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Dietary Supplementation of Poly-β-Hydroxybutyrate on the Growth, Digestive Enzymes Activity and Body Composition of Rainbow Trout (*Onchorynchus mykiss*)

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Research Article

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ABSTRACT

Poly-β- hydroxybutyrate (PHB) is short chain fatty acids and accumulated by many of microbes and utilizes as source of energy and carbon, the present study is planned to check the effect of dietary supplementation of poly-β-hydroxybutyrate on the growth, gut enzymatic activity and body composition of Rainbow Trout (*Onchorynchus mykiss*). The fish were fed with four types of feeds containing different levels of poly-β-hydroxybutyrate (PHB1%, PHB2%, PHB3%) and a control (PHB0%) feed without addition of any agent three times a day at feeding rate of 3% of the fish wet body weight. The results of current study revealed that dietary supplementation of poly-β-hydroxybutyrate increased the growth performance, improves body composition and immune response in rainbow trout (*Onchorynchus mykiss*) fingerlings. Overall growth performance was found satisfactory in all the treatment groups with significant difference. PHB1 group showed significant difference with the other entire group regarding FCR (1.23 ± 0.01), %weight gain (503.92 ± 14.02), SGR (3.10 ± 0.04) and weight gain (26.15 ± 0.92). There was significant difference observed in PHB1 group for crude protein, crude lipid. It was observed that PHB groups showed significantly lower value for crude fat and significantly higher value of crude protein as compare to control (PHB0) group. There was a non-significant differences present between PHB fed group and control group for fatty acid analysis There was a significant differences present between PHB fed group and control group for digestive enzymes activity. Specifically PHB1 showed significant difference with all other treatment groups. There was a significant differences present between PHB fed group and control group for hematological studied. PHB2 showed significant difference with all other treatment groups. In conclusion, the current study indicated that PHB is a valuable dietary supplement for improving growth performance, body composition and immune responses of rainbow trout fingerlings.

INTRODUCTION

Aquaculture has been always remained as an important and low-cost quality protein source for humans. During past few years it has been recognized as a fastest growing industry with an average growth rate of about 12% and Asia is playing leading role contributing 90% of the global production [1]. This growth needs to be sustainable to meet future protein demand. Fisheries

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and aquaculture has a key role in Pakistan's economy and no doubt it is the basic source of income for people of coastal areas. Instead of sea capture inland fish culture also plays a key role in increasing the country economy.

The farming history of trout is quite old among all *Salmonidae* members. Firstly it was reared in water in 1874, in 19th century first time rainbow trout farmed with artificial conditions [2]. Because of long farming history rainbow trout was considered as truly domesticated specie of present era.

Poly-β-hydroxybutyrate (PHB) is compound which is stored by bacteria normally and also has been showed positive effect as prebiotic and useful for a range of aquaculture species [3]. Poly-β-hydroxybutyrate (PHB) is a natural compound that is stored by a large variety of micro-organisms. As an intracellular energy and carbon reserve compound many microorganisms produce this compound of the fatty acid βeta-hydroxybutyrate [4]. Poly-β-hydroxybutyrate (PHB) is existing in a non-crystalline unstructured form within a bacterial cell but when lysis PHB converts to a (moderately) crystalline. The introduction of poly-β-hydroxybutyrate (PHB) for aquaculture use was first tested in 2007 when Halet et al. [5] and Defoirdt et al. [6] defined poly-β-hydroxybutyrate compound efficiency to protect *Artemia franciscana* larvae from Vibrio infection. Their results proposed that PHB protection was due to the intestinal formation of the monomer 3-hydroxybutyrate (3-HB) that had the ability to act as an anti-pathogenic compound. Since after that, the application of short chain fatty acids specially poly-β-hydroxybutyrate (PHB) was positively tested on a variety of aquaculture on different parameter such as gut microflora, gut enzymatic activity, growth, development, fatty acid profile and disease resistance immunity within giant freshwater prawn (*Macrobrachium rosenbergii*) by Nhan et al. [7] and of juvenile European sea bass (*Dicentrarchus labrax*) by Schryver et al. [3], Siberian sturgeon (*Acipenser baerii*) fingerlings [8], Chinese mitten crab (*Eriocheir sinensis*) against *Vibrio anguillarum* [9] and giant freshwater prawn larvae against Vibrio harveyi infection [10]. In all these studies the use of poly-β-hydroxybutyrate (PHB) showed significantly positive response [11].

MATERIALS AND METHODS

Study Station

The study was conducted at the indoor fish nutrition experimental station of the Department of Aquaculture, Faculty of Fisheries, Atatürk University, Erzurum, Turkey.

Experimental Setup

The experiment was carried out in twelve 50 L capacity glass aquariums for 60 days. To maintain the stable environment water was supplied at 1 L/min rate from a well with a semi-recirculation system. Water temperature in each aquarium was maintained at 13.0 ± 0.2°C. About 15 rainbow trout (*Onchorynchus mykiss*) with an average weight of 5.18 ± 0.08 g were stocked in each tank. The tanks were cleaned twice a day by siphoning and fecal matter was assembled and kept at -20°C daily. There were 3 replicates of each treatment. The fish were fed three times a day (9:00 am, 1:00 pm and 5:00 pm) at feeding rate of 3% of the fish wet body weight for first 30 days and increased to 4% fish wet body weight for next month (31st-60th day). After every three days feeding rate was readjusted for every aquarium by using expected increases in weight based on feed conversion efficiency. Feed was readjusted after every fortnight after biomass determination.

Feed Formulation

Three types of feeds were prepared containing different 1%, 2% and 3% level of poly-β- hydroxybutyrate (PHB) and a control/basal feed without addition of any stimulant. Basal diet contained around 47% protein and 20% of lipids. All the ingredients were weighed and crushed by using the grinding machine. All ingredients of feed and PHB as an additive was well mixed before extrusion in a machine mixer. The diets were kept at 35°C temperature for 24 hours and stored at -20°C.

Growth Parameters

Fish morphometric measurements (total body weight and length) were recorded at the time of initial stocking and then after fortnightly interval and fish were caught by using hand nets from aquarium. After taking measurements the fishes were transferred to their respective aquariums.

The following parameters were used to evaluate the growth of fish:

Net weight gain=final body weight (g)-initial body weight (g)

Specific growth rate (SGR)=lin (final mean body weight (g)-lin (initial mean body weight (g) × 100/duration of the experimental period (days)

Feed conversion ratio (FCR)=feed intake (g)/weight gain (g)

Proximate Analysis Fish

To check the complete fatty acid profile and complete body composition samples were collected at the termination of feeding trial. 5-6 fish samples were taken out from each tank and put in liquid nitrogen until samples have a cryogenic effect and died.

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The cryogenized sample was immediately stored at -80°C. The parameters like, dry matter, ash contents, % crude fat and crude protein of the feed and fish samples were carried out by following AOAC [12].

Total Lipids and Complete Fatty Acids Analysis

At end of trial the body tissue was taken for lipid and fatty acid analysis by following the protocol of Folch et al. [13], modified by Ways and Hanahan.

Hematological Studies

At the termination of trial, two samples from each container were captured randomly and blood samples were collected from the caudal vein. Pooled blood samples were immediately moved into vials coated EDTA. The samples were stored at -20°C until evaluated. The samples were further examined for complete blood count by using PE6800-Prokan- Hematology Analyzer.

Statistical Analysis

The data thus obtained were entered to statistical software SAS 9.1 and variance (ANOVA) under Duncan's Multiple Range Test. Data was presented as mean ± SD.

RESULTS AND DISCUSSION

The present study showed that there was a significant difference present between rainbow trout (Onchorynchus mykiss) fingerling fed with PHB-containing diets (PHB1%, PHB2%, PHB3%) and control diets (PHB0) in terms of growth parameters as PHB-enhanced percentage weight gain, FCR and SGR comparable to previous studies. It was observed that until second week of the trial poly-β-hydroxybutyrate (PHB) showed no effectiveness as a growth promoting substance for rainbow trout fingerling growth. But it may be due to the fact that in initial stages fingerlings were deprived of poly-β-hydroxybutyrate (PHB) hydrolyse enzyme and microbial community or may be a decrease in intestinal pH also played a role, as discussed by De-Schryver et al. [14]. So our finding supports the research on PHB by Hung et al. as they found that PHB enhanced growth in blue mussel, Defoirdt et al. [15] as they found that PHB can promoted the growth in brine shrimp (*A. franciscana*) larvae, De-Schryver et al. [14] also found that crystalline poly-β-hydroxybutyrate fed group showed significant increase in growth as compare to control group. Same results were reported by Nhan et al. [7] and Thai et al. [10] as both found poly-β-hydroxybutyrate in crystalline and amorphous form respectively increased growth and development in that giant fish water prawn larvae when fed with poly-β-hydroxybutyrate (PHB) enriched *Artemia nauplii*. But our finding are contradict by the findings of Situmorang et al. [16] as they found that PHB did not promote the growth in Tilapia but this is may be due to varying effect of PHB from specie to specie.

Several studies showed that chemicals which were used as prebiotics agent enhanced body protein level in rainbow trout and tilapia (*Oreochromis niloticus*). In agreement with these findings our results also indicated that PHB as a supplement in diet for rainbow trout showed significant difference between control and PHB fed groups regarding ash, crude lipids and crude protein level. Our results are also supported by Duan et al. [17] as they observed that for shrimp crude protein level in poly-β-hydroxybutyrate groups were significantly higher than control group. Our results are also in agreement with Situmorang et al. [16] as they found that in Tilapia PHB supplementation showed significantly increased in whole body composition specially crude lipid level of whole body as compare to control group. But the results of present study were contradicted with the finding of Najdegerami et al. [18] as they found that PHB did not showed any significant difference in body composition of rainbow trout fry.

For total fatty acid analysis it was observed that there was a non-significant difference present between PHB fed groups with control as supported by the findings of Situmorang et al. [16] as they found that PHB did not have significant effect on complete fatty acids analysis in Tilapia also supported by the findings that have been reported in PHB studies in rats, Nile tilapia [19], rainbow trout [20] and all the ornamental fishes [21]. But our finding are contradictory with the findings of Najdegerami et al. [11] as they found that PHB showed significant difference in total fatty acid composition of rainbow trout fry and Najdegerami et al. [8] as they found that in Siberian sturgeon fingerling PHB supplementation significantly increased total fatty acids composition of liver.

The result of present research showed that PHB can alter the digestive enzyme activity in rainbow trout fingerlings same as supported by Najdegerami et al. [11] and Duan et al. [17], they both observed that as compare to control group PHB groups showed significant difference regarding digestive enzyme activity in rainbow trout fry and shrimp respectively. But our results are contradictory with the finding of Situmorang et al. [16] as they found that high level of PHB showed non-significant effect on digestive enzyme activity of Tilapia.

Our study reveals that PHB has positive effect on the hematological parameters (WBC's, RBC's, Platelets, Hbg) response also supported by other researchers, as Uriel et al. found that group with polyhydroxybutyrate acid on rainbow have low phagocytic activities (low level of WBC's), further it was also supported by the findings in brine shrimp [22], Chinese mitten crab [9] and Mozambique tilapia [23-40].

CONCLUSIONS

In conclusion, the current study indicated that poly-β- hydroxybutyrate (PHB) is a valuable dietary supplement for improving

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growth performance, body composition, fatty acid profile and hematological parameters of rainbow trout fingerlings. By keenly observing previous studies with PHB, it was found that the fish species, size and dose of poly-β- hydroxybutyrate (PHB) supplementation are the factors that regulate the effect of PHB in aquatic species. Hence, this present study promotes further research on supplementation of poly-β- hydroxybutyrate (PHB) in trout culture as well as on the determination of the mechanisms of action and the effects on the hindgut microbial community.

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