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In Vivo and *in Vitro* Protein Digestibility of Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1972) Fed Steam Pressured or Extruded Feeds

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Abstract

In vivo and *in vitro* protein digestibility of rainbow trout (*Oncorhynchus mykiss*) fed four different commercial feeds produced by means of extrusion or steam pressure methods was examined. At the end of the feeding trials, the live weight gain (LWG), feed conversion ratio (FCR), specific growth rate (SGR) and feed consumption (FC) were examined. According to the results at the end of the feeding trials, the difference between the feed consumption values was found significant ($P \geq 0.05$). The feed conversion ratio values were 1.1 in the groups fed extruded feed (A, B) and 1.3 - 1.6 in the groups fed with steam pressured (C, D) feed. The feed samples and the feces obtained from feeding tanks were analyzed in order to determine the proximate composition and also calculate *in vitro* protein digestibility. Relative protein digestibility for *in vitro* values was found by using porcine-tripsin enzyme. According to the results, the relation between *in vivo* and *in vitro* protein digestibility values was compared. *In vivo* digestibility ratios were found 94.9% and 89.1% in the fish fed extruded feed groups and 94.4% and 93.4% in the fish fed steam pressured feed groups. *In vitro* protein digestibility values were 66.7%, 63.6% in extruded feeds and 57.6%, 51.5% in steam pressured feeds, respectively.

As a result, protein digestibility and feed efficiency ratio values in the rainbow trout fed extruded feeds were found better than the steam pressured feeds in this study.

Key Words: Rainbow trout (*Oncorhynchus mykiss*), protein digestibility, *in vivo* and *in vitro* digestibility method.

Introduction

The increase in the world's population is accepted as the most important factor accelerating the development of the aquaculture industry. Aquaculture is the most rapidly developing sector in the world and is now one of the most rapidly developing sectors in the Turkish food industry. And it has been greatly influenced by global developments.

Rainbow trout can produce intensive or semi intensive conditions in the land, in the sea net cages and in the lake. The reasons for the widespread existence of the rainbow trout are its endurance to diseases and acquired experience.

Fish require some main nutrients such as protein, fat, carbohydrate, vitamins and minerals, but these requirements vary by species. Proteins are the most required nutrients for the animal. Not only it is needed for growth but also it is used in energy requirements. Fish use proteins as their energy source, but because of the high cost of proteins, fats and carbohydrates are preferred as energy source in feeds. Proteins must be used only for growth in fish (Şener and Yıldız, 1998; Demir, 1996; Nose, 1989).

In extruded feeds produced with the extrusion technology, the protein ratio is increased over 55 % and fat ratio goes to 30 %. It can be thought that these feeds are very expensive, but the accurate method of evaluating fish food is the high digestibility ratio of feedstuffs. Thus, the cost of each digestible unit decreases. Extrusion technology has been used for 60 years as a feed processing method. It began to be used in 1980s and became widespread in net cages in aquaculture. By the development of intensive aquaculture everywhere in the world during the past two decades, the qualified feeds such as water stable pellet feeds that float in the water, slowly sinking and sinking feeds are required to produce and the extrusion technology is contributed a very important advantage to the fish feed industry.

It is well known that the feeds used in aquaculture must be friendly to fish, to the consumer, and to the environment. Extrusion technology enables the production of friendly aquaculture feeds by means of heating with extrusion and fat addition. Heating with extrusion project provides attractive possibilities to the feed production that is required by aquatic species (Şener and Yıldız, 1998).

Like rainbow trout, all carnivore fish have HCl that encourages digestive enzymes to be produced. Pepsin makes proteins to transform into amino acids and short-chained polypeptides (Nose, 1989). Protein digestibility also depends on the processing methods (Aksness *et al.*, 1996).

During the digestion experiments in fish, fish are held in a test tank. Collection and distribution of the fish feces are quite difficult. The digestibility of food in land animals is determined by two ways: First is the direct measurement method by weighing feed ingredients going to the digestive system and

extracted by feces. Second is the measurement of the food ratio and indicator material in diets and feces. Implementation of the first method on fish is not easy due to certain reasons such as the difficulty of distinguishing the feces and unconsumed feeds, loss of feces and the difficulty of feedstuff measurement. Consequently, it is better to measure the digestibility by using an indicator material in fish (Nose, 1989).

In recent years, some of the feces collecting mechanisms for digestibility experiments were developed. The aim of collecting feces by help of a filter that is placed on the outlet pipe of the fish tanks is appreciable. Then, Cho and Covey (1991) and Nose (1985) developed this mechanism (Figure 1). By means of these mechanisms, fish feed and float normally and it is possible to collect the fish feces without causing any stress. Also, carcass analyses can be measured for in vivo digestibility tests and growth performance (Nose, 1989).

Collection of feces must generally be done at least 2 hours before feeding. The feces could become dissolved material in quite a short time. Some important problems appeared when some methods for the land animals were applied to the fish in order to measure the digestibility, such as:

1. Fish are the small size experimental animals
2. Decomposition difficulties of dissolved materials in water such as nitrogenous compounds (NH_3).
3. Excretion of most metabolic products by gills in fish; nitrogen amount excreted by gills in freshwater fish is approximately 80 %.
4. Since body heat of fish is dependent on the water temperature heat affects the feed absorption and digestion speed.

Digestion speed varies according to the fish species, feed type and quantity and temperature. There are several findings, which showed that small fish digested their feed in a shorter time than the bigger ones. The temperature affects the speed of digestion enzymes secretion during feed absorption, and functions of the digestive system.

In digestibility studies and in the studies on finding the feeding values of the feedstuff, the most popular method is feeding trials (*in vivo* method), but these experiments are very slow and expensive and take a long time. And also private techniques are required to keep the animals alive. A faster and cheaper method of finding the feeding values of the feedstuff must be found for fish. Therefore, in vitro digestibility experiments for fish have been developed. By this way, faster and cheaper experiments can be done and also the feeding values and protein quality can be found by using a very small amount of crude materials (Lanari *et al*., 1995; Morales *et al*., 1999).

In this (*in vitro* digestibility) method, the conditions were provided to be as similar as the digestion channel conditions. This method was developed by examining the pH, water temperature and the pass time of the feeds through the intestine and stomach. By this method, firstly the in vivo conditions must be well known so as to measure the protein digestibility (Eid and Matty, 1988).

That is why; Grabner (1985) took samples of intestine content from rainbow trout (*O. mykiss*) and studied its physical and chemical situations. He examined the water temperature, pH, amount of digestion liquid in the intestine and stomach, protease activity and pass time through the intestine and stomach. It was determined that the optimum temperature is 15°C and the pH in intestine is 7.75 for rainbow trout. He founded out that there was a similarity between the in vivo and in vitro situations. Carp and rainbow trout were observed to benefit from the protein like land animals. In addition, he determined that the protein had a high (90%) digestibility.

While the aquaculture industry grows, the requirement of the special feeds to produce some species increases, too. Diet specialists and feed producers started to work intensively on feed materials used in feed industry in order to produce cheap aquaculture feeds. Many scientists advise making further research in developing simple in vitro digestion experiments to determine the feed digestibility rapidly (Alarcon *et al*., 1997).

The objective of the present experiment was to investigate protein digestibility of four feeds that included different feedstuffs produced with steam pressure or extrusion technology in rainbow trout by *in vivo* and *in vitro* methods.

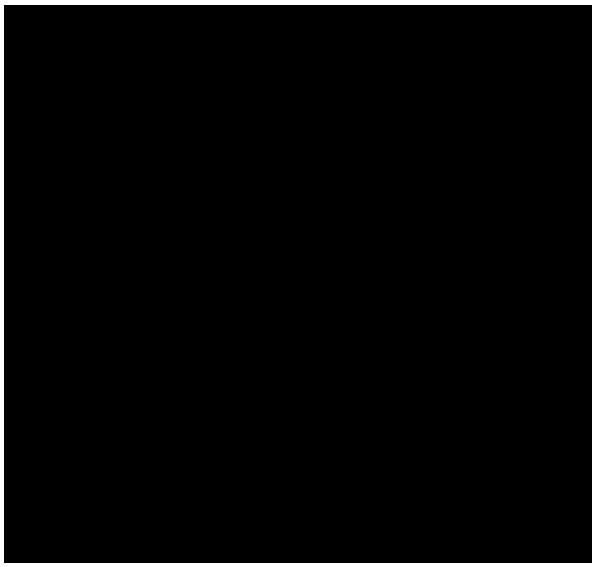

Figure 1. Feces collecting mechanism (From Cho and Cowey, 1991).

Material and Methods

Feeding Trials

Feeding experiments of this research were conducted in Sapanca Inland Waters Research Institute of Istanbul University, Faculty of Fisheries starting from July 12, 1999 until September 20, 2000.

176 rainbow trout that weighted approximately 29.07 g were placed randomly into eight 325 L. experiment tanks. Each of the tanks included 22 fish and totally 4 groups had two parallels according to the experimental feeds. The amount of water was adjusted to 0.3 L/sec. according to the fish sizes and number. Water temperature was measured as 12-14°C, the amount of dissolved oxygen was determined as 9.3 mg/L and pH was 7.3-7.8. In the experiments, totally four commercial feeds were used, the two of which were produced by steam pressure and the two other were produced with extrusion technology. Experimental feeds were named as A, B, C and D. Feed A is the imported extrude feed which had been produced abroad; the B is the extrude feed produced in Turkey; and the C and D are pelleted feeds produced with steam pressure technology in Turkey.

Fish feeding was fulfilled by hand twice a day. The fish were fed with a ratio of 1.6% according to the water temperature and the live weight. Experimental fish were weighed in every two weeks during the feeding trials in order to determine weight gain. In every period, weight gain of fish (WG) was calculated by subtracting the initial weight (G_1) from the last weight (G_2), ($WG= G_2 - G_1$). Specific Growth Rate was calculated by the formulae:

$$SGR= 100 \times (\ln (wf) - \ln(wi)) / t.$$

In this formulae; wf: final live weight of the period; wi: initial live weight of the period; t: period duration (day).

The Feed Conversion Ratio (F.C.R) was calculated as this formula (Arnesen and Krogdahl, 1995; Gomes *et al*., 1995; Ricker, 1979):

FCR = Consumed feed per period (g) / Relative Growth Rate per period (g) + dead fish weight (g)

Condition factor was calculated in respect to the formula

$$K = W / L^3 \times 100$$

in order to show the relationship between height and weight. The Relative Growth Rate in fish was determined with the variance analyzed by the importance control of the difference between different groups concerning the feed efficiency, and done according to t test (Sümbüloğlu, 1998; Zar, 1984).

Chemical Analyzes

The quantities of the feeds used in the research were determined at Istanbul University Faculty of Fisheries, Feed Analysis Laboratory according to Proximate Analyses (AOAC, 1968) methods. Dry matter, crude protein (CP), crude fat (CF), crude ash (CA) and crude cellulose (CC) amounts were appropriate to AOAC methods; nitrogen free extracts (NFE), total energy (GE) and metabolic energy (ME) value (Kcal / kg.) were calculated as to the results of the chemical analyses (Halver, 1972). Proximate analyses were made as parallel for two groups and calculated the means of the results.

Dry matter: 3-4 hours at 105°C in dry cupboard;

Crude protein: with the Kjeldahl nitrogen method;

Crude fat: in a soxhelet system with ether extract;

Crude cellulose: with AOAC (wet burning) method;

Crude ash: 4-6 hours at 550°C in ash furnace;

Nitrogen free extracts: 100 – (Moisture%+ CP% + CF% + CC% + CA%);

Gross energy (Kcal/kg): (5.65 (CP%) + 9.45 (CF%) + 4.10 (NFE%)) x 1.000 / 100;

Metabolic energy: 3.9 (CP) + 8.0 (CF) + 1.6 (NFE).

Digestibility Experiments

100µm plankton nets were placed on the outlet pipe of the experimental fish tanks in order to collect feces that would be used in the measurement of in vivo and in vitro protein digestibility. Feces were collected 2 hours later after the feeding and dried at room temperature for 8 days, and then held in aluminium folio in deep freezer at -20°C until the analyses were done (Lazo *et al*., 1998). Enzyme experiments were conducted at Istanbul University, Faculty of Fisheries and Aquaculture, Feed Analyses Laboratory between December 8, 1999 and January 7, 2000. *In vivo* apparent digestibilities were measured in four different kinds of feeds and *in vivo* apparent protein digestibilities of the feeds were calculated with the formula reported in the previous researches (Storebaken *et al*., 1998; Hillestad *et al*., 1999; Robainna *et al*., 1999; Sugiura *et al*., 1998; Aksness *et al*., 1996):

Protein Digestibility (%) = 100 – [(In feed Crude Cellulose% x In feces Protein amount%) / (In feces Crude Cellulose% In feed Protein amount%)]x 100

In *in vitro* enzyme experiments, -the Lazo single- enzyme assay (Lazo, 1998)- were conducted with each feed sample in order to determine the value of protein digestibility in the gut of rainbow trout. The enzyme used (Sigma Chemical Company, St. Louis, Missouri, USA) was trypsin type IX (14,900 BAEE

unites/mg protein) from porcine pancreas. Feed samples were ground so as to be sifted through the 180µm meshes and weighted 312.5 mg and stirred with 50ml. distillated water. Thus, it's adjusted by feed weighting to 6.25 mg crude protein per ml. The mixture was held at 15°C water bath and was diluted with HCl or NaOH (0.001 – 0.01 N) in order to make pH 7.75. 5 ml (1.5 mg/ml) of porcine trypsin solution. It was added to each feed and pH values were measured in 1 minute intervals for 10 minutes. In each assay, the magnitude of the pH drop (-ΔpH) during the enzyme treatments was used as an index of a feed protein digestibility. Casein was chosen as the reference protein due to its high in vivo apparent protein digestibility (about 99%). In vitro protein digestibility was calculated with the following formula (Lazo *et al*., 1998):

Relative Protein Digestibility (RPD) = $[(-\Delta pH_{ingredient}) - (-\Delta pH_{casein})] \times 100$

Results

The experimental feeds used in this study were named A, B, C and D. Nutrients of the experimental feeds were analyzed according to the proximate analyses method. Total and metabolized energy values were determined according to Halver (1972) (Table 1). It was evaluated that these values were enough for rainbow trout to meet nutritional requirements. Nutrients values, total and metabolic energy values of the experimental feeds are shown in Table 1.

Mean live weight gain was measured in every two weeks during period 5 of feeding trials. Mean individual weights were found by dividing the total fish weight by the number of fish in the tank. Growth performance results of the fish fed experimental diets are shown in Table 2.

The condition factor of fish was calculated according to the weight and length measurements of

Table 1. Nutrients value of experimental feeds

Nutrients (%)	Extruded Feeds		Steam Pressured Feeds	
	A	B	C	D
Dry matter	93.8	91.8	90.9	90.9
Crude Protein	42.3	45.3	45.2	39.1
Crude Fat	12.1	18.1	13.2	10.9
Crude Ash	9.8	6.8	10.1	10.1
Crude Cellulose	0.5	1.9	2.9	2.1
Nitrogen Free Extracts	29.1	19.7	19.6	28.7
Gross Energy (Kcal/kg)	47.3	50.8	46.1	43.8
Metabolizable Energy (Kcal/kg)	30.8	35.3	31.3	28.4

Table 2. Growth performance results of the fishes fed with experimental diet*

Parameters	Extruded Feeds		Steam Pressured Feeds	
	A	B	C	D
Initial Weight (g)	26.8 ± 0.12^{c}	26.7 ± 0.34^{c}	28.6 ± 0.04^{b}	33.2 ± 0.17^{a}
Final Weight (g)	69.1 ± 0.92^{a}	69.7 ± 0.55^{a}	69.8 ± 0.15^{a}	70.5 ± 0.55^{a}
Weight Gain (g)	42.2 ± 1.04^{a}	43.2 ± 0.89^{a}	41.2 ± 0.19^{a}	37.3 ± 0.72^{b}
Specific Growth Rate (%)	1.3 ± 0.1^{a}	1.3 ± 0.05^{a}	1.2 ± 0.1^{a}	1.1 ± 0.06^{a}
Feed Consumption (g)	47.3 ± 0.7^{c}	48.2 ± 0.6^{c}	50.9 ± 0.7^{b}	55.0 ± 0.4^{a}
Feed Conversion Ratio	1.1 ± 0.1^{b}	1.1 ± 0.1^{b}	1.3 ± 0.03^{ab}	1.6 ± 0.05^{a}
Condition Factor	1.2	1.2	1.2	1.2

* : Mean value for each group (n=3), Values in each row with different superscript differ at P<0.05.
Mean were tested by ANOVA and ranked by Duncan's multiple range test.

Table 3. *In vivo* and *in vitro* protein digestibility coefficient values of the experimental feeds*

Experimental Feeds		*In vivo* digestibility (%)	*In vitro* digestibility (%)
Extruded	A	94.9 ± 0.7^{a}	66.7 ± 0.7^{a}
Extruded	B	89.1 ± 0.9^{b}	63.6 ± 0.6^{b}
Steam Pressured	C	94.4 ± 0.5^{a}	57.6 ± 0.6^{c}
Steam Pressured	D	93.4 ± 0.6^{a}	51.5 ± 0.7^{d}

* : Values (n=3) in each row with different superscript differ at P<0.05.
Mean were tested by ANOVA and ranked by Duncan's multiple range test.

the fish samples obtained from each group in the beginning and at the end of the experiments. 10 fish samples were taken from each group for weight measurements and condition factor values are also shown in Table 2.

There was no mortality and any disease in the experimental groups during the feeding trials. In vivo protein digestibility values according to the feeding experiments and in vitro values according to Lazo enzyme assay are shown in Table 3.

Discussion and Conclusion

Relative growth rate (RGR) is a growth index that shows whether the feed given to the fish is efficient. Following the consumption of the feedstuffs as energy source, they gather in muscles, lungs and tissues, and it results in weight gain later.

At the end of the experiment, weight gain values were found 42.2 g in diet A group; 43.2 g in diet B group; 41.2 g in diet C group and 37.3 g in diet D group. It was observed that fish fed extrude feeds grew more and the difference between the groups in variance analyses concerned with the relative growth rates ($P \geq 0.05$). Although the diet A and the diet B groups that were fed with extrude feed consumed less feed than the diet C and the diet D groups, feed conversion was determined as following: diet A group: 1.1; diet B group: 1.1; diet C group: 1.3; diet D group: 1.6 (Table 2). This shows us that the feed efficiency rate of extrude feeds is better than that of the pelleted feeds. Billard (1990) told that the feed efficiency rate of rainbow trout was 2. Stevenson (1987) reported that in case the feed efficiency rate is greater than 2, then the efficiency falls down. The results of variance analyses about the feed efficiency rates showed that the difference between the groups was not noteworthy ($P \geq 0.05$). The condition factors in test groups were found 1.2 orderly in the beginning and at the end of the experiment and are shown in Table 2. These very close values are inside of the Tacon's (1993) boundaries. Nose (1989) reported that an indicator usage is an obligation in fish digestibility studies because of the difficulties in separating feces from uneaten feed. And Nose (1989) told that there might be loss of feces. Hillestead *et al*. (1999) and Morales *et al*. (1999) determined that cellulose could be used as an alternative of chromic oxide (Cr_2O_3), which is the most used indicator in the digestibility studies. Cellulose was used as the indicator in our study, too.

In vivo protein digestibility values in A, B, C and D feeds were calculated as 94.9 %, 89.1%, 94.4% and 93.4%, respectively (Table 3). These values were found parallel to those of the early studies by Eid and Matty (1988), Grabner (1985) and Lazo *et al*. (1998). However, one of the extruded feeds (Diet B) was produced in Turkey and the other was imported. Protein digestibility of these feeds was found different since the extrusion technologies for aquaculture feeds are new concepts in Turkey and probably some problems occurred when feeds were produced.

Lazo single-enzyme assay was implemented in order to determine the *in vitro* protein digestibility. Lazo *et al*. (1998) calculated *in vivo* and *in vitro* protein digestibility using porcine trypsin enzyme and shown a parallelism between the results. Lazo *et al*. (1998) preferred the casein as the reference protein due to the high digestibility value (approximately 99%) in rainbow trout. As the result of the *in vitro* protein digestibility experiments, we obtained the following values: 66.7% for imported extrude feed (A); 63.6 % for domestic extrude feed (B); 57.6% for steam pressured pellet feed (C) and 51.5% for steam pressured pellet fed (D). These results are similar to the *in vitro* protein digestibility values of Lazo *et al*. (1998).

According to the results of this research, the extrude feeds are better than the pelleted feeds with vapour pressure on account of protein digestibility and the growth performance in fish. It's determined that there is a parallelism between the results in feeding experiments and those in the experiments made in laboratory. And it's realized that Lazo single-enzyme experiment is an easy and rapid method in calculating the protein digestibility in fish feeds.

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