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Research Article:

Effect of egg flour as an alternative protein source on biochemicial parameters of rainbow trout

Tuna Kelestemur G.[1*]; Çambay Z.[2] ; Kelestemur N.[2]; Uslu A.A.[3]

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Abstract

In this study, the effects of diets supplemented with 13.5%, 22.5%, 31.5% and 51.7% (diet groups; D_1, D_2, D_3, D_4, respectively) egg flour instead of fish meal on blood proteins, lipids and ion levels pigmentation of rainbow trout were investigated. Fish (initial weight and length, 72.87±0.73 g, 18.11±0.06 cm, respectively) were distributed into 15 fiberglass rectangular (200 cm×40 cm ×40 cm) tanks in a 5×3 experimental design (5 diet groups×3 replicate groups) with a density of 30 juvenile rainbow trout (*Oncorhynchus mykiss*) per tank. Fish were fed with four different diets containing egg flour, and a basal diet (not supplemented) for 12 weeks. The results of oneway ANOVA test showed that blood proteins, lipids and ion levels of fish were affected by dietary supplementation of egg flour. Serum glucose level in the D_4 group was significantly lower (p<0.05) than the other diet groups. Serum total protein (TP) levels in D_4 and D_3 group were significantly higher (p<0.05) than the other diet groups. Serum uric acid, creatinin, blood urea nitrogen (BUN), and urea levels increased in the D_4 group. It was concluded that replacing with 50% egg flour in rainbow trout diet decreased the serum ion levels of magnesium (Mg^+), calcium (Ca^+), sodium (Na^+), potassium (K^+) and phosphorus (P) %. However, serum Cl^- levels were not statistically different among the groups (p>0.05). On the other hand, serum lipid levels concluded an increasing tendency with the increasing percentage of supplement in the fish diet. The results of the present work indicate that compared to the experimental diets, it can also be concluded that when egg flour is used in rations of over 50% in rainbow trout it has significantly negative effects on blood protein, lipid and ion concentration.

Keywords: Rainbow trout, Animal protein, Egg flour, Serum protein, Serum lipid, Serum ion

1-Fisheries Faculty, Firat University, 23119, Elazig, Turkey
2-Fırat University Health Services Vocational High School Elazig, Turkey
3-Aquaculture Research Institute, Elazıg, Turkey
*Corresponding author's Email: gkelestemur@firat.edu.tr

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Introduction

Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish (NRC, 1993). Control of the physiological state of the fish, focused on biochemical examinations of the blood, is an integral part of the complex methods of examining the health of the fish and at the same time, plays a role in feeding experiments in testing diets of different composition or testing the properties of substances having a specific effect (Kopp *et al*., 2011). The blood ions (Ca^+, Na^+, K^+ and P^-) are commonly used to determine the physiological characteristics, toxicity and health status of fish (Percin *et al*., 2010). Monovalent ions namely, Na^+, and K^+ play an important role in osmoregulation and homeostasis. In vertebrates, the Na concentration in the extracellular fluid surpasses that in the cytosol whereas K^+ is higher in the intracellular fluid compared to the plasma. Thus, the levels of serum electrolytes offer important knowledge concerning the health status of diseases of and impact of stress on fish (Burnett *et al.*, 2000; Wurst and Stickney, 1989; Percin *et al.*, 2010). Blood proteins and lipids in fish are used to determine physiological, toxicity and healthy status. Total protein (TP) is used as an indicator of damage to the liver. Increased concentrations cause structural changes in the liver. If starvation and absorption is small or defective, it results in an increase in TP amount and a decrease in dehydration (Elliott *et al*., 2006).

The amount of serum protein is a parameter related to the nutritional quality and quality of fish individuals which indicates a condition related to malnutrition of the fish for any reason (McDonald and Milligan, 1992; Mayer, 1998). Most of the lipids taken with food are triglycerides. Few of them are composed of phospholipids and ester cholesterol. The main lipids found in clam are cholesterol, triglycerides, phospholipids and unesterified (free) fatty acids. Cholesterol in the bloodstream comes from two sources: some is synthesized by the body, and some comes from food. In most instances, when dietary cholesterol intake increases, the body compensates by decreasing its cholesterol production. Conversely, when cholesterol intake decreases, synthesis increases. The existence of this compensatory mechanism is the reason why changes in dietary cholesterol intake have only a limited effect on blood cholesterol levels (Applegate, 2000).

In recent years, there has been a great deal of studies carried out on different animal and plant protein sources for aquaculture (Pratoomyot *et al*., 2010;

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Brinker and Reiter, 2011; Kader *et al.*, 2012) but none of these studies investigated the effect of egg flour as a new protein source on serum biochemical parameters of juvenile rainbow trout. Thus, this study aimed to investigate the effects of egg flour as an alternative animal protein source on serum lipid, protein and electrolyte in rainbow trout.

Materials and methods

Fish culture

Fish (initial weight and length, 72.87±0.73 g, 18.11±0.06 cm, respectively) were distributed into 15 fiberglass rectangular (200 cm×40 cm× 40 cm) tanks in a 5×3 experimental design (5 diet groups×3 replicate groups) with a density of 30 juvenile rainbow trout (*Oncorhynchus mykiss*) per tank. After the acclimation, fish were selected and randomly stocked. Fish were adapted to experiment conditions for 2 weeks prior to the start of the trial. Fish were fed with four different diets containing egg flour, and a basal diet without supplementation for 12 weeks. The investigation was initiated after the levels of dissolved oxygen had reached 7.6 mg L^{-1} at a flow rate of 2.1 L min^{-1}. The pH of the water in the tanks in which the fish were stocked was determined by a portable Checker brand pH meter and the dissolved oxygen and the temperature values were recorded through the use of a portable YSI 55 Model 51/12 oxygen probe throughout the duration of the experiment. The water temperature was between 8.8 and 9.4 °C and water pH was 8.4 throughout the experiment. Before the fish were anesthesised (15 mg L^{-1} Quinaldine sulfate), their body weight was measured every 2 weeks.

Diet preparation and experimental feeding

The composition of the basal diet is shown in Table 1. The basal composition of the experimental diets was based on the National Research Council NRC (1993). Experimental diets supplemented with 13.5%, 22.5%, 31.5%, and 51.7% egg flour instead of fish meal (D_1, D_2, D_3, D_4, respectively), and a basal diet (C) (not supplemented with egg flour) were prepared.

Table 1: Composition (%) and proximate analysis (%, dry matter) of the experimental diets.

Ingredients	C	D_1	D_2	D_3	D_4
Fish meal	45	36	29	20	-
Egg Flour	-	13.5	22.5	31.5	51.7
Soybean meal	18.3	18.3	18.3	18.3	18.3
Wheat meal	16	14.5	15.5	18.5	22.3
Fish Oil	13	10	7	4	-
Boncalit	5	5	5	5	5
Antioxidant (a)	0.1	0.1	0.1	0.1	0.1
Vitamin Karması (b)	1	1	1	1	1
Mineral Karması (c)	1	1	1	1	1
C Vitamini	0.6	0.6	0.6	0.6	0.6

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Table 1 continued:					
Toplam	**100**	**100**	**100**	**100**	**100**
Proximate composition and Total energy (kcal kg^{-1})					
Crude protein	40.83	40.14	39.89	39.87	35.52
Crude fat	17.60	17.60	17.14	17.14	18.27
Ash	13.10	12.14	14.10	13.03	13.16
N-free matter	91.02	89.52	94.14	90.15	92.07
Total energy	3230	3245	3260	3290	3310

a) Butilen Hydroxytoluene (BHT); 125.000 mg kg^{-1}
(b)Vitamin premix (IU or mg kg^{-1} dry diet): Vitamin A; 12.000.000 IU, vitamin D3; 120 IU, vitamin E; 30.000 IU, vitamin K3; 0.9 mg, vitamin B1; 1.2 mg, vitamin B2; 1.5 mg, vitamin B6; 1.2 mg, vitamin B12; 0.003 mg, niacin; 12 mg, cal-D- pant.; 2.4 mg, folik asit; 1000 IU, D biotin; 0.03 mg, inositol; 15 mg, vitamin C; 15 mg, antioxidant; 0.75 mg, choline cloride; 72 mg.
(c)Mineral premix (mg kg^{-1} dry diet); Mn 80.000, Fe 35.000, Zn 50.000, Cu 5.000, I 2.000, Co 400, Se 150

Dry matter (105°C, overnight), ash (650°C,6 h), crude protein (nitrogen×6.25), ether extract, crude fibre of diets were analyzed by methods of AOAC (1995). The experimental fish were fed there times a day. Daily feed allowance was 3% body weight per day.

Blood Sampling

At the end of the experiment, 10 fish were sampled randomly from each tank. Blood samples were obtained from the caudal vein of individual fish after anesthesia with Qinaldine (15 mg L^{-1} Quinaldine). The blood samples were centrifuged (3500 rpm for 7 min) to obtain serum and serum samples were stored at -20 °C until analysis (Sahan *et al.,* 2003). The blood parameters were determined using an Autoanalyzer (Roche Hitachi Cobas 6000) device in the Fırat University Health Services Vocational High School Laboratory. Total serum protein was determined by the biuret reaction (Doumas *et al.,* 1981). Glucose concentration was determined by the glucose hexokinase method (Barham and Trinder, 1972). Calcium (Ca) and magnesium (Mg) concentration were determined using the arzenazo III method (Ichaylova and Ilkova, 1971). Phosphorus (P) was determinad by an endpoint method with a blank sample using ammonium molybdenate reagent (Kratochvila and Garcic, 1977). Urea concentrations were determined by the kinetic enzymatic method with urease (Fidaleo and Lavecchia 2003). Cholesterole was determined by the CHOD-PAP method after enzymatic hydrolysis and oxidation (Bakker and Mücke, 2007). Ions levels (Na, K, Cl) were analysed by ion selective electrolides (Burnett *et al.,* 2000), and triglycerides were assessed by the Fossati three-step enzymatic reaction (Fossati and Prencipe, 1982).

Statistical methods

All the values were presented as mean ± SE. All data were analyzed by one-way analysis of variance (ANOVA) Duncan's test was used to compare differences among means (SPSS, Inc.).

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Results

There has been an increasing interest to find out cost-effective and practical approaches to utilize alternative protein sources more efficiently in carnivorous fish species towards sustainable aquaculture practice. In the study, the effect of egg meal, which is an important source of animal protein, which will be an alternative to fish based protein, on different blood parameter values of trout rations was determined. Serum TP, urea, uric acid, creatinin, BUN, glucose, trigliceride (TG), VLDL and cholosterol (CHOL) values of fish fed with the various experimental diets are presented in Table 2. The serum glucose level was significantly affected by D_4 diet group compared to other groups (p<0.05). The highest serum total protein levels were obtained in the D_4 and D_3 diet group (p<0.05). Serum uric acid, creatinin, BUN and urea levels increased in the D_4 group while they decreased in the other groups (p<0.05). The highest TG level was obtained in the D4 group (p<0.05). The lowest VLDL and CHOL levels were found in the D1 and control groups (p<0.05).

Table 2: Serum glucose, TP, uric acid, creatinin, BUN, urea, trigliceride (TG), VLDL and cholosterol (CHOL) levels levels of experimental groups at the end of the experiment.

Biochemical Parameters	C	D_1	D_2	D_3	D_4
Glucose	95 ± 2.41^{a}	99 ± 2.35^{a}	92 ± 2.17^{a}	94 ± 2.14^{a}	77 ± 2.12^{b}
TP	2.9 ± 0.41^{b}	3 ± 0.25^{b}	3 ± 0.22^{b}	3.7 ± 0.23^{a}	3.9 ± 0.11^{a}
Uric Acid	0.43 ± 0.02^{c}	0.54 ± 0.03^{c}	0.85 ± 0.01^{cb}	1.47 ± 0.01^{b}	2.41 ± 0.04^{a}
Creatinin	0.11 ± 0.14^{c}	0.17 ± 0.15^{c}	0.95 ± 0.19^{b}	1.04 ± 0.15^{b}	2.89 ± 0.22^{a}
BUN	2 ± 0.34^{c}	2.5 ± 0.15^{c}	2.5 ± 0.27^{cb}	3 ± 0.32^{b}	4.5 ± 0.13^{a}
Urea	2.8 ± 0.21^{d}	4.7 ± 0.15^{dc}	5.8 ± 0.17^{c}	$8.3\pm0.13^{b*}$	$12.7\pm0,10^{a}$
TG	$338\pm7.49^{d*}$	$450\pm6.89^{c*}$	$538\pm5.17^{bc*}$	$546\pm6.19^{b*}$	$669\pm7.15^{a*}$
VLDL	$110\pm5.41^{c*}$	$120\pm3.35^{c*}$	$220\pm5.17^{b*}$	$256\pm4.37^{b*}$	334 ± 4.19^{a}
CHOL	339 ± 2.41^{d}	357 ± 5.35^{d}	529 ± 3.17^{c}	614 ± 6.16^{b}	678 ± 5.79^{a}

[a-d] Means in the same line with different supscripts are significantly different (ANOVA, p<0.05). n=3

Serum Mg^{+}, Ca^{+}, Na^{+}, K^{+}, and P^{-} levels of fish fed with the various experimental diets are presented in Table 3. The test results showed that blood ion levels of fish increased with dietary supplementation of egg flour in particular in the D_3 group while they decreased in the D_4 group. Serum Ca^{+}, level was significantly affected by D_3 and D_2 diet groups, followed by the control and D_1 diet groups (p<0.05). On the other hand, Mg^{+}, Ca^{+}, Na^{+}, K^{+}, and P^{-} levels decreased with dietary supplementation of egg flour in particular in the D_4 group (p<0.05). Serum Ca^{+} level was higher in the Control group (p<0.05) than in the other diet groups. Serum Cl^{-} level was not statistically different among all dietary treatments (p>0.05).

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Table 3: Serum ion levels of experimental groups at the end of experiment.

Biochemical Parameters	C	D_1	D_2	D_3	D_4
Mg	0.97±0.43^{b}	1.39±0.15^{b}	1.71±0.17^{b}	2.43±0.32^{a}	0.65±0.17^{c}
Ca	3.5±0.41^{b}	3.9±0.15^{ab}	4.2±0.21^{a}	4.5±0.19^{a}	2.6±0.56^{c}
Na	175±0.48^{b}	186±0.75^{b}	204±0.34^{ab}	218±0.57^{a}	154±0.36^{c}
K	4.2±0.41^{bc}	4.7±0.35^{b}	5.3±0.19^{ab}	5.8±0,31^{a}	3.9±0.18^{c}
Cl	161±0.40	162±0.32	166±0.13	165±0.18	167±0.15
P	3100±19.14^{d*}	3300±25.35^{c}	3500±21.17^{b}	3700±26.1^{a}	3000±32.2^{e}

$^{a-c}$ Means in the same line with different supscripts are significantly different (ANOVA, p<0.05). n=3

Discussion

Protein is one of the most expensive components of aquaculture diets. Animal protein sources, especially fish meal have relatively high costs, limited supply, and variable quality. In addition fish meal is also an excellent source of essential fatty acids, digestible energy, minerals, and vitamins. (Tacon, 1993; Nguyen and Allen, 2009). However, the limited supply of FM, coupled with its increased demand in feeds for livestock and poultry, is likely to reduce the dependence on FM in aquatic feeds (El-Sayed, 1999). Many studies have investigated to evaluate the replacement of FM in practical diets for culture fish species with cheap, locally available plant and animal protein sources (Samano, 1997; El-Sayed, 1998; Fasakin *et al*., 1999; Abdelghany, 2003; Borgeson *et al*., 2006; Gaber, 2006; Nguyen and Allen, 2009). In this study the effects of egg flour evaluation instead of fish meal on serum electrolite, protein and lipid levels of rainbow trout were determined. Similarly, egg flour is as valuable as FM for diets. All of the research on eggs published since ACSH last reported on this subject in 1996 has confirmed and strengthened the conclusion that eggs can have a place in a healthy diet and can make an important contribution to good nutrition. Eggs are a highly nutritious food. They are an excellent source of high-quality protein and are far less expensive than most other animal protein foods. Eggs also provide significant amounts of several vitamins and minerals (Meister, 2002).

Evaluating the results of biochemical tests from literature, it can be said that the level of protein and lipid metabolism of rainbow trout corresponded to the contents of protein and lipid components in the diet similar to that determined in our study. The changes of biochemical parameters in the blood glucose of the rainbow trout also depend on factors such as stress, chemical factors, and diets (Řehulka and Parova, 2000b; Kopp *et al.*, 2011). In this study, the fish fed with diets completely supplemented with egg flour showed decreased concentration of blood glucose. On the other hand, TP, urea, uric acid, BUN, creatinin, VLDL, cholesterol and trigliceride levels showed increasing tendency with increasing proportions of egg flour in diets. Similarly, some researchers reported that the inclusions of high levels of protein ad lipid in diets have quite effective for fish metabolism.

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(Kopp *et al.*, 2011; Řehulka and Parova, 2000a; Řehulka and Parova, 2000b). Ion (Na^+, K^+, P^-, and Ca^+) levels indicate the operation of a variety of homeostatic mechanisms in the body. Sodium (Na^+), potassium (K^+) and Chloride, play an important role in osmoregulation and homeostasis (Clark, 1977). Based on our results it is clear that high protein supplemented in diet such as egg flour have an influence on the changes of serum ion concentration. However, serum Cl^- level was not statistically different among the groups (p>0.05). In this study was determined that fish serum ion levels increased in rainbow trout fed on egg flour partly substituted (respectively, % 13.5, % 22.5, % 31.5) with fish meal. In this study viewed that similarly some researchers reported that the effect of diet ingredient and nutrition regime have effect on fish blood chemistry (Keleştemur, 2011; Tuna Keleştemur, 2012).

In conclusion, the results of this study showed that egg flour can be used to a certain limit in rainbow trout diets as other plant and animal protein sources. Egg flour seems to be the cheapest and most easily available protein source to replace fish meal. It can be safely used up to 50%. However it can also be concluded that when egg flour is used over 50% in rations of rainbow trout, it has significantly negative effects on blood protein, lipid and ion concentrations of *O. mykiss.*

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