

The Effects of Using Peanut Meal in Rainbow Trout (*Oncorhynchus mykiss*) Diets on the Growth Performance and Some Blood Parameters

Ümit Acar^{1,2*} , Ali Türker³

¹ Department of Forestry, Bayramiç Vocational School, Çanakkale Onsekiz Mart University, Çanakkale.

² Department of Fisheries, Graduate School of Natural and Applied Sciences, Muğla Sıtkı Koçman University, Muğla, Turkey.

³ Muğla Sıtkı Kocman University, Faculty of Fisheries, Department of Aquaculture, 48000- Muğla, Turkey.

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Corresponding Author

Tel.: +90 286773 25 13/182

E-mail: umitacar@comu.edu.tr

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Abstract

In this trial, rainbow trouts (71.69±1.21 g) were fed with feeds containing peanut meal at different levels for 60 days and their growth performance, body compositions and some blood parameters were examined. Accordingly, 4 different trial feeds as the control feed (PNM0) that contains FM as the main source of protein, and feeds containing 10% (PNM10), 20% (PNM20) and 30% (PNM30) peanut meal as an alternative to fishmeal were prepared. At the end of the feeding period, fish that were fed with PNM10 gained as much weight as those fed with the control feed. Feed conversion ratio (FCR) and specific growth rate (SGR) findings of rainbow trouts fed with trial feeds differed significantly from the control group as the peanut meal level used in feeds exceeded 10% (P<0.05). No significant differences were detected in terms of fillet proximate compositions (P>0.05). In the examination of the hematological parameters obtained from rainbow trouts fed with feeds containing different peanut meal levels, it was detected that there were no significant differences compared to the control group (P<0.05), whereas the serum biochemical parameters generally worsened as the level of peanut meal in the ration exceeded 10% (P<0.05). The present study demonstrated that utilization of new protein source peanut oil cake meal can be effectively used to replace up to 10% of FM in diets of rainbow trout without any adverse effects of growth performance, feed utilization, hematological and serum biochemical parameters.

Introduction

Aquaculture is an essential industry which supplies important part of the world' food need. It has been identified by FAO as the fastest growing food sector in the world. Especially in the last 10-15 years, our country has been significant progress in aquaculture parallel with rapid growth of aquaculture in the world and be considered as an alternative food source. Aquaculture of rainbow trout realized in 109.657 tonnes (Turkish Statistical Institute) according to the data of 2018 (TÜİK, 2018). Fish meal (FM) production quantities are fluctuating because FM is produced from the fish obtained by fishing, while the importance of use of FM in fish feeds is so high. As FM production was 6,084 million tons in 2004, it decreased to 4,672 million tons in 2013 (IFFO, 2015). When the sectoral use of FM in the world in 2012 is examined, it is seen that 68% of FM is used in aquaculture feeding, 23% in pig feeding and 7%

in poultry industry (Boserup, 2017). Considering that production figures of FM, which is highly depended on by aquaculture, has been decreasing gradually and the aquaculture industry has been growing continuously, continued use of FM as the main protein source in fish feeds may negatively affect the sustainable development of aquaculture industry. For this reason, the potential use of protein sources, which can be used in feeds as an alternative to FM, has been intensively studied, and remarkable results have been obtained in reducing the proportion of FM in fish feeds in recent years. Potential use of alternative protein sources in feeds of carnivorous fish such as rainbow trout (*Oncorhynchus mykiss*), sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) were studied and FM proportion was reduced from 45% to 24% in the feeds of salmon species and from 50% to 30% in the feeds of marine fish (Tacon & Metian, 2008).

Among the main properties required in alternative

protein sources that can be used in fish feeds are showing no fluctuations in annual production quantities and having sufficient production quantities, being affordable, easily suppliable and transportable, being storable for a long time and being easily integrated into the feeds. As an alternative to FM, residuary oil cake from seed oil extraction from oily seeds, residuary products from poultry and animal production can be used. The oilseed peanut, *Arachis hypogaea* is a member of Fabaceae. The crop originated in South and Central America, but it has recently been cultivated in more than 60 countries in the world (Carrín & Carelli, 2010). World peanut production amounted to 38 million tons in 2005, but reached 45 million tons in 2013 (Boserup, 2017).

Peanut by-products, which remain after the extraction of peanut oil, can be used in animal feeds and contain lower levels of lysine and higher levels of arginine compared with soybean meal (Batal *et al.*, 2005). Defatted peanut meal (DPNM) has been used in aquatic animal feeds (Liu *et al.*, 2012; Garduno-Lugo and

Olvera-Novoa, 2008). As a result of the previous studies, it was determined that oil cakes can be used in fish feeds as an alternative to FM (Naylor *et al.*, 2009; Hardy, 2010; Kaushik & Seilliez, 2010; Yıldırım, Acar, Türker, Sunar, & Kesbiç, 2014).

In this study, effects of the feeds containing different levels of peanut oil cake proteins (0.0, 10, 20, and 30%) instead of FM protein on growth performance, feed consumption, feed conversion, body composition, and some blood parameters were investigated in order to determine the availability of a plant protein source such as peanut oil cake in rainbow trout (*Oncorhynchus mykiss*) feeds.

Material and Method

Experimental Fish and Conduction of Experiment

Rainbow trouts with an average weight of 71.69 ± 1.21 grams were used in the present experiment. Experimental fish were supplied from Selina Su Ürünleri

Table 1. Ingredients (g kg⁻¹) and composition of the experimental diets

g/100 g			PNM ₀	PNM ₁₀	PNM ₂₀	PNM ₃₀
Fish Meal ¹			40.2	36.18	32.16	28.14
Soybean meal ²			30	30	30	30
Wheat meal ²			7	5	4	3.5
Corn starch ²			6.8	5.82	5.1	4
Fish oil ³			12	12	12	12
Peanut meal ⁴			0	7	12.74	18.36
Vitamin-Mineral mix. ⁵			4	4	4	4
Total			100	100	100	100
Diets composition (%)	Fish meal	Peanut meal				
Crude Protein	65.2	30.6	42.5	42.3	42.3	42.1
Crude Lipid	10.5	10.35	17.3	17.6	17.7	17.8
Crude Ash	19.2	7.50	7.55	7.43	7.24	7.05
NFE ⁶			29.6	30.2	31.2	32.1
Amino acids (g kg ⁻¹)		Rainbow trout*				
Arginine		14	35.2	33.7	32.4	30.0
Phenylalanine		13	29.7	29.3	28.1	27.5
Histidine		6	26.8	19.1	18.2	16.2
Isoleucine		10	25.3	19.5	19.8	18.0
Lysine		21	26.5	21.0	22.4	20.5
Leucine		18	46.0	42.3	35.6	33.5
Methionine		8	29.4	26.0	25.6	25.1
Threonine		17	24.4	21.7	20.1	18.4
Valine		19	31.9	30.1	28.8	26.7
Cystine		4	6.10	5.88	5.21	4.70

PNM₀; non peanut meal added groups as control; PNM₁₀, %10 peanut meal added group; PNM₂₀, 20% peanut meal added group; PNM₃₀ 30% peanut meal added group

¹Anchovy FM. Koptur Balıkçılık. Trabzon.Turkey

²Soybean meal.Agromarin Yem San. ve Tic. A.Ş.. İzmir.Turkey

³Anchovy fish oil.Agromarin Yem San. ve Tic. A.Ş.. İzmir.Turkey

⁴Başpınar Fıstıkçılık Toprak Mahsülleri Ltd. Şti.. Osmaniye.Turkey

⁵Vitamin Mixture: Vitamin A. 18000 IU kg⁻¹feed; Vitamin D₃. 2500 IU kg⁻¹feed; Vitamin E. 250 mg kg⁻¹feed; Vitamin K₃. 12 mg kg⁻¹feed; Vitamin B₁. 25 mg kg⁻¹feed; Vitamin B₂. 50mg kg⁻¹feed; Vitamin B₃. 270 mg kg⁻¹feed; Vitamin B₆. 20 mg kg⁻¹feed; Vitamin B₁₂. 0.06 mg kg⁻¹feed; Vitamin C. 200 mg kg⁻¹feed; Folic acid. 10 mg kg⁻¹feed; Calcium d–pantothenate. 50 mg kg⁻¹feed; Biotin. 1 mg kg⁻¹feed; Inositol. 120 mg kg⁻¹feed; Choline chloride. 2000 mg kg⁻¹feed.

Mineral Mixture (mg kg⁻¹): Fe. 75.3 mg; Cu. 12.2 mg; Mn. 206 mg; Zn. 85 mg; I. 3 mg; Se. 0.350 mg; Co. 1 mg.

⁶ Nitrogen–free extracts (NFE) = matter – (crude lipid+crude ash+crude protein+crude fiber).

* Rainbow trout amino acid requirement according to Ogino & Nanri, 1980

Co. Ltd. (Fethiye, Muğla, Turkey). Fish used in the study were distributed among 12 experimental cages (100×100× 100 cm) as 50 fish/cage. In order to provide adaptation of the fish to the experimental environment, they were fed with commercial trout feed for 15 days. Trials of each experimental group were conducted in 3 repetitions. In the 60-day experiment, fish were fed by hand at 2% of their body weight twice a day.

Experimental Feeds and Analyses

Experimental feeds were prepared at Muğla Sıtkı Koçman University, Faculty of Fisheries, Fish Feeding and Feed Laboratory. FM, soy meal, wheat meal, corn starch, fish oil, vitamin and mineral mixtures used in experimental feeds were obtained from fish feed factory, peanut oil cake was obtained from Başpınar Fıstıkçılık Toprak Mahsulleri Co. Ltd. (Osmaniye, Turkey). Formulations with similar protein (43.5%) and oil (17.3%) content were prepared following the nutritional analyses such as moisture, protein, oil and ash on feedstuffs. For the study, peanut oil cake was added to the control feeds corresponding to 0.0, 10, 20, and 30% of FM protein. All raw materials were sieved prior to feed preparation, then passed through feed mill. Primarily, dry raw materials and then liquid raw materials were homogenized in laboratory type feed mixer. Thereafter, pellets obtained by passing through a laboratory type pelletizing machine were dried to a moisture of 10% in an air-circulated room condition. Prepared feeds were stored in locked polyethylene bags at -20°C until the beginning of feeding experiment. Amino acid composition analyses of prepared feeds are given in Table 1.

Calculations, proximate composition analysis in fish fillets and feeds

After the feeding trial, fish were collected, counted, and weighed. Growth performance and feed utilization were calculated using following equations;

$$\text{FCR (Feed conversion ratio)} = \text{feed consumed} / \text{weight gain};$$

$$\text{Relative growth rate (RGR; \%)} = 100 [(\text{final wet weight} - \text{initial wet weight}) / \text{initial wet weight}];$$

$$\text{Specific growth rate (SGR; \%g/day)} = 100 [(\text{Ln final wet weight} - \text{Ln initial wet weight}) / \text{days}].$$

Feed and fish samples (five fish per tank) were analyzed for proximate and amino acids composition at the end of the trial. Following AOAC's (2003) methods, dry matter (AOAC, 934.01), ash (AOAC, 942.05), and proteins ($N \times 6.25$; AOAC, 955.04) were determined.

Amino acid analysis of trial feeds was performed on a gas chromatograph using an EZ: Faast (Phenomenex Inc., USA) amino acid analysis kit. The

samples were first hydrolyzed according to AOAC (2003). The samples brought to constant weight were cooled and placed in 30 mg sample hydrolysis tubes and 10 ml of 6 HCl was added. After the helium gas was put on it, the hydrolysis tube which was quickly turned off was hydrolyzed at 110 ° C for 24 hours and finally filtered. The acid in the samples was diluted using an evaporator at 65 ° C by adding 20 ml of dilution solution onto the evaporator. Amino acid quantities of the diluted samples were analyzed by gas chromatography on Canakkale Onsekiz Mart University, Marine Sciences and Technology Laboratories. The conditions under which the reading is carried out; GC Column ZV-AAA (10 cm x 0.25 mm); oven temperature: 1 minute in 320 ° C increments from 110 ° C to 30 ° C; detector: FID 320 [deg.] C; injector 250 ° C; air 300 ml / min; transport gas: 1.5 ml / min He gas; sampling injection 2µl; kit: Phenomenex EZ: Faas GC-FID Hydrolyzed amino acid kit. The amount of amino acid was calculated in g / kg of sample.

Blood Sampling and Analyses

At the end of the experiment, 5 fish from each cage were used for blood analysis. Fish were anaesthetized with clove oil, (25 mg L⁻¹), which is a natural and commonly used product (Mylonas, Cardinaletti, Sigelaki, & Polzonetti-Magni, 2005), and blood samples were taken with a 5 ml plastic syringe from the caudal vein without harming the fish as soon as possible following the thorough cleaning of back side of the anal fin with alcohol in order to prevent blood from be contaminated by with mucous (Val, De Menezes, & Wood, 1998). Hematological and biochemical analyses were performed by placing the blood samples in K₃EDTA and gelled serum tubes.

Red blood cells (RBCs, 10⁶ mm³), hematocrit (Hct, %) and hemoglobin (Hb, gL⁻¹) was determined by using the method of Blaxhall and Daisley (1973). The RBCs was counted with a Thoma hemocytometer with the usage of Dacie's diluting fluid. The Hct was determined by using a capillary hematocrit tube. The Hb concentration was determined with spectrophotometry (540 nm) by using the cyanomethaemoglobin method. The Mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were calculated by using the following formula (Bain, Bates, & Laffan, 2016):

$$\text{MCV } (\mu\text{m}^3) = [(\text{Hct, \%}) \times 10] / (\text{RBC, } \times 10^6 \text{ per mm}^3);$$

$$\text{MCH (pg)} = [(\text{Hb, g/dL}) \times 10] / (\text{RBC, } \times 10^6 \text{ per mm}^3);$$

$$\text{MCHC (\%)} = [(\text{Hb, g/dL}) \times 100] / (\text{Hct, \%}).$$

Biochemical Analyses

After blood sample taken for biochemical analyses was centrifuged at 4000 rpm for 10 minutes and the blood serum was separated (Bricknell, King, Bowden, & Ellis, 1999), analyses of serum samples were carried out by spectrophotometer using kit (Bioanalytic). In the experiment, biochemical parameters of glucose (GLU), albumin (ALB), globulin (GLO), total protein (TPROT), triglyceride (TRI), cholesterol (CHOL), alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) were determined with bio analytic test kits (Bioanalytic Diagnostic Industry, Co.) and absorbance value was measured by using spectrophotometer (Optizen POP UV/VIS).

Statistical Analyses

In the study, Tukey multiple comparison test was used to evaluate the relationships between the data obtained from the experimental groups. Statistical analyzes were assessed at $P < 0.05$ significance level using the SPSS 19 (IBM SPSS Statistics 19) program.

Results

Growth performance and fish fillet proximate composition

No mortality was recorded during the experiment.

Dietary PNM treatment significantly affected the weight gain and the best growth performance was obtained in PNM₀ and PNM₁₀ groups as compared to PNM₂₀ and PNM₃₀ ($P < 0.05$; Table 2). A reduction in specific growth rate with an increase in the PNM level in the diets was observed ($P < 0.05$). The feed conversion rate in PNM₂₀ and PNM₃₀ groups have been found significantly higher than the other fish groups ($P < 0.05$, Table 2). Fish fillet proximate composition such as moisture, crude protein, crude lipid and crude ash was not affected by dietary PNM treatments in the diets ($P > 0.05$; Table 2).

Hematology and serum biochemical profiles

Dietary PNM treatment had no effect on erythrocytes count (RBC), hematocrit (Hct) and mean cell volume (MCV) in the all experimental groups ($P > 0.05$; Table 3). On the other hand, hemoglobin (Hb), mean cellular hemoglobin (MCH), and mean cellular hemoglobin concentration (MCHC) were lowest in PNM₃₀ group ($P < 0.05$; Table 3) as compared with PNM₀ group.

The biochemical parameters were found significantly differed as affected by PNM levels in fish feeds ($P < 0.05$; Table 4). Serum glucose levels were lower in PNM₀ and PNM₁₀ groups than those of the other groups ($P < 0.05$; Table 4). On opposite, at the end of 60 days feeding period serum total protein, globulin, and albumin values were higher in PNM₀ and PNM₁₀ groups than those of the other groups ($P < 0.05$; Table 4). The serum triglyceride values showed significant different in PNM included groups compared with PNM₀ ($P < 0.05$).

Table 2. Growth performance and fillet proximate composition of rainbow trout fed with experimental diets for 60 days

	PNM ₀	PNM ₁₀	PNM ₂₀	PNM ₃₀
Initial weight (g)	72.31±0.80	71.04±0.69	72.00±0.79	71.62±1.75
Final weight (g)	126.84±3.18 ^a	126.27±2.36 ^a	116.12±2.66 ^b	112.36±2.33 ^b
Relative growth rate %)	75.43±4.52 ^a	77.27±1.01 ^a	62.95±2.98 ^b	56.92±3.69 ^b
Specific growth rate	0.94±0.04 ^a	0.95±0.01 ^a	0.81±0.03 ^b	0.75±0.04 ^b
Feed conversion rate	1.01±0.07 ^a	0.95±0.02 ^a	1.19±0.04 ^b	131±0.07 ^b
<i>Fillet proximate composition</i>				
Moisture (%)	73.28±0.92	73.96±0.74	73.33±0.63	73.96±0.96
Crude protein (%)	18.22±0.22	17.99±0.31	18.35±0.24	18.09±0.96
Crude lipid (%)	4.98±0.10	5.10±0.12	5.02±0.08	5.12±0.14
Crude ash (%)	2.88±0.15	2.91±0.12	2.95±0.08	2.75±0.12

Means with different alphabetical characters in the same row are statistically different ($P < 0.05$). PNM₀; non peanut meal added groups as control; PNM₁₀, %10 peanut meal added group; PNM₂₀, 20% peanut meal added group; PNM₃₀ 30% peanut meal added group

Table 3. Hematological parameters of rainbow trout fed with experimental diets for 60 days

	PNM ₀	PNM ₁₀	PNM ₂₀	PNM ₃₀
Erythrocytes count (×10 ⁶ mm ³)	4.71±0.59	4.92±0.36	5.12±0.45	4.98±0.51
Hematocrit (%)	34.40±0.91	34.40±1.24	34.87±0.99	34.53±1.06
Hemoglobin (g x dL ⁻¹)	6.64±0.74 ^a	5.95±1.12 ^{ab}	5.84±0.71 ^{ab}	5.25±1.01 ^b
Mean cell volume (mm ³)	74.38±11.41	70.23±5.74	68.64±7.05	70.00±7.52
Mean cellular hemoglobin (pg)	14.42±2.88 ^a	12.10±2.28 ^b	11.51±1.87 ^b	10.61±1.97 ^b
Mean cellular hemoglobin concentration (g x dL ⁻¹)	19.33±2.21 ^a	17.27±3.15 ^{ab}	16.75±1.98 ^{ab}	15.26±3.18 ^b

Within a row mean values with different letters are significantly different at Tukey post hoc test ($P < 0.05$). PNM₀; non peanut meal added groups as control; PNM₁₀, %10 peanut meal added group; PNM₂₀, 20% peanut meal added group; PNM₃₀ 30% peanut meal added group

The serum cholesterol level showed reduced value with increasing of PNM level in diets ($P<0.05$), meanwhile LDH values showed no significant differences among the experimental groups ($P>0.05$). Serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP) values were increased as PNM levels increased and their highest values were observed in PNM30 and found statistically different from PNM₀ ($P<0.05$; Table 4).

Discussion

Another important part of the studies on fish feeding is to found alternative protein sources to FM, which is generally used as the basic protein source in fish feeds (Yiğit *et al.*, 2006; Ergun, Yigit, Türker, & Harmantepe 2008; Yigit, Ergün, Türker, Harmantepe, & Erteken, 2010).

In this study, the effects of peanut oil cake protein used instead of FM protein in rainbow trout feeds on growth performance, feed conversion ratio, body composition, and some blood parameters were investigated. When the protein from FM was substituted with 10, 20, and 30% of peanut oil cake protein, growth performances of the fish were determined to be negatively affected as peanut oil cake levels in the ration increased. The optimum PNM level is 10%. There are not many studies aiming to determine the use rate of peanut oil cake in fish feeds. Yıldırım *et al.* (2014) reported that 20% peanut oil cake could be used in substitution for FM in tilapia (*Oreochromis mossambicus*) feeds. Doğan (2012) stated that use of 15% hazelnut oil cake did not cause any negative effects on growth performance of the fish. Ustaoglu-Tiril, Karayucel, Alagil, Dernekbası and Yagci (2009) reported that growth performance of rainbow trout was adversely affected when 30% red lentil meal was used instead of FM in feeds. Despite the use of plant protein

sources in rainbow trout feeds are limited to 10% for cotton oil cake (Cheng & Hardy 2002), 20% for canola and pea meal (Thiessen, Campbell, & Adelizi, 2003), and 42% for sunflower oil cake (Sanz, Morales, De la Higuera, & Gardenete, 1994); it is an indisputable fact that this will reduce feed production costs.

When amino acid compositions of the feeds used in the experiment were examined, it was observed that the total amount of EAA among the groups gradually decreased with increased proportions of plant protein sources used in the feeds. As shown in previous studies, fish require 10 EAA. Feeds prepared for the experiment were observed to have met the general EAA requirements of rainbow trout (Wilson, 2003). Reduce growth performance of experimental fish could be explained by the quality of dietary protein.

Hematologic and biochemical parameters in fish are important guides in determining the health of fish, physiological effects of the environment they are in, and the feed given (Campbell, 2004; Fazio *et al.*, 2013; Faggio, Fedele, Arfuso, Panzera, & Fazio, 2014; Abdel-Tawwab, 2016; Abdel-Tawwab, El-Sayed, Monier, & Shady, 2017; Abdel-Tawwab, El-Sayed, & Shady, 2017; Yaghoobi *et al.*, 2017). Hematological parameters in fish vary depending on undernutrition (Hofer, Stoll, Romani, Koch, & Sordyl, 2000). When changes in hematological parameters of rainbow trout depending on the amount of FM and plant proteins are examined, it can be suggested that peanut oil cake used instead of FM does not have a negative effect in general. While RBC, HCT and MCV values did not differ as a result of hematological analyses at the end of the experiment, the lowest HB value was obtained in the group in which PNM30 was used. MCH and MCHC values of the fish were generally adversely affected by increased peanut oil cake in the feed because they varied depending on HB values.

Rinchart *et al.* (2003) fed rainbow trout with an

Table 4. Serum biochemical parameters of rainbow trout fed with experimental diets for 60 days

	PNM ₀	PNM ₁₀	PNM ₂₀	PNM ₃₀
GLU (mg dL ⁻¹)	128.95±14.06 ^a	122.96±21.32 ^a	158.29±8.99 ^b	162.03±14.01 ^b
TPROT (g dL ⁻¹)	3.53±0.67 ^a	3.14±0.57 ^a	1.74±0.37 ^b	1.70±0.48 ^b
ALB (g dL ⁻¹)	0.29±0.05 ^a	0.24±0.06 ^a	0.10±0.02 ^b	0.13±0.03 ^b
GLO (g dL ⁻¹)	3.25±0.66 ^a	2.90±0.53 ^a	1.64±0.36 ^b	1.57±0.48 ^b
TRIG (mg dL ⁻¹)	110.42±35.40 ^b	175.55±11.75 ^a	187.54±11.07 ^a	184.24±15.37 ^a
CHOL (mg dL ⁻¹)	315.28±68.07 ^a	174.50±49.58 ^b	109.36±19.59 ^c	111.84±20.32 ^c
GOT (U L ⁻¹)	108.06±14.66 ^{ab}	99.72±10.63 ^b	117.77±20.33 ^{ab}	129.32±29.19 ^a
GPT (U L ⁻¹)	9.95±2.33 ^b	10.77±1.31 ^{ab}	12.32±3.02 ^{ab}	14.10±3.02 ^a
LDH (U L ⁻¹)	156.07±21.15	144.11±20.37	162.47±19.83	158.32±38.04
ALP (U L ⁻¹)	186.54±23.54 ^b	198.94±17.78 ^{ab}	190.80±33.17 ^b	231.15±27.20 ^a

Values are least squares mean (n = 15 with common superscripts in the same line are not significantly different ($P>0.05$)).

GLU. glucose; Trig. triglyceride; CHOL. cholesterol; TPROT. total protein; ALB. albumin; Hct. hematocrit; Hb. hemoglobin; MCV. mean cell volume; MCH. mean cell hemoglobin; MCHC. mean cell hemoglobin concentration; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase. PNM₀, non peanut meal added groups as control; PNM₁₀, 10% peanut meal added group; PNM₂₀, 20% peanut meal added group; PNM₃₀, 30% peanut meal added group

average weight of 223 ± 33 g for 9 months with feeds prepared using cottonseed oil cake instead of FM with increasing percentages as 0, 25, 50, 75, or 100%. At the end of the experiment, they found that levels of hemoglobin and hematocrit significantly decreased as the proportion of plant proteins in the ration increased. They stated that the most important reason for this decrease is that gossypol, which is an antinutritional factor found in cottonseed oil cake, damages red blood cells and prevents iron from reaching the organisms by binding it.

Kumar, Makkar and Becker (2011) used the meal they obtained from seeds of *Jatropha curcas* in feeds of rainbow trout in proportions of 50 and 62.5% of FM and reported that there was no difference between the groups in terms of hematological parameters. In a study carried out with Atlantic salmon (*Salmo salar*) by Hemre, Sanden, Bakke-Mckellep, Sagstad and Krogdahl (2005), it was stated that hematological parameters were adversely affected with increased soy meal proportion used in the ration. At the end of the study, researchers reported that spleen sizes of the fish were normal, however, the reason for low erythrocyte indices might be that plant protein sources used in the feed might have led to premature release of erythrocyte cells from the spleen. In another study, Soltan, Hanafy and Wafa (2008) determined that when they used plant protein mixture instead of FM in Nile tilapia feeds, hematocrit levels of fish decreased significantly. They reported the reason for that as phytate enzyme found in plant feedstuffs binding to minerals and amino acids and decreasing their availability in the body and increasing erythrocyte fragility. Decrease in levels of red blood cells, hemoglobin and hematocrit was determined when percentage of cottonseed oil cake, which was used instead of FM in hybrid tilapia (*O. niloticus* × *O. aureus*) feeds, exceeded 30%. Similar results were obtained in different fresh water fishes (El-Saidy, & Gaber, 2004).

In the present study, serum glucose concentration in the fish groups in which plant protein sources were used was found to be higher than that in the control group. Plant protein sources contain high proportions of carbohydrate in their structures. High proportions of carbohydrate taken by fish in feeds cause serum glucose level to increase by being reduced to small sugars. Similar observations were obtained in many studies in which plant protein sources that may be an alternative to FM were investigated. For example, Kumar, Makkar, Amselgruber, & Becker, (2010) used meal obtained from seeds of *Jatropha curcas* and soy meal in proportions of 50% and 75% instead of FM in carp feeds and reported at the end of the study that plasma glucose concentration was higher than that of the control group. Similarly, at the end of the study which was conducted by substituting plant protein source containing equal amounts of sesame oil cake and corn meal for FM in feeds of sturgeon (*Huso huso*), serum glucose

concentration increased as plant protein level in the ration increased (Jahanbakhshi, Imanpoor, Taghizadeh, & Shabani, 2013). By contrast with these studies, Glencross, Hawkins, and Curnow (2004) reported that the use of yellow lupine meal in rainbow trout feeds had no negative effects on serum glucose levels.

Serum total protein concentration in fish is an important parameter used to monitor health and nutritional status (Olesen, & Jorgensen, 1986). Albumin and globulin are important components of immune system of fish. Therefore, determination of changes in protein metabolism of plant protein sources used in fish feeds is important. These changes may be in the form of increased or decreased protein synthesis, as well as activation and inhibition of enzymes (Canli, 1996). In the present study, a significant decrease in serum total protein, albumin and globulin concentrations was determined when peanut oil cake percentage in the ration exceeded 10%. Lin and Luo (2011) stated that fermented soy meal added to fish decreased serum total protein. In a study conducted with rainbow trouts, Kumar *et al.* (2011) used meal obtained from *J. curcas* seeds instead of FM and determined that total protein, albumin and globulin concentrations did not differ from the control group. Researchers emphasized that the reason for the difference in the results obtained in our study may be due to immunostimulant effect of *J. curcas* plant. Similar results were obtained in the studies carried out with *J. curcas* plant on different fish species (Kumar *et al.*, 2010).

Plant protein sources are known to affect cholesterol metabolism (Forsythe, 1995). Similar to our study, studies conducted on rainbow trout reported decreased serum cholesterol levels when plant protein sources were used instead of FM in feeds (Romarheim *et al.*, 2006; Yamamoto *et al.*, 2007). In addition, plant products were determined to decrease serum cholesterol levels of land animals (De Schrijver, 1990) to isoflavones found in their structures (Setchell and Cassidy, 1999). Cholesterol-lowering effect of plant protein sources is because they inhibit the absorption of cholesterol from the intestines by increasing the excretion of bile salts from the body (Kumar *et al.*, 2011). The amount of serum triglyceride is an indicator used to monitor the physiological changes caused by short terms of feeding in fish (Bucolo & David, 1973). In our study, serum triglyceride level increased as plant proteins in the feed increased. It was determined that this increase is directly proportional to body fat percentage in the present study. Similar results were obtained in previous studies on mirror carp (Kumar *et al.*, 2010), Nile tilapia (Akinleye, Kumar, Makkar, Angulo-Escalante, & Becker, 2012) and rainbow trout (Kumar *et al.*, 2011).

Serum enzymes are important indicators used to determine organ damages (Racicot, Gaudet, & Leray, 1975). Serum enzymes increase in the presence of

biliary obstruction or any disorder in the liver (Goel, & Agrawal, 1984). In the present study, liver enzymes were found to be higher than in the control and other groups when 30% peanut oil cake was used in the ration instead of FM.

Conclusions

In this study potential use of peanut oil cake instead of FM in feeds of rainbow trout was investigated. Since blood parameters directly affect fish growth and feed conversion, some blood parameters were also examined in order to confirm the effects of plant sources added to feeds. It was determined that the feeds did not adversely affect survival rate of the fish, and growth performance, feed conversion ratio and blood parameters were found to deteriorate with increasing plant material levels in the ration. According to these results, the partial use of peanut oil cake in feeds of rainbow trout may provide an economical production.

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