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The Influence of Replacing Fish Meal Partially in Diet With Soybean Meal and Full-fat Soya on Growth and Body Composition of Rainbow Trout (*Oncorhynchus mykiss*)*

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Abstract.- Rainbow trouts were fed for five months with iso-nitrogenous (average 50.36 % crude protein) diets in which dietary protein was supplied by fish meal (FM), soybean meal (SBM) or full-fat soya (FFS). Diet 1 (control diet) contained 43% FM and 25% SBM. Those of experimental diets were 28% FM and 40% SBM for Diet 2, 13% FM and 55% SBM for Diet 3, 28% FM and 40% FFS for Diet 4, and 13% FM and 55% FFS for Diet 5, respectively. It was found that an increase in plant protein in diets adversely affected growth performance and feed conversion ratio (FCR) in rainbow trout. It was also found that the fish fed with the diet containing FFS had significantly higher FCR than the fish fed with SBM ($P<0.05$). In comparison control diet to fish fed with Diet 2 and 3 there were no significant differences ($P>0.05$) in body protein content. In addition, the lowest cost to produce 1 kg fish can be obtained from experimental Diet 2. There was not a significant ($P>0.05$) difference in specific growth rate, protein efficiency ratio, protein productive value, lipid productive value, energy productive value and FCR between control diet and Diet 2, and SBM can be used up to 40% in rainbow trout growth rations. In conclusion, this study showed that Diet 2 is optimum for rainbow trout.

Key words: rainbow trout, *Oncorhynchus mykiss*, soy bean meal, full fat soy bean meal, growth, nutrition.

INTRODUCTION

Aquaculture are one of the fastest growing food producing activities in the world. Food and Agriculture Organization (FAO) statistics show that nearly half of the fish consumed as food worldwide are raised on fish farms rather than caught in the wild (Pickova and Morkore, 2007). Similarly, production of cultured fish in Turkey has increased remarkably over the past decade. The ratio of cultured fish production to total fish production was 1.5% in 1990s, 14% in 2000 and more than 20% in 2005 (Anonymous, 2006). For example, in 1996, cultured fish production was approximately 33,000 tonnes. It reached more than 125,000 tonnes in 2006, and forecasts indicate further increase in fish production (Köprücü, 2007).

Ongoing intensification of aquaculture in Turkey has made it essential to develop suitable and supplemental diets for fish using alternative protein sources. Traditionally, fish meal (FM) has been the main source of dietary protein for fish in Turkey because of its protein quality and palatability. Although there was a fluctuation between years more than 24,000 tonnes of FM was produced in Turkey. Productions of FM and fish oil (FO) between 1999 and 2004 were given in Table I.

Table I.- Productions of fish meal and fish oil in Turkey between 1999 and 2004 (Yıldırım, 2006).

Years	Fish meal production (tonnes)	Fish oil production (tonnes)
1999-2000	17,542	14,604
2000-2001	5,407	3,485
2001-2002	14,732	6,344
2002-2003	24,248	15,038
2003-2004	18,000	10,800

However, increasing demand, high cost and uncertain availability of FM has severely restricted its use in Turkey as a protein source for diets. For example, to produce FM *Engraulis encrasicolus* was mainly used by at least 25 factories in 1995s, but fluctuations in *E. encrasicolus* catch severely reduced FM production. In 2003-2004, FM and FO were produced by only 9 factories, and total FM production was 18,000 tonnes (Yıldırım, 2006). In 2006, approximately 60,000 tonnes of FM were used in fish food production (Anonymous, 2006;

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Köprücü, 2007). It means that rest of the demand was imported.

Mainly three fish species, *O. mykiss*, *Dicentrarchus labrax* and *Sparus auratus*, are reared in Turkey. Production of *O. mykiss* takes the first place. For example, in 2007 total reared fish production (sea + freshwater) was 139,873 tonnes. *O. mykiss* contained 41.8% of the total production. That of *D. labrax* and *S. auratus* was 30 and 24%, respectively (Baki and Dalgıç, 2009).

It is clear that alternative protein sources for fish diets (especially for trout) should be used to support aquaculture development in Turkey. At the farm level, the substitution of FM and FO could have a positive effect on production costs. Therefore, nutritional studies should be undertaken on a range of plant protein resources.

Among plant proteins soybean meal (SBM) is the most promising candidate for partial or total replacement of FM in fish diets (Mambrini *et al*., 1999; Carter and Hauler, 2000; Glencross *et al*., 2004; Morris *et al*., 2005; Francesco *et al*., 2007; Karalazos *et al*., 2007). For example, SBMs is extensively used in fish feeds. The reason for this is that high content of available protein with a well balanced amino acid profile in soybeans, constant composition, reasonable price and steady supply of SBMs. On the other hand, SBMs also contain approximately 30% indigestible carbohydrates and several compounds that may disturb the digestive process (Refstie *et al*., 2000; Derjant-Li, 2002; Francis *et al*., 2001).

The objective of this study was to investigate changes in growth, feed conversion and body composition when 40% or 55% SBM or full fat soya (FFS) incorporated in diet formulation of rainbow trout.

MATERIALS AND METHODS

Experimental diets

Solvent (hexane) extracted and heat treated (the temperature was increased gradually from 25°C to 150 °C), SBM [48.12% crude protein (CP), 1.23% crude fat (CF), 6.04% ash], FFS (37.61 % CP, 18 % CF, 5.5 % ash), maize gluten (60.4 % CP, 1.7 % CF, 2.2 % ash) and wheat bran (15.7 % CP, 2.7 % CF, 5.2 % ash) were purchased from Özuğur Feed Producer Company, Elazığ, Turkey. FM (64.45% CP, 9.81% CF, 10.24% ash) was purchased from İzmir Feed Producer Company, İzmir Turkey. FO was purchased from Sinop-Sürsan Company, Sinop Turkey. Vitamin and mineral premixes were obtained from Roche Products.

All diets were isonitrogenous and isoenergetic, contained average 50.36 % CP and average 4294 kcal/kg total energy. Thirty percentage of Diet 1 (control) consisted of SBM as a partial replacement for FM as was referred by Cho *et al*. (1985). While Diet 2 and 3 contained 40% or 55% SBM and 28% or 13% FM, Diet 4 and 5 contained 40% or 55% FFS and 28% or 13% FM, respectively. Crystalline amino acid was added to the diets to provide the IAA requirement profile according to the NRC (1993). The composition and proximate analysis of experimental diets are given in Table II.

SBM was ground to powder form in a mill. All dry ingredients were mixed thoroughly for 5 min in a food mixer. Then, oil was added and the diets were mixed again for 10 min. Diets were processed by addition of water to the mash whilst mixing to form dough. The moist feed mixture was passed through a meat grinder, broken into pellets, dried in an air-forced oven at 60˚C for 20 h to reduce the moisture. After this process, experimental feeds were allowed to cool to ambient temperature in an oven. All feeds were packed in plastic bags and stored at 4˚C.

Experimental procedure

Hatchery reared rainbow trout (*O. mykiss*) were transferred from grow-out ponds to experimental tanks. This study was carried out in Government Water Management Affairs of IX. Area Directory, Keban, Elazığ. At the start of the growth trial, uniform sized fish (49.95±0.32 body weight) were randomly distributed into 15 tanks (200 x 40 x 40 cm) with each treatment having three replicates for each diet. Thirty fish were stocked in each tank.

Water temperature varied between 8.8-9.4˚C, and pH ranged from 7.7-7.9 during the experimental period. Dissolved oxygen content of water changed between 7.2-7.7 mg L^{-1}. Rainbow trouts were fed with the experimental diets for five months. They were weighed and total length was measured

Table II.- Diet composition in experiment (%), proximate analysis of experimental diets (g/100g diet, dry matter), total energy (kcal/kg).

Ingredients	Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal (FM)	43	28	13	28	13
Soyabean meal (solvent extracted)	25	40	55	-	-
(SBM)	-	-	-	40	55
Full-fat Soya (FFS)	6	11	16	17	25
Maize gluten	12.83	8.66	4.62	10.81	3.70
Wheat bran	11	10	9	2	1.05
Fish oil (FO)	0.07	0.24	0.28	0.09	0.15
Methionine	0.10	0.10	0.10	0.10	0.10
Antioksidan [(a)]	1.00	1.00	1.00	1.00	1.00
Vitamin pre-mix[(b)]	1.00	1.00	1.00	1.00	1.00
Mineral pre-mix[(c)]					
Proximate composition					
Energy (kcal/kg)	49.52	50.79	50.85	50.83	49.85
Crude protein (CP)	12.87	12.92	11.28	10.44	10.12
Crude fat (CF)	11.51	10.68	9.82	9.73	8.34
Ash	5.04	4.12	4.04	4.51	5.12
Crude fibre	13.60	13.86	16.13	17.00	19.46
N-free extract	92.54	92.37	92.12	92.51	92.89
Dry matter	4287	4302	4275	4313	4292
Total energy					

[a] Butilen Hidroksi Toluen (BHT); 125.000 mg/ kg

[b] Vitamin pre-mix (mg kg^{-1}); Riboflavin 4 000, Pyridoxine 3 000, cyanocobalamin 10, Ascorbic acid 50 000, Niasin 10 000, Biotin 150, Thiamin 1 000, Folic acid 1 000, Kolin 1 000, Pantothenik acid 20 000, Myoinositol 300 000, Retinol 2 500 000 IU, Kalsiferol 2 400 000 IU, Tocopherol 50 000 IU.

[c] Mineral pre-mix (mg kg^{-1}); Mn 13 000, Fe 60 000, Zn 30 000, Mg 5, K 70, Na 60, Cu 3 000, I 1100, Co 400, Se 300.

every month to follow fish growth rate. The fish were weighed individually at the end of the experiment. Those fish handled individually (weighed or measured) were anaesthetized (100 mg benzocaine/L) before handling. At the end of the trial ten fish from each tank were used for analysis.

Chemical analyses and calculations of fish performance

After five months of feeding and following 24 h starvation period, ten fish from each tank were randomly selected for proximate analysis. Diets and fish carcass samples were analyzed in triplicate for crude protein (CP), crude fat (CF), ash and moisture according to the methods described by the Association of Official Analytical Chemist (AOAC, 1995). Moisture content was measured by drying samples at 105˚C for 24 h to constant weight in an oven. CP (Nitrogen x 6.25) was determined by Kjeldahl method autoanalyzer (Gerhardt VAP 40), CF was estimated using a soxhlet apparatus (Gerhardt) with petroleum ether and ash by heating at 550˚ for 5-6 h in a Shimadzu type ashing furnace. The energy content of the diets was determined by a bomb calorimeter (Julius Peters type).

Specific growth rate (SGR) was determined as described by Halver (1989):

SGR = Final weight (g) - initial weight (g) / time (months) x 100

Protein efficiency ratio (PER) was determined as described by Mazid *et al*. (1972).

PER = Wet weight gain (g) / protein consumed

Weight gain rate (WGR) was determined as described by NRC (1990).

WGR = Final weight (g) - initial weight (g) / initial weight (g) x 100

Feed conversion ratio (FCR) was determined as described by Halver (1989).

FCR = Dry feed consumed (g) / Wet weight gain.

Condition factor (CONF) was determined as described by Halver (1972).

CONF = Weight (g) / total length $(cm)^3$ (PPV) x 100

Protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV) was calculated as described by Steffens (1989):

PPV (%) = final fish body protein (g) - initial fish body protein (g) / total protein consumed (g) x 100

LPV (%) = (final fish body lipid (g) - initial fish body lipid (g) / total crude lipid consumed (g) x 100

EPV (%) = (final fish body energy - initial fish body energy / gross energy intake) x 100

Ingredient costs of feedstuffs were based on 2008-2009 averages of market prices in Turkey. Economics cost analysis of experimental diets was determined as described by FAO/UNDP (1985).

Data were analyzed by one-way analysis of variance (ANOVA) using Duncan's test was used to compare differences among means. The level of significance was chosen at $p<0.05$ and the results are presented as means±standard error of the mean (SEM).

RESULTS

In this study final body weight (FBW), weight gain rate (WGR) and SGR decreased in relation to the increases level of SBM and FFS in the diets. Mean final weight ranged from 74.61 to 108.41 g for fish fed the control and other diets. These means were significantly different ($P<0.05$). The mean final weight of fish fed with FFS was lower ($P<0.05$) than that of fish fed with SBM. Growth performance and nutrient utilization parameters of rainbow trout fed different experimental diets are presented in Table III.

The results showed that FCR ranged from 1.58 to 3.35 and PER varied from 1.28 to 0.62 among treatments. No significant difference was observed for SGR among Diets 1, 2 and 4 ($P>0.05$). The WGR of Diet 1 was significantly higher than the fish fed with the other diets ($P<0.05$). In addition, PER and FCR were not significantly different among fish fed with Diet 1 (Control) and Diet 2 ($P>0.05$). However, rainbow trout fed with Diets 3 and 5 had a significantly lower PER than the fish fed with Diet 1. The FCR of diet 5 was the lowest ($P<0.05$). Among the treatments, the condition factor of fish fed with the diets were not significantly different from each other ($P>0.05$).

The results also showed that PPV ranged from 13.43 to 34.14%; LPV varied from 9.43 to 19.40 and EPV varied from 9.96 to 26.13% among treatments (Table III). There was not a significant ($P>0.05$) difference in PPV between Diet 1 and Diet 2 but there was significant ($P<0.05$) difference with other diets.The lowest ($P<0.05$) LPV (9.43%) and EPV (9.96%) were observed for Diet 5.

Crude protein content of carcass was the highest in fish fed with Diet 1 and it was not significantly ($P>0.05$) different from that of fish fed with Diets 2, 3. However, CP content of Diet 4 and 5 were significantly lower ($P<0.05$) than that of the fish fed with the Diet 1.

Body lipid content was decreased as the amount of FFS in the diets increased, but lipid content was increased as the amount of SBM in the diets increased. However, except between Diet 3 and Diet 5, no significant differences ($P>0.05$) were observed in body lipid content between Diets 1, 2 and 4. There was not a significant difference in ash content between control and experimental diets ($P>0.05$). In addition, the lowest ash content (3.71%) was observed for Diet 3. Chemical composition of whole body of rainbow trout (*Oncorhynchus mykiss*) of different experimental groups at the start and at the end of the experiment are presented in Table IV.

Cost analysis (Table V) revealed that the cost per unit gain of growth was least for Diet 2 and Diet 1 (1,358 $ and 1,368 $). That of Diet 3, Diet 4 and Diet 5 was 1,827 $, 1,404 $ and 2,364 $, respectively.

DISCUSSION

The results of present investigation demonstrate that the inclusion of SBM and FFS up to 40% in the Diet (Diet 2 and Diet 4) did not affect significantly the SGR when compared with the control diet containing 43% FM. This is similar with results obtained with some other species. For

Table III.- Growth performance and nutrient utilization parameters of rainbow trout (*Oncorhynchus mykiss*) fed different experimental diets[1]

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight(g)	49.95±0.28[a]	49.56±0.33[a]	50.18±0.25[a]	49.80±0.33[a]	50.41±0.30[a]
Final weight (g)	108.41±0.24[a]	102.08±0.29[b]	84.61±0.19[d]	97.45±0.19[c]	74.61±0.17[e]
SGR	0.51±0.02[a]	0.47±0.02[a]	0.34±0.05[b]	0.44±0.02[a]	0.25±0.02[b]
PER	1.28±0.04[a]	1.16±0.02[ab]	0.81±0.10[c]	1.07±0.04[b]	0.62±0.05[c]
WGR	117±1.45[a]	105.97±1.15[b]	68.61±1.20[d]	95.68±1.32[c]	48.00±1.04[e]
FCR	1.58±0.01[d]	1.70±0.02[d]	2.60±0.04[b]	1.85±0.04[c]	3.35±0.05[a]
CONF	1.16±0,01[a]	1.18±0.01[a]	1.18±0.04[a]	1.20±0.10[a]	1.20±0.02[a]
PPV	34.14±0.59[a]	32.00±0.57[a]	21.83±0.72[c]	27.66±0.88[b]	13.43±0.80[d]
LPV	17.83±0.92[a]	19.40±0.64[a]	17.20±0.75[a]	18.33±0.88[a]	9.43±0.72[b]
EPV	26.13±1.14[a]	24.34±1.09[ab]	14.83±0.66[c]	22.96±0.60[b]	9.96±0.78[d]

[1]Means in a line with different superscripts are significantly different ($p<0.05$) Standard error, calculated from residual mean square in the ANOVA.
[2]CF, crude fat; EPV, energy productive value; FCR, feed conversion rate; LPV, lipid productive value; PER, protein efficiency ratio; PPV, protein production value; SGR, specific growth rate; WGR, weight gain rate.

Table IV.- Chemical composition of whole body of rainbow trout (*Oncorhynchus mykiss*) of different experimental groups at the start and at the end of the experiment [total energy (kcal/kg) (% dry matter)[1]]

Component body and Energy	N[2]	Initial	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Crude protein (CP)	9	77.51±0.97	81.79±0.57[a]	80.60±0.58[ab]	79.76±1.14[ab]	78.14±1.02[b]	74.58±1.56[c]
Crude fat (CF)	9	10.05±0.47	10.37±0.63[ab]	11.40±0.59[ab]	11.97±0.62[a]	10.39±0.66[ab]	9.91±0.53[b]
Ash	9	4.59±0.21	4.05±0.47[a]	4.24±0.45[a]	3.71±0.44[a]	4.82±0.40[a]	4.34±0.44[a]
N-free extract	9	7.02±0.57	2.90±0.26[c]	2.99±0.29[c]	3.65±0.45[c]	5.82±0.30[b]	10.45±0.68[a]
Dry Matter	9	22.74±0.67	22.15±0.80[a]	22.17±0.81[a]	22.54±0.98[a]	22.97±0.95[a]	22.42±0.70[a]
Total Energy	3	5595±47.00	5640±52.60[a]	5671±56.20[a]	5318±44.00[b]	5666±38.00[a]	5115±46.30[c]

[1]Means in a line with different superscripts are significantly different each other ($p<0.05$) Standard error, calculated from residual mean square in the ANOVA.
[2]Replicate numbers.

Table V.- Cost analysis of experimental feed diets fed to *O. mykiss*

Dietary treatment	Cost of feed per tonnes ($/tonnes)	Cost of feed/weight gain ($/1 tonnes fish)
Diet 1 (control)	857	1.368
Diet 2	799	1.358
Diet 3	734	1.827
Diet 4	754	1.404
Diet 5	719	2.364

example, Elangovan and Shim (2000) demonstrated that SBM up to 37% in the diet did not affect the growth rate for *Barbodes altus*. Sanz *et al.* (1994) suggested that sunflower meal protein could replace up to 40% of FM protein in the diet for rainbow trout. Mambrini *et al*. (1999) stated that about 50% of the FM could be replaced with soy protein concentrate without reducing performance in *O. mykiss*. In addition, Morris *et al.* (2005) also stated that a combination of extracted and FFS provided between 18% and 36% of the CP without adverse effects on growth, survival or basic quality attributes.

On the other hand, the results of present investigation showed that the inclusion of SBM and FFS up to 55% in the diet (Diet 3 and Diet 5) reduced significantly ($P<0.05$) the SGR and WGR in *O. mykiss* when compared with the control diet containing 43% FM. In earlier studies on various fish species, a decrease in growth performance was observed in fish fed diets containing high levels of plant protein sources (Fracesco *et al*., 2007; Kissil *et al*., 2000; Burel *et al*., 2000; Fagbenro and Davies, 2001; Cheng *et al*., 2003). For instance, sea bream (*Sparus aurata*) fed with soybean or rapeseed protein concentrates at 60% of inclusion reached a final body weight lower when compared with the control fed a FM diet. Cheng *et al*. (2003) reported that FM replacement levels had significant effects on fish weight gain, FCR and body composition of rainbow trout.

The present study also revealed that inclusion of high levels of SBM and FFS (55%) gave rise to higher FCR and lower PER, PPV and EPV. In addition, higher FCR values were found for the fish fed with FFS than the fish fed with SBM. Similar results were also found by Morris *et al*. (2005). They demonstrated that FCR increased in response to levels of FFS in feed, which was partly explained by increased feed intake at higher levels of soya inclusion. Lower PER and PPV values were obtained for the sunflower diet and SBM diet than those for the control diet by Sanz *et al.* (1994).

Different results are reported in the literature regarding the effects of plant protein sources on body fat content for fish fed with plant proteins. In the present trial, no significant ($P>0.05$) difference was observed between control and SBM or FFS. Similar results was observed by Francesco *et al*. 2007 and Cheng *et al*. (2003), they have reported that no effects on whole fat contents compared to control diet. Luo *et al*. (2006) also reported that rainbow trout fed on cottonseed meal in diets were no significantly differences on the body composition. In addition, Morris *et al*. (2005) found that FFS meal represented 25% of the formulation had no significant effects on body proximate composition. On the other hand, in the present study it was found that the body lipid content decreased as the amount of FFS increased. Similar results were obtained by Robaina *et al*. (1998) and by Kissil *et al*. (2000) for *Sparus aurata*, both reported a decrease in body fat for fish fed with plant protein. According to Francesco *et al*. (2007) the effect of plant protein sources on fat in fish is still under investigations and it is probably related to a different lipid metabolism and to be different protein sources.

The present study revealed that the body protein content of fish decreased with increasing SBM and FFS in diets. Efficient protein synthesis requires sufficient availability of all essential amino acids (Dabrowski and Guderly, 2002). Unbalanced amino acid concentrations in a diet resulted in increased protein degradation (Langar *et al.,* 1993; von der Decken and Lied, 1993), and thereby increased protein turnover (Martin *et al.* 2003). Many researchers (Refstie *et al.,* 2000; Pack *et al.,* 1995; Cheng *et al.,* 2003) reported that the plant protein (SBM) based diets lower nitrogen retention in salmon and trout because these diets have less digestible energy and an amino acid profile that is suboptimal for muscle growth. Cheng *et al*. (2003) reported that fish meal replacement levels (25%, 50%, 75%, and 100% replaced with SBM and wheat gluten) had significant differences in body CP and ash content ($P<0.05$) in rainbow trout. These results are in agreement with previous studies for rainbow trout fed with diet containing SBM, and could be due to the presence of the antinutritional factors in soybean. Upper inclusion levels for carnivorous fish such as salmonids are restricted to 20-30% due to the presence of antinutrients, which eventually leads to reduced growth and feed utilization (Heikkinen *et al*., 2006). Furthermore, the replacement of fish meal protein with soybean protein reduced the apparent digestibility of CP, fat energy and almost all amino acids, independent of type of SBM processing (Forde-Skjservik *et al.,* 2006).

It is highly likely that as aquaculture continues to expand FM and FO will become progressively more expensive. This problem will be exacerbated by climatic events, by growing environmental pressure to decrease exploitation pressure on finite marine resources, and by increasing consumer perception that levels of pollutants such as dioxin (a toxic compound) in fish have now reached unacceptable levels (Turchini *et al*., 2005). Therefore, finding alternatives to FM and FO for farmed fish feeds is becoming an increasingly necessary.

In conclusion, this study shows that solvent extracted SBM could be used up to 40% in rainbow trout rations without statistically significant adverse effects on nutrient utilization and body composition of carcass. In addition, it was also found that it is the most cost-effective and affordable feed.

Nevertheless, based on the results obtained from this research it can also be concluded that when FFS used up to 40% in rations of rainbow trout it has significantly (P<0.05) adverse effects on PER, PPV, EPV and FCR in comparison to the control diet.

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