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Effect of Different Plant and Animal Origin (Fishmeal) Feeds on Digestive Enzyme Activity and Haematology of Juvenile *Labeo rohita*

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ABSTRACT

This study was aimed at determining the impact of sources of ingredients on the intestinal enzyme activity and haematology of juvenile *Labeo rohita*. Five isocaloric treatment diets *viz.*, T_1 (guar meal and canola meal), T_2 (soybean meal and cottonseed meal), T_3 (guar meal and cottonseed meal), T_4 (soybean meal and canola meal), T_5 (fishmeal and canola meal) control T_0 (control which received rice polish only) were prepared and fed to *Labeo rohita* juveniles. At the end of the experiment, amylase and protease concentrations in the whole intestine were found highest in the T_3 diet ($p \leq 0.05$). Leukocyte and erythrocyte counts were higher in T_0 low-protein diet ($p \leq 0.05$). The concentrations of albumin and protein were found highest in the T_4 diet ($p \leq 0.05$). It can be concluded that the diet T_3 containing guar and cottonseed meals remained effective for the activity of digestive enzymes. However, the non-protein diets are more effective in improving haematological traits of juvenile *Labeo rohita*.

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Authors' Contributions

KJI and MA conceived and planned the study. KJI, FA, MHR and MKR analyzed enzyme activities. KJI, FR and HA did hematological studies. KJI, AJ and NK wrote the article. MA and I statistically analyzed the data.

Key words

Digestive enzymes; *Labeo rohita*, plant and animal protein sources, haematology.

INTRODUCTION

Aquaculture is anticipated to be a fastest emerging field to fulfill protein requirement of humans in some parts of the world especially Pakistan. Due to excellent nutrient profile and high palatability, fishmeal is a major source of animal protein generally used for all types of fishes and specifically for carnivorous fishes (El-Saidy and Gaber, 2003; Siddhuraju and Becker, 2003; Wu *et al.*, 2004). However, increasing fishmeal cost and competition with other livestock feed industries are the main factors that hinder the development of fish industry (New and Csavas, 1995; Xie *et al.*, 2001; FAO, 2002). The issue has been addressed and successful substitutes of fishmeal has been explored (partially if not completely) with more success rate in herbivorous fishes (Shiau *et al.*, 1987; El-Saidy and Gaber, 1997; Wilson *et al.*, 2004). Moreover, the aquaculture nutritionists are in a continuous search to reduce the use of fishmeal and look for more cost effective substitutes (Craig, 2004).

The process of digestion determines the accessibility of nutrients needed for all body functions and enzymatic activity and is the basic tool to observe feeding acceptability and its contribution towards the growth and maintenance of fish body (Gisbert *et al.*, 2009). Metabolic adaptations to the changing feed ingredients and in turn enzymatic secretions result in better feed utilization (Caruso *et al.*, 2009). Digestive enzyme activities vary in different fish species which may be due to differences in digestive potential and feeding habits. The study of enzyme functioning is helpful in understanding the mechanism of digestion in fish and changes in ambient environment (Sunde *et al.*, 2004; Chakrabarti and Sharma, 2005). Proteolytic and amylase enzyme activities can disclose the ability of different fish species to use protein and carbohydrates (Hidalgo *et al.*, 1999). Therefore, the examination of digestive physiology is of major concern to evaluate the whole digestive process efficiency mostly depends upon the type and function of the digestive enzymes. The comparative studies of these enzymes and their activities in different parts of the digestive system and in different fish species are well documented (Jonas *et al.*, 1983;

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Munilla-Morán and Saborido-Rey, 1996a,b; Sethuramalingam and Haniffa, 2002; Xavier *et al.*, 2012).

Haematological parameters are influenced by variations in quality of diets thus are key health indicators (De Pedro *et al.*, 2005; Ferguson *et al.*, 2010). The variations in haematological parameters are associated with changing feed ingredients which probably be due to the presence of a wide variety of nutritional and anti-nutritional factors (Osuigwe *et al.*, 2007). These parameters are also closely related to the environmental and biological factors (Steinhagen *et al.*, 1990; Fernandes and Mazon, 2003). The present study was, therefore, planned to envisage the effect of plant and animal origin feed by-products on enzymatic activities and hematological parameters of juvenile *Labeo rohita*.

MATERIALS AND METHODS

Experimental design

Fish, *Labeo rohita* was used as an experimental animal. The experiment was conducted in twelve earthen ponds, each having 0.03 ha area and depth of two meters. Each pond was filled with water up to 1.5 meters and stocking density of each pond was 100 fish with an average weight of 200±4.43g. Five isocaloric diets were prepared by combining guar meal and canola meal, soybean meal and cottonseed meal, guar meal and cottonseed meal, soybean meal and canola meal, and fishmeal and canola meal and were designated as T_1, T_2, T_3, T_4, and T_5 experimental diets, respectively, while control pond (T_0) received rice polish only (Table I). There were two replicates for each of the control and treatment diets. The fish were fed @ 4% of their wet biomass twice daily. The proximate analysis of experimental diets (Table I) was completed through Near Infra-Red Technology (Martinez *et al.*, 2003; Iqbal *et al.*, 2014; Iqbal *et al.*, 2015). The physico-chemical parameters *viz.*, temperature, pH, salinity, dissolved oxygen and total dissolved solids were recorded throughout the experiment on daily basis.

Estimation of digestive enzymes

At the end of the research trial, six fish samples from each of the treatment and control ponds were collected at random. Three fish samples were degutted to remove the whole intestine while three to remove anterior and posterior parts of the intestine. Whole intestine, anterior part and posterior part of the intestine were homogenized in chilled Tris-HCl separately. The homogenates were centrifuged at 6000×g at 4°C for 15 min and the supernatant was collected and stored at -4°C for further analysis (Ismat *et al.*, 2013).

Amylase

Three test tubes for amylase activity were prepared; (i) sample test tube containing 1% starch solution, phosphate buffer and sample at a ratio of 1:1:1, (ii) blank test tube having 1 ml starch solution only (incubated both the test tubes at 37°C for 15 min) and (iii) standard test tube containing 1ml of 0.1% glucose solution. 1 ml reagent dinitrosalicylic acid was added in the blank and standard test tubes and kept them in boiling water bath for 1 min and then cooled at room temperature. 2 ml distilled water was added in standard and blank test tubes. Absorption of blank, standard and samples was taken on the spectrophotometer at 540 nm (Ismat *et al.*, 2013). A unit (U) of activity was defined as the 1 µmol of substrate hydrolyzed per min per ml of enzyme extract.

Protease

Protease enzyme activity was assessed by using a substrate solution containing 1% azocasein in 50mM Tris–HCl has pH 7.5 (Garcia-Carreno, 1992; Ismat *et al.*, 2013). Ten µL of enzyme extract was mixed with 0.5 ml of buffer (pH 7.5), then added 0.5 ml of substrate solution and incubated the sample at 25°C for 10 min. The reaction was stopped by adding 0.5 ml trichloro-acetic acid (20%) and centrifuged at 14,000 × g for 5 min. The absorbance of the supernatant was recorded at 366 nm. A standard curve was prepared by using different concentrations of azocasein (0, 2, 4, 5, 6, 8, 10 mg). A unit (U) of activity was defined as the 1 µmol of substrate hydrolyzed per min per ml of enzyme extract.

Haematological analysis

Blood samples were taken from six fish samples from each treatment and control ponds; three of these were reserved for haematological analysis and the blood was collected in vials having EDTA as an anticoagulant, while the other three for biochemical estimation of blood and the blood was stored in vials without EDTA. Red blood cells (RBC, 10^{-6}/µL) and white blood cells (WBC, 10^{-3}/µL) were counted using Neubauer Haemocytometer. RBC reagent (2 ml reagent/10µL blood) and WBC reagent (950µL reagents/50µL of blood) were used for counting of the respective cells in blood samples. Haematocrit (HCT) was determined using micro HCT capillaries filled with blood and centrifuged at 8,700×g for 5 min and expressed as percentage of total blood volume (Wintrobe, 1974). Erythrocyte sedimentation rate (ESR) was determined in mm/h by using ESR tubes containing 50 mm sodium citrate in 200 mm of blood. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined following Penev

and Dukova-Peneva (2007).

Estimation of biochemical components of blood

Clinical Chemistry Analyzer (Metro Lab, 1600-DR) was used for the biochemical analysis *viz*., blood hemoglobin (Hb, g/dl), total protein (PROT, g/dl), and albumin (ALB, g/dl) of blood. Globulin (GLOB, g/dl) was computed by subtracting the values of ALB from total PROT.

Statistical analysis

The data were subjected to ANOVA Technique using SPSS (Version 16.0) and level of significance was based on $p \leq 0.05$. Duncan's Multiple Range Test (DMRT) was applied to compare means for detection of the level of variation among treatments.

RESULTS

A non-significant difference in amylase activity was noticed in the anterior and posterior parts of the intestine ($p>0.05$) whereas a slightly higher amylase activity was observed in the whole intestine among the treatment groups ($p<0.05$; Table II). Highest amylase activity (1.76 U/ml.min^{-1}) was observed in the diet containing guar and cottonseed meal (T_3) whereas it was lowest (0.50 U/ml.min^{-1}) in the diet containing fish and canola meal (T_5) in the whole intestine.

The protease activity was found significantly different throughout the intestinal tract in different treatment groups ($p<0.05$; Table II). The diet containing guar and canola meal (T_1) showed least protease activity (3.18 U/ml.min^{-1}) whereas diet containing guar and cottonseed meal (T_3) showed highest protease activity (4.13 U/ml.min^{-1}) in the whole intestinal tract. Moreover, the diets containing different ingredients also showed different protease activity in the anterior and posterior part of the intestine. The diet containing guar and cottonseed meal (T_3) showed highest protease activity (4.01 U/ml.min^{-1}) whereas diet containing soybean and canola meal (T_4) showed lowest protease activity (1.13 U/ml.min^{-1}) in the anterior part of the intestine. The diet containing soybean and canola meal (T_4) showed highest protease activity (3.99 U/ml.min^{-1}) whereas diet containing fish and canola meal (T_5) showed lowest protease activity (1.19 U/ml.min^{-1}) in the posterior part of the intestine (Table II).

A significantly higher WBCs and RBCs were observed in the fish having a control diet (T_0; $p<0.05$; Table III). There was no difference in Hb and ESR concentration among all the dietary groups. Higher concentration of ALB was detected in the diet containing soybean and canola meal (T_4) whereas lowest in the diet containing guar and cottonseed meal (T3; $p<0.05$). A significantly higher GLOB concentration was observed in blood samples of fish having diet containing fish and canola meal (T_5), whereas non-significant differences for PROT were determined for T_1, T_4, T_5 and T_0 dietary groups (Table III).

DISCUSSION

Feed and its constituents are the major factors which influence the activities of digestive enzymes in fish (Fernandez *et al.,* 2001). During present study, a higher amylase activity in the whole intestine of fishes was recorded in the diet containing guar meal and cottonseed meal. Lazzari *et al*. (2010) observed variations in amylase activity in different parts of intestine fed with different diets. De Almeida *et al.* (2006) observed higher amylase activity in the posterior part of the intestine in tambaqui (*Colossoma macropomum*) while Jancarik (1974) and Fisher (1973) observed higher amylase secretions in anterior part of the intestine when *Cyprinus carpio* and *Ctenopharyngodon idella* were fed with animal origin feed stuffs. It has been suggested that the increasing dietary PROT in feed also enhances amylase activity in rainbow trout (*Oncorhynchus mykiss*; Kawai and Ikeda, 1973; Plantikow 1981) while Das and Tripathi (1991) reported opposite findings in *C. idella*. Debnath *et al*. (2007) observed non-significant variations in amylase activity in *Labeo rohita* fed with diets containing varying levels of crude PROT. Melo *et al*. (2012) stated that amylase activities were opposite to the dietary concentration of PROT and maximum values were observed in anterior portion of intestine in juvenile silver catfish.

During the present study, the difference in protease activity in different parts of the intestine is in line with the results of Lazzari *et al*. (2010) who observed variations in protease activity in jundiá (*Rhamdia quelen*) due to differences in the diets. Recently, Rodiles *et al*. (2012) also observed the influence of different diets on the proteolytic activities in juvenile Senegalese sole, *Solea senegalensis*. It has been reported in several studies that the higher protease activity in the intestine is associated with the higher PROT contents in the diet (El-Saidy *et al.,* 2000; Eusebio and Coloso 2002; Mohanta *et al.,* 2008; Melo *et al.,* 2012; Xiong *et al.,* 2011). According to Lopez-Lopez *et al*. (2005) no relation exists between the dietary proteins and the activity of protease enzymes.

The lower concentrations of WBC and RBC in high PROT diets are in agreement with the findings of Yue and Zhou (2008) observed decrease in WBC and RBC concentrations in juvenile hybrid tilapia by

Table I.- Analyzed nutrient composition of feed combinations (% or otherwise stated).

Analysis	T_1	T_2	T_3	T_4	T_5	T_0
Protein	36.76	38.44	37.64	37.58	40.35	6.07
Moisture	7.08	9.68	7.14	9.62	7.33	4.92
Fat	1.77	1.42	1.6	1.35	4.87	3.15
Ash	8.22	12.48	12.36	8.35	15.60	6.30
ME (kcal/kg)	4090	4072	4082	4069	4248	4161

T_0, Rice polish; T_1, guar meal and canola meal; T_2, soybean meal and cotton seed meal; T_3, guar meal and cotton seed meal; T_4, soybean meal and canola meal; T_5, fishmeal and canola meal.

Table II.- Effect of different plant and animal origin feeds on enzymes activity in an intestine of juvenile *Labeo rohita.*

Treatment	Amylase (U/ml.min^{-1})			Protease (U/ml.min^{-1})		
	Anterior	Posterior	Complete	Anterior	Posterior	Complete
T_1	1.09 ± 0.06^{ab}	0.65 ± 0.24^{b}	1.22 ± 0.05^{ab}	2.23 ± 0.01^{d}	1.44 ± 0.13^{e}	3.18 ± 0.23^{c}
T_2	0.78 ± 0.23^{b}	0.93 ± 0.55^{b}	1.26 ± 0.35^{ab}	2.29 ± 0.08^{d}	2.19 ± 0.07^{d}	3.80 ± 0.18^{ab}
T_3	0.42 ± 0.02^{b}	0.79 ± 0.49^{b}	1.76 ± 0.03^{a}	4.01 ± 0.16^{a}	3.07 ± 0.11^{c}	4.13 ± 0.17^{a}
T_4	0.91 ± 0.34^{b}	0.8 ± 0.22^{b}	1.05 ± 0.67^{ab}	1.13 ± 0.11^{e}	3.99 ± 0.13^{a}	3.70 ± 0.14^{ab}
T_5	0.50 ± 0.64^{b}	0.84 ± 0.07^{b}	0.50 ± 0.64^{b}	2.28 ± 0.08^{d}	1.19 ± 0.05^{e}	3.43 ± 0.46^{bc}
T_0	0.76 ± 0.04^{b}	0.62 ± 0.09^{b}	0.93 ± 0.35^{b}	3.19 ± 0.12^{c}	3.93 ± 0.24^{ab}	3.76 ± 0.61^{ab}

A column containing different superscripts are significantly different ($p<0.05$)
For other abbreviations see Table I.

Table III.- Effect of different plant and animal origin feeds on haematology of juvenile *Labeo rohita.*

Parameters	T_1	T_2	T_3	T_4	T_5	T_0
WBC ($10^3/\mu L$)	13.73 ± 0.18^{b}	13.70 ± 0.14^{b}	14.05 ± 0.07^{b}	13.80 ± 0.85^{b}	13.40 ± 0.28^{b}	16.30 ± 0.14^{a}
RBC ($10^6/\mu L$)	1.10 ± 0.07^{d}	1.45 ± 0.07^{c}	1.57 ± 0.03^{bc}	1.70 ± 0.02^{b}	1.48 ± 0.35^{c}	1.84 ± 0.78^{a}
Hb (g/dL)	6.40 ± 0.14^{a}	6.70 ± 0.14^{a}	6.60 ± 0.42^{a}	6.65 ± 0.07^{a}	6.85 ± 0.07^{a}	6.50 ± 0.14^{a}
ESR (mm/hr)	ND	ND	0.50 ± 0.03^{a}	ND	ND	0.50 ± 0.03^{a}
ALB (g/dl)	6.40 ± 0.14^{abc}	5.65 ± 0.07^{cd}	5.40 ± 0.57^{d}	6.65 ± 0.21^{a}	5.80 ± 0.14^{bcd}	6.55 ± 0.35^{ab}
GLOB (g/dl)	4.45 ± 0.07^{b}	4.00 ± 0.28^{b}	4.55 ± 0.35^{b}	4.35 ± 0.07^{b}	5.19 ± 0.13^{a}	4.30 ± 0.28^{b}
PROT (g/dl)	10.85 ± 0.07^{a}	9.65 ± 0.21^{b}	9.95 ± 0.21^{b}	11.00 ± 0.28^{a}	10.99 ± 0.01^{a}	10.85 ± 0.07^{a}

Similar superscript in a row showed (≤0.05) non-significant difference
ND, not detected; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; ESR, erythrocyte sedimentation rate; PROT, protein; ALB, albumin; GLO, globulin.
For other abbreviations see Table I.

replacing protein sources. Contrarily, Nasir and Al-Sraji (2013) observed higher RBC values on higher PROT diets as compared to low PROT diets. Probably these haematological variations are associated with a wide variety of nutritional and anti-nutritional factors of the ingredients (Osuigwe *et al.,* 2007). Khara *et al.* (2013) observed decrease in WBC and RBC counts with increase in estradiol-17 hormones in fish diets. Ozovehe (2013) observed RBC and Hb decrease by increasing level of *Moringa oleifera* leaf meal in diet due to presence of toxic substances. Moreover, higher number of RBC and WBC are indicators of good health.

ALB is synthesized by the liver using dietary proteins and its presence in the plasma creates an osmotic force that maintains fluid volume with the vascular space. The higher concentration of ALB from soybean and canola meal diets might indicate higher availability of PROT from these sources. The immune status of fish is linked with the presence of GLOB which is also the indicator of some kind of immunostimlant in the diets as per Choudhury *et al*. (2005). Significantly higher concentrations of GLOB was observed in blood samples

of fish from T_5 ponds while non-significant differences for PROT were determined for T_1, T_4, T_5 and T_0 fish samples. Metwally (2009) found significant increase in blood PROT when fish were fed with garlic in diet. Anyanwu *et al.* (2011) found significant variations in various blood parameters when fish were fed with varied dietary levels of plant feed. Similarly Ighwela *et al.* (2012) observed variations in the haematological parameters of Nile tilapia (*O. niloticus*) fed with higher levels of maltose. Nasir and Al-Sraji (2013) observed significant increase ($p<0.05$) in blood serum protein by increasing protein in feed. Yousefian *et al*. (2013) observed significant differences in total PROT when larvae were fed with artificial feed. However, Hosseini and Khajepour (2013) found non-significant differences in blood total PROT by increasing dietary soybean meal. Similarly, El-Kasheif *et al.* (2011) observed non-significant differences in haematological parameters of *O. niloticus* fed with artificial feed with limited inclusion of fish oil. Metwally and Elgella (2009) observed non-significant differences in total plasma PROT in Nile tilapia when fed on different plant waste materials.

CONCLUSION

It can be concluded that the response of body organs varies with varying feedstuffs and the feed items have pronounced effect on enzymatic activities, hematological and histological parameters in juvenile *Labeo rohita*. The diet containing guar and cottonseed meals remained effective for the activity of digestive enzymes. However, the non-protein diets are more effective in improving haematological traits of juvenile *Labeo rohita*. In future formulations these factors would be of paramount importance in the selection of ingredients for biologically efficient and cost effective diets.

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Statement of conflict of interest

The authors have no conflict of interest to declare.

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