

## Evaluation of Extruded Chickpea, Common Bean and Red Lentil Meals as Protein Source in Diets for Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

<sup>1</sup>Serap Ustaoglu Tiril, <sup>1</sup>Ismihan Karayucel, <sup>1</sup>Fikret Alagil,  
<sup>1</sup>Seval Dernekbası and <sup>2</sup>Feraye Berkay Yagci  
<sup>1</sup>Faculty of Fisheries, Sinop University, 57000, Sinop, Turkey  
<sup>2</sup>SIBAL Feed Company, 57000, Sinop, Turkey

**Abstract:** An experiment was conducted to evaluate, the use of extruded chickpea, common bean and red lentil meals as dietary protein source for juvenile rainbow trout. Three experimental diets were formulated based on a reference diet, which composed of 70% reference diet and 30% test ingredient. The diets were given as triplicate groups of juvenile rainbow trout (initial weight of 50 g) to apparent satiation twice daily. There were no significant differences among the groups fed chickpea, common bean and reference diet, for weight gain, specific growth rate and feed conversion ratio, but there were significant differences among these groups and red lentil group in these respects. There were no significant differences in digestibility of protein between the fish fed chickpea and reference diets and chickpea and common bean diets, but the red lentil diet gave lower protein digestibility. Apparent protein digestibility coefficients of the ingredients were determined high for extruded chickpea (80.65%), mid-range for extruded common bean (72.91%) and low for extruded red lentil meals (50.07%). The results indicated that extruded chickpea and common bean meals have higher potential than red lentil meal for use as dietary protein source in diets for juvenile rainbow trout.

**Key words:** Chickpea meal, common bean meal, red lentil meal, protein source, digestibility, juvenile rainbow trout

---

### INTRODUCTION

The use of plant proteins in aquaculture diets has become more common in recent years, because of increases in cost and global demand of fish meal, which is the primary ingredient of aquaculture diets. This situation forces the nutritionists to investigate alternative protein sources such as grain legumes. However, the use of grain legumes in aquaculture diets is potentially limited by some inadequacies in their protein composition, relatively high levels of carbohydrate e.g., starch and non-starch polysaccharides and the presence of a variety of anti-nutritional factors. Many of their nutritional deficiencies (e.g., limiting levels of sulfur amino acids and tryptophan) can be overcome by the addition of low cost synthetic amino acids and enzymes, or relevant forms of processing (Booth *et al.*, 2001).

Extrusion is a cost-effective processing method that is now widely used to improve the nutritive value of legumes, primarily as a mean of reducing the levels of heat-labile, non-nutritive compounds (Marzo *et al.*, 2002). This method, using a combination of moisture, pressure, temperature and mechanical shear, results in physical and chemical changes, such as ingredient particle

size reduction, starch gelatinization and inactivation of enzymes (Cheng and Hardy, 2003). Extrusion processing also enhances widely protein digestibility of plant ingredients (Watanabe, 2002).

The use of grain legumes in aquaculture diets has great potential and was used successfully in many experimental aquaculture diets (Booth *et al.*, 2001). Soybean products were mostly used as protein sources in feeds for rainbow trout (Oliva-Teles *et al.*, 1994; Kaushik *et al.*, 1995; Olli and Krogdahl, 1995; Refstie *et al.*, 1997, 1998, 2000; Vielma *et al.*, 2002). Other legumes such as peas (Gomes *et al.*, 1993; Pfeffer *et al.*, 1995; Thiessen *et al.*, 2003; Gouveia and Davies, 1998; 2000), lupin (De la Higuera *et al.*, 1988; Hughes, 1988; 1991; Robaina *et al.*, 1995), faba beans (Pfeffer *et al.*, 1995), canola (Thiessen *et al.*, 2003) and others (sunflower meal) (Sanz *et al.*, 1994) were also used in trout feeds. However, there is little or any known research on the use of chickpea, common bean and red lentil meal in rainbow trout diets.

The determination of the nutrient digestibility is the first step in evaluation the potential of ingredients for use in the feed for fish species (Allan *et al.*, 2000; Tibbets *et al.*, 2006).

Thus, the aim of this study was to determine the apparent protein digestibility of extruded chickpea, common bean and red lentil meals, as dietary protein source for juvenile rainbow trout. Furthermore, the effects of experimental diets on growth performance, feed efficiency and muscle composition of juvenile rainbow trout were also determined.

## MATERIALS AND METHODS

**Fish and maintenance:** The experiment was conducted at Sinop University Fisheries Faculty in Sinop (Turkey) in an indoor facility. Experimental fish were obtained from a commercial farm, Kuzey Su Urunleri Inc., in Bafra-Samsun and acclimated for 2 weeks under experimental conditions prior the experiment. During the acclimation, the fish were fed with a commercial diet twice per day to satiation.

The experiment was performed in twelve 150 L rectangle fiberglass tanks in a flow-through water system. The experimental fish (Avg. 50 g) were subjected to a fast of 24 h, weighed to the nearest 0.1 g and randomly distributed at a stocking rate of 20 fish per tank with three replicates. The average initial weight of fish was uniform: there was no statistical difference in weight between treatments ( $p > 0.05$ ). Water inflow was adjusted to 3 L  $\text{min}^{-1}$  and supplemental aeration was provided via airstone diffusers. Water quality parameters were monitored daily and average temperature, dissolved oxygen and pH were  $16.5 \pm 0.9^\circ\text{C}$ ,  $6.8 \pm 0.2 \text{ mg L}^{-1}$ , 7.5, respectively. The light: dark cycle was 12 h: 12 h. The experiment lasted for 45 days.

At the beginning (15 fish) and the end (5 fish) of the study, fish from each tank were homogenized and analyzed for muscle composition.

**Diet preparation:** Diet ingredients were provided by a local fish feed manufacturer (SIBAL Inc., Sinop-Turkey). Extruded chickpea (*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.) and red lentil meals (*Lens culinaris* L.) were provided by SARMAKSAN Inc. (Adana, Turkey). Digestibility assessment of the ingredients was undertaken on the diet-substitution basis (Aksnes *et al.*, 1996; Glencross *et al.*, 2004). A reference diet was formulated and prepared to satisfy the nutrient requirements of juvenile rainbow trout (protein level 450 g  $\text{kg}^{-1}$  DM, fat level 160 g  $\text{kg}^{-1}$  DM). The reference diet contained fish meal, solvent-extracted soybean, full fat soybean and corn gluten meals as principal protein sources and fish oil as lipid source. The ingredients was thoroughly mixed, forming the basis for all experimental diets in this study. The test diets were prepared by mixing

70% of the reference diet mixture and 30% of the plant ingredient to be tested. The chemical and amino acid composition of the test ingredients were shown in Table 1. Formulation and chemical composition of the reference and the test diets were shown in Table 2.

Chromic Oxide ( $\text{Cr}_2\text{O}_3$ ) was incorporated into the diets at a concentration of 0.5% as a marker to assess apparent digestibility of the diets. Feed ingredients were thoroughly mixed, homogenized, moistened by the addition of 35% boiling water and pelleted (3.0 mm) in a mincing machine. The pellets were dried at  $70^\circ\text{C}$  for 18 h, cut into pieces approximately 5 mm in length and stored in plastic bags in a refrigerator.

**Feeding and fecal collection:** Fish were fed diets by hand to apparent satiation twice a day (09:00 and 15:00), 6 days a week. Feed for each tank was weighed daily to a constant amount (100 g) and feed consumption in each tank was determined by subtracting unconsumed feed from the ration. All possible care was taken during feeding so that no uneaten feed settled on the tank bottoms. The tanks were thoroughly cleaned after each feeding. Fish were submitted to a 1 week adaptation period to the experimental diets. Then, fecal samples were collected daily between 11:00 and 12:00 and between 16:00 and 17:00 by slow siphoning with an 8 mm plastic tube. Then, the fecal samples were immediately frozen and stored at  $-20^\circ\text{C}$  for pending analysis.

**Chemical analyses:** Chemical composition of dried samples of fish, diets and feces were analyzed by standard methods (AOAC, 1995). Dry matter after drying at  $105^\circ\text{C}$  for 24 h; crude protein by the Kjeldahl method after acid digestion ( $\text{N} \times 6.25$ ), crude lipid after extraction with petroleum ether by the Soxhlet method and ash by incineration at  $550^\circ\text{C}$  in a muffle furnace for 12 h were analyzed. Chromic oxide in the diet and faeces was determined spectrophotometrically according to Bolin *et al.* (1952). The Apparent Digestibility Coefficients ( $\text{ADC}_{\text{diet}}$ ) was based on the following equation (Glencross *et al.*, 2004):

$$\text{ADC}_{\text{diet}} = 1 - \left[ \left( \frac{\text{Cr}_{\text{diet}}}{\text{Cr}_{\text{faeces}}} \right) \times \left( \frac{\text{Nutrient}_{\text{faeces}}}{\text{Nutrient}_{\text{diet}}} \right) \right]$$

Where:

$\text{Cr}_{\text{diet}}$  and  $\text{Cr}_{\text{faeces}}$  = The chromium content of the diet and faeces, respectively

$\text{Nutrient}_{\text{diet}}$  and  $\text{Nutrient}_{\text{faeces}}$  = The nutritional parameter of concern (dry matter, protein or energy) content of the diet and faeces, respectively

Table 1: Chemical and amino acid compositions of the test ingredients

Compositions	Extruded chickpea meal	Extruded bean meal	Extruded red lentil meal
<b>Proximate (%)</b>			
Dry matter	94.59	97.02	90.82
Crude ash*	3.66	5.16	3.00
Crude lipid*	5.18	1.20	0.61
Crude protein*	21.03	23.56	27.96
NFE+crude fiber <sup>1</sup>	70.13	70.08	68.43
<b>Amino acids (ingredient %)</b>			
Methionine	0.21	0.14	0.11
Histidine	0.21	0.71	0.54
Isoleucine	0.99	0.98	1.18
Leucine	1.45	1.18	2.01
Lysine	1.16	1.97	2.09
Phenylalanine	0.96	1.20	1.21
Threonine	0.78	1.31	1.10
Valine	0.91	1.73	1.44
Arginine <sup>2</sup>	ND	ND	ND
Tryptophan <sup>2</sup>	ND	ND	ND
Tyrosine	0.53	0.86	0.84
Alanine	0.82	0.96	1.19
Serine	0.83	1.05	1.49
Proline	0.86	1.08	1.32
Glutamic acid	4.57	3.18	5.42

\*Dry matter basis, <sup>1</sup>NFE+Crude fiber: Nitrogen-Free Extract (calculated by difference), <sup>2</sup>Not Determined

Table 2: Formulation and chemical compositions of the reference and the experimental diets

Formulation and chemical compositions	Reference diet	Chickpea diet	Common bean diet	Red lentil diet
<b>Ingredients (g kg<sup>-1</sup>)</b>				
Fish meal	350	245	245	245
Extracted soybean meal	190	133	133	133
Full fat soybean meal	110	77	77	77
Maize gluten	140	98	98	98
Wheat meal	76.5	53.55	53.55	53.55
Fish oil	125	87.5	87.5	87.5
Vitamin premix <sup>1</sup>	2	1.4	1.4	1.4
Mineral premix <sup>2</sup>	1.5	1.05	1.05	1.05
Cr <sub>2</sub> O <sub>3</sub>	5	3.5	3.5	3.5
Extruded chickpea meal	-	300	-	-
Extruded commonbean meal	-	-	300	-
Extruded red lentil meal	-	-	-	300
Total	1000	1000	1000	1000
<b>Proximate composition (%)</b>				
Dry matter	92.6	94.1	96.7	96.9
Crude protein*	45.4	40	40.4	43.4
Crude lipid*	16.3	14.4	13.7	13.3
Crude ash*	7.9	6.9	7.3	7
NFE+Crude fiber* <sup>3</sup>	30	36	38.6	36.3
Gross energy (kJg <sup>-1</sup> )	22.4	21.5	21.7	21.9
<b>Essential and non-essential amino acids (diet%)</b>				
Methionine	1.11	0.77	0.81	0.42
Histidine	1.12	0.9	ND	1.1
Isoleucine	2.36	1.78	1.88	1.9
Leucine	4.53	3.39	3.9	3.84
Lysine	2.83	1.86	1.64	2.63
Phenylalanine	2.23	1.71	1.83	2.33
Threonine	1.96	1.57	1.85	1.51
Valine	2.44	1.92	2.56	2.22
Arginine <sup>4</sup>	ND	ND	ND	ND
Tryptophan <sup>4</sup>	ND	ND	ND	ND
Tyrosine	1.86	1.33	1.29	1.78
Alanine	2.7	2.02	2.22	1.87
Serine	1.75	1.55	1.6	1.58
Proline	2.85	2.1	3.07	2.66
Glutamic acid	7.92	5.98	7.38	6.03

\*Dry matter basis, <sup>1,2</sup>Provided per kg of feed: Vitamin A 12500 IU; Vitamin D3 2500 IU; Vitamin K3 10 mg; Vitamin B1 10 mg; Vitamin B2 20 mg; Vitamin B6 15 mg; Vitamin B12 0.03 mg; Vitamin C 250 mg; Niacin 200 mg; Biotin 1 mg; Folic acid 10 mg; Pantothenic acid 60 mg; Ca 1000 mg; Ethoxyquin 130 mg; Magnesium 600 mg; Potassium 450 mg; Zinc 90 mg; Manganese 12 mg, Cu, 5 mg. <sup>3</sup>NFE+fiber: Nitrogen-Free Extract (calculated by difference), <sup>4</sup>Not Determined

The digestibility values for each of the test ingredient in the test diets were calculated according to the equation (Bureau *et al.*, 1999):

$$ADC_1 = ADC_T + \left[ \frac{(1-s) D_R}{s D_1} \right] (ADC_T - ADC_R)$$

Where:

- ADC<sub>1</sub> = Apparent digestibility coefficient of test ingredient
- ADC<sub>T</sub> = Apparent digestibility coefficient of test diet
- ADC<sub>R</sub> = Apparent digestibility coefficient of the reference diet
- D<sub>R</sub> = Percent nutrient of the reference diet mash
- D<sub>1</sub> = Percent nutrient of the test ingredient
- s = Proportion of test ingredient in test diet mash (i.e., 0.3 in this study)
- 1-s = Proportion of reference diet mash in test diet mash (i.e., 0.7 in this study)

Amino acids were analyzed by a hydrolysis method using a Phenomenex EZ Faast GC-FID at the TUBITAK Marmara Research Center in Gebze. All chemical analyses were carried out in duplicate.

**Statistical analysis:** Minitab Release 13.1 was used for statistical analyses. One-way ANOVA was performed to test for significant differences among treatment groups. Differences among treatments were compared by the Tukey's multiple range test and considered significant at p<0.05.

## RESULTS

No mortality was observed and the fish accepted all the experimental diets. This showed no unpalatability and

unacceptability effect of tested feedstuffs. Growth and feed utilization efficiency shown in Table 3 indicated that there were significant differences in final body weight, weight gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) (p<0.05). The highest weight gain was obtained in fish fed chickpea diet, but the differences between groups fed chickpea, common bean and reference diets were not statistically significant (p>0.05). No significant differences were found in SGR of fish fed chickpea, common bean and reference diets (p>0.05). However, there was a significant difference between red lentil diet and other groups (p<0.05). The best feed conversion ratio was obtained in the reference and chickpea diets, but there were no significant differences in FCR between these groups and common bean diet (p>0.05). However, there were significant differences between these groups and red lentil diet (p<0.05). The highest protein efficiency ratio was obtained in fish fed chickpea and the reference diets and no significant difference was found among these groups (p>0.05). However, significant differences were found between the red lentil diet and the other groups (p<0.05).

Apparent digestibility coefficients of dry matter, crude protein, crude lipid, NfE+Crude fiber and gross energy were shown in Table 4. Results indicated that the ADCs for dry matter, crude protein, crude lipid, NfE+Crude fiber and gross energy were significantly affected by the composition of the test ingredients. Dry matter digestibility was significantly higher in the reference diet than the other groups (p<0.05). Dry matter digestibility coefficient of red lentil meal was significantly lower than the dry matter ADC of the chickpea diet (p<0.05). There were no significant differences in the digestibility of protein and lipid among chickpea and reference diets (p>0.05). However, the protein digestibility

Table 3: Growth performance and feed efficiency in fish fed the experimental diets (mean±SEM, n = 3)

Parameters	Chickpea diet	Common bean diet	Red lentil diet	Reference diet
Initial body weight (g)	49.7±0.190 <sup>a</sup>	51.0±0.070 <sup>a</sup>	50.6±0.720 <sup>a</sup>	50.8±0.350 <sup>a</sup>
Final body weight (g)	88.3±2.410 <sup>a</sup>	86.3±2.860 <sup>a</sup>	78.2±2.070 <sup>b</sup>	90.0±3.100 <sup>a</sup>
Weight gain (%) <sup>1</sup>	77.7±7.140 <sup>a</sup>	69.2±5.370 <sup>a</sup>	54.5±4.460 <sup>b</sup>	77.2±4.730 <sup>a</sup>
SGR (%) <sup>2</sup>	1.27±0.10 <sup>a</sup>	1.16±0.12 <sup>a</sup>	0.96±0.11 <sup>b</sup>	1.27±0.15 <sup>a</sup>
FCR <sup>3</sup>	1.24±0.02 <sup>a</sup>	1.43±0.07 <sup>ab</sup>	1.61±0.14 <sup>b</sup>	1.09±0.06 <sup>a</sup>
PER <sup>4</sup>	2.01±0.04 <sup>a</sup>	1.73±0.08 <sup>b</sup>	1.44±0.12 <sup>c</sup>	2.02±0.06 <sup>a</sup>

<sup>1</sup>Weight gain (%) = [(final weight-initial weight)/initial weight] ×100, <sup>2</sup>Specific Growth Rate (SGR) = 100 × [(ln final body weight-ln initial body weight)/45 days], <sup>3</sup>Feed Conversion Ratio (FCR) = Total diet fed (g)/total weight gain (g), <sup>4</sup>PER, Protein Efficiency Ratio = weight gain (g)/protein intake (g)

Table 4: Apparent digestibility coefficients (%) of the diets<sup>1</sup>

Parameters	Chickpea diet	Common bean diet	Lentil diet	Reference diet
Dry matter	84.63±0.18 <sup>b</sup>	79.07±1.58 <sup>c</sup>	76.66±2.26 <sup>c</sup>	90.01±0.01 <sup>a</sup>
Crude protein	92.41±0.20 <sup>ab</sup>	90.78±0.79 <sup>b</sup>	85.42±1.38 <sup>c</sup>	94.75±0.06 <sup>a</sup>
Crude lipid	96.46±0.11 <sup>a</sup>	95.11±0.43 <sup>b</sup>	94.89±0.15 <sup>b</sup>	97.16±0.12 <sup>a</sup>
NfE + crude fiber	75.21±0.77 <sup>b</sup>	66.41±2.48 <sup>c</sup>	68.34±3.19 <sup>c</sup>	84.87±0.07 <sup>a</sup>
Gross energy	88.41±0.17 <sup>b</sup>	84.27±1.18 <sup>c</sup>	83.65±1.54 <sup>c</sup>	93.12±0.03 <sup>a</sup>

<sup>1</sup>Values are means from triplicate groups of fish where the means in each row with a different letters are significantly different (p<0.05) (mean±SEM, n = 3)

Table 5: Apparent protein digestibility coefficients (%) of the test ingredients<sup>1</sup>

Parameter	Extruded chickpea meal	Extruded common bean meal	Extruded red lentil meal
Crude protein	80.65±1.23 <sup>a</sup>	72.91±4.34 <sup>a</sup>	50.07±6.61 <sup>b</sup>

<sup>1</sup>Values are means from triplicate groups of fish where the means in each row with a different letters are significantly different (p<0.05) (mean±SEM, n = 3)

Table 6: Chemical composition (wet weight %) of dorsal muscle of rainbow trout fed the experimental diets<sup>1</sup>

Parameters	Chickpea diet	Common bean diet	Lentil diet	Reference diet
Dry matter	26.72±0.23	26.76±0.50	27.87±0.07	27.60±0.25
Crude protein	19.16±0.20	19.03±0.15	18.86±0.18	17.36±0.83
Crude lipid	6.41±0.14	5.25±0.48	4.96±0.43	5.92±0.06
Ash	1.71±0.01	1.65±0.02	1.51±0.09	1.69±0.07

<sup>1</sup>The values are given as mean of three replicates±SEM

of red lentil diet was significantly lower than the other diets (p<0.05). No significant difference was found between the protein ADCs of chickpea and common bean diets (p>0.05).

Apparent protein digestibility coefficients of the test ingredients were shown in Table 5. There were no significant differences in protein digestibility of chickpea and common bean meals (p>0.05), although the protein digestibility of chickpea meal tended to be higher. Protein digestibility of red lentil meal was significantly lower than chickpea and common bean meals (p<0.05).

The chemical composition of experimental fish was given in Table 6. No significant differences were found in dorsal muscle composition of rainbow trouts fed the experimental diets (p>0.05).

## DISCUSSION

Use of plant products as protein source in fish feeds shows considerable potential application for the aquaculture worldwide. There are many researches especially on the evaluating of soybean products of various forms in diets for rainbow trout (Oliva-Teles *et al.*, 1994; Kaushik *et al.*, 1995; Olli and Krogdahl, 1995; Refstie *et al.*, 1997, 2000). From these studies, it was shown that soybean protein had high protein digestibility and efficient available in feeds for rainbow trout. Other grain legumes were also used successfully in many experimental fish diets. Grain legumes such as pea, lupin, faba bean or rapeseed, whose protein is lower than soybean, were also confirmed as potential protein source in diets for rainbow trout, turbot and sea bass (De la Higuera *et al.*, 1988; Hughes, 1991; Gomes *et al.*, 1993; Pfeiffer *et al.*, 1995; Burel *et al.*, 1998, 2000; Gouveia and Davies, 1998, 2000; Thiessen *et al.*, 2003). Many studies present only dietary ADCs for diets which test ingredient replace either a ingredient from a control diet or a proportion of fish meal. Furthermore, there is little information about the use of chickpea (Allan *et al.*, 2000; Booth *et al.*, 2001) and no information about the use of common bean and red lentil meal in fish diets. Reference to digestibility values for these ingredients for fish is practically rare or non-existent in the study.

De la Higuera *et al.* (1988), Hughes (1991) and Burel *et al.* (2000) reported that lupins were useful feed ingredients in diets for rainbow trout. Robaina *et al.* (1995) also reported that replacement of 30% fishmeal protein by lupin meal in gilthead sea bream diet did not affect the growth performance. Gomes *et al.* (1993) found any adverse effect on growth performance of rainbow trout by the inclusion of co-extruded rapeseed and field peas at up to 15% replacement of the protein. Gouveia and Davies (1998) also found successful results about protein, energy and carbohydrate digestibility for pea seed meal in juvenile sea bass. Pereira and Oliva-Teles (2002) showed that the growth performance of gilthead sea bream fed diets with pea seed meal representing up to 20% of total protein was not significantly different from that of fish fed fishmeal-based diet. These researchers also found no significant differences in apparent protein digestibility by the inclusion of pea seed meal in the diets (10 or 20%), even though, the ADC in the fishmeal-based diet was slightly higher in the experimental diets. Gouveia and Davies (2000) showed that the inclusion up to 30% of extruded dehulled pea meal, replacing fish meal, had no adverse effect on the growth performance or body composition of juvenile European sea bass. The whole body composition did not differ among the fish fed the experimental diets, suggesting a similar utilization of the nutrients.

Nutrient digestibility coefficients of some legumes or for diets containing these legumes were often investigated. The findings of the present study are generally consistent with the observations of the above mentioned studies, which also noted successful nutritional responses of rainbow trout or other fish species when diets contained grain legumes. The apparent protein and lipid digestibility coefficients of test and reference diets in the present study were quite high (85.42-94.75% for protein, 94.89-97.16% for lipid), indicating the adequate efficiency of the ingredient composition in the diets. Digestibility values in fish normally range 75-95% for protein and 85-95% for lipid (NRC, 1993). The values in this study are similar to those of diets containing fish-meal as well as plant protein (Kaushik *et al.*, 1995; Refstie *et al.*, 1997; Aksnes and Opstvedt, 1998).

Morales *et al.* (1999) studied the nutrient digestibility of different feeds in which 40% of the fish meal protein was replaced by corn gluten, cotton seed, lupine seed, soybean or sunflower meals for rainbow trout. They reported that ADCs of protein of these diets were ranged between 81.18-88.93%. The protein digestibility values of our study are similar or higher than these results. Gouveia and Davies (1998) tested diets containing 20 and 40% pea meal in European sea bass fingerlings and found no differences for protein ADCs (89%). This result is also comparable to our results although, the fish species was different.

Juvenile rainbow trouts were capable of digesting protein quite effectively in extruded chickpea and common bean meals in this study. Because, the effects of extrusion processing on ADCs of nutrients were also known, extruded form of the tested feedstuffs were used in this study. Hence, the results of the present study showed that ADC of crude protein in chickpea was quite high (80.65%) for juvenile rainbow trout. Pfeffer *et al.* (1995) also showed that treatments (such as autoclaving) increase protein digestibility of field peas and field bean for rainbow trout. These researchers also reported that the protein digestibility of 86% for field peas and 76% for field beans, increased to 91 and 89% after cooking, respectively. Allan *et al.* (2000) found a similar result to our result for protein digestibility of chickpea (82.2%) for Australian silver perch. These authors reported higher protein digestibility results for lupins (95.9-97.1%), cow peas (96.5%), faba bean (90.5), similar result for field pea (81%) and lower result for vetch (71.2%). The protein digestibility value of vetch (71.2%) is similar to our result which reported for common bean (72.91%). Protein digestibility of red lentil meal (50.07) was significantly lower than the chickpea (80.65%) and common bean meals (72.91%). Although, a positive correlation between ingredient protein content and protein digestibility was reported for rainbow trout and red drum (Smith *et al.*, 1995; McGoogan and Reigh, 1996), lower digestibility for red lentil meal which included 27.96% crude protein content was found in this study.

Thiessen *et al.* (2003) evaluated the use of raw or extruded pea and canola products in rainbow trout diets. Apparent protein digestibility of different ingredients in rainbow trout varied between 90.9 (raw whole pea) to 94.6 (autoclaved air classified pea protein), which are higher than our results. They found high protein digestibility values (93.5%) for extruded/dehulled peas. These researchers concluded that dehulled peas and air-classified pea protein are suitable ingredients for use in trout diet formulation at a level of 20%.

Burel *et al.* (2000) evaluated extruded pea, lupin and rapeseed meals in rainbow trout diets and reported that

extruded lupin was a promising substitute for fish meal with the high protein ADC of 96%. However, extruded pea had lower protein ADC of 88%. Cheng and Hardy (2002) also found high protein digestibility for canola meal (94.8%) in rainbow trout. These results are higher than the protein ADC's of the present study. This may be attributed to the different protein content of the tested feedstuffs.

In the present study, no growth differences were observed in rainbow trout fed chickpea, common bean and reference diets, while fish fed the red lentil meal diet exhibited lower growth performance. FCR, SGR and PER of rainbow trout fed chickpea diet and the reference diet were similar, however, the ratios of the fish fed common bean and red lentil diets were lower. The results demonstrated that juvenile rainbow trout were more efficient in digesting and utilizing the protein of chickpea and common bean meal than the protein of red lentil meal. Lower growth rate in rainbow trout fed red lentil diet may be attributed to the lower methionine content in this diet.

## CONCLUSION

All three legumes tested, especially chickpea and common bean, proved to have potential as feed ingredients for juvenile rainbow trout. This study is the first examining the digestibility value of common bean and red lentil as plant protein source for rainbow trout. Future work should be directed towards to the availability of amino acids of tested ingredients for fish. Further studies are needed to find out the acceptable inclusion level of tested ingredients in rainbow trout diets.

## ACKNOWLEDGEMENTS

We would like to thank the Owners of the Sibal Feed Company, Mr. Engin Savas and Mr. Ahmet Celen, Sarmaksan, Adana, for supplying diet ingredients as well as Mr. Osman Parlak, the Owner of the Kuzey Su Urunleri fish farm, for supplying experimental fish. We also would like to thank Hamide ERDURDU for analyses of the feed ingredients.

## REFERENCES

- Aksnes, A., T. Hyertnes and J. Opstvedt, 1996. Comparison of two assay methods for determination of nutrient and energy digestibility in fish. *Aquaculture*, 140: 343-359.
- Aksnes, A. and J. Opstvedt, 1998. Content of digestible energy in fish feed ingredients determined by the ingredient-substitution method. *Aquaculture*, 161: 45-53.

- Allan, G.L., S. Parkinson, M.A. Booth, D.A.J. Stone, J.R. Stuart, J. Frances and R. Warner-Smith, 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, 186: 293-310.
- AOAC (Association of Official Analytical Chemists), 1995. *Official Methods of Analysis*. 16th Edn. Arlington, VA.
- Bolin, D.W., R.P. King and E.W. Klosterman, 1952. A simplified method for the determination of Chromic Oxide ( $\text{Cr}_2\text{O}_3$ ) when used as an inert substance. *Science*, 116: 634-635.
- Booth, M.A., G.L. Allan, J. Frances and S. Parkinson, 2001. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus* IV. Effects of dehulling and protein concentration on digestibility of grain legumes. *Aquaculture*, 196: 67-85.
- Bureau, D.P., A.M. Haris and C.Y. Cho, 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 180: 345-358.
- Burel, C., T. Boujard, G. Corraze, S.J. Kaushik, G. Boeuf, K.A. Mol, S. Van der Greyten and E.R. Kuhn, 1998. Incorporation of high levels of extruded lupin in diets for rainbow trout (*Oncorhynchus mykiss*). Nutritional value and effect on thyroid status. *Aquaculture*, 163: 325-245.
- Burel, C., T. Boujard, F. Tulli and S.J. Kaushik, 2000. Digestibility of extruded peas, extruded lupin and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Aquaculture*, 188: 285-298.
- Cheng, Z.J. and R.W. Hardy, 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr.*, 8: 271-277.
- Cheng, Z.J. and R.W. Hardy, 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr.*, 9: 77-83.
- De la Higuera, M., M. Garcia-Gallego, A. Sanz, G. Cardenete, M.D. Suarez and F.J. Moyano, 1988. Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout *Salmo gairdneri*. *Aquaculture*, 71: 37-50.
- Glencross, B.D., C.G. Carter, N. Duijster, D.R. Evans, K. Dods, P. McCafferty, W.E. Hawkins, R. Maas and S. Sipsas, 2004. A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 237: 333-346.
- Gomes, E.F., G. Corraze and S.J. Kaushik, 1993. Effects of dietary incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 113: 339-353.
- Gouveia, A. and S.J. Davies, 1998. Preliminary nutritional evaluation of pea seed meal *Pisum sativum* for juvenile European sea bass *Dicentrarchus labrax*. *Aquaculture*, 166: 311-320.
- Gouveia, A. and S.J. Davies, 2000. Inclusion of an extruded dehulled pea seed meal in diets for European sea bass *Dicentrarchus labrax*. *Aquaculture*, 182: 183-193.
- Hughes, S.G., 1988. Assessment of lupin flour as a diet ingredient for rainbow trout *Salmo gairdneri*. *Aquaculture*, 71: 379-385.
- Hughes, S.G., 1991. Use of lupin flour as a replacement for full-fat soy in diets for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 93: 57-62.
- Kaushik, S.J., J.P. Craedi and J.P. Lalles, 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 133: 257-274.
- Marzo, F., R. Alonso, E. Urdaneta, F.J. Arricibita and F. Ibanez, 2002. Nutritional quality of extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. *J. Anim. Sci.*, 80: 875-879.
- McGoogan, B.B. and R.C. Reigh, 1996. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. *Aquaculture*, 141: 233-244.
- Morales, A.E., G. Cardenete, A. Sanz and M. De la Higuera, 1999. Re-evaluation of crude fiber and acid-insoluble ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 179: 71-79.
- NRC (National Research Council), 1993. *Nutrient Requirements of Fish*. National Academy Press. Washington D.C., pp: 114.
- Oliva-Teles, A., A.J. Gouveia and E. Gomes, 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 124: 343-349.
- Olli, J.J. and A. Krogdahl, 1995. Nutritive value of 4 soybean products as protein sources in diets for rainbow trout *Oncorhynchus mykiss*, Walbaum reared in freshwater. *Acta Agric. Scand. Sect. A: Anim. Sci.*, 44: 185-192.
- Pereira, T.G. and A. Oliva Teles, 2002. Preliminary evaluation of pea seed meal in diets for gilthead sea bream (*Sparus aurata*). *Aquacult. Res.*, 33: 1183-1189.

- Pfeffer, E., S. Kinzinger and M. Rodehutschord, 1995. Influence of the proportion of poultry slaughter by-products and of untreated or hydrothermally treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* Walbaum, on apparent digestibilities of their energy and organic compounds. *Aquacult. Nutr.*, 1: 111-117.
- Refstie, S., S.J. Helland and T. Storebakken, 1997. Adaptation to soybean meal in diets for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 153: 263-272.
- Refstie, S., T. Storebakken and A.J. Roem, 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fishmeal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture*, 162: 301-312.
- Refstie, S., O. Korsoen and T. Storebakken, 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 190: 49-63.
- Robaina, L., M.S. Izquierdo, F.J. Moyano, J. Socorro, J.M. Vergara, D. Montero and H. Fernandez-Palacios, 1995. Soybean and lupin seed meals in diets for gilthead seabream *Sparus aurata*: Nutritional and histological implications. *Aquaculture*, 130: 219-233.
- Sanz, A., A.E. Morales and M. de la Higuera, 1994. Sunflower meal compared with soybean meal as partial substitutes for fish meal in rainbow trout *Oncorhynchus mykiss* diets-protein and energy utilization. *Aquaculture*, 128: 287-300.
- Smith, R.R., R.A. Winfree, G.W. Rumsey, A. Allred and M. Peterson, 1995. Apparent digestion coefficients and metabolizable energy of feed ingredients for rainbow trout *Oncorhynchus mykiss*. *J. World Aquacult. Soc.*, 26: 432-437.
- Thiessen, D.L., G.L. Campbell and P.D. Adelizi, 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. *Aquacult. Nutr.*, 9: 67-75.
- Tibbets, S.M., J.E. Milley and S.P. Lall, 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture*, 261: 1314-1327.
- Vielma, J., K. Ruohonen and M. Peisker, 2002. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 204: 145-156.
- Watanabe, T., 2002. Strategies for further development of aquatic feeds. *Fish Sci.*, 68: 242-252.