THE EFFECT OF PROBIOTIC DIET ON GROWTH AND HEMATOLOGY PARAMETERS OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS*, WALBAUM 1792)

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Abstract

This study was undertaken to examine the effect of dietary probiotics on rainbow trout, based on growth performance and hematological profile. The 2 species of endospore bacillus, B. licheniformis and B. subtilis, as a blend of probiotic bacteria, were employed in 3 concentrations for diet supplementation (V_1:22.4x10^9, V_2:38.4x10^9 and V_3: 70.4x10^9 CFU g^{-1}). The effects were compared with those of control diet, containing no probiotic. The trout with initial mean body weight of 101.96±2.26 g was fed with 3 percentage of their body weight, 3 times per day. After feeding with supplemented diets for 4 weeks, growth performance, feed utilization and health status were assessed. The comercial probiotic mixture showed an improvement of feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) at concentration of 22.4x10^9 CFUg^{-1}. The hematological indices (hemoglobin, red blood cells and hematocrit) showed differences among experimenatal variants. In the present study a higher levels of probiotics does not correlates with the best results in terms of growth and physiological status. Thus is demonstrated that the appropriate doze of probiotic should be established before application in intensive aquaculture in order to prevent any unwanted effects.

Key words: probiotic, growth performance, hematology indices, recirculating aquaculture

INTRODUCTION

The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. Probiotics are usually defined as live microbial feed supplements, that are administered in such a way as to enter the gastrointestinal tract and to be kept alive; this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health [3] [5].

The use of probiotics in human and animal nutrition is well documented [2],[16] and recently, they have begun to be applied in aquaculture [3], [5], [15], [8].

Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes [15].

Because Bacillus bacteria secrete many exoenzymes [11], these bacteria have been widely used as putative probiotics.

Commercial "BioPlus®2B" probiotic consists of Bacillus licheniformis and Bacillus subtilis. Both bacterial species are characterized by a high proteases, lipases, and amylases production, in the small intestine. They also accelerate the metabolism and increase nutrient utilization. At the same time, they produce volatile fatty acids that decrease the pH of digestive tract, enhance the development of lactacidogenic microflora and reduce the number of pathogenic E. coli.

The present study aimed to determine the effects of dietary supplementation with commercial probiotic BioPlus®2B: mixture of Bacillus licheniformis (DSM 5749) and

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Bacillus subtilis (DSM 5750) in ratio 1:1.) on growth performance and blood hematological parameters of rainbow trout, at the same stocking densities, for 30 days.

MATERIAL AND METHOD

The experiment took place at the recirculating aquaculture system station, of Aquaculture, Environmental Science and Cadastre Department, from "Dunarea de Jos" University of Galati and lasted 30 days, between February and March 2011, in four rearing units, with a capacity of 0.320 m^3 each (40×80×100 cm). The starting date was 17th of February, when the system was populated with a number of 38 rainbow trout fingerlings per rearing unit. Classic Extra 1P pellets, 41% protein content, having incorporated a probiotic product, BioPlus®2B, of different concentrations, consisting of Bacillus licheniformis (DSM 5749) and Bacillus subtilis (DSM 5750) in a ratio of 1:1, was used. The feeding ratio was 3% of the body weight. The feeding frequency was 2 times per day and was done manually. Four experimental variants were established and compared:

- V_0 - pellets with 41% crude protein, without probiotics;
- V_1 - pellets with 41% crude protein, with probiotics - $22,4\times10^9$ CFU/kg food;
- V_2 - pellets with 41% crude protein, with probiotics - $38,4\times10^9$ CFU/kg food;
- V_3 - pellets with 41% crude protein, with probiotics -$70,4\times10^9$ CFU/kg food.

The general behavior and welfare (feeding behavior, swimming behavior and survival rate) were registered and evaluated daily, for further examination. Temperature, pH and dissolved oxygen concentration were also daily monitored. Every four days ammonium, nitrite and nitrate concentrations were checked and if the values exceeded the critical levels, the water was exchanged (but not more than 10% daily) in order to maintain the values within the optimal range. The following equipment was used to measure the water quality parameters: oxygen concentration and percentage saturation were measured with the Hanah HI 98186 oxymeter. Determination of $N\text{-}NH_4$, $N\text{-}NO_2$, and $N\text{-}NO_3$ was carried out using the of the Spectroquant Nova 400 photometer. At the end of the experimental trial, 1 ml of blood was sampled from a number of 5 fish, for each experimental variant, by caudal venous puncture, using lithium heparin as anticoagulant. The red blood cell counts ($RBC_c x10^6/\mu l$) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian dilute solution. The hematocrit (PVC,%) was determined, in duplicate, by using heparinised capillary tubes and centrifuged for 5 minutes at 12000 rpm in a micro hematocrit centrifuge. Haemoglobin concentration (Hb, g/dl) was determined by photometrical cyanmethemoglobin method. Using standard formulas according to [6], the red blood indices were calculated: the mean corpuscular volume (MCV, µm3), the mean corpuscular haemoglobin (MCH, pg) and the mean corpuscular haemoglobin concentration (MCHC, g/dl).

At the end of the experiment the fish were weighed, and the following growth performance indicators were calculated: biomass growth (WG), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). The formulas that were used are shown below:

- Weight gain (WG) = Final weight (Wt) Initial weight (W_0) (g);
- Food conversion ratio (FCR) = Total feed (F) / Total weight gain (W) (g/g);
- Specific growth rate (SGR) = 100 x (ln Wt – ln W0) / t (% BW/day);
- Protein efficiency ratio (PER) = Total weight gain (W) / amount of protein fed;

The water quality paramaters and haematological parameters of the experimental groups were expressed by mean and standard deviation (M±St. Dev.) and the differences between the values were statistic analyzed with t-Student test.

RESULTS AND DISCUSSIONS

Rearing water quality properties:

As it is presented in Table 1, all tested water quality values were within the optimal range for rearing rainbow trout. To maintain water parameters within the allowable limits,

we proceeded to remove organic waste and uneaten feeds. Average water temperature, DO, pH, N-NH_4, N-NO_2, and N-NO_3, during the experiment, were: 13,56±0,89ºC, 6,83±0,50 mg/l^{-1}, 7,02±0,37 pH units, 0,05±0,07 mg/l, 0,11±0,08 mg/l, 77,08±18,64 mg/l.

Table 1 Synthetic table with temperature, DO ,nitrogen and phosphate compounds average values (± SD) during the experimental period

Parameter	V0	V1	V2	V3
Temperature (^{0}C)	13,63±0,91	13,54±0,9	13,58±0,86	13,49±0,92
DO (mg/ l^{-1})	6,84±0,52	6,92±0,48	6,58±0,61	6,95±0,60
pH (unit.pH)	7,10±0,31	7,02±0,41	7,01±0,39	6,98±0,39
N-$N0_2$ (mg/l)	0,11±0,06	0,08±0,07	0,14±0,15	0,14±0,12
N-NO_3 (mg/l)	82,20±16,6	77,28±18,34	73,95±15,92	74,42±18,67
N-NH_4 (mg/l)	0,09±0,11	0,04±0,06	0,06±0,09	0,06±0,05

Growth performance:

Data related to rainbow trout growth performances, including initial weight, final weight and daily weight gain are presented in Table 2. No significant difference was observed for initial weight betwen the treatment group and control ($p<0,05$). During the feeding trial, no mortalities were recorded in any of the experimental or control groups.

Table 2 The growth parameters for all experimental variants

Growth performance / Experimental variant	V_0	V_1	V_2	V_3
	(control)	$2.24x10^9$ CFU g^{-1}	$3.84x10^9$ CFU g^{-1}	$7.04x10^9$ CFU g^{-1}
Number of fish	38	38	38	37
Initial biomass (g)	3843.05	3861.74	3796.62	3891.55
Mean individual weight (g/fish)	101.13	101.62	99.91	105.18
Initial stocking density (kg/m^3)	12,00	12,06	11,86	12,16
Final numbers of fish	38	38	38	37
Final biomass (g)	7156,45	7340	6927,30	6991,12
Mean final fish weight (g/fish)	188,33	193,16	182,30	188,95
Final stocking density (kg/m^3)	22,36	22,93	21,65	21,84
Individual weight gain (g)	87,19	91,53	82,39	83,77
Total weight gain (g)	3313,40	3478,26	3130,68	3099,57
Specific growth rate (SGR) (%/day)	2,14	2,21	2,07	2,02
Daily growth rate - (g/kg/day)	3,01	3,16	2,84	2,89
Feed conversion ratio FCR (g/g)	1,01	0,96	1,07	1,08
Protein efficiency ratio PER (g/g)	2,40	2,53	2,28	2,25

Daily growth rate (GR), which is in fact the technological indicator, shows a linear growth of fish, ranging from 2,84 (V_2) to 3,16 g/day (V_1). Regarding the SGR and FCR, both indicators registered better values at V_1 variant (SGR value of 2,21 g%/day and an FCR of 0,96 g fodder/g weight gain), compared with the control grup.

The mean final weight of the control group was lower (188,33 g) than that of V_1 trial (193,16 g), but were almost similar with the V_2 (182,30 g) and V_3 (199,95 g) variant.The protein efficiency ratio (PER) also recorded an important value of 2,53 (g/g) for V_1variant and almost similar values were observed between V_2 (2,28 g/g) and V_3 variants (2,25 g/g).

The addition of probiotics to diets did not exert any effect on the growth rate of rainbow trout in the particular stages of the growth test. Similar results were reported by [7], who found that growth and feed

conversion of juvenile dentex were not significantly influenced by probiotics and [14] who revealed that probiotic used on juvenile channel catfish diet had no positive effect on specific growth or immune stimulating aspects.

Blood hematological parameters:

Hematological parameters analyzed from fish blood samples are shown in figure 1-6. The hematological exam of rainbow trout consisted of the examination of the following hematological parameters, together with the values obtained (see Fig. 1-3): erythrocyte counts (RBCc), hemoglobin (Hb) and haematocrit values (PCV) in fish fed diets containing probiotics were higher than that of the control grup, but no significant differences were recorded between the experimental variants (p>0.05).

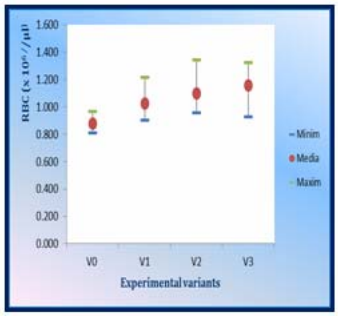

Fig. 1 Red blood cell counts variation

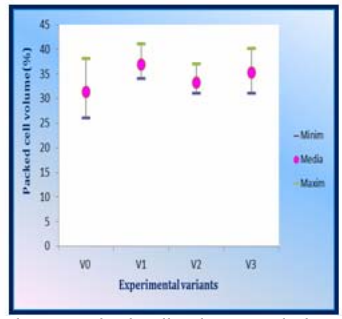

Fig. 2 Packed cell volume variation

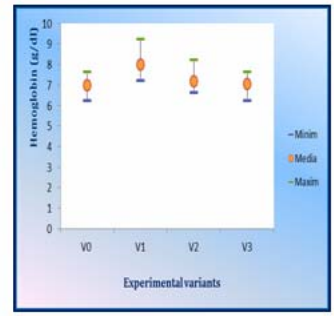

Fig. 3 Hemoglobin variation

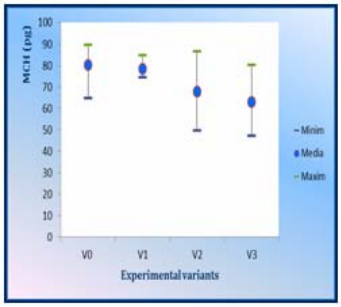

Fig. 4 Mean corpuscular volume variation

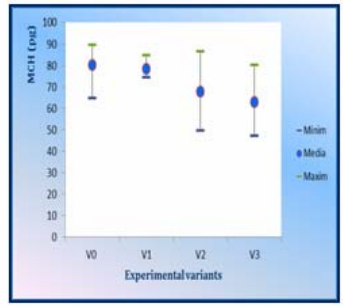

Fig. 5 Mean corpuscular hemoglobin variation

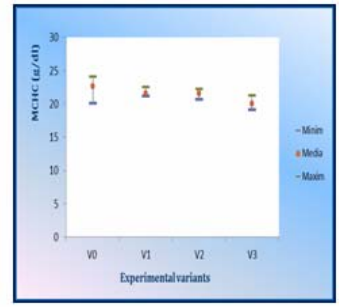

Fig. 6 MCHC variation

These results suggest an improvement of fish health in case of administrating probiotic supplement feed diets. The present findings confirm those reported by [9] who revealed that blood hematological parameters (hemoglobin, erythrocytes count) in fish fed diets containing Biogen® (commercial probiotic consists on Bacillius licheliformes and Bacillius subtilis) were significantly higher compared to the control group. Also, [10] find a positive effect represented by a significant increase in RBCs count and Hb conc. in both fish groups fed with probiotics supplemented diets (Saccharomyces cerevisae yeast and both live Bacillius subtilis and Saccharomyces cerevisae), compared with the control group, fed with probiotic free diet. By analyzing the data values regarding red blood constants (MCV, MCH and MCHC), we can notice that no significant differences (p>0,05) were recorded between the contol group and the experimental variants. So, in case of V1variant, the mean corpuscular volume (MCV, µm3) increased with 1,20% compared with the control grup, but in V2 and V3

trials, we registred a sligh decrease, with an average value of 312,246±61,56µm3 at V3, respectively 313,252±59,43 at V4 variant. Regarding the mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dl), a sligh decrease was observed, directly proportional to the increase of probiotics concentration in diet, but statistically insignificant (p>0,05). All the haematological values recorded in this study, among all treatments, ranged within the mentioned references for rainbow trout (see table 3).

Table 3 Reference interval of the hematological indices for rainbow trout (*O. mykiss*)

References	Hematological indices					
	PCV (%)	Hb (g/dl)	RBCs ($x10^6$/µl)	MCV ($µm^3$)	MHC (pg)	MCHC (g/dl)
[4]	24 - 55	7,6 - 16	0,8 – 1,5	276 - 476	55 - 82	14 - 26
[17]	47	9,4	1,33	353	71	20
[13]	32,10	7,0	1,49	216,64	47,04	21,83
[1]	34	47,8	1,18	278,96	32,96	124,82

CONCLUSIONS

The current experiment indicates that by adding a commercial probiotic "BioPlus®2B" (Bacillus licheniformis and Bacillus subtilis) in rainbow trout diet no significant differences were found in terms of fish growth. After 30 days of experimental period, the values of fish growth performance indicators, specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER), did not show any significant differences between the particular experimental variants, but it was observed a slight improvement of physiological functions and health status of the fish fed with probiotic diets, compared with those from the control grup.

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