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

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Vitamin D is an important dietary additive for fish in terms it can boost up the immune system of fish and unfortunately fish cannot synthesize vitamin D itself.

**Methods:** The present study was planned to determine the effect of vitamin D as a feed additive on the immunity, haematology, and body composition of *Labeo rohita*. Four diets (T₁, T₂, T₃, T₄) containing different concentrations of vitamin D (250mg, 500mg, 1.0g and 1.5g) in food and a control diet were administered for 90 days trial.

**Result:** At the termination of experiment there were significant differences in growth and haematological parameters between control and test diets. T₁ (1.0g of vitamin D) showed the maximum weight gain with lowest FCR value (1.43±0.33) however, the RBC count of T₂ group was highest (1.85±0.07) than the control, T₃ and T₄ 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.
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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<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> 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).

\[
WG(\%) = \frac{100 \times Final\ BW - Initial\ BW}{Initial\ BW}
\]

\[
FCR = \frac{Total\ feed\ given\ (g)}{Weight\ gain\ (g)}
\]

\[
SGR = \frac{In\ (Final\ wet\ BW) - In\ (Initial\ BW)}{Number\ of\ Periods} \times 100
\]

**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.

\[
Moisture\ (%) = \frac{Wet\ weight - Dry\ weight}{Wet\ weight} \times 100
\]

\[
Dry\ matter = 100\ -\ %\ moisture
\]

\[
Crude\ protein\ % = \frac{Sample \times 0.875}{Sample\ weight}
\]

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

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

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

**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).

\[
MCV = \frac{RBC\ in\ millions/mm3}{PCV/1,000\ ml\ blood}
\]

\[
MCH = \frac{Hb\ in\ g/1,000\ ml\ blood}{RBC\ in\ millions/mm3}
\]

\[
MCHC = \frac{Hb\ in\ g/100\ ml\ blood \times 100}{PCV/100\ ml}
\]

**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.
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**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. T1 (250 mg vitamin D), T2 (500 mg vitamin D), T3 (1g vitamin D) and T4 (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, hematomical parameters and physicochemical parameters. The experimental trial start in hapas, the total initial body weight for control was 277.64±2.9, 296.96±1.8 for T1, 356.1±8.5 g for T2, 391.5±1.8g for T3 and 387.1±9.4g for T4. The total final body weight for control was 294.50±3.2g, for T1 was 364.40±5.8g, for T2 was 410.0±9.4g, for T3 was 465.9±1.9 g and T4 was 450.8±9.8 g. The maximum weight gain was observed in T3 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 T3 (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 trial 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 T2 to T4, the highest protein contents were observed in T3 and lowest was observed in T1. Fat, Ash and fiber did not show significant difference. The highest fat and ash was observed in T1. Statistical analysis showed significant difference between treatments with highest content in T1. Similarly the values for dry matters were significantly different between the group and highest value was recorded in T1 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 (T4) showed the highest overall acceptability, flavor, odor and whiteness as compared to other treatments.

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**Table 1:** Contribution of each Ingredient in following formula.

| Ingredients                  | Control | T1 (250mg vit D) | T2 (500mg vit D) | T3 (1g vit D) | T4 (1.5g vit D) |
|------------------------------|---------|------------------|------------------|---------------|----------------|
| 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) | T1 (250mg vit D) | T2 (500mg vit D) | T3 (1g vit D) | T4 (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±3.2a        | 364.40±5.8b      | 410.0±9.4ab      | 465.9±1.9a    | 450.8±9.8a     |
| Net gain in weight (g)         | 84.30±3.82a        | 182.20±4.01c     | 269.8±8.02c      | 372.0±7.95c   | 322.8±2.88c    |
| % weight gain(g)               | 6.01±2.68a         | 11.26±4.01c      | 17.30±9.59a      | 19.73±4.28b   | 17.1±3.34c     |
| Total length(cm)               | 27.94±0.63a        | 28.1±1.13b       | 34.2±8.86a       | 32.68±8.4a    | 30.8±2.51ab    |
| FCR                           | 5.76±2.64a         | 2.47±0.601b      | 2.01±0.59a       | 1.43±0.33b    | 1.64±0.32c     |
| FCE                           | 19.48±11.69a       | 41.80±9.26ab     | 45.3±13.7ab      | 33.99±16.7b   | 54.2±23.6a     |
| SGR                           | 7.7±0.26c          | 7.12±1.209ab     | 7.59±0.22a       | 7.70±0.26a    | 7.68±0.23c     |

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

| Parameters   | Control (0% vit D) | T1 (250mg vit D) | T2 (500mg vit D) | T3 (1g vit D) | T4 (1.5mg vit D) |
|--------------|--------------------|------------------|------------------|---------------|-----------------|
| Protein      | 33.61±0.26a        | 31.57±0.45a      | 38.43±0.36a      | 38.25±0.62a   | 36.11±0.77b     |
| Fat          | 2.07±1.02a         | 1.91±0.09a       | 1.65±0.21a       | 2.14±0.50a    | 1.71±0.21b      |
| Ash          | 2.35±0.28a         | 2.60±0.35a       | 2.30±0.14a       | 2.70±0.28a    | 2.51±0.26a      |
| Fiber        | 2.08±0.30a         | 1.40±1.62a       | 1.82±0.19a       | 1.88±0.02a    | 2.02±0.11a      |
| Moisture     | 71.82±0.91c        | 78.77±0.7a       | 74.38±0.59a      | 74.06±0.33b   | 71.36±1.47c     |
| Dry Mater    | 18.67±0.60bc       | 22.28±0.7a       | 16.57±0.74a      | 17.54±1.93bc  | 19.93±0.80ab    |
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Table 4: Organoleptic/sensory score of fish flesh against various treatments.

| Character  | Control (0% vit D) | T₁ (250mg vit D) | T₂ (500mg vit D) | T₃ (1g vit D) | T₄ (1.5g vit D) |
|------------|--------------------|------------------|------------------|--------------|----------------|
| Odor       | 6.79±0.7ᵇ          | 7.67±0.3ᵃ        | 7.23±0.6ᵇ        | 7.59±0.5ᵇ    | 6.29±0.7ᵇ      |
| Texture    | 6.41±0.1ᵃ          | 7.24±0.9ᵃ        | 7.59±0.9ᵃ        | 7.19±0.7ᵃ    | 7.43±0.4ᵃ      |
| Flavor     | 5.85±0.7ᶜ          | 6.9±0.7ᵇ         | 6.9±0.2ᵇ         | 8.45±0.7ᵃᵇ  | 7.44±0.6ᵇ      |
| Whiteness  | 6.8±0.8ᵃ           | 7.14±0.3ᵃ        | 6.77±0.17ᵃ       | 7.18±0.7ᵃ    | 7.07±1.0ᵃ      |
| Oiliness   | 6.93±0.6ᵃ          | 7.03±0.5ᵃ        | 6.94±0.2ᵃ        | 7.35±0.7ᵃ    | 6.88±0.1ᵃ      |
| Overall acceptance | 5.6±0.9ᵇ       | 6.34±0.5ᵃᵇ      | 5.93±0.7ᵇ        | 7.50±0.2ᵃ    | 7.48±0.4ᵃ      |

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

| Parameter | Control (0% vit D) | T₁ (250mg vit D) | T₂ (500mg vit D) | T₃ (1g vit D) | T₄ (1.5g vit D) |
|-----------|--------------------|------------------|------------------|--------------|----------------|
| Hemoglobin g/dl | 5.55±0.07ᵇ        | 7.05±0.21ᵃ      | 2.95±0.21ᵈ      | 4.3±0.2ᵇ     | 5.8±0.8ᵇ      |
| TLC cmm    | 48300.0±848.5ᵃ     | 46200.0±2545.5ᵈ | 56750.0±4313.3ᵇ | 64000.0±1414.2ᵇ | 467000.0±7919.6ᵇ |
| Neutrophile % | 4.5→7.0ᵇ         | 2.5→0.7ᵇ       | 2.0→0.7ᵇ       | 5.5±0.7ᵃ     | 6.0±1.4ᵃ      |
| Lymphocytes % | 89.5±3.5ᵃ         | 96.5±0.7ᵃ       | 92.5±4.9ᵃ       | 94.0±5.6ᵃ    | 91.5±2.1²      |
| Monocytes%  | 2.0±1.4ᵃ           | 1.5±0.7ᵃ        | 1.5±0.7ᵃ        | 3.0±1.4ᵃ     | 2.5±0.7ᵃ      |
| Eosinophils% | 4.5±0.7ᵃ          | 1.5±0.7ᵃ        | 1.5±0.7ᵃ        | 1.5±0.7ᵃ     | 2.5±0.7ᵃ      |
| Platelets Count | 450000.0±1414.2¹ᵇ | 325000.0±1213.3¹ᵇ | 52000.0±1414.2ᵇ | 40000.0±2828.3ᵇ | 126500.0±4949.75ᵇ |
| Total RBC (x106 µl) | 0.71±0.07ᵐᵈ    | 0.89±0.12ᵃ     | 0.54±0.03ᵃ     | 1.45±0.07ᵇ   | 1.85±0.07ᵇ   |
| HCT        | 7.85±0.07ᵈ        | 12.8±0.8ᵃ       | 31.2±2.6ᵃ       | 6.9±0.4ᵈ     | 23.5±0.7ております |
| MCV fl     | 154.6±0.5²ᵃ       | 126.2±11.3ᵇ     | 161.0±8.4ᵃ      | 131.1±7.4²ᵃ  | 159.8±7.1ᵃ    |
| MCH pg     | 47.45±0.77ᵐᵇ⁵     | 51.8±1.6³ᵃ     | 47.5±2.2¹ᵇ     | 43.0±0.9ᵇائه | 41.5±3.5³ᵃ    |
| MCHC g/dl  | 28.9±1.1³ᵃ        | 31.25±1.76ᵇᵇ   | 33.15±1.20ᵇᵇ   | 32.5±1.48ᵇᵇ | 41.25±2.19ᵃᵇ |

**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 hematological parameters related to WBCs showed slight increase in different white blood cell however; RBCs and related parameters did not show 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, research aimed at evaluating the costs/benefits ratio and the effects on organisms and environments are increasing. In general, additives are nutritive or non-nutritive ingredients that are added to the diet alone 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 metabolism 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 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 catfish. 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 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 D₃ supplemented diet (Tavekkjåkn 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 T₄ which was similar to the results of (Lock et al., 2010) and (Graff et al., 2002) but the level decrease with further increase (T₅). 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 (Ibrahim et al., 2010 and Rahman et al., 2006).

Organoelotopic evaluation of the study did not reveal 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 hematolgy 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 T3 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, (Andrade et al., 2007) 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 increament 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 hematolgy 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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