in red sea bream fed on diet supplemented with 3, 6, 9 or 12 % carboxy methylcellulose level. Previous studies substantiate ours and further verify that Tilapia, red sea bream and *Labeo rohita* have probably similar response and tolerance capability to carboxy methylcellulose.

Digestibility

Dry matter digestibility was uniform among treatments. Protein digestibility declined significantly when we moved from 4% to 16% diet. Protein digestibility was significantly lower in treatment 3 and 4 than treatment 2. When later two diets were compared with each other diet 4 again showed significantly poor digestibilities than diet 3. Lipid digestibility remained same from diet 1 to 3 but it drastically fell down when we moved towards higher cellulose levels. Carbohydrates however did not show any difference. Though there was minor variation in digestibility values of carbohydrates between treatment 3 and 4 but statistically differences were not discernable (Table 3).

Table 3- Apparent digestibility coefficients (%) of the experimental diets

Treatment	Dry Matter	Crude Protein	Crude Fat	Carbohydrates
4% cellulose diet	79.8±7.5[a]	82.5±4.2[a]	90.3±6.1[a]	62.3±4.1[a]
8% cellulose diet	78.8±8.1[a]	80.3±5.1[a]	85.4±7.3[a]	60.4±3.9[a]
12% cellulose diet	78.7±6.2[a]	72.2±3.9[b]	78.4±3.5[a]	60.3±4.5[a]
16% cellulose diet	77.9±5.6[a]	63.5±4.1[c]	67.3±5.2[b]	58.5±4.7[b]

In the current studies fat digestibility was same in control (4% cellulose) and treatment 1(8% cellulose) and 2(12% cellulose) but decreased in 16% cellulose containing diet. Protein digestibility followed the same trend but digestibility started to decline even in 12% cellulose containing diet. Contradictory to our findings Dias *et al*., (1998) showed that addition of two levels of cellulose(10 and 20%) did not affect digestibility of protein, growth and feed utilization in sea bass but higher levels did impart negative effects in current studies on *Labeo rohita* fingerlings. Lekva *et al*., (2010) while working on Atlantic cod*(Gadus morhua* L.) observed decreased digestibility of fat and dry matter when α-cellulose level was increased in diet having no effect on liver index, protein digestibility and protein retention. On the other hand Velortas *et al*., (2011) while working on penaeoid shrimp observed decreased digestibility coefficients from 83.7% to 51.2% (*A. longinaris*) and from 71.9% to 7.6% (*P. muelleris*) as the dietary starch levels increased. It seems that the physiological effects of cellulose in fish are not fully understood and quite inconsistent from specie to species.

Dietary plant ingredients can affect gastrointestinal transit time of feed as a result of presence of fibers and sugars and alter the digestibility of nutrients ingested by fish (Eusebio *et al*., 2004); Zhou *et al*., 2004). According to Eusebio *et al*., (2004) as dietary fiber is part of the carbohydrate component of plant ingredients, most fish can not utilize it. However, low dietary concentrations of fiber (3-5%) may have beneficial effects on fish growth. High dietary fiber (>8%) however may decrease dry matter digestibility of the diet and reduce availability of other nutrients (Altan and Korkut, 2011). Similarly Pavasovic *et al*., (2006) investigated that diet with either 30% α-cellulose or fullers earth significantly reduced apparent dry matter and protein digestibility's in red claw crayfish, *Cherax quadricarinatus*. The same workers in another study on the same animal explored that dietary levels above 12% α-cellulose were correlated with significant reductions in survival rate, specific growth rate and feeding efficiency. These studies are quite in line with ours and further confirm that higher levels of cellulose in fish or crustacean diets have deleterious effects on growth and nutrient digestibility's.

CONCLUSIONS

It can therefore be concluded that *Labeo rohita* fingerlings can tolerate complex carbohydrates up to 16% if included in the diet but there is a gradual reduction in growth performance and nutrient digestibility if level exceeds 12% of the diet.

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