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Gibel Carp (Carassius auratus gibelio) Meal as an Alternative Major Protein in Feeds for Rainbow Trout Juveniles (*Oncorhynchus mykiss*)

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Abstract

In this study, the effects of gibel carp meal (GFM) application as a protein source on growth performance, fillet composition, feed digestion and haematological and serum biochemical indices of rainbow trout (*Oncorhynchus mykiss*) were evaluated. Replacement of anchovy fish meal (AFM) with GFM was performed at 0%, 50% and 100% levels (GFM_0, GFM_{50} and GFM_{100}). After 60 days of feeding the best nutritional performance was obtained in the GFM_{100} group. There was no significant difference in crude moisture, crude protein, crude lipid or crude ash contents in fish fillet between the GFM_0, GFM_{50} and GFM_{100} groups. No side effects were observed in hematological and serum biochemical indices of rainbow trout. Dry matter, crude protein and crude lipid digestibility coefficients did not differ significantly in experimental groups. As a conclusion, the results of the study suggested that the GFM could be used totally as a replacer of AFM in diets for rainbow trout without the adverse effects on growth performance, feed use, feed digestion, hematological and serum biochemical parameters of fish.

Introduction

Fish meal (FM) is noted the most preferred animal origin protein component in aquaculture feed by the producers due to its valuable features (Hardy & Tacon, 2002). FM is derived from baitfish for large-scale fish, which is important in human consumption (Alder, Campbell, Karpouzi, Kaschner & Pauly, 2008). In addition, fish caught for FM production potentially represent a loss of economic value of fisheries for high trophic level species such as salmon, cod and tuna (Cashion, Le Manach, Zeller & Pauly, 2017). Moreover, fishing fluctuations are subject to negative feed production for aquaculture.

The use of FM in fish feeds has caused increasing price and demand. Due to concerns about high FM cost, in the coming period it has become imperative to reduce or eliminate FM for the fisheries sector. The first objective for the sustainable development of aquaculture is to reduce FM dependency. One of the methods adopted by nutritionists is to replace FM with cheaper animal or plant protein sources, partly or wholly. However, up to now, any plant-based raw material used for carnivorous species has become an alternative to fish. The reasons for these limitations include anti-nutritional factors, lack of amino acid composition, and low digestibility for species (Gaylord *et al*., 2007).

The production of world food fishes has increased day by day. Most of this production (60% of total production) was based on freshwater species (FAO, 2016). Rainbow trout is an important species of aquaculture (Munakata, Ogihara, Schreck & Noakes, 2017). This type has high profitability due to its low feed protection rate when appropriate culture conditions are provided. For this reason, the rainbow

trout (FAO, 2016), provide the world with an estimated 24.5% aquaculture production.

In general, observing physiological reactions is not enough to determine the effects of nutrition on the health status of fish. Thus, changes in blood parameters based on nutritional activities provide a more reliable clue in determining the health status of fish Farhangi & Carter, (2001).

In general, the presence of non-indigenous fish species in habitats has been admitted as negative for the protection of indigenous fish species (Lusková, Lusk, Halačka & Vetešník, 2009). One of the most common progeny in Europe is Carassius auratus gibelio (Szczerbowski, 2002). There is no commercial value of such invasive cyprinid, up to the present it has not been taken into consideration by the public. In addition, it is rich in protein as a feed additive (65% in the current study). In terms of the freshwater fishery sector in Turkey, carp fishery constituted 16% (5,500 tons) of total fishery (Atalay, Kırankaya & Ekmekçi, 2017). Plant feeds are commonly used alternative protein source in the aquaculture (Tacon & Metian, 2008). However, vegetable-based raw materials have some basic amino acid deficiencies and nutritional inhibitors, which limit their use in fish diets.

In conclusion, this study was conducted to evaluate the growth performance and fish health of the rainbow trout juveniles fed with meal obtained from the invasive species (Carassius auratus gibelio) in Europe.

Materials and Methods

Gibel Carp Meal and Experimental Diets Preparation

Gibel carps were obtained from local fisherman. All fish, after removing their internal organs, were separated in small pieces and boiled. They were then dried in the oven at 40°C for 24 hours. Dried fish were ground, stored in airtight plastic containers passed through a 595 µm sieve and kept in a refrigerator at 4°C until required for use in diet production.

The experimental diets were produced in the laboratory. The formulation and major nutrients composition of the 3 experimental diets are presented in Table 1. Diets were prepared by replacing 0% (GFM0), 50% (GFM50), and 100% (GFM100) of the added anchovy meal of the control diet with gibel carp fish meal (GFM). All diets contained approximately 38 % crude protein (CP) and 15 % crude lipid (CL) (Table 1)

Feeding Trial

This study was carried out at the Kastamonu University aquarium unit (Kastamonu, Turkey). The rainbow trout were obtained from the local facility and fed commercial pelleted diet (45 % CP, 18 % CL) for 2 weeks until the start the feeding trial. Then fish were weighted (average weight 4.58±0.09 g) and randomly selected 25 fish per aquarium were distributed to 9 equally sized experimental aquarium (60 × 40 × 50 cm). Fish were hand-fed with experimental diets (in triplicates) to apparent satiation, twice a day for 60 days. During the feeding experiment the average water temperature was 12.8±0.3°C, the dissolved oxygen was 8.2±0.2 mg L-1 and the pH was 7.9±0.1.

Calculations, Proximate Composition Analysis in Fish Fillets and Feeds

Growth performance and feed utilization of experimental fish were calculated described by Kesbiç *et al*., (2016).

The digestibility study was carried out during the last week of the experiment. According to Furukawa (1966), digestion tests were included in 0.5% chromic oxide experimental diets. After seven adaptation periods, the stools were collected using a modified stool collection system for 7 days, centrifuged (4 ° C, 4000 rpm, 15 minutes), freeze-dried and analyzed. Apparent digestibility (AD) coefficients were calculated according to the following formula:

ADDry Matter (%)

ADNutrient (%)

Feed and fish samples (five fish per tank) were analyzed for proximate and fatty acids composition according to AOAC (2003) at the end of trial.

Blood Collections and Analyses

After a 60-day growth period, five fish from each aquarium were collected to draw blood. For blood sampling, fish were anesthetized with phenoxyethanol (Sigma-AIdrich Co. LLC, Germany). Blood samples were collected from the tail vein using a 2 mL syringe then centrifuged at 4000 rpm to separate serum for 10 minutes for biochemical assays. Biochemical indices including glucose (GLU), total protein (TPROT), albumin (ALB) triglyceride (TRI), cholesterol (COL), alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) in serum were analyzed using bioanalytical test kits (Bioanalytic Diagnostic Industry, Co) and measured with a Shimadzu spectrophotometer (PG Instruments, UK).

Nudo & Catap (2011) reported the method for determining the lysozyme activity used. Briefly, 25 µl serum sample is added to 175 µl Micrococcus luteus suspension (pH 5.8) and samples on 96 plates were incubated at room temperature for 30 minutes It allowed. Readings were made at 450 nm multisense microplate reader and standard (L6876 Sigma, Lysozyme from chicken egg white) in µg / mL as standard calculated from the curved line.

Myeloperoxidase activity analysed with some changes of the methods reported in the literature (Quade & Roth 1997; Kumari & Sahoo 2006). For analysis, 10 µl of the serum sample was diluted with 90 µl of HBSS solution. Subsequently, this mixture was added with a solution containing 3,3 ', 5,5'-tetramethylbenzidine dihydrochloride and hydrogen peroxide, and the reaction was stopped with 35 µl of sulfuric acid after 2 minutes. Readings were made at 450 nm multisense microplate reader.

Blood samples were allocated for hematological assays and tuber heparin was added containing the rest for other hematological analysis. Red blood cells (RBC, 106 mm3), hematocrit (Hct, %) and hemoglobin (Hb, g dL-1) was determined by using the method of Blaxhall & Daisley (1973). RBC was counted with a Thoma hemocytometer with the usage of Dacie's diluting fluid. Hct was determined by using a capillary hematocrit tube. Hb concentration was determined with spectrophotometry (540 nm) by using the cyanomethahemoglobin method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated by using the following formula (Bain, Lewis & Bates, (2006):

$$MCV(\mu m3) = [(Hct, \%) \times 10]/(RBC, \times 106 \text{ per } mm3),$$

$$MCH(pg) = [(Hb, g/dL) \times 10]/(RBC, \times 106 \text{ per } mm3) \text{and}$$

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

Statistical Analyses

Values of all measured variables are expressed as least square means and standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Tukey multi comparison

Table 1. Ingredients (g kg^{-1}), composition of the experimental diets and essential amino acids (AA) profile (as % of protein) in anchovy fish meal (AFM), gibel carp fish meal (GFM), and experimental diets containing graded replacement levels of AFM with GFM

Ingredients composition (g kg^{-1})			GFM_0	GFM_{50}	GFM_{100}
Fish meal[1]			470	235	-
Gibel carp meal			-	235	470
Soybean meal[2]			200	200	200
Wheat meal[2]			110	110	110
Corn starch[2]			75	80	85
Fish oil[3]			125	120	115
Vitamin – Mineral[4]			40	40	40
Total			1000	1000	1000
Gross composition(g kg^{-1}DM)					
	AFM	GFM	GFM_0	GFM_{50}	GFM_{100}
Protein	57.02	65.05	384.0	388.7	389.4
Lipid	13.76	10.63	155.3	153.9	154.7
Ash	15.44	17.02	111.7	122.1	136.6
NFE	11,15	6,06	329.0	304.5	284.3
Aspartic Acid	1.88	2.20	0.88	0.96	1.00
Glutamic Acid	0.96	1.27	0.45	0.52	0.58
Serine	13.93	13.44	6.54	6.43	6.12
Histidine	1.50	1.27	1.03	0.98	0.91
Glycine	8.35	9.34	4.38	4.39	4.25
Alanine	8.26	8.86	4.16	4.16	4.03
Tyrosine	1.61	1.82	0.85	0.86	0.83
Valine	2.47	2.55	1.86	1.88	1.86
Methionine	1.05	1.09	0.68	0.70	0.69
Tryptophan	5.29	5.11	2.66	2.62	2.50
Phenylalanine	1.42	1.73	1.34	1.42	1.46
Isoleucine	1.63	2.08	1.47	1.58	1.65
Leucine	0.31	0.54	1.22	1.28	1.32
Lysine	6.83	6.49	3.16	3.32	3.45

[1]Anchovy fish meal. Koptur Balıkçılık. Trabzon.Turkey

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

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

[4]Vitamin and Mineral Mixture: Vitamin A. 18000 IU kg^{-1}feed; Vitamin D_3. 2500 IU kg^{-1}feed; Vitamin E. 250 mg kg^{-1}feed Vitamin K_3. 12 mg kg^{-1} feed; Vitamin B_1. 25 mg kg^{-1}feed; Vitamin B_2. 50mg kg^{-1}feed; Vitamin B_3. 270 mg kg^{-1}feed; Vitamin B_6. 20 mg kg^{-1}feed; Vitamin B_{12}. 0.06 mg kg^{-1} feed; Vitamin C. 200 mg kg^{-1}feed; Folic acid. 10 mg kg^{-1}feed; Calcium d–pantothenate. 50 mg kg^{-1}feed; Biotin. 1 mg kg^{-1}feed; Inositol. 120 mg kg^{-1}feed; Choline chloride. 2000 mg kg^{-1}Fe. 75.3 mg kg^{-1}; Cu. 12.2 mg kg^{-1}; Mn. 206 mg kg^{-1}; Zn. 85 mg kg^{-1}; I. 3 mg kg^{-1}; Se. 0.350 mg kg^{-1}; Co. 1 mg kg^{-1} feed.

test. Statistical significance was established at P<0.05.

Results

Growth Performance

No deaths occurred during the experiment. GFM treatment in the diet, significantly influenced the weight gain and the best growth performance was obtained in GFM100 group and was significantly different compared to GFM0 (P<0.05). The specific growth rate was increased by the amount of GFM in the feed and found significantly different between treatment groups (P<0.05). The lowest feed conversion ratio was obtained in GFM100 groups and found significantly different from GFM0 group (P<0.05). There were no significant differences between treatment groups for hepatosomatic index (P>0.05). Viscerosomatic index in GFM100 diets was significantly higher than GFM0 diet (P<0.05). (Table 2)

Digestibility of Feedstuffs

The dry matter, protein and lipid digestibility coefficients are given in Table 3. The apparent digestibility coefficients for dry matter, protein and lipid were not affected by different GFM levels in tested diets (P>0.05).

Fish Fillets Composition

The fillet proximate composition of fish is shown in Table 4. Partial or complete replacement for AFM diets with GFM were also unaffected (P<0.05) in protein, lipid and ash content.

Blood Parameters

Changes in hematological and biochemical variables when the AFM was partially or completely substitute with GFM are shown in Table 5. The replacement of AFM with GFM in diets for rainbow trout did not significantly affect the hematological parameters such as erythrocytes counts, hemoglobin, cell hemoglobin and mean cell hemoglobin concentration compared to the mean control group (p> 0.05). Only the hematocrit value was slightly decreased in the GFM100 group and was significantly different from other groups (P<0.05).

Although the hematological parameters were not

Table 2. The growth performance of rainbow trout fed formulated diets containing GFM at graded replacement levels for AFM on protein unit basis over 60 days feeding trial

	GFM_0	GFM_{50}	GFM_{100}
Initial weight (g)	4.51±0.07^{a}	4.66±0.09^{a}	4.67±0.05^{a}
Final weight (g)	8.42±0.99^{a}	9.01±1.67^{ab}	10.04±0.56^{b}
Weight gain (%)	84.77±3.65^{a}	92.76±1.18^{ab}	99.30±6.40^{b}
Spesific growth rate	1.28±0.08^{a}	1.45±0.03^{b}	1.62±0.06^{c}
Feed conversion rate	1.15±0.05^{b}	1.09±0.01^{ab}	1.02±0.03^{a}
Hepatosomatic index	1.76±0.05^{a}	1.55±0.26^{a}	1.78±0.29^{a}
Viscerosomatic index	17.90±1.97^{a}	15.87±2.20^{a}	23.09±1.76^{b}

Values with the same letters as superscripts in the same row are not significantly different (P > 0.05).

Table 3. Apparent digestibility coefficients (ADCs) of experimental diet components

	GFM_0	GFM_{50}	GFM_{100}
Dry matter	75.04±5.10	77.27±8.64	74.89±6.21
Protein	93.42±4.14	95.22±6.49	94.78±5.69
Lipid	89.02±2.47	90.44±3.44	90.55±5.74

Values with the same letters as superscripts in the same row are not significantly different (P > 0.05).

Table 4. Proximate composition of rainbow trout fillets fed with experimental diets for 60 days

	GFM_0	GFM_{50}	GFM_{100}
Protein	17.45	18.02	18.65
Lipid	14.50	13.96	14.01
Ash	1.16	0.97	1.03

affected have changed with the substitution of AFM with GFM, some biochemical parameters were found significantly different (Table 6). Serum glucose (GLU) value was higher in GFM100 group compared with GFM0 (P<0.05). Serum triglycerides (TRIG) value was found lower in GFM50 and GFM100 groups and significantly different from GFM0 (P<0.05). Albumin (ALB) was higher in GFM100 group compared with GFM0. Serum total protein (TPROT), cholesterol (CHOL), globulin (GLO), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), lysozyme activity (LIZ), myeloperoxidase activity (MPO) and super oxidase dismutase (SOD) values did not demonstrate significant differences between the experimental groups (P> 0.05).

Discussion

The effects of dietary vegetable or animal protein source on growth performance, fatty acid profiles and some hematological and biochemical variables of rainbow trout have been evaluated in earlier studies (Güllü, Acar, Tezel & Yozukmaz, 2014). However, using low level of FM in carnivorous fish diets is more complicated without reducing growth performance and animal health. Also, FM replacement by alternative plant stuffs may reduce amino acid content of diets and this can also effect the immune response (Li, Wang, Zheng, Jiang & Xie, 2009). The current work is the first study addressing the effect of dietary GFM on growth performance, fatty acid profile, hematological and serum biochemical variables as a protein source in the diets of rainbow trout. The results of the present study showed that the totally replacement of AFM with GFM successfully implemented in rainbow trout diets without adverse effect on growth performance and feed utilization or fish health status. These results are overlap with Gümüş, (2010) who replaced the FM with sand smelt meal in diets for common carp, Cyprinus carpio up to 75%. Also, Spinelli, Mannken & Stemberg, (1979) found no significant differences in growth performance of Coho salmon (Oncorhynchus kisutch) fed with fly larvae meal as a source of protein in diets compared with FM based diet. Several other raw material of animal origin have been tested with various fish species for replacement of FM with limited rate. (Abdelghany, 2003; Güllü *et al*., 2014; Mugo-Bundi *et al*., 2015; Gasco *et al*., 2016). The limited use reason of these ingredients compared with FM was that the essential amino acid contents were poor and presence

Table 5. Blood parameters of rainbow trout fed formulated diets containing gibel carp fish meal at graded replacement levels for anchovy fish meal on protein unit basis over 60 days feeding trial

	GFM_0	GFM_{50}	GFM_{100}
Erythrocytes count (cel×10^{12}/L)	1.85±0.13^a	1.71±0.05^a	1.72±0.23^a
Hematocrit (%)	32±1^b	32.67±0.57^b	29.33±0.57^a
Hemoglobin (g/L)	36.90±2.80^a	45.00±10.40^a	44.60±2.90^a
Mean cell volume (fL)	864.30±38.3^a	954.60±25.40^a	862.40±100.1^a
Mean cellular hemoglobin (pg/cell)	20.04±3.02^a	26.38±6.54^a	26.43±5.16^a
Mean cellular hemoglobin concentration (g/L)	115.60±12.10^a	138.00±30.40^a	152.30±12.50^a

Values with the same letters as superscripts in the same row are not significantly different (P>0.05).

Table 6. Serum biochemical parameters of rainbow trout fed formulated diets containing gibel carp fish meal at graded replacement levels for anchovy fish meal on protein unit basis over 60 days feeding trial

	GFM_0	GFM_{50}	GFM_{100}
GLU (µmol/L)	3.27±0.15^a	2,96±0.41^a	3.99±0.42^b
TPROT (g/L)	32.70±3.50^a	37.40±4.20^a	35.10±2.50^a
TRIG (µmol/L)	0.75±0.03^b	0.56±0.06^a	0.59±012^a
CHOL (µmol/L)	5.43±0.15^a	5.57±0.44^a	5.36±0.27^a
ALB (g/L)	6.10±0.70^a	7.10±0.70^{ab}	7.40±0.05^b
GLO (g/L)	28.10±3.00^a	29.70±3.00^a	27.70±2.00^a
GOT (µkat/L)	1.67±0.28^a	1.71±0.19^a	1.90±0.26^a
GPT (µkat/L)	0.39±0.03^b	0.29±0.05^a	0.29±0.02^a
ALP (µkat/L)	0.86±0.07^a	0.96±0.12^a	0.90±0.13^a
LIZ (nkat/L)	173.17±7.67^a	174.33±23.17^a	174.17±20.17^a
MPO (nkat/L)	17±1.17^a	17.16±0.33^a	17.17±1.00^a
SOD (nkat/L)	1.51±0.07^a	1.52±0.05^a	1.51±0.07^a

Values with the same letters as superscripts in the same row are not significantly different (P> 0.05). GLU.glucose; TPROT.total protein; Trıg. triglyceride; CHOL.cholesterol; ALB.albumin; GLO = globulin; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; ALP = alkaline phosphatase; LIZ = lyzosme activity; MPO = myeloperoxidase activity; SOD = Superoxide dismutase activity

of chitin to FM. The advantage of GFM used in the present study compared with AFM was that the essential amino acid contents were similar without anti-nutritional factors. No significant differences were observed in dry-matter digestibility when rainbow trout fed different GFM rates. To the best of our knowledge, there is no study on the digestibility of diets formulated using GFM. However, previous studies reported the low dry-matter digestibility of feedstuffs related with fiber and ash content (McGoogan & Reigh, 1996; Bureau, Harris & Cho 1999). The current values of the study are comparable with those reported for herring meal (94.9%), menhaden meal (89.9%) and poultry by-product meal (95.9%) in rainbow trout. (Sugiura, Dong, Rathbone & Hardy,1998). The high protein digestibility values in this study explain with the availability coefficients of amino acids tend to reflect the apparent protein digestibility of highly digested feed ingredients (Zhou, Tan, Mai & Liu, 2004; Wu, Liu, Tian, Mai &Yang, 2006).

Haematological and serum biochemical parameters help to understand fish health status, nutritional quality and rearing conditions of fish (Cnaani, Tinman, Avidar, Ron & Hulata 2004; Fazio, Filiciotto, Marafiotti, Di Stefano, Essenza, Placenti, Buscaino, Piccione &Mazzola 2012; Shi, Zhang, Gao, Peng & Zhang, 2015). In the present study, replacement of AFM with GFM showed no haematological abnormalities in rainbow trout juveniles. Similar results were obtained by Abdelghany, (2003), when used gambusia meal in tilapia diets. Some biochemical parameters such as glucose level in GFM100 groups showed differences compared with other experimental groups. Increased serum glucose level showed that GFM have no adverse effect on energy metabolism in fish (Zhou, Mai, Tan & Liu, 2005). Total serum protein is a key indicator in the metabolism of diet, and its reduction is likely due to decrease in digestion and dietary metabolism. In the present study, serum total protein values showed no significant differences between the experimental groups. These findings consistent with previous results on the substation of animal based protein in rainbow trout diets (Güllü *et al*., 2014). Also, a decreasing trend were obtained by Farhangi & Carter (2001) when FM substitute with lupin meal in rainbow trout diets. These caused by digestible amino acid content of protein sources in raw material used in diets. There were no significant differences in total cholesterol across the all treatments. This suggests the culture system provided optimal conditions for growth. Blood cholesterol levels are affected by various factors such as cholesterol metabolism and feed consumption (Tocher, Bendiksen, Campbell & Bell, 2008). The combination of animal protein in the test diet might have avoided hypocholesterolemic effects as reported in other studies involving plant-based fish meal replacement (Chen, Wooster, Getchell, Bowser & Timmons 2003; Borgeson, Racz, Wilkie, White & Drew, 2006; Soltan, Hanafy & Wafa, 2008; Lim & Lee, 2009). Liver enzymes rise in the blood serum due to case of damage to the cell membrane. Also, some physiological and environmental factors including diets, ambient temperature, fish age, and salinity affect the levels and activities of serum enzymes (Costillas & Smith 1997). In aquaculture, these enzymes (such as GOT, GPT, ALP) can be used to determine the liver lesion (Soltanzadeh, Esmaeili Fereidouni, Ouraji & Khalili, 2016). In the present study, there were no significant differences in the liver enzymes activity in the experimental groups. Studies on Nile tilapia, Oreochromis niloticus showed no significant differences on liver enzymes when fish meal replace with knife fish Chitala ornata meal in diets (Abarra *et al*., 2016).

The main goal of the present study were GFM obtained from a invasive species can be used totally in diets for rainbow trout juveniles without adverse effect on growth performance, fillet proximate composition and fish health. Hereby, non-economic species can be used industrially. Further studies is highly suggested can totally controlled production of this invasive species and used in aqua feed industry to reduce FM price.

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