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Effect of Vitamin D on the Growth, Haematological and Bio-Chemical Profile of *Labeo rohita*

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ABSTRACT

Background: Fish health and disease management is the key factor in modern aquaculture where intensification manifolds the infestation chances of parasites. Irrespective of antibiotics, the use of dietary additives gaining attention in development of aqua feed. Vitamin D is an important dietary additive for fish in terms it can boost up the immune system of fish and unluckily fish cannot synthesize vitamin D itself.

Methods: The present study was planned to determine the effect of vitamin D as feed additive on the immunity, hematology and body composition of *Labeo rohita.* Four diets (T_1, T_2, T_3, T_4) containing different concentration of vitamin D (250mg, 500mg, 1.0g and 1.5g) in food and a control diet were administrated for 90 days trial.

Result: At the termination of experiment there were significant differences in growth and hematological parameters between control and test diets. T_3 (1.0g of vitamin D) showed the maximum weight gain with lowest FCR value (1.43±0.33) however, the RBC count of T_4 group was highest (1.85±0.07) than the control, T_1, T_2 and T_3 groups. The body composition of *Labeo rohita* growth factor and survival rate were significantly higher ($P < 0.05$) in fish fed on diets containing vitamin D at 1.00g/Kg concentration.

Key words: Dietary additives, Growth factor, Hematology, Proximate analysis, Vitamin D.

INTRODUCTION

The development of fisheries and aquaculture to attain all out profits from resources of fish production, it is compulsory to provide an artificial feed to fish by which fish growth and weight gain can be maximized in the shortest possible time (Bhosale *et al.*, 2010).

In Pakistan, Chinese and major carps are commercially important cultivated fish species. In Asian countries, *Labeo rohita* is most important consumable and culture able fish species and has a potential for marketable production but slow growth rate is a major constraint when compared with Chinese carps. Commonly, these species are reared on usual diet with low protein. Due to low nutritional imbalance and nutrient level, these feeds cannot boost up its growth. So the introduction of nutritionally balanced diet or ingredients is the immediate need of the fish culturist and is one of the limiting factors for optimal growth of carps (Jhingran and Pullin, 1985; Khan *et al.*, 2004).

Fat soluble vitamins are naturally present in few foods out of which vitamin D can be used as a nutritional supplement. Ultraviolet rays from sunlight attack the skin and generate vitamin D (Ross, 2011). In gut, vitamin D endorses calcium absorption, concentration of phosphate to permit usual bone mineralization, regulates sufficient serum calcium and to evade hypocalcemic tetany. Furthermore, vitamin D is necessary for proper growth of bone and bone renovation by osteoclasts and osteoblasts (Cranney *et al.*, 2007).

A large quantity of vitamin D is stored in fish fat tissue and liver, fish is most significant nutritive source of vitamin D due to the fat associated with muscle. Fish do not produce vitamin D, so vitamin D is always available for fish via their diet. Vitamin D absorbed directly from diets including all vertebrates (Dusso *et al.*, 2005). In fat containing tissue such as liver and muscle makes fish tremendous dietary source of vitamin D_3 for humans due to large quantities of vitamin D_3 (Takeuchi *et al.*, 1987; Calvo *et al.*, 2005). In human's growth and development fish oil is very important due to excessive vitamin D (Lock *et al.*, 2010).

Haematological parameter is important for the detection of physiological and pathological changes in fishes. Various researchers have been recognized the normal ranges for various blood parameters in fish physiology and pathology (Rambhaskar and Srinivasa Rao, 1987; Xiaoyun *et al.*, 2009). For analyzing the health status of farmed animals the investigation of blood indices has verified to be a valuable approach (Bahmani *et al.*, 2001). To the detection of fish

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heath, blood biochemistry parameters can be used. In blood composition; exogenous factors, such as diseases, stress (Cnaani *et al.*, 2004) and management (Svobodova *et al.*, 2008) are continuously induce major changes. The objective of this study is to evaluate the growth performance and hematology and meat quality of *Labeo rohita*, by using of vitamin D under different treatments.

MATERIALS AND METHODS

Experimental setup

The experiment was conducted in hapas (6Lx6Wx5Dft) at earthen pond, the fish farm complex, Department of Fisheries and Aquaculture, UVAS, Ravi campus, Pattoki. The duration of experimental trial was three months. Completely randomized design was selected with four treatment groups and one control group, each with 3 replicas. Experimental fish (*Labeo rohita*) was stocked in each hapa (15 fish/hapa) weighing 200g and fed on formulated pelleted feed @ 2% fish wet body weight twice a day.

Feed formulation

For fish 30% CP feed was prepared by using cotton seed meal, rice polish, guar meal, maize gluten, Soya bean meal vitamin premix and vitamin D. To estimate the effects of vitamin D on growth and haematology of *Labeo rohita* reared in freshwater, five experimental diets (T_1, T_2, T_3, T_4 and control) containing different vitamin D concentration (250mg, 500 mg, 1.0g, 1.5g and 0mg). Feed formulation was given in Table 1. All the ingredients were mixed thoroughly to a homogenous mixture. For binding 1% molasses and 600 ml water was added in to the mixture, dough was extruded through mincer and then desired pellets were obtained. The dried feed was stored in air tight plastic bags and stored at 35°C until use.

Growth performance

On the day of stocking morphometric measurements *viz*. fish weight and total body length were recorded and then every fortnightly to adjust the feed consequences. At the completion of trial growth parameters of experimental fish such as Feed conversion ratio (FCR), average weight gain, Specific growth rate (SGR) was calculated by formulas given by (Bekcan *et al*., 2006).

$$WG\ (\%) = \frac{100\times \text{Final BW} - \text{Initial BW}}{\text{Initial BW}}$$

$$FCR = \frac{\text{Total feed given (g)}}{\text{Weight gain (g)}}$$

$$SGR = \frac{\ln(\text{Final wet BW}) - \ln(\text{Initial BW})}{\text{Number of Periods}} \times 100$$

Bio-Chemical study

At the completion of experiment, 5 fish from each experimental group were sampled and subjected to the proximate analysis after taking blood. Proximate composition was evaluated according to the (AOAC, 2010). Hot air oven was used to determine the moisture, by drying the sample at 105°C. Kjeldahl's method (N×6.25) was used to determine the crude protein content by converting nitrogen contents. Crude fat was determined by the Soxhlet apparatus. Ash content was determined by muffle furnace at 550°C for 4 hours. Crude fiber contents were determined by crucible furnace.

$$\text{Moisture}\ (\%) = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

$$\text{Dry matter} = 100 - \%\ \text{moisture}$$

$$\text{Crude protein}\ \% = \frac{\text{Sample} \times 0.875}{\text{Sample weight}}$$

$$\text{Fat}\ \% = \frac{\text{Fat weight (g)}}{\text{Weight of sample (g)}} \times 100$$

$$\text{Ash}\ (\%) = \frac{\text{Ash weight (g)}}{\text{Weight of dry sample (g)}} \times 100$$

$$\text{Crude fiber}\ (\%) = \frac{\text{Fiber weight (g)}}{\text{Weight of dry sample (g)}} \times 100$$

Haematological parameters

At the end of experimental trial, fish samples from each treatment were randomly selected for the collection of blood. Blood was collected with the help of syringes in EDTA containing vacutainers. Blood samples were loaded on Neubauer haemocytometer to determine erythrocytes (RBC) and leukocytes WBC count (Blaxhall and Daisley, 1973). Haematocrit or PCV was determined by using capillary method. Haemoglobinometry (Sahlis's method) was employed for estimating hemoglobin content of blood. To estimate the different leukocyte blood smears were prepared, air dried fixed in methanol and stained with Giemsa solution (Blaxhall and Daisley, 1973). Mean corpuscular hemoglobin (MCH), mean corpuscular Volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) were computed by following formulas (Dacie and Lewis, 1991).

$$MCV = \frac{\text{PCV/1,000 ml blood}}{\text{RBC in millions/mm3}} = f$$

$$MCH = \frac{\text{Hb in g/1,000 ml blood}}{\text{RBC in millions/mm3}} = pg$$

$$MCHC = \frac{\text{Hb in g/100 ml blood} \times 100}{\text{PCV/100 ml}} = g/dl$$

Organoleptic evaluation

Sensory evaluation was conducted in clean environment with white florescence light. At the completion of experiment 5 fish were randomly collected from each treatment and then well cleaned, degutted and uniformly filleted. Iodized salt was sprinkled on each fillet and then cooked in Dawlance microwave oven at 65°C for 15 min. A panel of 10 experts was invited to evaluate the sample on the following parameters; overall acceptability, texture, flavor, whiteness, oiliness and odour.

Statistical analysis

The SAS (Statistical Analysis Software) version 9.1 was used for all statistical analyses due to its wider applications. Duncan's multiple range tests were performed. Probability level was set at $P<0.05$.

RESULTS AND DISCUSSION

During the research trial, *L. rohita* were grown under controlled conditions with five different feeding groups' *viz*. T_1 (250 mg vitamin D), T_2 (500 mg vitamin D), T_3 (1g vitamin D) and T_4 (1.5 g vitamin D) and control group without vitamin D for a period of 90 days. There was no statistical difference at the start of experiment between control and experimental groups. After 90 days of trial there were statistical differences in growth parameters, hematological parameters and physicochemical parameters. The experimental trial start in hapas, the total initial body weight for control was 277.64±2.9, 296.96±1.8g for T_1, 356.1±8.5 g for T_2, 391.5±1.8g for T_3 and 387.1±9.4g for T_4. The total final body weight for control was 294.50±3.2g, for T_1 was 364.40±5.8 g, for T_2 was 410.0±9.4g, for T_3 was 465.9±1.9 g and T_4 was 450.8±9.8 g. The maximum weight gain was observed in T_3 under the influence of artificial feed having 1.00g vitamin D/ Kg of feed with lowest FCR and highest SGR value. The total % age weight gain for *L. rohita*) was also higher in T_3 (19.73±4.28g). Statistical analysis showed that control group had highest FCR and lowest SGR as compared to diet containing vitamin D levels showed in (Table 2).

At the end of trail bio-chemical test were performed to analyze the fiber, ash, dry matter, fats, protein and moisture contents. Although the protein contents increase in treatments groups as compared to control group however the values were variable, there was slide decrease in protein contents from T_2 to T_4, the highest protein contents were observed in T_3 and lowest was observed in T_1. Fat, Ash and fiber did not show significant difference. The highest fat and ash was observed in T_3. Statistical analysis showed significant difference between treatments with highest content in T_1.Similarly the values for dry matters were significantly different between the group and highest value was recorded in T_1 showed in (Table 3).

Organoleptic evaluation

Among various dietary treatments organoleptic evaluation indicated that texture, odor, whiteness, flavor, oiliness and overall acceptability of fish flesh revealed significant differences (Table 4). Results indicated that the fishes fed on 1 gm vitamin D (T_3) showed the highest overall acceptability, flavor, odor and whiteness as compared to other treatments.

Table 1: Contribution of each Ingredient in following formula.

Ingredients	Control	T_1	T_2	T_3	T_4
Cotton seed meal	20%	20%	20%	20%	20%
Guar meal	30%	30%	30%	30%	30%
Maize gluten meal	20%	20%	20%	20%	20%
Soybean meal	15%	15%	15%	15%	15%
Rice polish	14%	14%	14%	14%	14%
Vitamin D	0%	250mg	500mg	1g	1.5g
Vitamins premix	0%	1%	1%	1%	1%

Table 2: Growth performance of *Labeo rohita* in all treatments.

Parameter	Control (0% vit D)	T_1 (250mg vit D)	T_2 (500mg vit D)	T_3 (1g vit D)	T_4 (1.5g vit D)
Initial weight (g)	277.64±2.9	296.96±1.8	356.1±8.5	391.5±1.8	387.1±9.4
Final weight (g)	$294.50±3.2^{c}$	$364.40±5.8b^{c}$	$410.0±9.4^{ab}$	$465.9±1.9^{a}$	$450.8±9.8^{a}$
Net gain in weight (g)	$84.30±3.82^{d}$	$182.20±4.01^{c}$	$269.8±8.02^{b}$	$372.0±7.95^{a}$	$322.8±2.88^{a}$
% weight gain(g)	$6.01±2.68^{c}$	$11.26±40.1^{b}$	$17.30±9.59^{a}$	$19.73±4.28^{a}$	$17.1±3.34^{a}$
Total length(cm)	$27.94±0.63^{b}$	$28.1±1.13^{b}$	$34.2±8.86^{a}$	$32.68±8.4^{a}$	$30.8±2.51^{ab}$
FCR	$5.76±2.64^{a}$	$2.47±0.601^{b}$	$2.01±0.59^{b}$	$1.43±0.33^{b}$	$1.64±0.32^{b}$
FCE	$19.48±11.69^{c}$	$41.80±9.26a^{b}$	$45.3±13.7^{ab}$	$33.99±16.7^{b}$	$54.2±23.6^{a}$
SGR	$7.7±0.26^{d}$	$7.12±1.209^{b}$	$7.59±0.22^{c}$	$7.70±0.26^{a}$	$7.68±0.23^{b}$

Table 3: Proximate analysis of *Labeo rohita* for all treatments.

Parameters	Control (0% vit D)	T_1 (250mg vit D)	T_2 (500mg vit D)	T_3 (1g vit D)	T_4 (1.5mg vit D)
Protein	$33.61±0.26^{c}$	$31.57±0.45^{d}$	$38.43±0.36^{a}$	$38.25±0.62^{a}$	$36.11±0.77^{b}$
Fat	$2.07±1.02^{a}$	$1.91±0.09^{a}$	$1.65±0.21^{a}$	$2.14±0.50^{a}$	$1.71±0.21^{a}$
Ash	$2.35±0.28^{a}$	$2.60±0.35^{a}$	$2.30±0.14^{a}$	$2.70±0.28^{a}$	$2.51±0.26^{a}$
Fiber	$2.08±0.30^{a}$	$1.40±1.62^{a}$	$1.82±0.19^{a}$	$1.88±0.02^{a}$	$2.02±0.11^{a}$
Moisture	$71.82±0.91^{c}$	$78.77±0.7^{a}$	$74.38±0.59^{b}$	$74.06±0.33^{b}$	$71.36±1.47^{c}$
Dry Mater	$18.67±0.60^{bc}$	$22.28±0.7^{a}$	$16.57±0.74^{c}$	$17.54±1.93^{bc}$	$19.93±0.80^{ab}$

Table 4: Organoleptic/sensory score of fish flesh against various treatments.

Character	Control (0% vit D)	T_1 (250mg vit D)	T_2 (500mg vit D)	T_3 (1g vit D)	T_4 (1.5mg vit D)
Odor	6.79±0.7^{ab}	7.67±0.3^{a}	7.23±0.6^{ab}	7.59±0.5^{a}	6.29±0.7^{b}
Texture	6.41±0.1^{a}	7.24±0.9^{a}	7.59±0.9^{a}	7.19±0.7^{a}	7.43±0.4^{a}
Flavor	5.85±0.7^{c}	6.9±0.7^{bc}	6.91±0.2^{bc}	8.45±0.7^{ab}	7.44±0.6^{ab}
Whiteness	6.84±0.8^{a}	7.14±0.3^{a}	6.77±0.17^{a}	7.18±0.7^{a}	7.07±1.0^{a}
Oiliness	6.93±0.6^{a}	7.03±0.5^{a}	6.94±0.2^{a}	7.35±0.7^{a}	6.88±0.1^{a}
Overall acceptability	5.60±0.9^{b}	6.34±0.5^{ab}	5.93±0.7^{b}	7.50±0.2^{a}	7.48±0.4^{a}

Table 5: Haematology parameters of *Labeo rohita* among different treatments.

Parameter	Control (0% vit D)	T_1 (250mg vit D)	T_2 (500mg vit D)	T_3 (1g vit D)	T_4 (1.5g vit D)
Hemoglobin g/dl	5.55±0.07^{b}	7.05±0.21^{a}	2.95±0.21^{d}	4.3±0.2^{c}	5.8±0.8b^{a}
TLC cmm	48300.0±848.5^{a}	46200.0±2545.5^{d}	56750.0±4313.3^{bc}	64000.0±1414.2^{b}	46700.0±7919.6^{c}
Neutrophile %	4.5±0.70^{ab}	2.50±0.70^{bc}	2.0±0^{c}	5.50±0.7^{a}	6.0±1.4^{a}
Lymphocytes %	89.5±3.53^{a}	96.5±0.70^{a}	92.5±4.94^{a}	94.0±5.65^{a}	91.5±2.12^{a}
Monocytes%	2.0±1.41^{a}	1.50±0.70^{a}	1.50±0.7^{a}	3.0±1.41^{a}	2.5±0.7^{a}
Eosinophils%	4.5±0.70^{a}	1.5±0.70^{b}	1.5±0.7^{b}	1.5±0.7^{b}	2.5±0.7^{b}
Platelets Count	45000.0±1414.21^{bc}	32500.0±2121.3^{d}	52000.0±1414.2^{b}	40000.0±2828.38^{c}	126500.0±4949.75^{a}
Total RBC (x106 µl)	0.71±0.07^{cd}	0.89±0.12^{c}	0.54±0.03^{d}	1.45±0.07^{b}	1.85±0.07^{a}
HCT	7.85±0.07^{d}	12.8±0.84^{c}	31.2±2.26^{a}	6.9±0.42^{d}	23.5±0.7^{b}
MCV fl	154.60±4.52^{a}	126.2±11.38^{b}	161.0±8.48^{a}	133.1±7.42^{b}	159.8±7.14^{a}
MCH pg	47.45±0.77^{ab}	51.8±1.69^{a}	47.5±2.21^{ab}	43.0±0.98^{b}	41.5±3.53^{a}
MCHC g/dl	28.9±1.13^{c}	31.25±1.76^{bc}	33.15±1.20^{b}	32.5±1.48l^{bc}	41.25±2.19^{a}

Haematology parameters

Haematology of experimental fish was done for WBCs (Monocytes, Neutrophils, Lymphocytes and Eosinophils) and RBCs and their related parameters (hemoglobin, mean corpuscular hemoglobin, platelet count, TLC, HTC, MCV, MCH and MCHC) were performed at the end of trial (Table 5). Statistical analysis of hematology parameters related to WBCs showed slight increase in different white blood cell however; RBCs and related parameters did not showed any specific trend with respect to the vitamin D concentration. Control group showed higher values then the treatment group indicating that vitamin D had no any effect on the hematology of fish.

Diet additives have recently attracted attention and investments of the aquaculture industry and, consequently, researches aimed at evaluating the costs/benefits ratio and the effects on organisms and environments are increasing. In general, additives are nutritive or nonnutritive ingredients that are added to the di*et al*one or in combination with each other and that are present in small quantities. An improper or incomplete diet can result in nutrient and vitamin deficiencies and the onset of serious conditions such as stunted or improper growth, a weakened immune system, or death. Vitamin supplements, along with a varied diet, are an ideal way to fill in nutritional gaps. (Gasco *et al.*, 2018) fish need vitamins for health just like any animal especially Vitamin D that is necessary for calcium and phosphorus metabolization that aids in normal development of bones and scales and immune system of fish.

In the present study, effect of vitamin D supplemented feed was assessed in *L. rohita.* Although, the fish fed on control diet revealed lower growth and SGR than diets supplemented with vitamin D, indicated that vitamin D is favorable for the growth of *L. rohita.* Vitamin supplementation improved growth response and feed utilization. Our studies were in contradictory to the (Graff *et al.*, 2002) who fed various vitamin D_3 levels to Atlantic salmon fry and observed no significant results for weight, length, SGR and mortality. (Poston, 1968; Hilton and Ferguson, 1982; Horvli *et al.*, 1998) did not observe any effect on the growth of brook trout, rainbow trout and Atlantic salmon. Similarly, channel catfish (Brown and Robinson, 1992) also reported normal growth when fed on diets supplemented with vitamin D. Andrews *et al.,* (1980) reported inhibited growth in channel cat fish. The inconsistency in the results is may be due to the different age groups, environments and species used for experimentation. Fishes may not require vitamin D supplemented diets at their early life stages and each species has its own specific vitamin D requirement. The vitamin D_3 deficient fish exhibited a thin epidermis and extensively necrotized underlying musculature and hypocalcaemia however, these pathological changes largely reversed after 4 weeks of feeding with a vitamin D_3 supplemented diet (Taveekijakarn *et al.*, 1996).

During the experimental period in the present trial there was a tendency of increasing Crude Fat with increasing dietary levels of vitamin D in T_3 which was similar to the results of (Lock *et al.,* 2010) and (Graff *et al.,* 2002) but the level decrease with further increase (T_4). The reason for this is unclear and more research is needed on this field. Studies

with other vitamins supplementation diets also support our results. Vitamin C with basal diet supplement given to *O. niloticus* for 1 month results increase in condition factor, body weight gain, survival rate and specific growth rates (Ibrahem *et al.,* 2010 and Rahman *et al.,* 2006)

Organoleptic evaluation of the study did not revealed any specific significant difference in term of odor, texture, flavor, whiteness, overall acceptability and oiliness of *L. rohita* fish flesh. However, supplementation of vitamin D has improved overall acceptability. It seems that there is little effect of vitamin D on the sensory evaluation but the reason is not clear.

Hematology results revealed different trends for different parameters at varying vitamin concentrations. An increasing trend in white blood cells and red blood cells was observed as the concentration increase. Our results were in accordance to the results of (Tatina *et al.*, 2010) and (Lin and Shiau, 2005) who studied the effect of vitamin C and E on the fish hematology and immune response for grouper. Like our study, (Falahatkar, 2005) also studied the effect of different levels of diet Vitamin C on some of hematological parameters of great sturgeon found that there were significant differences in WBC among the treatments. The highest number of RBC was observed in vitamin D supplemented diets especially in T_3 which indicates that diets containing different levels of Vitamin D have significant influence on RBC value. Similar results were obtained by (Lim *et al.,* 2000) for channel catfish fed with different vitamin C and iron concentrations, (Falahatkar,2005) for great sturgeon fed with different vitamin C levels, (Montero *et al.,* 2001) for *S. aurata* fed with different vitamin C and E concentrations, (Andrade *et al.,* 2007) for pirarucu fed with different vitamin C and E concentrations and (Tatina *et al.*, 2010) for Sterlet (*Acipenser ruthenus*). Vitamin A also has great influence on the RBC concentration as the feed having lower concentration then the required values can leads to the anemia in the Nile tilapia. The increment in the RBC, WBC and Hb of Nile tilapia is also similar to our study (Guimarães *et al.*, 2014). In conclusion, Vitamin D has significant effect on the growth of fish and hematology however; body composition and organoleptic studies did not showed any specific effect of Vitamin D supplemented diet in *L. rohita*.

CONCLUSION

Feed supplements can enhance the production of aquaculture and vitamin D could be a good supplement as it has significant effect on the growth and immunity of fish. In the present study vitamin D @ 1g of feed produced positive effect on the fish immune parameters and growth.

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