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Effects of riboflavin on growth, hematological and immunological parameters of rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Abstract

The fishing industry is deeply dependent on complex and high quality diets consisting of dry feed, vitamins and micro/macro-elements. The suitable dose of each essential nutrient is different based on the fish species, their age and physiological conditions. In this study, the effects of two recommended doses of riboflavin (20 and 30 mg vitamin per each kg dry feed) on growth, hematological and immunological parameters of rainbow trout (*Oncorhynchus mykiss*) fingerlings were investigated. The results showed that although treatment with riboflavin could improve biometric and immunological parameters of the fishes but the higher concentration (30mg) of riboflavin had no extra-positive effects on these factors. Furthermore, both doses decreased hematopoietic activity of this fish. Overall, the results of this study showed that treatment with 20 mg of riboflavin is more effective than treatment with 30 mg of riboflavin per kg of dried food.

Keywords: Rainbow trout, Riboflavin, Growth, Haematological, Immunological

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Introduction

Malnutrition is defined as shortage in nutritional compositions and elements required for normal activity and growth of body (Black *et al.,* 2003). Although eradication of hunger is too far from reality, its severity can be reduced by improvement in the farming industries. Among them, aquaculture is considered as a suitable way to decrease the protein demands in different cultures. Therefore, improvements in the fishing industry in terms of increases in the fish productivity and reduction in costs are two critical factors to provide cost friendly protein sources (Powers, 2003). In addition to employment of advanced fishery technologies, the fishes themselves need to feed on essential nutritional factors, including vitamins (Hughes *et al.,* 1981). Vitamin supplementation in fish diets has been used widely to improve fish production in aquaculture. Vitamins improve the immunity system, meat quality, survival, growth, resistance against diseases and stressors, fecundity, and reproductive efficiency (Conklin, 1989, Gapasin *et al.,* 1998, Samocha *et al.,* 1998, Racotta *et al.,* 2004). Riboflavin is an essential nutrient for all animals including fishes (NRC, 1993). Anorexia, poor growth, and high mortality are common signs of riboflavin (vitamin B2) deficiency in various species of fish (Murai and Andrews, 1974; NRC, 1993). The recommended riboflavin dose for Salmonids is 20-30 mg per each kg dry diet (Halver, 1972). Generally, hematological parameters are used as indicators of health in fish. This study was directed to determine the effects of the recommended doses of riboflavin on biometric and hematologic parameters of rainbow trout (*Oncorhynchus mykiss*) fingerlings.

Materials and methods

Rearing tank preparation

The tests were performed in 9 sterile fiberglass tanks (100-L) (3 treatments and 3 replicates) equipped with a continuous aeration system. All effective physical parameters of the tanks, including temperature, dissolved oxygen, salinity and pH were controlled throughout the test period (10-12°C, 7.5 ppm oxygen, pH 7.8 and salinity 0.5%).

Rainbow trout preparation

After adaptation of the healthy fingerlings (*O. mykiss)* with the tank conditions for a week, fish weighing 6-7 g were chosen for the tests (33 fishes per tank).

Food preparation and treatment scheme

Based on the calculated average daily food intake (Sedgwick, 1995) for the fishes, they were fed with commercial foods (containing 42% protein, 14% fat, 10% ash, 3.5% fibre and 1.2% phosphor; prepared from Cheane Company, Iran) 5 times a day for 2 months. In addition to the control tanks, the containers were divided into three groups: group one and two were treated with 20 and 30 mg vitamin per each kg taken dry feed, respectively, while no riboflavin was added to the control group. The tests were repeated three times.

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Determination of growth parameters
The biometric factors, including length, weight, body weight gain index (BWI), the specific growth rate (SGR), daily growth rate (DGR), condition factor (K), food conversional rate (FCR) and survival percentage (S) for each treatment were calculated based on existing formula (Hung and Slinger, 1982; Ebrahimi, 2011).

Hematological parameters
The blood samples were taken from the fish artery vessel. The samples were heparinised and were analysed for red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell count (WBC) based on routine procedures (Blaxhall and Daisley, 1973; Vutukuru, 2005).

Immunological parameters
The serum samples were stored at -20°C before beginning the test. IgM concentration was measured by Nephelometery method (Gopalakannan and Arul, 2006; Rezkar Jaafar *et al.*, 2011). In this method, Antigen (IgM) was mixed with anti-IgM polyclonal antibody and the mixture was irradiated at 400 to 840 nm.

In order to prevent inactivation of lysozyme, first the sera were immediately kept at -70 °C. The lysozyme activity of sera was measured based on lytic action on *Micrococcus lysodeikticus* (Sigma, St. Louis, USA). The changes in concentration of bacterial cells were determined based on optical density (OD) of the suspension in 450nm (Jaafar *et al.*, 2011).

The total protein of the samples was measured by Tietz' procedure (Tietz, 1986). Finally, the total haemolytic complement (CH50) of the sera was assayed based on the bulletin in the used kit (DiaMetra kit, Italy) (Miller and Nussenzweig, 1975).

Statistical analysis
Based on the case, different statistic programs (SPSS Ver. 20, Shapiro-Wilk, Kruskal Wallis, One Way ANOVA and Mann-Whitney tests) were used.

Results
Following treatment of the fish with riboflavin (20 and 30 mg kg^{-1} dry feed) for 60 days, different biometric parameters of fish were measured. Although the results showed that treatments of the fish with 20 mg riboflavin could significantly increase their length and weight, the higher concentration had no extra-effect on the parameters (p>0.05) (Table 1). Also, the same patterns of improvement were seen for body weight gain (%BWI), specific growth rate (SGR) and DGR of the fish (p<0.05). Indeed, even though the treatment improved the indexes, increase in concentration of riboflavin was not effective. Furthermore, significant decreases in FCR were recorded (p<0.05). Again, no differences between the effects of these two doses on FCR were observed. Based on the results, only the higher concentration (30 mg) had a meaningful

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positive effect on the condition factor (K) (p<0.05). No meaningful differences in the survival rate of the control and treated fish were recorded (p<0.05) (Table 1).

Table 1: Changes in biometric and blood factors of fingerling rainbow trout fishes (*Oncorhynchus mykiss*) treated with 20 and 30 mg Riboflavin per kg food (p<0.05).

Parameter	Control	20mg	30mg
Length (cm)	12.4 ± 0.7^{a}	13.1 ± 0.5^{a}	12.9 ± 0.8^{a}
Weight (g)	20.0 ± 3.4^{a}	22.7 ± 2.9^{a}	23.6 ± 4.54^{a}
Body weight gain (%BWI)	217 ± 10^{a}	257.7 ± 3.26^{a}	258.7 ± 22.1^{a}
Condition Factor (K)	1.07 ± 0.02^{b}	1.06 ± 0.01^{b}	1.11 ± 0.01^{b}
Specific growth rate (SGR)	2.0±0.05	2.12±0.02	2.13±0.1
Daily growth rate (DGR)	3.6 ± 0.2^{a}	4.3 ± 0.05^{b}	4.31 ± 0.37^{b}
Food conversional rate (FCR)	1.5 ± 0.1^{d}	1.3 ± 0.01^{bc}	1.3 ± 0.1^{c}
Survival Rate	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
Erythrocyte cell count (10^{8})	0.8±0.1	0.6±0.02	0.74±0.06
Hb (g $100mL^{-1}$)	7.2±0.7	5.4±0.3	6.96±0.5
Haematocrit	35±6	36±2	33.66±4.5
MCV	421±17.2	450.4±4.5	457.3±3.3
MCH	89±0.6	92.7±1.66	94.3±2.4
MCHC	20.7±0.3	20.0±0.6	20.33±0.3
WBC (10^{4})	0.35±0.01	0.72±0.06	0.77±0.08
Neutrophil	37.7 ± 0.9^{c}	24.3 ± 0.3^{ab}	25.0±1.5 ab
Lymphocyte	59 ± 1.7^{c}	71.33 ± 1.5^{b}	72±2.6
Monocyte	2.3±0.3	2.3±0.3	2.7±0.3
Eosinophil	1.0±0.6	1.7±0.3	1.0±0.6
Total Immunoglobulin	14.5±0.5	16.8±0.6	14.6±1.1
IgM	15.7±0.9	25.3±4.8	24.3±3.4
Lysozyme	26.0±2.7	47.33±8.9	45.33±11.9
CH50	31.7±3.7	40.0±4.04	24.3±0.9

On the other hand, the riboflavin treatment could differently affect RBC parameters. Although the RBC counts and haemoglobin concentration decreased at 20 mg, the parameter returned to control levels at 30 mg. No meaningful differences were observed in haematocrit level, MCH and MCHC between the control and cases. Also, the treatment increased MCV, and the higher doses of this vitamin enlarged the index (p<0.05) (Table 1).

Although the treatments could positively affect both total WBC and the lymphocyte count, it caused a significant decrease in neutrophils (p<0.05). The treatment had no effect on eosinophils and monocytes (p<0.05). Despite the complex results obtained from the analysis of white blood cells, the same results were seen when the effects of these two doses of riboflavin on different white blood cells were analyzed (p<0.05) (Table 1).

Furthermore, both total immunoglobulin and IgM level showed upward trends when the fish were treated with riboflavin. However, higher concentration of the treatment decreased the level of total immunoglobulin (without any positive or negative effect on IgM) (p<0.05) (Table 1). Also, both concentrations (20 and 30 mg) had the same positive effects on the lysozyme activity of the serum. Finally, CH50 level first showed

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a meaningful increase at 20, but its level dropped at 30 mg and reached lower than the control level.

Discussion

The intensive aquaculture is mentioned as an efficient system for reducing the protein source shortage for human beings worldwide (FAO, 2011). Over the five decades, based on developments in both fish production methods and transportation systems, the industry has had intensive impacts on reduction of this demand. Although fish products provide valuable sources of amino acids and other essential nutrients, the consumption rate of this source has declined in recent years in both developed and developing countries, and people prefer to use other protein sources because of their quite lower costs (FAO, 2010). Different reasons have been reported for the high expenses of fish farming. The business is time-consuming and comes with high-risk, and feeding the fishes is too expensive. In addition to the dry feeds, the food must to be enriched with different minerals and vitamins, including riboflavin (Brown and Gratzek, 1980).

In order to minimize the expenses of growing this fish, which in turn directly affects the actual cost of this fish, we tried to investigate the effects of two doses of riboflavin (20 and 30 mg per kg weight) on different growth parameters (biometric performance, blood factors and immunological system) of rainbow trout fingerlings.

The results showed that although treatment with these two doses of riboflavin could improve biometric parameters of the fish in terms of weight, length, body weight gain percentage, specific growth rate and daily growth rate, the treatment with 20 mg riboflavin was as effective as treatment with 30 mg. These results confirmed that taking extra-doses of riboflavin has no positive effects on the production of rainbow trout. Furthermore, both of the treatments led to a decrease in FCR. It means that these fish need more food for their growth than the controls in these conditions. However, it is important to notice that only the treatment with a higher concentration (30 mg) of riboflavin had a meaningful positive effect on the condition factor (K).

Moreover, the treatment had an inverse effect on hematopoietic activity of the fishes, and significant decreases have been shown in both RBC counts and Hb concentration at 20 mg riboflavin. The treatment with 30 mg could recover the situation, and both RBC count and Hb concentration improved reaching the control levels. Also, the treatment with both doses failed to increase haematocrit level, MCH and MCHC levels. Naturally, as a result of reductions in the concentrations of RBC and its related factors, fish have to increase their blood cell volume (MCV). In the biological view, any decline in the blood elements is a critical situation, leading to a variety of clinical manifestations. Since other nutrients, including different vitamins and minerals, play important roles in the hematopoietic activity of animals, it appears that any change in

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their balance interrupts this activity (Hien and Doolgindachbaporn, 2011).

Also, we found that treatment with 30 mg riboflavin had no extra-effects on immunological parameters of the fishes, and 20 mg riboflavin per Kg dry feed had the same effects as the higher dose in terms of total WBC count, total immunoglobulin, IgM concentration and lysozyme activity in the serum samples. Both of the treatments were ineffective on eosinophil and monocyte levels. Also, total immunoglobulin and CH50 level dropped at 30 mg and reached lower than that in the control. The results showed that although treatments with both doses of riboflavin (20 and 30 mg per each kg dry feed) had negative outcomes on the fingerlings of rainbow trout in terms of red blood production, they could improve biometric and immunological parameters of the fishes. The reduction in red blood cell production, however, had no detrimental impacts on the fish survival index.

In conclusion, treatment with 20 mg riboflavin per each kg dry feed had the same result as 30 mg of this vitamin in terms of improvements in both immunological and biometric parameters and thus it is not necessary to supply the farms with extra riboflavin more than 20 mg Kg^{-1} dry feed.

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