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Replacement of Fish Meal with Fish Processing by-Product Silage in Diets for the Rainbow Trout, *Oncorhynchus mykiss*

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Abstract.- This study evaluated the effects of the dietary fish silage (FS) used as a replacement for fish meal (FM), on growth performance, fatty acid composition and serum variables of rainbow trout, *Oncorhynchus mykiss*. Four experimental diets, including 0% (FS0), 20% (FS20), 40% (FS40) and 60% (FS60) of fish silage, were formulated. Diets were given to triplicate groups of 40 fish/cage (average weight 52.90±1.88 g) for 8 weeks. The results showed that there were no significant differences in growth performance and nutrient utilization between control and FS20 group ($p>0.05$). Fish fed with the FS20 diet grew significantly better than others ($p<0.05$). Fillet protein and lipid content decreased with increasing of fish silage addition ($p<0.05$). FM replacement by FS did not affect the serum glucose level, but significantly increased total protein, triglyceride and total cholesterol levels ($p<0.05$). Fatty acid composition of fish fillet was no significantly different between groups ($p>0.05$). Results showed that fish processing by-product silage has potential to replace fish meal up to 20% in rainbow trout diets without adverse effects on growth performance, fatty acid composition and serum biochemical variables.

Key words: Rainbow trout, *Oncorhynchus mykiss*, fish silage, growth performance, fatty acid composition.

INTRODUCTION

The availability, cost and environmental sustainability of feed fish are some of the main bottlenecks preventing the expansion of aquaculture industry (Worm *et al.,* 2006). Farmed carnivorous fish are traditionally fed with diets containing large amounts of marine fish meal (FM) (Torstensen *et al.,* 2008). Meanwhile, price of fish meal keeps increasing because of the diminishing fish stock and increasing demand for the product. The increasing demand and progressive scarcity of FM in the international market boosted its price and launched the quest for reduction of FM in fish diets and the consequent search for alternative, acceptable protein sources (Yıldırım *et al.,* 2014). By far, many studies have been conducted to find out alternative raw materials to replace FM for rainbow trout (Yoshitomi *et al.,* 2006; Hunt *et al.,* 2014). However, the use of an important protein source such as fish silage has not yet been widely studied for rainbow trout. Waste management is an important research field addressing ecological balance through the diminution of environmental problems and provision of sustainable production and economic benefits. Fish silage obtained by using organic acid is called acid silage to differentiate it from fermented silage that is usually mixed with molasses or lactic acid bacteria (Mach *et al.,* 2010). To give an example, approximately, 100.000 tons of seafood is processed in Turkey every year. As a result, a significant amount of unused waste piles up. Reprocessing this waste into fish silage and using the silage to replace FM in feed has been tested in different species such as Atlantic salmon, *Salmo salar* (Heras *et al.,* 1994), tilapia *Oreochromis niloticus* (Fagbenro, 1994). With regards to rainbow trout *Oncorhynchus mykiss*, the only studies available were conducted by Stone *et al.* (1989) and Güzel *et al.* (2011). Their results showed as fish silage could be considered a good protein sources for aquaculture. However, they did not evaluate fatty acids profiles and serum biochemical variables of fish.

The main aim of present study was to determine the growth performance, fillet fatty acid composition and some serum biochemical variables of rainbow trout when fish fed diets produced from

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fish processing by-product silage.

MATERIALS AND METHODS

Silage production via acid application from fish processing waste

Marine fish processing by-products (inedible parts such as head, skin, fins and viscera) were obtained from Marenostro Fish Processing Facility located in Milas (Mugla, Turkey). The marine fish by-products were minced by an electrical mincer with a 6 mm mirror diameter hole. In accordance with Turkish Food Regulations, 85% formic acid (Tekkim trademark) was added at 3.0% of weight and then homogenized with mixer. The pH of the resulting mince was reduced below 4 and maintained at this level. The acid was blended to the mince homogenously to achieve dispersion of acid in all parts of the waste. Also, at this phase, 300 ppm BHT (butyl hydroxy toluene) was added into the mince as an antioxidant (Tatterson and Windsor, 1982; Beveridge, 1987). The obtained mixture put into tight-head acid proof 60L plastic storage containers and kept at an ambient temperature. The silages (FS) were left to ripen and only stirred once a day for the first 10 days. Silage pH was measured and recorded daily and acid was added as required in order to control pH levels below 4 (Tatterson and Windsor, 1982).

Experimental diets

Four experimental diets, each containing a different level of replacement of FM protein by FS (47% Crude protein, 26% Crude lipid) protein: 0%, 20%, 40% and 60% (Table I), were prepared. The dry ingredients were carefully mixed with a laboratory food mixer. The mixtures were primed with water to yield a suitable pulp. Wet diets were made into 4-mm pellets, dried at 40°C in a drying cabinet, and stored at -20°C until use.

Experimental animal, experimental set-up and growth, nutrient utilization

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Selina Aquaculture Facility located in Fethiye, (Mugla, Turkey). Fish were kept in a pond (5000 l) for acclimatization period of 1 wk. During acclimatization they were fed by Agromey standard fish diet containing 43% protein and 17% lipid of dry matter. Fish (average weight 52.90±1.88 g) were randomly distributed in four treatment groups with three replicates in with 4 mm mesh size net cages (80×80×80 cm). The duration of the experiment was

Table I.- Percentage and proximate composition of the experimental diets containing supplement of different FS rate

	FS-0	FS-20	FS-40	FS-60
Ingredients (%)				
Fish meal	47.00	37.60	28.20	18.80
Fish silage	-	16.00	28.20	45.80
Soybean meal	20.00	20.00	20.00	20.00
Fish oil	12.50	9.40	7.10	3.40
Wheat meal	11.00	9.00	9.00	6.00
Corn starch	7.50	6.00	5.50	4.00
Vitamin–mineral mix[1,2]	2.00	2.00	2.00	2.00
Total	100	100	100	100
Chemical analyses (%, DM)				
Protein	45.00	45.20	45.50	45.60
Fat	17.90	17.50	16.90	17.30
Ash	8.37	7.42	6.47	5.44
NFE[3]	24.70	25.90	27.40	28.70
Gross energy, MJ/kg[4]	20.01	20.07	20.15	20.14
Fatty acid composition of diets (%, DM)				
14:00	7.90	5.81	5.19	5.21
15:00	0.67	1.09	1.38	1.87
16:00	21.31	19.93	19.82	17.93
18:00	6.02	5.98	5.45	4.81
20:00	1.05	1.12	1.12	1.22
16:1n-7	8.19	6.60	6.69	5.25
18:1n-9	14.30	18.79	21.91	24.55
20:5n-4	0.43	0.41	0.40	0.44
20:1n-9	1.50	1.69	1.79	2.04
18:2n-6	12.75	12.67	11.56	10.49
18:3n-3	1.36	2.17	2.75	3.64
22:2n-6	0.55	0.44	0.33	0.22
22:5n-3	8.42	6.50	6.26	6.47
22:6n-3	14.38	15.68	14.33	14.96
ΣSFA	36.95	33.93	32.96	31.04
ΣMUFA	24.42	27.49	30.79	32.28
ΣPUFA	37.46	37.46	35.23	35.78
Total n-3	24.16	24.35	23.34	25.07
Total n-6	13.30	13.11	11.89	10.71

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

[2]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.

[3]Nitrogen–free extracts (NFE) = matter – (crude lipid+crude ash+crude protein).

[4]Energy calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, and 17.0 kJ g^{-1} NFE.

8 weeks. Fish were fed handed *ad libitum* twice a day (10:00–17:00 h). The proximate composition of diet ingredients, diets and fillet of fish was determined using the standard methods of the Association of Official Analytical Chemist (AOAC, 2000).

Growth performance and feed utilization were calculated by the following formulas:

FCR = feed consumed / weight gain

RGR, % = [(final wet weight - initial wet weight) / initial wet weight] × 100

SGR = [(ln final wet weight - ln initial wet weight) / days] × 100

HSI, % = (100 × liver weight ×BW^{-1})

VSI, %= (100 × carcass weight ×BW^{-1})

Lipid extraction and fatty acid analysis

Dietary and whole body lipids were extracted with chloroform/methanol (2:1 v/v) according to the procedure of Folch *et al.* (1957). Fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% boron trifluoride-methanol (AOAC, 2000). Fatty acid methyl esters (FAME) were analyzed using a flame ionization gas chromatograph (Shimadzu GC-2014) equipped with a Omegawax 250 capillary column (30 ml X 0.25 mm internal diameter), a flame ionization detector (FID), and a split injection system with nitrogen carrier gas. The injector port and detector temperatures were maintained at 250°C and 260°C, respectively. The column temperature program was held at 140°C for 5 min, then elevated at a rate of 3°C/min to 200°C. Total run time was 60 min per sample. Fatty acids were identified by comparing their retention times to authentic standard fatty acid standards (Sigma-Aldrich Co., USA).

Blood collection

After day 60, blood sampling was conducted to assess the effects of dietary FS on biochemical variables. Twelve randomly selected fish from each group were not fed for a period of 24 h and then anesthetized with clove oil. Blood samples of fish were collected from the caudal vein with a syringe, added to the tubes containing EDTA (BD Microtainer®, UK). Blood serum was separated by centrifugation (4000 (g, 10 min) in plastic biochemistry tubes (Kima-vacutest®, Italy) and stored at -20°C until biochemical analysis.

Biochemical analysis

Biochemical indices, including glucose (GLU), total protein (TPROT), triglyceride (TRI) and cholesterol (CHO) in serum were analyzed using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) and measured by a Shimadzu spectrophotometer (PG Instruments, UK).

Statistical analyses

Values of all measured variables were expressed as mean ± SD. Statistical significance was determined by one-way ANOVA; when differences between treatment found, Tukey's test (in SPPS version 17.0) was used to compare means. Differences were considered significant at *p*-values less than 0.05.

RESULTS

Growth performance, feed efficiency, biological indices results and blood serum variables are shown in Table II. At the end of the 60 day feeding trial survival was 100% in all treatments groups. There was no significant difference in weight gain (RGR %) between fish fed FS-0 and FS-20 diets. However, a significant reduction of this variable occurred when FM replacement level was increased by FS ($p<0.05$). Specific growth rate (SGR) and feed conversion rate (FCR) showed the same trend as RGR while the proportion of FS increased in the diets ($p<0.05$). There were no significant differences in hepatosomatic index (HSI) and viscera somatic index (VSI) among all groups ($p>0.05$). Fillet protein, lipid and ash content were affected by dietary treatment. They showed decreasing trend with increasing of FS level in diets ($p<0.05$). Biochemical variables of fish are shown in Table II. By the end of 60 day feeding there were no significant differences in glucose (GLU) among all groups ($p>0.05$). The dietary FS supplementation increased TRIG and CHO level, the highest values obtained in FS-60 groups ($p<0.05$). TPROT level were affected by the FS supplementation ($p<0.05$). TPROT increased by increasing dietary FS level in the diets. Fillet fatty acids composition of fish fed

Table II.- Growth, biological indices and blood serum variables of the rainbow trout fed with experimental diets for 8 week growth trial.

	FS0 (Control)	FS20	FS40	FS60
Growth and feed efficiency				
RGR (%)[1]	137.13±7.47^{a}	139.60±6.32^{ab}	117.31±2.76^{bc}	99.99±12.60^{c}
FCR[2]	1.31±0.07^{ab}	1.18±0.11^{a}	1.39±0.03^{ab}	1.56±0.20^{b}
SGR (%day $^{-1}$)[3]	1.44±0.05^{a}	1.46±0.04^{a}	1.29±0.02^{ab}	1.15±0.10^{b}
HSI (%)[4]	1.36±0.26^{a}	1.21±0.13^{a}	1.36±0.16^{a}	1.32±0.09^{a}
VSI (%)[5]	12.35±3.90^{a}	10.84±1.29^{a}	12.87±1.52^{a}	13.60±1.52^{a}
Body composition (wet wt basis)				
Moisture	72.66±1.17^{a}	73.36±0.90^{a}	71.60±1.90^{a}	74.00.1.20^{a}
Protein	19.43±0.16^{b}	18.26±0.28^{ab}	18.12±0.81^{ab}	16.98±0.05^{b}
Lipid	4.52±0.62^{ab}	5.85±0.89^{b}	4.88±0.40^{ab}	3.66±0.28^{a}
Ash	3.08±0.50^{b}	2.48±0.51^{ab}	2.11±0.20^{ab}	2.03±0.23^{a}
Blood serum				
Glucose (mg/dl)	19.26±4.16^{a}	24.30±6.76^{a}	19.66±3.86^{a}	27.29±7.25^{a}
Total protein (g/dl)	1.61±0.72^{a}	3.09±0.88^{b}	2.87±0.76^{b}	3.58±0.76^{b}
Triglycerides (mg/dl)	21.12±3.39^{a}	22.64±8.14^{a}	71.11±14.26^{b}	92.25±11.54^{c}
Cholesterol (mg/dl)	50.06±14.08^{a}	52.46±5.38^{a}	164.00±31.88^{b}	188.17±27.21^{b}

Values are provided as mean ± SD. Means in a row with different superscripts significantly differ ($p<0.05$).
[1]Relative growth rate = 100[(final wet wt - initial wet wt)/initial wet wt]
[2]Feed conversion ratio = feed intake/wt gain
[3]Specific growth rate = 100(ln final fish wt - ln initial fish wt)/days
[4]Hepatosomatic index = 100(liver wt/body wt)
[5]Viserosomatic index = 100(carcass wt/body wt)

with experimental diets for 8 weeks were shown in Table III. Total saturated fatty acids (SFA) and total polyunsaturated fatty acids (PUFA) were not significantly different among groups ($p>0.05$).

DISCUSSION

Substitution of FM with less expensive protein sources would be beneficial in reducing feed costs for fish feed industry. The results of this study clearly showed that marine fish processing by-products silage can be included in diet of rainbow trout at up to 20% of fish meal protein without negative effects. The high protein content of FS makes it a good source of animal protein in fish diets (Fagbenro, 1994) being also economically and more environmental friendly. Growth performance and feed utilization were affected by the fish meal replacement level. Growth performance decreased while fish meal substitution level increasing with FS in diets. These results were comparable with those obtained by Goncalves *et al.* (1989) who, feeding young eels (*Anguilla anguilla*) with fish silage obtained from sardines, reported that the best growth was obtained in the group fed with feed containing 20% silage. Crampton *et al. (*1982) found that salmon fed 25% silage based diet grew faster compared with commercial dry pellet. The same result was also observed in Atlantic salmon (*Salmo salar*) when 10% fish meal protein was replaced by fish silage protein in diet (Espe *et al.*, 1992). A higher growth performance was reported for mrigal carp (*Cirrhinus mrigala*) fed with 53.1% of fish silage based diet than fish fed with a fish meal based diet (Ali *et al.*, 1994). FCR and SGR were also influenced by dietary treatment. FCR values slightly improved up to 20% fish meal replacement, then decreased to 60%. These results were in agreement with other studies when a partial or total fish meal was substituted with different raw materials in rainbow trout diets (Farhangi and Carter, 2001; Yoshitomi *et al.*, 2006; Shafaeipour *et al.*, 2008). These results can be attributed with palatability and or attractiveness of the feed

Table III.- Flesh fatty acid profiles (% fatty acids) of rainbow trout fed by experimental diets for 60 days.

	FS-0	FS-20	FS-40	FS-60
Fatty acid composition of fillets				
14:00	3.16±0.21[b]	2.99±0.08[ab]	2.75±0.06[a]	2.75±0.09[a]
15:00	0.24±0.02[a]	0.26±0.02[a]	0.25±0.02[a]	0.26±0.03[a]
16:00	14.28±0.73[a]	14.82±0.62[a]	14.53±0.66[a]	14.19±0.76[a]
18:00	4.45±0.07[b]	4.07±0.15[a]	4.08±0.05[a]	3.78±0.15[a]
20:00	0.73±0.02[a]	0.73±0.01[a]	0.66±0.03[a]	0.70±0.05[a]
16:1n-7	4.54±0.08[a]	4.37±0.11[a]	4.31±0.09[a]	4.52±0.11[a]
18:1n-9	30.70±0.81[a]	30.35±0.86[a]	30.23±0.82[a]	30.27±0.84[a]
20:5n-4	0.31±0.01[a]	0.44±0.05[ab]	0.50±0.07[b]	0.48±0.05[b]
20:1n-9	2.06±0.04[ab]	1.98±0.06[a]	1.99±0.05[a]	2.20±0.12[b]
18:2n-6	20.24±0.75[a]	20.86±0.71[a]	20.93±0.57[a]	20.58±0.74[a]
18:3n-3	3.58±0.06[a]	3.85±0.08[b]	3.99±0.08[b]	4.06±0.16[b]
22:2n-6	2.97±0.02[b]	2.70±0.08[ab]	2.54±0.06[ab]	2.30±0.10[a]
22:5n-3	0.91±0.04[a]	0.88±0.06[a]	0.97±0.08[a]	0.92±0.05[a]
22:6n-3	10.30±0.24[a]	10.45±0.32[a]	10.10±0.12[a]	10.60±0.21[a]
ΣSFA	22.84±1.05[a]	22.87±0.88[a]	22.27±0.82[a]	21.68±1.08[a]
ΣMUFA	37.61±0.78[a]	37.14±1.08[a]	37.03±1.03[a]	37.47±1.12[a]
ΣPUFA	38.00±1.12[a]	38.74±1.26[a]	38.53±1.12[a]	38.46±1.26[a]
n-3	14.79±0.35[a]	15.18±0.47[a]	15.06±0.49[a]	15.58±0.42[a]
n-6	23.21±0.77[a]	23.56±0.79[a]	23.47±0.84[a]	22.88±0.84[a]

Values are provided as mean ± SD. Means in a row with different superscripts significantly differ ($p<0.05$).

ingredients. Partial replacement of FM with FS influenced the fillet protein and lipid composition, decreased with increasing dietary FS inclusion. This may be explained by the less efficient utilization of protein in the FS groups. Similar results were obtained in common carp *(Cyprinus carpio)* when tuna liver meal was used as a fish meal replacer in diets (Gümüş *et al.*, 2009).

Information on the effects of dietary animal protein sources on serum total protein, glucose, total cholesterol and triglyceride levels of rainbow trout were still limited in the literature. Increased glucose level is a well-known stress indicator in fish (Morgan and Iwama, 1997) and nutritional status can affect the glucose response in fish (Martínez-Porchas *et al.*, 2009). The results of the present study showed no significant differences among the groups for GLU level. These results were similar to that found for Siberian sturgeon (*Acipenser baerii*) when fish meal in diets was replaced with rendered animal protein (Zhu *et al.*, 2011). Serum protein, levels are thought to be related to a strong innate immune response of fish (Magnadóttir, 2006). The present study showed an increased serum total protein levels with increasing of FS in dietary, suggesting that dietary FS had no negative effects on the immune system of rainbow trout. For this reason, increase in TP level will enable the fish to be more resistant to stressful conditions. Cho *et al.* (2007) reported that when the animals fed high quality protein sources, they had higher concentrations of TP in serum. At the end of the 8 week, results of the study showed that total cholesterol and triglyceride levels increased with the augment of dietary FS. However, decreased of CHO and TRIG levels in rainbow trout have been reported when used plant protein sources in diets (Kaushik *et al.*, 1995; Romarheim *et al.*, 2006). On the contrary, CHO level did not show differences in Siberian sturgeon (*Acipenser baerii*) when FM was replaced with rendered animal protein source (Zhu *et al.*, 2011). These results could indicate that either cholesterol metabolism in fish was different involved in the control of cholesterolemia (Kaushik *et al.*, 1995). Further study investigating this aspect will be able to clarify this hypothesize. Fatty acid composition of fish reflects the fatty acid composition in the diet (De Francesco *et al.*, 2004). In the present study, the fatty acid profile in the fish was impacted by that of the feed, as in other studies

on rainbow trout (Morris *et al.,* 2005). The experimental diets used in the present study contained both n-3 and n-6 highly unsaturated fatty acids such as linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (22:6n-3; DHA) acids and may have satisfied the essential fatty acid requirements of rainbow trout (Sargent *et al*., 2002). Shafaeipour *et al.* (2008) performing a study to determine the effects of canola meal in rainbow trout, found a lower level of n-3 and n-6 ratios of fish fed vegetable protein source than that of the present study.

In conclusion, the results of the present study showed that fish processing by-product silage can be safely used in rainbow trout diets up to 20% without adverse effects on growth, fatty acid profile and serum biochemical variables. At the light of these results, further future studies to evaluate the use of fish silage in fish diets are encouraged.

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