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A comparative study of beta glucan and plant stimulants on the growth, histology and immune response of *Labeo rohita*

Tayyab R.[1]; Khan N.[1]*; Ashraf M.[1]; Khalique A.[2]; Rasool F.[1]; Azmat H.[1]; Abbas S.[1]; Mahmood Anjum K.[3]; Hameed Mughal D.[4]; Javed Iqbal K.[5]; Dogar S.[1]; Kim H.[6]; De Zoysa M.[7]

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Abstract

The experiment was conducted to evaluate the effects of beta glucan, the plant extract, Vitabio A, and the plant extract with probiotic *Lactobacillus* spp. (Vitabio B) on the growth, histology and immune response of *Labeo rohita* (Rohu), reared for 120 days in a flow through system. Fish with an average body weight of 264±2.65g were stocked in cemented tanks at 10 fish/replicate. Four iso-nitrogenous artificial feeds containing 0.1% of beta glucan, plant extract, plant extract+probiotic and control (without any immunostimulants) were given to fish twice a day at 3% body weight of fish. A significant (p<0.05) difference in weight gain was observed between the control and plant extract fed group while it was non-significant (p>0.05) for the other treatments. The total serum proteins and cell counts (CBC) showed non-significant (p>0.05) difference among all the treatments except for RBC and Hb which were significantly higher in T1 (control) and T2 (plant extract + bacteria *Lactobacillus* spp.) compared to T3 (beta glucan) and T4 (plant extract). The analysis of fish body composition revealed that plant stimulant treatment had significantly higher crude protein and ash contents compared with the other treatment groups and lower fat content compared with the control (T1). In conclusion, the plant extracts and beta glucan can be used without any adverse effects on growth, total serum proteins, hematology and fish body composition.

Keywords: *Labeo rohita*, Immunostimulants, Growth, Hematology, Body composition.

1-Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore
2-Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore
3-Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore
4-Directorate of Quality Enhancement Cell, University of Veterinary and Animal Sciences, Lahore, Pakistan
5-Department of Zoology, The Islamia University of Bahawalpur, Pakistan
6-Vitabio Corporation, Daejeon 305-764, Republic of Korea
7-College of Veterinary Medicine, Chungnam National University, Daejeon305-764, Republic of Korea.
*Corresponding author's Email: noorkhan@uvas.edu.pk

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Introduction

The share of the aquaculture industry to fish production has been increasing since last few years. This increase in fish production can further be enhanced to overcome the problems related to intensification. With the emergence of the aquaculture industry, the stress on the environment is increasing, resulting in increased stress on the aquatic animals. To maintain higher growth, survival rate and to maximize profits the most crucial thing is to ensure a disease free environment for fish (Gatlin III, 2002). Therefore, appropriate methods are needed to control the disease outbreaks and maintain a healthy environment. Recently vaccination and immunostimulants are considered effective methods for controlling disease problems both in finfish and shellfish culture systems. Immunostimulants are regarded as a suitable tool to increase the immunity of both humoral and cellular response of cultured organisms (Dashtiannasab *et al*., 2012). Immunostimulants of major interest are divided into six types and comprise a group of biological and synthetic compounds. The application of immunostimulants is particularly important for fish which are raised in environments where the nature of the pathogen is not known. Beta glucan and plant extracts fall among these types. Beta glucan is one of the remarkable immunostimulant used in aquaculture (Meena *et al*., 2013). Use of herbal extracts in animal feeds is also getting interest nowadays because they are considered to be cost effective supplements, environmentally safe and biodegradable. Natural plant extracts have been used to promote growth, prevent stress, increase appetite, act as immunostimulants and have antimicrobial effects in fin fish and shrimp larviculture (Sivaram *et al*., 2004). Realizing the importance of plant stimulants and beta glucan, the present study was designed to investigate the efficacy of beta glucan and plant stimulants on growth, histology and immune response of *L. rohita* in a flow through system.

Materials and methods

Experimental protocol

The experimental fish *L. rohita* was collected from fish ponds at the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki and conformed in tanks and then treated with $KMnO_4$ in quarantine for 15 days. Before stocking, morphometric characteristics of fish viz. wet body weight and length were measured and recorded. The experiment was carried out in 4 cemented rectangular tanks with dimensions 2.9× 0.76×0.91m (Length×Width×Depth), partitioned by mesh fitted in a wooden frame with 2 feet water level in each tank. Electric aerator pumps were installed to keep the DO at a desirable level, while other parameters were maintained with the continuous supply of fresh tube well water. Ten (10) fish (*L. rohita*) with an average weight of 264±2.65g were stocked in each partition of the tank. There were 2 replicates of each treatment. The fish

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was fed twice a day at feeding rate of 3 % of the fish wet body weight.

Feed ingredients and formulation

Four types of artificial feeds having 30% crude protein (CP) were prepared. Treatment1 (T_1) was control feed without the addition of any stimulant, Treatment 2 (T_2) containing plant extract+bacteria *Lactobacillus* spp. Treatment 3 (T_3) beta glucan (Food Chem, China) and Treatment 4 (T_4) containing plant extract (Vitabio A). Additives were added to the basal diet at the rate of 0.1% (Table 1).

Table 1: Feed formulation and ingredient composition of experimental diets.

Ingredients and CP% composition	T1		T2		T3		T4	
	Inclusion%	CP%	Inclusion %	CP%	Inclusion %	CP%	Inclusion %	CP%
FM (62.50%)	16	10	16	10	16	10	16	10
SBM (46.60%)	20	9.32	20	9.32	20	9.32	20	9.32
RP (12%)	24	2.88	24	2.88	24	2.88	24	2.88
WB (14%)	24	3.36	24	3.36	24	3.36	24	3.36
MG (28.50%)	10	2.85	10	2.85	10	2.85	10	2.85
CSM (40.95%)	4.0	1.64	4.0	1.64	4.0	1.64	4.0	1.64
M. premix	1	0.00	0.9	0.00	0.9	0.00	0.9	0.00
Sunflower oil	1	0.00	1	0.00	1	0.00	1	0.00
Plant extract + bacteria (Vitabio B)	-	-	0.1	0.00	-	-	-	-
Beta glucan	-	-	-	-	0.1	0.00	-	-
Plant extract (Vitabio A)	-	-	-	-	-	-	0.1	0.00
Total	100	30.05	100	30.05	100	30.05	100	30.05

T1= Control; T2= Plant extract + bacteria (Mixture of Korean herbs(VitabioB) +bacteria *Lactobacillus* spp.); T3= Beta glucan; T4= Plant extract (Mixture of Korean herbs(Vitabio A)); FM= Fish meal; SBM= Soybean meal; RP= Rice polish; WB= Wheat bran; MG= Maize gluten; CSM= Cotton seed meal; M. Premix= Mineral premix

Proximate analysis

Proximate analysis of feed ingredients, feed and post-trial fish were performed by following AOAC (2006). All the samples were dried in an oven at 105°C for 18-20 hours to determine their dry matter. The crude protein was determined by Micro Kjeldhal method (prepared by Technico Scientific Supply). Ether extract/crude fat was determined by Soxhlet Apparatus (Model: Behrotest Reihenextraktions Geraete), using diethyl ether as a solvent. The ash content was determined by incinerating 1 g of the sample in a muffle furnace at 550°C overnight.

Growth studies

Fish growth parameters such as initial weight (g) and length (cm) were taken prior to stocking. Other parameters such as final weight, net weight gain, percentage weight gain, SGR% and FCR were determined according to the following formulae:

Net weight gain (NWG)=Average final weight (g)-Average initial weight (g)

Percent weight gain={Final weight (g)-Initial weight (g) / Initial weight (g)}×100

Specific growth rate was estimated by the formula given by Hopkins (1992).

SGR%={ln (Final weight)-ln (Initial weight) / No. of days}×100

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Feed Conversion Ratio (FCR)=Feed intake (g)/Wet weight gain (g)

Hematological studies

Erythrocytes and leucocytes were counted using Neubaur's hemocytometer chamber following Blaxhall and Daisley (1973). Hemoglobin was measured following Sahli (1969). Total serum proteins (albumin and globulin) were measured using a spectrophotometric method following Layne (1957).

Histological studies

The fish were dissected to remove the liver and kidney. The organs were preserved in 4% formalin. Fish samples (control and treated groups) were fixed in Bouin's fluid as described by Humason (1962). Samples were soaked for 20-30 minutes with xylene. It was replaced with 50:50 soft paraffin and xylene at 60^{o}C in an oven for 2 hours. After that it was substituted with soft paraffin at 60^{o}C in an oven for 2 hours and then with hard paraffin for 24 hours at 60^{0}C. A 2-3 µm microtome was used to cut sections of the specimen which were placed on the slide with Meyer's albumin as reported by Humason (1962).

Statistical analysis

The data was analyzed by using analysis of variance test (ANOVA) using SAS 9.1 version and Duncan Multiple Range test to compare the means.

Results

Fish growth studies

All the fish were collected throughout the experimental period to check the effect of experimental and control feeds on growth. Statistically significant (p<0.05) differences in weight gain were observed between the control and plant extract group (T4) while it was non-significant (p≥0.05) with the other treatments. A significant difference (p<0.05) of length increment was recorded among the control (T1), plant extract (T4) and plant extract+bacteria *Lactobacillus* spp. (T2) while a no significant difference (p>0.05) was observed between the beta glucan (T3) and plant extract+bacteria *Lactobacillus* spp. (T2) groups (Table 2).

Table 2: Growth studies of *Labeo rohita* under various treatments of herbal extracts and beta glucan immunostimulants.

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Initial weight (g)	252.55±0.45^{a}	260.35±1.05^{ab}	270.85±8.35^{b}	274.95±0.75^{b}
Final weight (g)	341.25±0.8^{a}	349.62±0.48^{ab}	355.92±6.78^{b}	353.82±0.02^{ab}
Gain in weight (g)	88.7±1.3^{a}	89.27±0.57^{a}	85.07±1.57^{a}	78.87±0.77^{b}
% weight gain	35.1±0.6^{a}	34.35±0.3^{ab}	31.4±1.5^{bc}	28.7±0.40^{c}
Initial length (cm)	27.87±0.17^{a}	27.22±0.50^{a}	28.13±0.41^{a}	28.92±0.53^{a}
Final length (cm)	31±0.0^{a}	32.25±0.25^{b}	32.05±0.05^{ab}	33.45±0.55^{c}
Increase in length(cm)	3.13±0.17^{a}	4.28±0.0^{b}	3.91±0.36^{ab}	3.48±0.03^{ab}
FCR	4.7±0.1^{a}	4.95±0.05b^{a}	5.15±0.15^{b}	5.60±0.10^{c}
SGR (%)	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{b}

*Means with the same letters in a row are not significantly different.

T1= Control; T2= Plant extract + bacteria (Mixture of Korean herbs (VitabioB) + bacteria *Lactobacillus* spp.); T3= Beta glucan; T4= Plant extract (Mixture of Korean herbs (Vitabio A));

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Hematological parameters

RBC and hemoglobin (Hb) contents of the plant extract (T4) and beta glucan (T3) groups were significantly lower than that of the control (T1), while no significant difference (p>0.05) was observed between T1 and T2 (plant extract+bacteria *Lactobacillus* spp.). Other types of cells showed non-significant differences among all the treatments. The total serum proteins also showed a non-significant difference among all the treatments. Similarly no significant differences (p>0.05) were observed for total proteins, albumins and globulins (Table 3).

Table 3: Hematological and serological parameters of *Labeo rohita* under various treatments of herbal extracts and beta glucan immunostimulants.

Parameters	T_1	T_2	T_3	T_4
RBCs (10^6)	2.31±0.3 ^{a}	1.59±0.1^{ab}	1.13±0.3^{b}	1.33±0.1^{b}
WBCS (10^6)	0.06±0.009^{a}	0.04±0.005^{a}	0.04±0.01^{a}	0.04±0.007^{a}
Lymphocytes (%)	91±2.9^{a}	92±1.5^{a}	92±2.12^{a}	85.75±3.3^{a}
Monocytes (%)	1.75±0.2^{a}	1.25±0.2^{a}	1.5±0.2^{a}	2.75±0.8^{a}
Neutrophils (%)	5.75±2.5^{a}	6±1.9^{a}	5.5±2.2^{a}	9±2.8^{a}
Eosinophils (%)	1.25±0.2^{a}	1.25±0.2^{a}	1.25±0.2^{a}	2.75±1.1^{a}
Platelets (10^6)	0.04±0.005^{a}	0.04±0.008^{a}	0.02±0.005^{a}	0.03±0.01^{a}
Hb (g dL^{-1})	10.97±0.6^{a}	9.87±0.8^{a}	8.35±0.8^{ab}	7.15±0.9^{b}
Total proteins (g dL^{-1})	8.0±1.17^{a}	8.0±1.17^{a}	7.55±1.39^{a}	8.62±1.18^{a}
Albumin (g dL^{-1})	2.81±0.46^{a}	2.70±0.35^{a}	2.72±0.62^{a}	2.77±0.44^{a}
Globulins (g dL^{-1})	5.19±0.78^{a}	4.01±0.99^{a}	4.83±0.82^{a}	5.85±0.74^{a}

*Means with the similar letters in a row are not significantly different

T1= Control; T2= Plant extract + bacteria (Mixture of Korean herbs (VitabioB) + bacteria *Lactobacillus* spp.); T3= Beta glucan; T4= Plant extract (Mixture of Korean herbs (Vitabio A));

Post-trial fish body composition

After the termination of the trial, fish were taken from each treatment for analysis of body composition. Crude protein, fat and ash contents of plant extract group (T_4) showed significant differences with the other treatments (Table 4).

Table 4: Post trial fish body composition under various treatments of immunostimulants.

Parameters	T_1	T_2	T_3	T_4
Crude Protein%	62.30±1.70^{a}	67.42±1.19^{b}	67.17±0.72^{b}	70.52±0.07^{b}
Crude Fat %	15.35±0.04^{a}	12.27±0.47^{b}	12.31±0.11^{b}	8.29±0.2^{c}
Ash contents %	10.65±0.25^{a}	17.78±0.44^{ab}	16.10±0.80^{b}	18.85±0.29^{c}

*Means with the similar letters in a row are not significantly different

T1= Control; T2= Plant extract + bacteria (Mixture of Korean herbs (VitabioB) + bacteria *Lactobacillus* spp.); T3= Beta glucan; T4= Plant extract (Mixture of Korean herbs (Vitabio A));

Histological studies

The control treatment (T_1) showed normal pattern of hepatocytes in the liver (Fig. 1), the plant extract+ bacteria *Lactobacillus* spp. group (T_2) showed congestion in blood vessels and degeneration in hepatic cords and hepatocytes (Fig. 2). Liver of the beta glucan (T_3) containing group showed normal hepatic parenchyma and no change was observed (Fig. 3) whereas

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normal liver parenchyma was observed in the plant extract group (T_4) (Fig. 4).

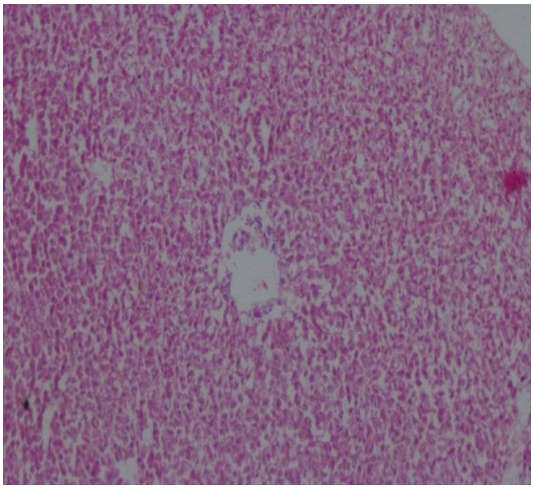


Figure 1: Hepatocytes are arranged in normal fashion (T1).

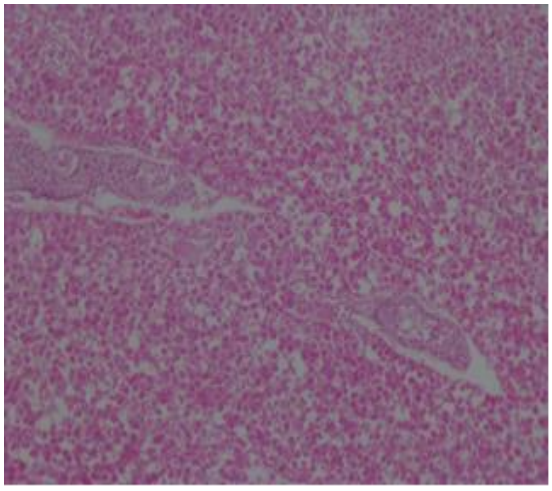


Figure 2: Congestion in blood vessels and degeneration in hepatic cords (T2).

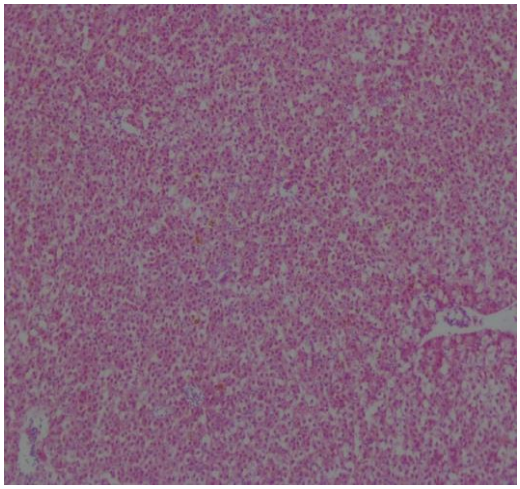


Figure 3: Normal hepatic parenchyma (T3).

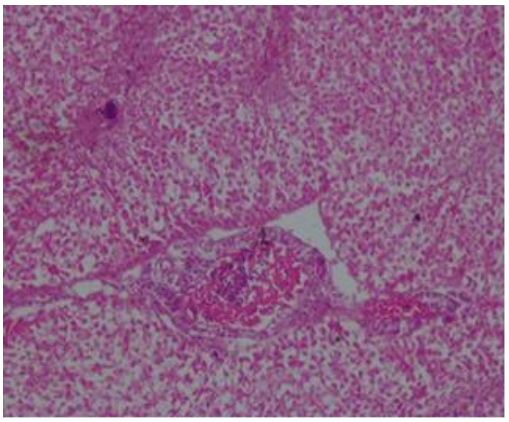


Figure 4: Normal liver (T4).

The control treatment showed normal arrangement of renal parenchyma, the plant extract + bacteria *Lactobacillus* spp. group (T_2) showed degeneration of renal tubules in the renal parenchyma (Fig. 5) in the kidney. However, in the beta glucan group (T_3) the kidney showed severe degeneration of tubular epithelium along with few intact tubules (Fig. 6), while in plant extract group (T_4) the kidney showed some degeneration in renal tubules (Fig. 7).

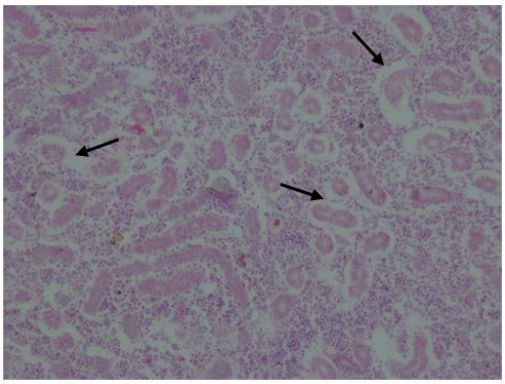


Figure 5: Degeneration of renal tubules (T2).

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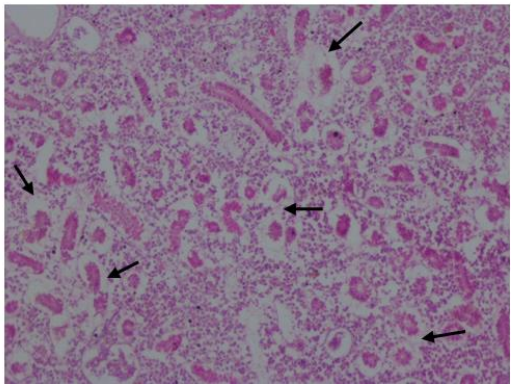


Figure 6: Severe degeneration of tubular epithelium.

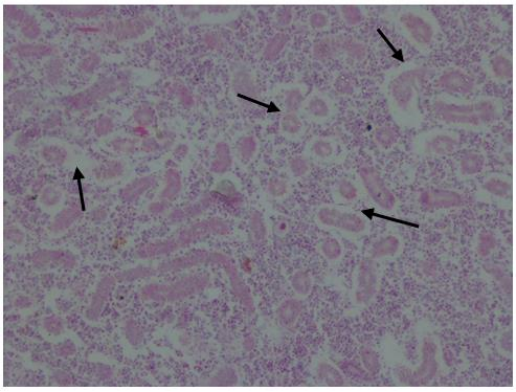


Figure 7: Degeneration of renal tubules epithelium (T3) of the renal parenchyma (T4).

Discussion

The weight gain and SGR% in the beta glucan treated group showed a non-significant difference with the control and other treated groups. The results of weight gain and SGR of the current study are in line with findings of Meshram *et al.* (2014) who observed similar results when he fed freshwater prawn with beta glucan. However a slight difference of FCR and % weight gain between the control and treated groups was observed. This difference can be due to poor efficiency of feed conversion resulting in lower weight gain. The lower weight gain is supported by the findings of Dalmo and Bogwald (2008). They suggested that many factors such as the concentration of β-glucan, its solubility and species of fish, temperature and duration of the feeding period affect growth and weight gain. They also determined that only soluble β-glucans are absorbed by the intestine. The gut contains no endogenous enzyme for their particulate degradation lowering the chances of absorption.

A significant decrease in weight gain, % weight gain, SGR% and increase in FCR was found in the herbal mixture treated group as compared to the control. This slower growth performance may be the result of various anti nutritional factors (gossypol, phytate, protease inhibitors, lectins, etc.) that adversely affect fish health (Krogdahl *et al.*, 1994; Vielma *et al.*, 2000; Francis *et al.*, 2001).This decrease in weight gain is in line with the Sotolu and Faturoti (2009) who fed the *Clarias gariepinus* with varying levels of *Leucaena leucocephala* plant seed meal based diet.

A non-significant difference in the growth parameters was found in the plants extract+bacteria treatment as compared to the control. This improvement in growth parameters is also supported by the results of Yanbo and Zirong (2006) and Ambas *et al.* (2013). They demonstrated that bacterial strains included in feeds led to an increase in enzymatic digestion, acted as growth promoting factors and boosted immune response.

The overall cell counts showed a non-significant difference among themselves in all treatments. The beta glucan showed no considerable

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difference of cells except RBCs. Similar findings about hematological parameters were reported by Welker *et al.* (2007) in their research on channel catfish (*Ictalurus punctatus*) and Hoseinifar *et al.* (2011) in their work on Beluga juveniles (*Huso huso*).The RBC and hemoglobin contents were found to have a decreasing trend in all the groups compared with the control. There was a significant decrease of RBC between the control and plant extract treatment groups. A similar decrease was reported by Soltan *et al.* (2008) in their work on Nile tilapia by the addition of plant protein sources. The same trend was reported by Bello and Nzeh (2013) who fed *Clarias gariepinus* with *Moringa oleifera* enriched diets. With the increasing levels of moringa, decrease in Hb and RBC was observed. The possible reason for this decrease was considered the binding of anti- nutritional molecules to the minerals viz. iron making their availability lesser in the body and increasing the chances of RBC frailty.

The total serum proteins showed a non-significant (p>0.05) difference among all the treatments. Similar results were reported by Amao *et al*. (2012) when he fed Hacco Cocks with cocoa bean shell supplemented with vitamin E and beta glucan. This appeared contrary to the findings of Abasali and Mohamad (2010) who fed common carp with plants extract and recorded an increase in total serum proteins of the treated groups.

The results of proximate composition of our study revealed an increase in protein levels and decrease in fat contents which are in corroboration with the results of Maniat *et al.* (2014) who fed benni fish with paprika plant and observed a significant increase in protein and decrease in fat with the increasing levels of plant powder. The fat contents in the current study are in line with the findings of Fallahpour *et al.* (2014) who fed common carp with marsh mellow extract and found a decreasing trend in fat contents compared to the control. While the results of crude protein and ash are slightly different from his findings.

Histological studies showed that the control group revealed normal configuration of the hepatocytes, central vein and blood sinusoids, while congestion and degeneration was observed among other treatments. This effect on liver parenchyma can be due to nutrient toxicity of the plant extracts. The same degenerations and vacuolation of the hepatocytes in Nile tilapia after its exposure to cypermethrin were reported previously by Karthigayani *et al*. (2014).

Histological studies showed normal patterns of cells in the kidney of control group. However treated groups showed degeneration of renal tubules and vacuolation. Russo *et al.* (2006) recorded no significant differences in growth rates among fish fed immunostimulants (beta-glucan and nucleotide) or the control diet when Red-Tail Black Shark got infected with *Streptococcus iniae*. Further, histological observation revealed leukocyte infiltration in the intestinal area, posterior kidney and brain.

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Necrosis and tissue degeneration were observed in the same organs in addition to degeneration of the renal tubules. Apines-Amar *et al.* (2013) studied growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown-marbled grouper, fed onion and ginger, β-glucan (1%), vitamin C (3%) and a control diet (without immunostimulants) and reported that kidney sections did not show significant pathology in the supplemented groups compared to the uninfected control. Besides minimal infiltration of inflammatory cells, the kidney tubules and glomeruli were also found intact.

Our findings suggest that plant extracts and beta glucan can be used safely to promote growth of *L. rohita* without any adverse effects on body composition and hematology of the fish.

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