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Evaluation of haematological and biochemical parameters in fingerlings of Indian major carp *Labeo rohita* fed with nutraceutical Stimulin

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Abstract
The present study was designed to evaluate haematological and biochemical effect of commercial nutraceutical Stimulin on the fingerlings of Indian major carp *Labeo rohita*. Therefore, the fingerlings with average weight of 7.203 ± 0.24 g were distributed randomly into four treatment groups T1, T2, T3 and T4. Experimental diets were prepared by mixing rice bran, deoiled mustard oil cake, deoiled soybean cake and vitamin mineral mixture. The nutraceutical Stimulin was incorporated into diet D2 @ 0.5%, D3 @ 0.75% andD4 @ 1.00%. In control diet D1, Stimulin was not incorporated. T1 group fishes were fed with D1 diet, T2 with D2, T3 with D3@ 5% body weight per day for 90 days. The water quality parameters were regularly monitored. Fingerlings fed with diet D4 in T4 group achieved higher value of total leukocyte counts (33.646×10^3/µl), total erythrocyte counts (3.26×10^6/µl), haemolglobin (9.9 g %), total serum protein (8.8 g/dl), albumin (1.445 g/dl) and globulin (7.358 g/dl) as compared to control and all other treatments. Haematological examination was carried out to estimate the effect of experimental diet on the fish to evaluate the growth, stress resistance and specific and non specific immunity of the fish. The obtained results indicated that T4 was the best treatment which realized significant ($P<0.05$) increase in all haematological and biochemical parameters. The results indicated that Stimulin has very good immunostimulating and does not have any adverse effect in health of raising *Labeo rohita* in culture system.

Keywords: Nutraceutical, Labeo rohita and Stimulin

Introduction

Development of aquaculture ismainly depended on availability of compatible and suitablediets. Nutrition and feeding play a central and essential role in the sustained development of aquaculture and, therefore, fertilizers and feed resources continue to dominate aquaculture needs.

Many of medicinal herbs and their chemical components are used as an immunostimulants which are used in artificial diet preparation, aquaculture research and their practices. Many of the herbal plants have the ability to inhibit the microbial pathogens and activate the immunity (Immanuel *et al*., 2004; Chansue *et al*., 2000: Dugenci *et al*., 2003) [12, 4, 10]. Medicinal plants have been used in various traditional systems, as they have immune potential against numerous diseases (Kottai Muthu *et al*., 2005) [15]. Remediation through plant materials would be cheaper, cost effective and eco-friendly with no deleterious effects. Immunostimulation is an alternative effective method against vaccination. It may be achieved through only feed supplement. Commercial vaccines are expensive for fish farming practices and are specific against particular pathogens (Raa *et al*., 1992) [22]. One of the most promising methods of controlling diseases in aquaculture is strengthening the defense mechanisms of fish through prophylactic administration of immunostimulants (Robertsen, 1999) [23].

The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al*., 2001) [2]. Blood biochemistry parameters can be also used to detect the health of fish (De Pedro *et al*., 2005) [9]. Exogenous factors, such as management (Svobodova *et al*., 2008) [28], diseases (Chen *et al*., 2005) [6] and stress (Cnaani *et al*., 2004) [7], always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress (Skjervold *et al*., 2001) [26]. The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions

(Lermen *et al.*, 2004) [17]. Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters (Coz-Rakovac *et al.*, 2005) [8]. Stimulin is a unique and powerful blend of immune stimulatory molecules that have been derived from natural sources and have the strong capability to activate the nonspecific defence mechanism in fish and shrimps. It modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens. Stimulin induce reactions such as change in haemocyte count, induction of encapsulation reactions, compete with bacteria such as *Vibrio / Aeromonas* for iron and prevents attachment of bacteria to gut and influence clotting reactions. Stimulin being an herbal extract from natural sources is thus an effective way out to increase the non- specific immunity in fish.

2. Material and methods

2.1 Fish collection and maintenance: The experimental work was carried out at Wet Lab of the College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar, in tarai region of Uttarakhand. One hundred and eighty specimens of healthy and disease free fingerlings of *Labeo rohita*, weighing with average body weight of 7.203±0.24 g were procuredand acclimatized for 2 days in cemented tanks under indoor captive conditions in aerated water and stocked in FRP tanks.

2.2 Experimental design: Four treatment combinations were made as: T1- Control diet without Stimulin, T2 - diet with 5 g of Stimulin per kg feed, T3- diet with 7.5g of Stimulin per kg feed and T4- diet with 10 g of Stimulin per kg feed. The experiment consisted of 4 groups with triplicates (4T×3R= 12). Twelve equal sized FRP tanks each having 400 L water, were used for the experiment. The water was aerated every day for 4 hours. Each tank was stocked with 15 fingerlings of *Labeo rohita*. Water quality parameters were monitored on daily basis. The physico-chemical parameters of water such as temperature (19.90-20.94 °C), dissolved oxygen (7.44-7.63 mg/l), free carbon dioxide (0.29-0.49 mg/l), pH (7.49-7.65) and total alkalinity (106.66-110.75 mg/l) were in optimum range during the experimental period. Fishes for growth estimation were sampled fortnightly. Haematological and biochemical analysis was done at the end of the experiment (90^{th} day).

2.3 Formulation of experimental diets: Four isoproteinous diets were prepared by mixing rice bran (49.50 %), deoiled mustard cake (24.75 %), deoiled soybean cake (24.75 %) and agrimin forte (1.00%). The experiment feed proximate composition were moisture (11.0%), ash (7.00%), crude protein (28.00%), crud fat (7.50%) and crude fibre (6.00%). The control treatment fishes were fed with normal diet while experimental fishes were fed with diet having Stimulin D2 (5 g /kg of feed), D3 (7.5 g /kg of feed) and D4 (10 g/kg of feed) @ 5% body weight per day in two equal installments for 90 days.

2.4 Calculation of Haematological parameters

Total Leukocyte Count (TLC) = (No. of cells in four corner grids × 50) / µl.

Total Erythrocyte Count (TEC) = (No. of cells in 5 small squares × 10,000) / µl.

Haemoglobin (Hb) = Reading was taken at the lower meniscus of Sahli's haemoglobinometer in terms of gm %.

2.5 Calculation of Biochemical parameters

Total serum protein (TSP) (in g/dl) = (Absorbance of test / Absorbance of standard) ×6.5

Albumin (in g/dl) = (Absorbance of test / Absorbance of standard) ×4

Globulin = Total serum protein – Albumin

3. Results and discussion

Water quality plays important role in growth and survival of aquatic organisms. It is determined by various physical chemical and biological parameters of water body. The values of water quality parameters presented in Table 3 were statistically non-significant to each other at 5% level of significance. The results are in accordance with earlier findings of Kumar *et al.* (2007) [16] in their study on impact of *Glycyrrhiza glabra* as growth promoter in the supplementary feed of an Indian major carp *Cirrhinus mrigala*.

The details of various hematological and biochemical parameters in different treatment groups have been included in Table 4. The data on various parameters showing the effect of Stimulin into diet D1 (no Stimulin), D2 @ 0.5%, D3 @ 0.75% and D4 @ 1.00% on blood in *Labeo rohita*.

3.1 Hematological analysis

3.1.1 Total leukocyte count (TLC): The highest TLC level was found in T4(diet with 1.00% of Stimulin) which was significantly different from T1, T2, T3($P<0.05$). The treatments means revealed that the best total leukocyte count was recorded in T4 (33.646 × 10^3/µl), followed by T3 (28.9× 10^3/µl), T2 (24.50× 10/µl) and minimum in T1 (21.135× 10^3/µl). Thus the treatment T4showed best TLC level which is significantly ($P<0.05$) high as compared to control T1 as well as other treatments (Table 1).The results are in conformity with the findings of Innocent *et al.* (2011) [13] who found significant increase in TLC ($P<0.01$) in *Coriandrum sativum* added diet of *Catla catla* for 14 days. Similar results are indicated by Sahu *et al.* (2007) [24] who reported that WBC counts were higher in *Lobeo rohita* fingerlings fed *Mangifera indica* kernel when compared to control. Jha *et al.* (2007) [14]also reported that WBC count was increased in *Catla catla* administered with yeast RNA, w- 3 fatty acid and b-carotene.

3.1.2 Total erythrocyte count (TEC): The highest TEC level was found in T4/D4 (diet with 1.00% of Stimulin) which was significantly different from T1, T2 and T3 ($P<0.05$). The treatments means revealed that the best total erythrocyte count was recorded in T4 (3.26× 10^6/µl) followed by T3 (2.98 × 10^6/µl), T2(2.86 × 10^6/µl) and T1control (2.20× 10^6/µl) (Table 1). Innocent *et al.* (2011) [13] also found same results in *Catla catla* fed with *Plumbago rosea* added diet, post challenged with *Aeromonas hydrophila*. He observed increase in TEC ($P<0.05$) as compared to control and other treatments. Harikrishnan *et al.* (2003) [11] also found increased levels of erythrocyte and haemoglobin content were observed in *A. hydrophila* infected *Cyprinus carpio* treated with *Azadiracta indica*.

3.1.3 Haemoglobin: The haemoglobin analysis of fish blood showed the highest haemoglobin level in treatment T4/D4 (diet with 1.00% of Stimulin). The treatments mean revealed that the best Hb level was recorded in T4(9.9 g %) followed by T3(8.6 g %), T2(7.3 g %) and least in T1control (6.9 g %).

The Hb concentration level in T4, T3 and T2 were significantly (P<0.05) high as compared to control (Table 1). The above findings are in agreement to Asadi *et al.* (2012) [1] who observed immunomodulatory effects of watercress extraction on immunological and haematological parameters of rainbow trout (*Oncorhynchus mykiss)*.Sivagurunathan *et al.* (2011) [25] investigated the immunostimulatory potential of ginger (*Zingiber officinalis*)and turmeric (*Curcuma longa*) in *Cirrhinus mrigala* exposed to *Pseudomonas aeruginosa*. Administration of turmeric and ginger not only prevented a drastic decline in TEC and Hb values thereby improving the general health of the fish to withstand the stress condition.

3.2 Biochemical analysis

3.2.1 Total serum protein (TSP): The biochemical analysis of the fish showed that the total serum protein level was highest in T4/D4 (diet with 1.00% of Stimulin) which was in the range of (8.8 g/dl). It was significantly (P<0.05) high as compared to control T1 as well as other treatments. T3 treatment showed second best TSP level (7.42 g /dl) which was also significantly (P<0.05) high as compared to T2 (6.638 g/dl) and control T1 (5.32 g/dl) treatment. This result was supported by the study of founding that serum protein values were always higher in the fish treated with different immunostimulant than those in the control. Increase in the serum protein levels is thought to be associated with a stronger innate immune response in fish. (Wiegertjes *et al.* 1996) [29].

3.2.2 Albumin: The best albumin level (1.445 g/dl) was found in T4 treatment fed with D4 (diet with 1.00% of Stimulin) which was significantly different from T1 control (1.186 g/dl) (P<0.05). Treatment T3 showed the second best albumin level (1.412 g/dl) followed by T2 with the albumin level of (1.332 g/dl), which was significantly different from control treatment. Mohsen Abdal-Tawwab *et al*., (2010) [18] observed an increase in serum protein, albumin and globulin levels in Tilapia fed with Green tea incorporated diet and infected with *A. hydrophila*. Similarly results were also observed by Sudagar and Hajibeglou (2010) [27] observed in *C. carpio* fed with feed incorporated with mixed plant extracts *(Inula helenium*, *Tussilago farfaro*, *Brassica nigra*, *Echinacea purpurea* and *Chelidnium majus*) for 60 days and infected with *A. hydrophila*.

3.2.3 Globulin: The biochemical analysis of fish blood showed that the best globulin level was in T4/D4supplemented with 1.00% of Stimulin. The treatments mean revealed that the best globulin level was recorded in treatment T4 (7.358 g/dl), followed by treatment T3 (6.008 g/dl), treatment T2 (5.306 g/dl) and minimum in T1 control treatment (4.134 g/dl). Thus treatment T4 showed best globulin level which was significantly (P<0.05) high as compared to control T1 as well as other treatments. Treatment T3 showed second best globulin level which was also significantly (P<0.05) high as compared to control T1 as well as other treatments. Pourmoghim *et al.* (2015) [21] found similar results in the dietary of *Origanum vulgare* extract supplementation in rainbow trout enhanced total plasma protein, albumin and globulin values in comparison with control group. Similar results were reported in rainbow trout fed with garlic and ginger by Nya and Austin (2009) [19, 20], *Laurus nobilis* by Bilen and Bulut (2010) [3] and *Coggyria coggyria* by Bilen *et al.* (2011) [4].

Table 1: Observations of haematological and biochemical parameters of *Labeo rohita*

Parameter	T_1/D_1 (Control) No Stimulin	T2/D2 (0.5% Stimulin in feed)	T3/D3 (0.75% Stimulin in feed)	T4/D4 (1% Stimulin in feed)
No. of fish stocked	45	45	45	45
No. of fish harvested	41	42	41	42
Initial mean weight (g)	7.11±0.54	7.23±0.36	7.24±0.48	7.20±0.51
Final mean weight (g)	14.33±0.73	15.83±0.90	19.14±1.23	21.25±1.06
Survival (%)	91.11	93.33	91.11	93.33
TLC$\times 10^3$µl	21.135 ± 0.044	24.500 ± 0.042	28.9 ± 0.419	33.646 ± 0.014
TEC$\times 10^6$µl	2.20 ± 0.003	2.86 ± 0.002	2.98 ± 0.434	3.26 ± 0.043
Haemoglobin (g%)	6.9 ± 0.0614	7.3 ± 0.521	8.6 ± 0.440	9.9 ± 0.280
Total serum protein (g/dl)	5.32 ± 0.54	6.638 ± 0.287	7.42 ± 0.057	8.8 ± 0.028
Albumin (g/dl)	1.186 ± 0.002	1.332 ± 0.004	1.412 ± 0.050	1.445 ± 0.057
Globulin (g/dl)	4.134 ± 0.330	5.306 ± 0.481	6.008 ± 0.187	7.358 0.069

4. Conclusion

It can be concluded from the present study that the inclusion of the commercial nutraceutical Stimulin @ 1.00% in diet of *Labeo rohita* is helpful to get good immunostimulating and survival without having any adverse impact on health of fish as well as on aquatic environment.

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