

## Effect of solvent extracted soybean meal and full-fat soya on the protein and amino acid digestibility and body amino acid composition in rainbow trout (*Oncorhynchus mykiss*)

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### Abstract

This study was carried out to investigate the apparent digestibility coefficients (ADCs) value of protein, amino acid and energy and body amino acid composition of rainbow trout fed solvent extracted soybean meal (SBM) and full-fat soybean meal (FFS) partly replacing fish meal (FM) in diets. Five iso nitrogenous (average 50.36% crude protein) and energetic (4294 kcal/kg total energy) diets were designed in 5 groups in triplicates. Diet one as control group with 43% FM and 25% SBM, Diet 2 with 28% FM and 40% SBM, Diet 3 with 13% FM and 55% SBM, Diet 4 with 28% FM and 40% FFS, Diet 5 with 13% FM and 55% FFS. Diets were fed to trout fishes with 49 g initial body weight for 20 weeks at  $8.8 \pm 0.45$  °C. It was found that ADCs of energy, protein and most amino acids particularly methionine and lysine and fish body muscle lysine levels, significantly decreased by increasing the amount of FFS and SBM in diets ( $P < 0.05$ ). On the other hand, valine, isoleucine, threonine, methionine, phenylalanine, lysine and histidine showed a decreasing tendency with the increasing proportion of SBM in fish body composition. It was concluded that increasing the SBM and FFS percent replacing FM in trout fish diet, caused reducing protein, amino acid and energy digestibility, compared with the control diet. However, it can also be concluded that the replacement of SBM was more efficient than the replacement of FFS as regarding growth, ADCs of protein and essential amino acid contents of *O. mykiss*.

**Keywords:** Rainbow trout, *Oncorhynchus mykiss*, Soybean meal, Full-fat soybean meal, Amino acid, Digestibility

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## Introduction

One of the fastest growing aquaculture industries of the world is that aqua-feed production with annual growth rates in excess of 30% per year (Francis et al., 2001). As aqua-feed production continues to expand, fish meal and fish oil will become increasingly more expensive and shortage in the future. Thus, replacement of fish meal with less expensive plant protein sources will be beneficial in reducing the cost of feed production (Carter and Hauler, 2000; Francis et al., 2001; Luo et al., 2006). A partial substitution of fish meal by plant protein sources is well supported by fish, so currently 30% is already replaced in most commercial fish feeds (Francesco et al., 2004).

Soybeans are one of the most important plant proteins used for 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). They have high protein content and well-balanced amino acid compositions for fish with methionine as first limiting amino acid among plant protein feedstuffs used as alternative protein sources in fish diets (Kaushik et al., 1995; Elangovan and Shim, 2000; Refstie et al., 2000; Fagbenro and Davies, 2001; Romarheim et al., 2006). However, any imbalances in essential amino acid composition will evidently affect feed conversion by reduced protein utilization. Furthermore, the dietary contents of amino acids may have to be balanced with respect to the nutritional requirement for optimal protein utilization and growth performance (Albrektsen et al., 2006).

Full-fat soya is also included into fish diets largely as a plant protein source. It also contributes to the dietary fat, containing about 18% lipid. The economic value of full-fat soya as a lipid source depends on the relative market price of fish and plant oils. Therefore, when fish oil prices are high, lipid- rich oil seed meals may be preferred as a favorable source of oil (Morris et al., 2005).

Generally the evaluation of the ADC values of feedstuffs used in fish diets is one of the most important steps in formulating properly balanced diets to satisfy the nutrient requirements of fish (Cho et al., 1982). In addition, the protein quality of dietary ingredients is the leading factor affecting fish performance and protein digestibility is the first measure of its availability by fish (Fagbenro and Davies, 2001; Halver and Hardy, 2002).

Fish meal (FM) has traditionally been the main source of dietary protein for fish in Turkey. Although there was a fluctuation between years more than 24,000 tonnes of FM was produced in 2002-2003 in Turkey (Yıldırım, 2006). However, increasing demand, high cost and uncertain availability of FM has severely restricted its use in Turkey. For example, *Engraulis encrasicolus* was mainly used to produce FM 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; Harlioğlu, 2011). Approximately 60,000 tonnes of FM were used in fish food production in 2006

(Anonymous, 2006; Köprücü, 2007). It means that rest of the demand was imported (Harlioğlu, 2011).

It is apparent that alternative protein sources for fish diets should be preferred to support aquaculture expansion in Turkey. In addition, the substitution of FM and FO could have a positive effect on production costs. The objectives of the present study were to determine apparent digestibility coefficient values for protein and amino acids in SBM and FFS and body amino acids composition of *O. mykiss* fed diets containing graded levels of SBM and FFS with methionine supplement, as partial replacement for fish meal.

## Materials and methods

### *Experimental conditions*

The experiment was conducted in Government Water Management Affairs of IX. Area Directory, Keban, Elazığ. Each treatment having three replicates, fifteen tanks (200 x 40 x 40 cm) were each stocked with 30 rainbow trout of initial average weight of  $49.95 \pm 0.32$  g. Fish were initially weighed at the end of the each month. Fish were cultured for five months (August- December) under natural photoperiod. The tanks were supplied with freshwater at constant temperature  $8.8 \pm 0.45$  °C. Dissolved oxygen content of water changed between 7.2-7.7 mg L<sup>-1</sup>, pH content of water changed between 7.7-7.9.

### *Experimental Diets*

Solvent (hexane) extracted SBM (48.12% crude protein, 1.23% crude fat, 6.04% ash), FFS (37.61 % CP, 18 % CF, 5.5 %

ash), Maize gluten (60.4 % CP, 1.7 % CF, 2.2 % ash), Wheat Bran (15.7 % CP, 2.7 % CF, 5.2 % ash) were obtained from Özüğür Feed Producer Company, Elazığ Turkey. Fish meal (64.45% CP, 9.81% CF, 10.24% ash) was obtained from İzmir Feed Producer Company, İzmir Turkey. Fish oil was purchased from Sinop-Sürsan Company, Sinop, Turkey.

The control diet was prepared as formulated 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 National research council (NRC) (1993). Proximate composition, total energy and amino acid composition of the dietary ingredients are given in Table 1. Diet formulation and proximate composition of experimental diets are given in Table 2. The amino acids tryptophan and arginine could not be analyzed.

All diets were isonitrogenous and isoenergetic, contained average 50.36% crude protein and average 4294 kcal/kg total energy. Small amount of corn gluten were added to make diets iso-nitrogenous and to improve the technical quality of the pellets. The fish were hand-fed the diets to satiation three times in a day. Daily feed intake was recorded. Chromium III oxide was included in all diets (1%) as inert digestibility marker.

SBM was ground to powder form in a mill. Diets were processed by addition of water (about 30% of mash dry weight) to the mash whilst mixing to form dough, which

was subsequently screw-pressed using a meat grinder through a 3 mm diameter die. The resultant moist pellets were then oven dried at 60°C for 20 hours 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. *Sampling of faecal matter for digestibility determinations and chemical analyses*

Faeces were collected from each experimental tank after 2 weeks of adaptation to feed. One hour after the feed administered, any feed and faeces present in the tank were removed. Fresh faeces produced by the fish after this period and before the second feed was given, were siphoned out. At least two siphoning were carried out from each tank during each collection period to minimize nutrient leaching from the faeces (Hossain and Jauncey, 1989; Spyridakis et al., 1989). The collected faeces were filtered onto filter paper and dried at 60 °C for 24 h and

were frozen at -20 °C (Beko BK 3405 CF, D).

After five months of feeding, 10 fish from each tank were randomly selected for chemical analysis. Proximate analyses of samples were made following procedure Association of official analytical chemists, (AOAC) (1995): dry matter, after drying at 105°C for 24 h to constant weight in an oven; protein (nitrogen x 6.25) were determined by Kjeldahl method autoanalyzer (Genhardt VAP 40); fat was using determined soxhlet apparatus (Genhardt) with petroleum ether; ash content was determined by heating at 550 °C for 5-6 h in a Shimadzu type ashing furnace. The energy contents of the diets and feces were analysed using an automatic adiabatic bomb calorimeter (Julius Peters, Berlin, West Germany). The chromic oxide content of the experimental diets and feces were determined by a spectrophotometer (Baush and Lomb, wavelength 350 nm) as described by Furukawa and Tsukahara (1966).

**Table 1: Proximate composition (% dry matter), total energy (kcal/kg) and amino acid content (% dry diet) of dietary ingredients**

Components	Fish meal	Soybean meal	Full-fat soya	Maize gluten	Wheat bran
Crude protein	64.45	48.12	37.61	60.55	15.74
Crude fat	9.81	1.23	18.25	1.41	2.71
Ash	10.24	6.04	5.52	2.48	5.26
Crude fiber	2.71	3.95	5.54	2.14	11.22
Nitrogen-free extracts	3.54	28.57	21.04	24.41	52.73
Moisture	9.25	12.09	12.04	9.04	12.34
Total energy	3426	2934	3809	4260	1200
<b>Amino acid content <sup>1</sup></b>					
Arginine	3.82	3.39	2.80	2.02	0.86
Histidine	1.45	1.19	1.01	1.31	0.39
Isoleucine	2.66	2.03	2.18	2.54	0.51
Leucine	4.48	3.49	2.80	10.2	0.92
Liysine	4.72	2.85	2.40	1.11	0.58
Methionine	1.75	0.57	0.54	1.63	0.19
Phenylalanine	2.41	2.22	2.10	3.96	0.55
Treonine	2.50	1.78	1.69	2.07	0.46
Tryptophane	0.65	0.64	0.52	0.43	0.25
Valine	3.22	2.02	2.02	3.09	0.69

<sup>1</sup>Essential amino acid values were obtained from the National Research Council, (1990) and (1993).

**Table 2: Diet formulation (%) and proximate composition (% , dry matter) of rainbow trout experimental diets**

	Diet1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
<b>Ingredients</b>					
Fish meal	43	28	13	28	13
Soyabean meal, (solvent extracted)	25	40	55	-	-
Full- fat Soya	-	-	-	40	55
Maize gluten	6	11	16	17	25
Wheat Bran	11.83	7.66	3.62	9.81	2.70
Fish oil	11	10	9	2	1
Methionine	0.07	0.24	0.28	0.09	0.15
Antioksidan <sup>(1)</sup>	0.10	0.10	0.10	0.10	0.10
Vitamin pre-mix <sup>(2)</sup>	1.00	1.00	1.00	1.00	1.00
Mineral pre-mix <sup>(3)</sup>	1.00	1.00	1.00	1.00	1.00
Cr <sub>2</sub> O <sub>3</sub>	1.00	1.00	1.00	1.00	1.00
<b>Proximate composition and</b>					
<b>Total energy (kcal/kg)</b>					
Crude protein	49.52	50.79	50.85	50.83	49.85
Crude fat	13.87	13.92	11.28	10.44	9.12
Ash	11.51	10.68	9.82	9.73	8.34
Crude fiber	5.04	4.12	4.04	4.51	5.12
N-free extract	12.60	12.86	16.13	17.00	20.46
Dry matter	92.54	92.37	92.12	92.51	92.89
Total Energy	4287	4302	4275	4313	4292

<sup>1</sup> Butilen Hidroksi Toluen (BHT); 125.000 mg/ kg. <sup>2</sup> Vitamin pre-mix (mg kg<sup>-1</sup>); 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 asit 20 000, Myoinositol 300 000, Retinol 2 500 000 IU, Kalsiferol 2 400 000 IU, Tokoferol 50 000 IU. <sup>3</sup> Mineral pre-mix (mg kg<sup>-1</sup>); Mn 13 000, Fe 60 000, Zn 30 000, Mg 5, K 70, Na 60, Cu 3 000, I 1100, Co 400, Se 300.

Amino acid composition of diets, faeces and trout muscle was determined after hydrolysis in accordance with AOAC (1995) in a gas chromatography (Phenomenex EZ Faast GC-FID Hydrolized Aminoacid Analysis Kit, Varian GC, CP-3800GC M) by Marmara Research Centre of the Scientific and Technological Research Council of Turkey.

#### Calculations

Apparent digestibility coefficient (ADC) was determined by the indirect method described by Cho and Slinger (1979) according to the following formula:

$$ADC = 100 \times [1 - (\% Cr_2O_3 \text{ in diet} / \% Cr_2O_3 \text{ in feces}) \times (\% \text{ faeces nutrient} / \% \text{ dietary nutrient})]$$

Apparent digestible energy (ADE) was determined by the difference between the total energy of the diet and the energy lost in the faeces described by Lovell (1988) according to the following formula:

$$ADE (\%) = [(Diet \text{ energy} - Faeces \text{ energy}) / Diet \text{ energy}] \times 100$$

All 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 $\pm$ standart error of the mean (SEM).

### Results

Amino acid composition of the experimental diets and amino acid requirements of rainbow trout are shown in Table 3. The present study showed that final body weight significantly decreased as the amount of SBM and FFS in diets increased (Table 4) ( $P<0.05$ ). Apparent digestibility coefficients (ADCs) of gross energy, protein and amino acids in

experimental diets for rainbow trout are shown in Table 4. The ADC of gross energy Diet 2 and Diet 4 had no significant difference compared with control diet (Diet 1) ( $P>0.05$ ). However, the ADC of gross energy showed tendency with increasing proportion of SBM and FFS, and there were significant differences between Diet 1, Diet 3 and Diet 5. Furthermore, the ADC of energy of Diet 5 was significantly lower than that of the other diets ( $P<0.05$ ).

**Table 3: Amino acid composition of the experimental diets (g/100g, dry matter) and amino acid requirements of rainbow trout according to Lovel (1998)**

Amino acid <sup>1</sup>	Requirement	Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
Valine	1.2	2.85 $\pm$ 0.05 <sup>c</sup>	2.95 $\pm$ 0.01 <sup>b</sup>	3.15 $\pm$ 0.01 <sup>a</sup>	2.76 $\pm$ 0.02 <sup>c</sup>	2.90 $\pm$ 0.06 <sup>b</sup>
Leucine	1.4	4.75 $\pm$ 0.01 <sup>d</sup>	3.74 $\pm$ 0.0 <sup>e</sup>	5.49 $\pm$ 0.01 <sup>b</sup>	5.08 $\pm$ 0.01 <sup>c</sup>	6.25 $\pm$ 0.01 <sup>a</sup>
Isoleucine	0.9	3.02 $\pm$ 0.02 <sup>a</sup>	2.93 $\pm$ 0.01 <sup>b</sup>	2.79 $\pm$ 0.02 <sup>c</sup>	2.42 $\pm$ 0.02 <sup>e</sup>	2.65 $\pm$ 0.04 <sup>d</sup>
Threonine	0.8	2.53 $\pm$ 0.08 <sup>ab</sup>	2.19 $\pm$ 0.03 <sup>c</sup>	2.68 $\pm$ 0.02 <sup>a</sup>	2.37 $\pm$ 0.02 <sup>b</sup>	2.46 $\pm$ 0.08 <sup>b</sup>
Methionine	1.0	1.43 $\pm$ 0.01 <sup>ab</sup>	1.39 $\pm$ 0.02 <sup>ab</sup>	1.47 $\pm$ 0.03 <sup>a</sup>	1.24 $\pm$ 0.02 <sup>c</sup>	1.23 $\pm$ 0.02 <sup>c</sup>
Phenylalanine	1.8	1.90 $\pm$ 0.02 <sup>d</sup>	3.08 $\pm$ 0.02 <sup>a</sup>	3.10 $\pm$ 0.05 <sup>a</sup>	2.37 $\pm$ 0.01 <sup>b</sup>	2.21 $\pm$ 0.03 <sup>c</sup>
Lysine	1.8	3.04 $\pm$ 0.07 <sup>b</sup>	3.50 $\pm$ 0.03 <sup>a</sup>	3.04 $\pm$ 0.04 <sup>b</sup>	2.47 $\pm$ 0.03 <sup>c</sup>	1.49 $\pm$ 0.01 <sup>d</sup>
Histidine	0.7	1.18 $\pm$ 0.03 <sup>b</sup>	0.54 $\pm$ 0.01 <sup>c</sup>	0.42 $\pm$ 0.02 <sup>c</sup>	1.38 $\pm$ 1.12 <sup>a</sup>	1.05 $\pm$ 0.01 <sup>b</sup>
Tyrosine	-	1.45 $\pm$ 0.02 <sup>c</sup>	1.89 $\pm$ 0.02 <sup>a</sup>	1.94 $\pm$ 0.02 <sup>a</sup>	1.93 $\pm$ 0.03 <sup>a</sup>	1.78 $\pm$ 0.04 <sup>b</sup>
Alanine	-	2.59 $\pm$ 0.03 <sup>d</sup>	2.86 $\pm$ 0.07 <sup>bc</sup>	2.75 $\pm$ 0.01 <sup>c</sup>	2.96 $\pm$ 0.03 <sup>ab</sup>	3.0 $\pm$ 0.02 <sup>a</sup>
Glycine	-	3.23 $\pm$ 0.01 <sup>a</sup>	2.55 $\pm$ 0.01 <sup>c</sup>	2.43 $\pm$ 0.02 <sup>d</sup>	2.28 $\pm$ 0.01 <sup>e</sup>	2.58 $\pm$ 0.01 <sup>b</sup>
Serine	-	2.05 $\pm$ 0.01 <sup>d</sup>	2.36 $\pm$ 0.07 <sup>bc</sup>	2.97 $\pm$ 0.02 <sup>a</sup>	2.45 $\pm$ 0.06 <sup>b</sup>	2.21 $\pm$ 0.04 <sup>cd</sup>
Proline	-	3.74 $\pm$ 0.01 <sup>a</sup>	3.29 $\pm$ 0.01 <sup>b</sup>	3.19 $\pm$ 0.01 <sup>c</sup>	3.07 $\pm$ 0.02 <sup>d</sup>	3.77 $\pm$ 0.03 <sup>a</sup>
Aspartic acid	-	7.38 $\pm$ 0.03 <sup>a</sup>	5.93 $\pm$ 0.03 <sup>c</sup>	6.50 $\pm$ 0.01 <sup>b</sup>	7.78 $\pm$ 0.02 <sup>a</sup>	6.43 $\pm$ 0.07 <sup>b</sup>
Hidroxyproline	-	0.45 $\pm$ 0.01 <sup>c</sup>	0.30 $\pm$ 0.01 <sup>d</sup>	0.50 $\pm$ 0.0 <sup>b</sup>	0.48 $\pm$ 0.00 <sup>b</sup>	0.55 $\pm$ 0.01 <sup>a</sup>
Glutamic acid	-	6.55 $\pm$ 0.10 <sup>d</sup>	10.01 $\pm$ 0.09 <sup>c</sup>	9.08 $\pm$ 0.05 <sup>b</sup>	9.30 $\pm$ 0.13 <sup>b</sup>	6.92 $\pm$ 0.05 <sup>c</sup>

<sup>1</sup>Means in a line with different superscripts are significantly different each other ( $p<0.05$ ) SE, calculated from residual mean square in the ANOVA. Replicate number is three ( $n=3$ )

In this study, protein and amino acids digestibility decreased in relation to the level of SBM and FFS. The highest protein digestibility coefficients were observed for the control diet. When the ADC values of

SBM and FFS protein were calculated by the indirect method, FFS used in Diet 4 and 5 found to have less digestible protein than the SBM used in Diet 2 and 3 ( $P<0.05$ ).

**Table 4: Weight performance of rainbow trout fed on experimental diets for five months and apparent digestibility coefficients (ADC) of the experimental diets**

	Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight (g)	49.95±0.28	49.56±0.33	50.18±0.25	49.80±0.33	50.41±0.30
Final weight (g)	108.41±0.24 <sup>a</sup>	102.08±0.29 <sup>b</sup>	84.61±0.19 <sup>d</sup>	97.45±0.19 <sup>c</sup>	74.61±0.17 <sup>c</sup>
Digestibility (%)					
Energy	86.07±0.20 <sup>a</sup>	85.37±0.40 <sup>ab</sup>	84.67±0.24 <sup>b</sup>	85.04±0.36 <sup>ab</sup>	83.10±0.39 <sup>c</sup>
Crude Protein	89.44±0.12 <sup>a</sup>	85.30±0.26 <sup>b</sup>	80.28±0.19 <sup>c</sup>	79.91±0.25 <sup>d</sup>	67.14±0.17 <sup>e</sup>
Amino Acid					
Valine	88.06±0.26 <sup>a</sup>	86.07±0.13 <sup>b</sup>	82.75±0.24 <sup>c</sup>	82.79±0.60 <sup>c</sup>	67.67±1.22 <sup>d</sup>
Leucine	88.54±0.79 <sup>a</sup>	82.76±0.20 <sup>c</sup>	78.54±0.16 <sup>d</sup>	84.97±0.11 <sup>b</sup>	69.56±0.29 <sup>e</sup>
Isoleucine	90.25±0.22 <sup>a</sup>	88.05±0.51 <sup>b</sup>	80.79±0.45 <sup>c</sup>	70.73±0.35 <sup>d</sup>	71.29±0.51 <sup>d</sup>
Threonine	89.97±0.31 <sup>a</sup>	83.94±0.84 <sup>bc</sup>	85.31±0.19 <sup>b</sup>	82.70±0.36 <sup>c</sup>	68.96±1.40 <sup>d</sup>
Methionine	95.36±0.27 <sup>a</sup>	88.86±0.32 <sup>b</sup>	87.54±0.32 <sup>c</sup>	83.17±0.32 <sup>d</sup>	75.59±0.25 <sup>e</sup>
Phenylalanine	80.83±0.83 <sup>c</sup>	87.26±0.07 <sup>a</sup>	74.71±0.35 <sup>d</sup>	85.65±0.34 <sup>b</sup>	62.33±0.45 <sup>e</sup>
Lysine	87.91±0.44 <sup>a</sup>	87.74±1.52 <sup>a</sup>	77.86±0.24 <sup>c</sup>	84.81±0.11 <sup>b</sup>	51.20±0.87 <sup>d</sup>
Histidine	100±0.00 <sup>a</sup>	83.42±1.02 <sup>b</sup>	84.81±2.17 <sup>b</sup>	97.15±0.09 <sup>a</sup>	75.19±0.41 <sup>c</sup>
Tyrosine	100±0.00 <sup>a</sup>	89.94±0.65 <sup>c</sup>	76.84±0.89 <sup>d</sup>	97.96±0.11 <sup>b</sup>	65.72±0.76 <sup>e</sup>
Alanine	86.84±0.07 <sup>a</sup>	85.82±0.40 <sup>a</sup>	77.36±0.06 <sup>c</sup>	80.18±0.22 <sup>b</sup>	65.78±0.59 <sup>d</sup>
Glycine	87.49±0.05 <sup>a</sup>	85.14±0.08 <sup>b</sup>	79.37±0.75 <sup>c</sup>	79.82±0.22 <sup>c</sup>	74.32±0.20 <sup>d</sup>
Serine	93.10±0.19 <sup>a</sup>	88.82±0.08 <sup>bc</sup>	90.50±0.34 <sup>b</sup>	88.00±0.60 <sup>c</sup>	73.01±1.30 <sup>d</sup>
Proline	95.98±0.34 <sup>a</sup>	93.02±0.05 <sup>b</sup>	90.50±0.34 <sup>c</sup>	88.00±0.60 <sup>d</sup>	73.01±1.30 <sup>e</sup>
Aspartic acid	90.16±0.06 <sup>a</sup>	88.89±0.07 <sup>b</sup>	89.10±0.02 <sup>b</sup>	86.69±0.11 <sup>c</sup>	64.97±0.52 <sup>d</sup>
Hydroxyproline	100±0.00 <sup>a</sup>	73.66±0.18 <sup>b</sup>	75.13±0.30 <sup>b</sup>	74.33±0.47 <sup>b</sup>	71.57±2.55 <sup>b</sup>
Glutamic acid	87.18±0.26 <sup>c</sup>	90.93±0.04 <sup>a</sup>	88.73±0.02 <sup>b</sup>	83.88±0.18 <sup>d</sup>	70.05±0.29 <sup>e</sup>

<sup>1</sup>Means in a line with different superscripts are significantly different each other (p<0.05) SE, calculated from residual mean square in the ANOVA, Each analyzed sample has been obtained grouping of the nine different fish.

The present study revealed that there were significant differences on ADCs of amino acids in all diets. The ADCs of valine, leucine, isoleucine, methionine, phenylalanine, lysine, tyrosine, alanine, glycine, proline, glutamic acid showed a decreasing tendency with increasing proportion of SBM. Similarly, except isoleucine and hydroxyproline, ADCs of all amino acids showed a reduction with increasing proportion of FFS. The ADCs of amino acids of Diet 5 (% 55 FFS) was lower than

that of the other diets. In addition, the ADCs of valine, isoleucine, methionine, phenylalanine, lysine, alanine, glycine, proline, aspartic acid and glutamic acid of Diet 2 (% 40 SBM) was significantly higher than that of Diet 4 (% 40 FFS) (P<0.05). Conversely, the ADCs of leucine, histidine and tyrosine of Diet 4 was significantly higher than that of Diet 2 (P<0.05). As regarding the ADCs of methionine, control diet was significantly higher than all diets. The greatest difference among diets was observed for

lysine, where the FFS diets revealed much lower values than the control diet. Data of the amino acid content of rainbow trout muscle at the beginning and at the end of the five months trial are presented in (Table 5). Data on muscle amino acid levels indicated some differences. For example, essential amino acid (EAA) levels of control diet were significantly higher than those of experimental diets and no significant difference was observed for methionine between the fish fed on diets SBM and FFS ( $P>0.05$ ). However, rainbow trout fed on diets SBM and FFS showed a lower lysine level than the control group. On the other hand, valine, isoleucine, threonine, methionine, phenylalanine, lysine and histidine showed a decreasing tendency with increasing proportion of SBM. No significant difference was observed for valine, leucine, isoleucine, treonine, methionine, phenylalanine and lysine between the fish fed on Diet 4 and Diet 5 ( $P>0.05$ ).

### Discussion

In the present study, it was found that the replacement of fish meal with SBM or FFS resulted in a depressing effect on fish growth. Similarly, some researchers reported that the inclusions of high levels of plant protein in diets have negative effects on salmonid fish performance (Kaushik et al., 1995; Refstie et al., 1997, 2000; Romarheim et al., 2006; Drew et al., 2007) and on sea bream (*Sparus aurata*) and turbot (Burel et al., 2000; Kissil et al., 2000). This reduced growth of fish species may be explained by differences in digestibility of protein, amino acids and energy.

Protein quality of dietary protein sources depends on the amino acid composition and their digestibility. The ADCs for protein in protein-rich feed ingredients are usually in the range of 75-95% (NRC, 1993). Although the data presented in this study suggest a reasonable agreement (Except Diet 5) between ADCs of protein (for Diet 1 (control), Diet 2, Diet 3 and Diet 4), ADCs of individual amino acids within a diet are variable. The ADCs of protein, energy and amino acid decreased as the amount of SBM and FFS. Decreasing in protein, amino acids and energy values in salmon at high dietary inclusion levels of plant protein were also found by Mundheim et al. (2004). Overland et al. (2009) found that SBM diet caused reduced digestibility for protein, fat, essential amino acids, and energy compared with FM diets in *Salmo salar*. The reduction in the digestibility of protein and amino acids in the fish fed with SBM or FFS diets in the present study is in agreement with earlier findings (Olli et al., 1994; Gomes et al., 1995; Romarheim et al., 2006, 2008). Methionine is the first limiting amino acid of SBM in fish (Mambrini et al., 1999; Fagbenro and Davies, 2001; Romarheim et al., 2006). In this study, all diets were supplemented with methionine to meet the amino acid needs estimated for trout according to NRC (1993). However, based on the result, the ADCs of methionine showed a decreasing tendency with increasing proportion of SBM and FFS. According to Romarheim et al. (2008) SBM diet may be associated with reduced digestibility of sulfur-containing amino acids.



**Table 5: Amino acid content of rainbow trout muscle at the beginning and at the end of the experiment**

Amino acids <sup>1</sup> (g/100g )	Initial <sup>2</sup>	Experimental diets <sup>3</sup>				
		Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5
Valine	1.40±0.01	1.15±0.01 <sup>b</sup>	1.29±0.03 <sup>a</sup>	1.12±0.01 <sup>b</sup>	1.08±0.07 <sup>b</sup>	1.09±0.05 <sup>b</sup>
Leucine	1.87±0.02	1.59±0.07 <sup>ab</sup>	1.43±0.16 <sup>ab</sup>	1.69±0.00 <sup>a</sup>	1.32±0.07 <sup>b</sup>	1.50±0.09 <sup>ab</sup>
Isoleucine	1.41±0.01	1.06±0.03 <sup>a</sup>	1.08±0.01 <sup>a</sup>	0.97±0.00 <sup>a</sup>	0.91±0.07 <sup>a</sup>	0.99±0.10 <sup>a</sup>
Threonine	0.95±0.03	0.93±0.01 <sup>a</sup>	1.02±0.02 <sup>a</sup>	0.97±0.01 <sup>a</sup>	0.96±0.05 <sup>a</sup>	0.99±0.04 <sup>a</sup>
Methionine	0.93±0.01	0.63±0.02 <sup>a</sup>	0.67±0.02 <sup>a</sup>	0.60±0.02 <sup>a</sup>	0.66±0.03 <sup>a</sup>	0.64±0.05 <sup>a</sup>
Phenylalanine	1.54±0.01	1.08±0.05 <sup>a</sup>	1.10±0.02 <sup>a</sup>	0.86±0.02 <sup>b</sup>	0.80±0.07 <sup>b</sup>	0.91±0.08 <sup>b</sup>
Lysine	1.42±0.03	2.04±0.03 <sup>a</sup>	1.66±0.08 <sup>b</sup>	1.50±0.02 <sup>c</sup>	1.51±0.04 <sup>c</sup>	1.43±0.02 <sup>c</sup>
Histidine	0.25±0.01	0.03±0.01 <sup>b</sup>	0.16±0.00 <sup>a</sup>	0.08±0.02 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.15±0.03 <sup>a</sup>
EAA <sup>4</sup>	9.77±0.01	8.51±0.04 <sup>a</sup>	8.41±0.07 <sup>b</sup>	7.79±0.01 <sup>c</sup>	7.30±0.06 <sup>e</sup>	7.70±0.09 <sup>d</sup>
Tyrosine	1.01±0.03	0.65±0.01 <sup>ab</sup>	0.68±0.01 <sup>a</sup>	0.60±0.02 <sup>ab</sup>	0.56±0.05 <sup>b</sup>	0.62±0.06 <sup>ab</sup>
Alanine	1.08±0.01	1.11±0.38 <sup>a</sup>	1.13±0.02 <sup>a</sup>	1.17±0.01 <sup>a</sup>	1.13±0.05 <sup>a</sup>	1.11±0.04 <sup>a</sup>
Glycine	1.06±0.00	0.99±0.04 <sup>a</sup>	0.94±0.01 <sup>a</sup>	0.95±0.01 <sup>a</sup>	0.97±0.09 <sup>a</sup>	0.92±0.03 <sup>a</sup>
Serine	0.54±0.00	0.63±0.03 <sup>a</sup>	0.62±0.03 <sup>a</sup>	0.71±0.01 <sup>a</sup>	0.71±0.04 <sup>a</sup>	0.69±0.02 <sup>a</sup>
Proline	0.86±0.00	0.62±0.06 <sup>ab</sup>	0.76±0.02 <sup>a</sup>	0.67±0.01 <sup>ab</sup>	0.49±0.10 <sup>b</sup>	0.55±0.09 <sup>ab</sup>
Aspartic acid	1.54±0.02	2.28±0.14 <sup>ab</sup>	2.12±0.10 <sup>b</sup>	2.60±0.05 <sup>a</sup>	2.49±0.13 <sup>ab</sup>	2.27±0.14 <sup>ab</sup>
Hidroxyproline	0.16±0.00	0.17±0.02 <sup>a</sup>	0.11±0.01 <sup>b</sup>	0.08±0.02 <sup>c</sup>	0.17±0.01 <sup>a</sup>	0.16±0.00 <sup>a</sup>
Glutamic acid	1.75±0.01	2.53±0.03 <sup>bc</sup>	2.69±0.04 <sup>ab</sup>	2.75±0.01 <sup>a</sup>	2.66±0.03 <sup>ab</sup>	2.47±0.12 <sup>c</sup>
NEAA <sup>5</sup>	8.00±0.04	8.98±0.34 <sup>d</sup>	9.05±0.06 <sup>c</sup>	9.53±0.03 <sup>a</sup>	9.18±0.11 <sup>b</sup>	8.79±0.20 <sup>e</sup>

<sup>1</sup>Means in a line with different superscripts are significantly different each other (p<0.05) Standart error, calculated from residual mean square in the ANOVA. <sup>2</sup>Each analyzed sample has been obtained grouping the of three different fish. <sup>3</sup> Each analyzed sample has been obtained grouping the of nine different fish. <sup>4</sup> EAA, Essential amino acids. <sup>5</sup> NEAA, Non-Essential amino acids

They found that the reduction in plasma levels of methionine in trout fed the SBM diet. Therefore, the decreasing tendency also may be associated with reduced digestibility of sulfur-containing amino

acids. Furthermore, the ADCs of lysine also decreased as the amount of SBM and FFS increased. Elangovan and Shim (2000) reported that decreased amino acid absorption in rainbow trout, especially

methionine when SBM was used above 50% of the total formulation of the diet. On the other hand, while feeding fish SBM and FFS included diets the content of muscle lysine reduced in comparison to the control diet ( $P<0.05$ ). This indicates that supplemental lysine may be advantageous when using FFS and SBM in diets for trout, especially when used at high inclusion levels in diet.

Albrektsen et al. (2006) reported that digestibility of lipids was significantly reduced with increased vegetable protein inclusion for fish meal, resulting in reduced digestible energy similar to results found in salmonids. Several studies have therefore concluded that dietary SBM lowers the lipid digestibility in salmonids (Olli et al., 1994; Refstie et al., 1999; Forde-Skjaervik et al., 2006; Romarheim et al., 2006; Yamamoto et al., 2007). For the same reason, energy digestibility was significantly reduced with increased SMB and FFS based on our result.

Forde-Skjaervik et al. (2006) reported that the lower crude protein and amino acid digestibility in SBM and FFS were related to different protein structure and processing of the fish meal and the soybean meals. This effect could not be overcome by bioprocess of the soybean meal. The different processing techniques have an important effect on the digestibility of diets (Ustaoğlu and Rennert, 2002). Plant meals also contain significant amounts of carbohydrates and numerous enzyme inhibitors that may have detrimental effects on fish performance (Davies and Morris, 1997; Carter and Hauler, 2000). Overland et al. (2009) reported that the reduction in digestibility

of protein and amino acids in the fish fed the SBM diet could be due to the high intake of anti-nutritional factors of these fish which may negatively affect nutrient utilization or due to heat-induced reduction in protein digestibility.

Many studies of growth and digestibility in rainbow trout fed on soybean meal partly substituted with FM, have shown varied results (Gomes et al., 1995; Kaushik et al., 1995; Refstie et al., 2000; Romarheim et al., 2006). As a result of these studies, it was commonly stated that the effectiveness of soybean products as protein source for fish depends on extraction and processing techniques, different inclusion level, differences among feed ingredients substituted by soybean (Ustaoğlu and Rennert, 2002; Romarheim et al., 2006). It was also stated that soybean contains antinutritional factors which lower the activity of digestive enzymes, thus reducing the digestibility of feedstuff (Mambrini et al., 1999; Refstie et al., 1999; Francis et al., 2001; Fagbenro and Davies, 2001; Dersjant-Li, 2002; Morris et al., 2005;).

It can be concluded that the inclusion of both SBM and FFS in feed for rainbow trout reduces growth, energy, protein and amino acid digestibility. In addition, the conclusion which may be drawn from the present study is that solvent extracted SBM could be used up to 40% in rainbow trout rations. Nevertheless, it can also be concluded that when FFS used up to 40% in rations of rainbow trout it has significantly adverse effects on the growth and essential amino acid contents of *O. mykiss*'s muscle in comparison to the SBM diet ( $P<0.05$ ).

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