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Evaluation of immunostimulatory effect of feed probiotic biosyn on *Labeo rohita*

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Abstract
The present study was carried out from February to May 2015 to evaluate the effects of dietary supplementation of commercial probiotic Biosyn in the fingerlings of Indian major carp (*Labeo rohita*) on haematological and biochemical parameters. Therefore, the fish with similar body weight (7.239 ± 0.232 g) were distributed randomly into four treatment groups T_0, T_1, T_2 and T_3. Experimental diets were prepared by mixing rice bran, deoiled mustard oil cake, soybean cake and vitamin mineral mixture. The biomedicine Biosyn was incorporated in to diet D_1 @ 0.2%, D2 @ 0.4%, D3 @ 0.6%. In control diet D_0, Biosyn was not incorporated in feed. The fishes were fed @ 5% body weight per day for 90 days. T_3 group of fishes showed higher value of TLC (35.114×10^3/µl), TEC (3.35×10^6/µl), Hb concentration (10.2 g%), Total serum proteins (9.1g/dl), Albumin(1.695 g/dl) and Globulin (7.405 g/dl) as compared to control and other treatments. The obtained results indicated that T_3 was the best treatment which realized significant ($P<0.05$) increase in all haematological and biochemical parameters. There were no adverse effects on water quality parameters among all the experimental treatments. Consequently, from the obtained results, it can be concluded that the inclusion of the commercial probiotic Biosyn @ 0.6% in diet of *Labeo rohita* is useful to get the best fish performance with friendly effects on the environment.

Keywords: biomedicine, haematology, probiotic, total serum protein

Introduction
The demand for animal protein in India is expected to increase progressively with the increase in human population. Aquaculture is one of the most important options in animal protein production and requires high quality feeds with high protein content as well as some complementary additives to keep organisms healthy and favour the growth. The use of probiotics as farm animal feed supplements dates back to the 1970. One of the best methods to reduce feed cost is through the use of feed additives (Lara Flores *et al*., 2003) [12]. Their primary effects are to improve feed efficiency and / or daily gain. The use of these additives allows fish farmers to maximize the performance through improvement in health, reproduction and feed efficiency (Marzouk *et al*., 2008) [13].

Probiotics are live microorganism which can affect the host animal by improving their intestinal flora as supplements to improve growth performance (Kesarcodi Watson *et al* 2008) [11] and they are used as antimicrobial agents (Moriarty 1997) [15]. On the other hand, they are effective agents to decrease the effects of stress and resulted in higher production in aquaculture (Ghazalah *et al* 2010) [9]. Mechanism of action of probiotics is very different. Some of these mechanisms are included as inhibition of pathogens via production of antagonistic compounds, competition for attachment to cell, competition for absorption of nutrients, alteration of enzymatic activity of pathogens (Bomba *et al* 2002) [4]. Many studies showed the positive effects of viable microorganisms as probiotic in diets of fish (Brunt & Austin 2005; Pangrahi *et al* 2005; Barnes *et al* 2006; Abo-State *et al* 2009) [6, 16, 3, 1]. Probiotics must be considered as potentially useful for the control of fish diseases (Irianto & Austin 2002) [10]. In aquaculture, persistent disease problems necessitate the use of bacteria as probiotics and as alternatives to antibiotic. The common probiotics used in aquaculture, belonging to *Lactobacillus sp., Bacillus* sp., *Bifidobacterium sp., Vibrio sp., Saccharomyces sp., Enterococcus sp. Bacillus subtilis*, are now being used for oral bacteriotherapy in aquaculture. Biosyn is an optimum blend of feed probiotics and it consists of optimum number of colony forming units, counts of selected strains of bacteria which are capable of creating a healthy gut flora in fish. Probiotics in Biosyn helps in decomposition of excess and waste organic matter and suppress the growth of undesirable microbes in the pond and there by helps to keep the water clean. The present study was carried out to evaluate the effect of commercial probiotic Biosyn on haematological and biochemical parameters in *Labeo rohita.*

Material and Methods

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 7.239±0.232 g were procured, acclimatized for 2 days in cemented tanks under indoor captive conditions in aerated water and stocked in FRP tanks.

Experimental design: Four treatment combinations were made as: T_0- Control diet without Biosyn, T_1- diet with 2 g of Biosyn (0.2%) per kg feed, T_2- diet with 4g of Biosyn (0.4%) per kg feed and T_3- diet with 6 g of Biosyn (0.6%) 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*. The control treatment fishes were fed with normal diet while experimental fishes were fed with diet having Biosyn D_1 (2 g /kg of feed), D_2 (4 g /kg of feed) and D_3 (6 g/kg of feed) @ 5% body weight per day in two equal instalments for 90 days. Four isoproteinous diet were prepared by mixing rice bran, deoiled mustard cake and deoiled soybean cake (Table1). The proximate composition of experiment feed is presented in (Table 2).

Table 1: Composition of experimental feed for *Labeo rohita*

Sl. No.	Ingredients	% Composition of ingredients
1.	Rice bran	49.5
2.	Mustard oil cake	24.75
3.	Soybean meal	24.75
4.	Agrimin Forte	1.00
	Total	100

Table 2: Proximate composition of experimental feed *Labeo rohita*

Sl. No.	Contents	Percentage (%)
1	Moisture	11.0
2	Ash	7.0
3	Crude protein	28.0
4	Crude fat	7.50
5	Crude fibre	6.0

The water quality parameters were also analysed during the study period (Table 3). Different methods of chemical analysis were carried out according to Boyd, (1984) [5] and APHA (1985) [2], and by using analysis of variance it was found that there was no significant difference amongst the treatments. The growth parameters were analyzed fortnightly.

Table 3: Profile of water quality parameters in the experimental tanks

Parameters	Mean ± SE			
	Control T_0	Treatment T_1	Treatment T_2	Treatment T_3
Water temperature	19.83 ± 0.01	20.63 ± 0.05	19.77 ±0.01	20.26 ± 0.05
Dissolved Oxygen	7.50 ± 0.01	6.85 ± 0.05	7.50 ± 0.01	7.45 ± 0.01
Free CO_2	0.55 ± 0.04	0.46 ± 0.05	0.37 ± 0.04	0.28 ± 0.03
pH	7.52 ± 0.05	7.62 ± 0.06	7.60 ± 0.04	7.51 ±0.04
Total alkalinity (mg/l)	104.40 ± 0.59	113.91 ± 0.64	103.83 ± 0.61	105.00 ± 0.62

Haematological and biochemical studies: 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. Haematological and biochemical analysis was done at the end of the experiment (90^{th} day).

Collection of blood samples: Each fish was individually caught by using scoop net, weighed and blood was collected from caudal peduncle using 2 ml syringe. Blood was collected in EDTA anti-coagulant vials. In order to collect the blood for serum protein blood was collected in sterilized ependorf tubes without addition of anti coagulant.

Estimation of Total Leukocyte Count (TLC): Leucocytes were counted by using improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1968) [7]. TLC implies the total number of WBC in 1 µl of blood. It was calculated by using haemocytometer.

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

Estimation of Total Erythrocyte Count (TEC): Total erythrocytes were counted with the help of improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1968) [7]. TEC implies the total number of RBC in 1 µl of blood. It was calculated by using haemocytometer.

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

Haemoglobin estimation: Graduated measuring tube of Sahli's haemoglobinometer was used for haemoglobin estimation. Reading was taken at the lower meniscus in terms of gm %.

Biochemical analysis

Total serum protein

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

Albumin

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

Globulin

Globulins = Total serum protein – Albumin

Result and Discussion

Haematological parameters

Total leukocyte count (TLC): The highest TLC level was found in treatment T_3 (diet with 0.6% Biosyn) which was significantly different from T_0, T_1 and T_2 ($P<0.05$). The treatments means revealed that the best total leukocyte count was recorded in T_3 (35.114×10^3 /µl), followed by T_2 (29.9×10^3 /µl), T_1 (25.3×10^3 /µl) and minimum in T_0 (22.240×10^3 /µl). The results are in confirmatory with the study of Sameh *et al.* (2011) [18] who incorporated some probiotics in the feed of Nile tilapia (*Oreochromis niloticus)* for 70 days. The results indicated that there was significant ($P<0.05$) increase in red blood cell count, haemoglobin and haematocrit count.

Total erythrocyte count (TEC): The lowest TEC level was found in T_0/D_0 (diet with 0% Biosyn). The treatments means revealed that the best total erythrocyte count was recorded in T_3 (3.35×10^6 /µl) followed by T_2 (3.12×10^6 /µl), T_1 (2.98×10^6 /µl) and T_0 control (2.45×10^6 /µl). The highest TEC level was found in T_3/D_3 (diet with 0.6% Biosyn) which was significantly different from T_0, T_1 and T_2 ($P<0.05$). Dahiya *et al.* (2012) [8] also found the comparable results in Indian Magur (*Clarias batrachus L.*) fed with probiotic diet. Their results indicated that probiotic had a positive effect on haemoglobin level and erythrocytes count.

Haemoglobin: The observations on haemoglobin in different treatment groups has been presented in (table 4)The haemoglobin analysis of fish blood showed the highest haemoglobin level in treatment T_3/D_3 (diet with 0.6% Biosyn). The treatments mean revealed that the best Hb level was recorded in T_3 (10.2 g %) followed by T_2 (9.8 g %), T_1 (7.9 g %) and least in T_0 control (7.6 g %). Treatment T_3 showed highest Hb concentration level which was significantly ($P<0.05$) high as compared to control as well as other treatments. Mehrim (2011) [14] incorporated probiotic supplement in the diet of monosex Nile tilapia (*Oreochromis niloticus)* for 14 weeks and found significant increase in haemoglobin level ($P<0.05$) in probiotic added diet fed fishes as compared to fishes fed on control diet.

Biochemical parameters

Total serum protein (TSP): The biochemical analysis of the fish showed that the total serum protein level was highest (9.1 g/dl) in T_3/D_3 (diet in 0.6% Biosyn) which was significantly ($P<0.05$) high as compared to control T_0 as well as other treatments. The level of TSP was followed by T_2 (8.7 g /dl), T_1 (7.325 g/dl) and T_0 (6.5 g/dl) treatment. (Table 4)

Albumin: The best albumin level (1.695 g/dl) was found in T_3 treatment fed with D_3 (diet with 0.6% Biosyn) which was significantly different from T_0 control (1.480 g/dl) ($P<0.05$). Treatment T_2 showed the second best albumin level (1.660 g/dl) followed by T_1 with the albumin level of 1.610 g/dl, which was significantly different from control treatment. (Table 4)

Globulin: The treatments mean revealed that the best globulin level was recorded in treatment T_3 (7.405 g/dl), followed by treatment T_2 (7.04 g/dl), treatment T_1 (5.715 g/dl) and minimum in T_0 control treatment (5.02 g/dl). (Fig. 6).Thus treatment T_3 showed best globulin level which was significantly ($P<0.05$) high as compared to control T_0 as well as other treatments. Treatment T_2 showed second best globulin level which was also significantly ($P<0.05$) high as compared to control T_0 as well as other treatments.

Mehrim (2011) [14] also found the similar results while assessing the growth and immunostimulatory effects of bio medicine Biogen R on monosex Nile tilapia, *Oreochromis niloticus*, under different stocking densities. Highly significant increase was observed in T_4 i.e. Biogen at a level of 3 g/kg diet at stocking density rate of 30 fish/m^3 of monosex Nile tilapia (*Oreochromis niloticus*) and it was regarded as the best treatment which significantly ($P<0.05$) increased all growth performances (final weight, AWG, ADG, SGR), haematological parameters (haemoglobin, RBC count, PCV, blood platelets and WBC count), plasma proteins (total proteins, albumin, globulin and albumin/globulin ratio) improved FCR, blood indicies (MCV, MCH and MCHC), differential leucocytes, carcass composition, histometric examination of fish dorsal muscle. Rajesh *et al.* (2006) [17] also found the comparable results while evaluating the potential of *Bacillus subtilis* on growth, haematology and biochemical parameters in Indian major carp *Labeo rohita* challenged with *Aeromonas hydrophila.* The TLC, Hb content, total protein and gslobulin content was significantly ($P<0.05$) higher as compared to control after two weeks of experimental period.

Conclusion

It can be concluded from the present study that the inclusion of the commercial probiotic Biosyn @ 0.6% in diet of *Labeo rohita* is helpful in having good immunostimulatory effects and good survival without having any adverse impact on health of fish as well as on aquatic environment.

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Table 4: Haematological and biochemical observations in *Labeo rohita*

Parameters	T_0/D_0(Control)	T_1/D_1 (0.2%)	T_2/D_2 (0.4%)	T_3/D_3 (0.6%)
No. of fish stocked	45	45	45	45
No. of fish harvested	42	42	41	43
Survival (%)	93.33	93.33	91.11	95.55
TLC $\times 10^3$ /µl	22.240±0.040	25.3±0.39	29.9±0.394	35.114±0.013
TEC $\times 10^6$ /µl	2.45±0.004	2.98±0.003	3.12±0.402	3.35±0.034
Haemoglobin g%	7.6±0.0514	7.9±0.520	9.8±0.032	10.2±0.0244
Total serum protein (g/dl)	6.5±0.052	7.325±0.310	8.7±0.041	9.1±0.023
Albumin (g/dl)	1.480±0.003	1.610±0.003	1.660±0.051	1.695±0.052
Globulin (g/dl)	5.02±0.384	5.715±0.429	7.04±0.549	7.405±0.582

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