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Replacement of Dietary Fish Oil with Flaxseed Oil and its Effects on Hematological and Biochemical Parameters of Rainbow Trout Fingerlings (*Oncorhynchus mykiss*)

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Abstract: The aim of the present study was to evaluate the effects of dietary fish oil substitution with flaxseed oil on the blood factors of rainbow trout fingerlings (*Oncorhynchus mykiss*). Fish were fed with diets containing different oils, including treatment with 100% fish oil (FOD), flaxseed oil (FxOD) and 1:1 ratio of fish and flaxseed oil (FFxOD). Finally, hematologic factors including hemoglobin (Hb), hematocrit (Hct), total count of white blood cells and neutrophil showed no significant differences between treatments. In the case of the lymphocytes number in the treatment of 100% of flaxseed oil, a significantly lower value was observed compared with the other treatments. In the blood biochemical parameters including Cholesterol (CHOL), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Triglycerides (TG), High Density Lipoprotein (HDL), Glucose (Glu) and Low Density Lipoprotein (LDL) no significant differences between treatments were found however, in the factors of Total Protein (TP), Albumin (Alb), Globulin (Glb) and Alb/Glb there were significant differences among treatments. The obtained results showed that the use of flaxseed oil in the diet of rainbow trout fingerlings can lead to changes in some hematological and biochemical parameters of blood.

Key words: Rainbow Trout · Fish Oil · Flaxseed Oil · Hematological and Biochemical Parameters

INTRODUCTION

Fish oil is a valuable component of diet for fish because it provides essential fatty acids including Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Arachidonic acid (AA) that are essential for the optimum growth and functional development. Fatty acids found in fish oil (PUFAs) are essential for structure maintaining, fluidity and biological performance of cell membranes. In addition, fish oil is an important source of lipid-soluble vitamins and highly digestible energy [1]. Essential fatty acid deficiency of diet has been known as a deterrent of the immune cells performance in human [2] and fish [3-7]. Much of the global production of fish oil is confirmed based on fishing from the seas whereas due to the limitation of marine reserves, there is not possibility of increasing the global annual harvest from the pelagic fish stocks and thus there is not fish oil production. Therefore, the possibility of providing industries with the ever growing demand of fish oil is far reaching [1, 4]. For this reason, these industries are seeking other fat sources to replace fish oil in the diet. Extensive research has been started and many of these researches reported that plant oils are potential candidate for partial replacement of fish oil in aquaculture diets [3]. Recent researches represented acceptable and satisfactory results for replacement fish oils with plant oils in Gilthead sea bream (*Sparus aurata*), European sea bass and Turbot (*Psetta maxima*) fishes [3-5, 13-15]. Current price of plants oils is generally lower than that of fish oil [1, 9-12]. Most researches in this field were done in marine fish and little information is available regarding freshwater fish.

Measurement of hematologic parameters in order to assess the health of fishes is one of the items that be evaluated in the field of changing composition of fish diet. Therefore, the aim of this study was to evaluate the effects of different oils of diet (fish and plant oil) on some biochemical and hematological parameters in rainbow trout fish species.

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MATERIALS AND METHODS

This research was conducted in aquaculture salon of Natural Resources Faculty of the Isfahan University. In this experiment, a closed CCTV System was used. Fish were acclimatized to the new environmental conditions and then commercial diet (SFT2 of Chine food production factory) was used for 20 days. At the end of acclimatization period, Healthy fish (appearance form) with a mean weight of 16.49 ± 0.46 g were selected to begin the experiment. This experiment was conducted in a completely randomized design (CRD) with three treatments and three replications including 9 experimental units and 10 fish per each unit during eight weeks. Treatments included 100% fish oil (control treatment) (FOD), 100% flaxseed oil (FxOD) and 1:1 blends of fish and flaxseed oils (FFxOD). Used diet was adjusted by nutrition requirement standard tables of Nutrient Requirement Council (NRC). Chemical compound of diet and the ratio of materials are presented in Table 1. During the nourishment period, feeding was performed constantly (3% of the weight of biomass in each tank and two times daily).

Studied Factors: At the end of experiment, four fish per unit were selected randomly (one day before blood sampling, feeding was discontinued). Blood samples of all fish (anesthetized using clove powder dissolved at the concentration level of 100 mgL^{-1}) using a syringe head (21) from the Caudal vein.

To measure the hematocrit (%) and hemoglobin concentration (gdL^{-1}), blood samples in tubes containing EDTA (as anticoagulant material) were gathered and analyzed immediately. Hct was determined using capillary tubes, centrifugal micro-hematocrit (10000 rpm for three minutes) of the model Hettich 210 models and the special ruler of hematocrit. Measurement of the concentration of Hb (two replicates for each fish) was carried out using cianomethemoglobin [6]. The total count of white blood cells was determined using hemocytometer lam (NeoBar). Differential count was measured after Smears (Giemsa staining) using a light microscope with 100x lens and with the aid of immersion oil [6].

Serum Biochemical Analyses: Blood samples (collected using Eppendorf tubes without anticoagulant substance) were kept in the refrigerator for 4 hours and then serum was separated by centrifuging (by 3000 rpm for 10 min) [6].

Table 1: Ingredient (%), proximate composition (% wet weight) and energy ($KJ\ g^{-1}$) of the experimental diets

	FOD	FxOD	FFxOD
Fish Meal	58	58	58
Soybean Meal	20	20	20
Wheat Meal	8.6	8.6	8.6
Fish Oil	8	0	4
Flaxseed Oil	0	8	4
Vitamin premix*	2	2	2
Mineral premix**	1.5	1.5	1.5
Lysine	0.07	0.07	0.07
Methionine	O.13	0.13	0.13
Choline chloride	O.2	0.2	0.2
Molasses	1	1	1
Salt	O.5	0.5	0.5
Proximate composition			
Moisture	9.80	9.10	9.63
Ash	14.59	14.76	15.29
Crude protein	30.66	30.15	29.58
Crude lipid	16.09	16.52	16.81
Crude fiber	2.16	2.42	2.01
NFE***	26.70	27.05	26.41
Energy****	18.56	18.71	18.57

Diet abbreviations, FOD: 100% fish oil; FxOD: 100% Flaxseed oil; FFxOD: 50% fish oil and 50% flaxseed oil.

*Contains (mg kg-1 food): E (30), K (3), niacin (40), thiamine (2), riboflavin (7),
pyridoxine (3), folacin (1.5), pantothenic acid (18), biotin (0.7) and cyanocobalamin (0.18).

**Contains (mg kg-1 food): Mg (100), Zn (60), Fe (40), Cu (5), Co (0.1), I (1) and Antioxidant (100).

***NFE: nitrogen free extract, calculated by difference (100 – moisture – ash – crude protein – crude lipid –crude fibers).

****Calculated on the basis of 23.6, 39.5 and 17.2 kJ g^{-1} of protein, fat and carbohydrate, respectively

Serum samples were maintained in the refrigerator at -20°C prior to the biochemical analysis. The whole Serum biochemical tests were carried out using the Roche COBAS MIRA auto analyzer and commercial kits manufactured by Pars Azmoon Co. Factors examined included: AST, ALT, TP, Alb, Glb, Alb/Glb, CHOL, TG, HDL and LDL.

Globulin is calculated by subtracting the albumin from the total protein.

Statistical Analysis: Each tank considered as an experimental unit and statistic data were reported as mean ± standard deviation. Data were analyzed using statistical packages SPSS v15. Normality of data is achieved by Kolmogorov-Smirnov (K-S) test. After confirmation of normal distribution of data and homogeneity of variances, differences between means were compared using Duncan's multiple range test at significance of differences ($P<0.05$) among dietary treatments.

RESULTS

Hematologic Parameters: The results of hematological parameters for rainbow trout fingerling is presented in Table 2. The results showed that different resources of dietary fat have not a significant difference (P>0.05) on different bloody parameters (Ht, Hb, WBCs) but white cells counting is affected by different sources of dietary fat. Lymphocyte frequency is lower than other treatments in 100% flaxseed oil treatment significantly (P<0.05) while no significant was observed in neutrophils frequency (P>0.05).

Biochemical Parameters: The results of biochemical parameters of serum are represented in Table 3. Significant difference was not observed between different treatments (P>0.05) in the rate of enzymes activities of ALT and ASL, lipoproteins of HDL and LDL, cholesterol, triglyceride and glucose in fish serums. The amount of Total protein, albumin, globulin and the ratio of albumin to globulin were not showed significant difference between different treatments (P<0.05).

DISCUSSION

Hematologic parameters provide overall information of fish health and condition. These indicators by a large extent are influenced by nutritional conditions (feed chemical composition and feeding manner), temperature and stress condition. In addition, hematological examination and analysis of serum components provide valuable information for the detection of metabolic disorders and disease in the fish [7, 8].

The lack of significant differences in hematologic parameters (Ht, Hb and WBCs) among different treatments explains that multiple sources of fat have been efficient in providing the fish fingerling with the demanded essential oils so that no signs of deficiency of essential fatty acids, like increase in hemoglobin and hematocrit were observed in fish fed with vegetable oils.

In confirmation of the results of this study Sobahadra *et al* [9] and Montero *et al.* [5] reported similar results that complete and partial replacement of fish oil with canola oil in the diet of largemouth bass

Table 2: Hemoglobin, hematocrit and leucocytes of rainbow trout reared on the experimental diets

Hematology parameters	Treatments		
	1	2	3
Hb (g/dl)	4.67± 0.66	0.52 ± 6.23	0.28 ± 5.37
Hct (%)	2.29± 38.98	2.26 ± 38.44	1.38 ± 33.78
WBCs (cell/mm^3)	10 * 10^3	12.5 * 10^3	12* 10^3
Lym (% of WBCs)	0.88^a ± 95. 66	00^b ± 93.00	0.67^a ± 96.67
Neut (% of WBCs)	0.33± 3.33	1.15 ± 4.00	0.58 ± 3.00

Means with the same letters at the same row are not significantly different (P>0.05)

Table 3: Biochemical parameters in serum of rainbow trout reared on the experimental diets

Biochemical parameters	Treatments		
	1	2	3
AST (U/L)	65.83± 568.33	75.96± 552.33	96.67 ±494.67
ALT (U/L)	4.63± 25.33	5.69 ± 21.67	0.88 ± 22.33
HDL (mg/dl)	10.97±243.00	7.05± 217.67	13.37± 246.33
LDL (mg/dl)	2.52±57.00	2.4± 60.67	5.84± 57.67
TP (g/dl)	0.11^a± 5.38	0.16^{ab} ± 5.09	0.04^b ± 4.75
Alb (g/dl)	0.05±2.34	0.07±2.34	0.03± 2.24
Glb (g/dl)	0.11^a ± 3.04	0.12^{ab} ±2.75	0.07^b ±2.50
Alb : Glb	0.07^b± 0.76	0.05^{ab} ± 0.85	0.03^a ± 0.9
CHOl (mg/dl)	19.7 ±337.00	4.09 ±324.44	23.24 ±339.67
TG (mg/dl)	26.06 ±234.00	15.6 ± 245.33	21.94± 234
Glu (mg/dl)	5.17 ± 74.66	1.20± 79.33	7.36±72.33

Means with the same letters at the same row are not significantly different (P>0.05)

(*M. salmoides*) and gilthead sea bream had had no significant effects on blood parameters. In contrast to these findings, Babalola *et al*. [10] reported that substitution of fish oil with sunflower oil increases the parameters like Hb, WBC and RBC in catfish (*Heterobranchus longifilis*). The reason had been explained as species differences, the higher ability of the fish to carry oxygen and higher ability of the fish to utilize the n-6 fatty acids found in vegetable oils [10]. Red and white blood cell counts are used as indicators of hematopoiesis. White blood cells play an important role in immune responses, particularly in inflammatory responses [11]. In this study use of flaxseed oil in diet has reduced the number of lymphocytes so that there has been made a significant difference with other treatments, but applying a combination of these oils (1:1 mixture of fish and flaxseed) and fish oil in the diet made no significant differences.This difference could be due to the low ability of fish that fed whit FXOD against stress caused by capture and manipulation before blood sampling. Because the change in white blood cells number is considered one of the most sensitive indices expressing the acute stress. Stress often leads to a decrease in the number of lymphocytes and monocytes and increased number of neutrophils. The end result of stress would be fish weakness and immune system suppression and increased susceptibility to infectious diseases [12]. We can say that the use of flaxseed oil alone in the diet can affect the health of fish. Soubahadra *et al*. [9] reported that a complete replacement of fish oil with soybean oil in a large mouth bass diet has not had any effect on total leukocyte differentiation percentage. The reasons for this discrepancy could be due to the result of species differences, fat sources applied and the level of fat used.

Biochemical parameters can be used to diagnose fish diseases and stress [12]. Serum protein is fairly sure parameters that reflect the health status of the organism. Booke [13] observed that gender, fish spawning, food, osmotic pressure, temperature, light, age, hormones responsible for hibernation, water hypoxia and seasonality are the factors on which serum total protein concentration depends. Tests for total protein, albumin and globulin are used to control disease pathways in immune disorders and abnormal kidney and liver function [12].

As the data in Table 3 suggests, the amount of TP, Alb and Glb in treatment 3 which is the combination of fish oil and flaxseed oil, was lower than other treatments. On this ground one could deduce that among different sources of fat only the 1:1 combination of fish oil and flaxseed oil has altered TP, Alb and Glb which has created an unsuitable milieu for the fish under study. Plasma proteins change in response to changes in plasma volume that may be the result of stress or prolonged starvation [12].Gary [12] say that low protein, albumin and globulin in blood plasma are due to infections, nutritional problems and renal failure.

CONCLUSION

In this study, using two different fat sources include fish oil and flaxseed, one can say that applying alternative fat sources other than fish oil in the diet can affect the blood factors such that in response to this, the number of lymphocytes and serum proteins have affected. The reason could be due to changes in the fatty acid composition of fish cells and tissues, which leads to the weakening of fish and liver damage in rainbow trout fingerlings.

It is recommended that any attempts to replace fish oil with flaxseed oil in the diet should be as a combination of both to create the proper balance of essential fatty acids and prevent severe changes in composition of these acids in the tissues.

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