Alternative role of sesame seed replacing fish meal in the diet of rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Abstract

Rainbow trout is the most important species of cold water fishes in Iran and the feeding diets which improve the quality and growth of this fish are of great importance. Rainbow trout *Oncorhynchus mykiss* (n=360) specimens with approximate weight of 2±0.02g were fed with different concentrations of sesame seed in their diet instead of fish meal for 120 days. Feeding diets were prepared in 3 sesame seed concentration levels (10, 15, 20 percent compared with control (normal diet), completely random design was used for all triplicate experiments. The greatest rate of weight gain (74±0.04g.), length gain (18.5±0.01cm), specific growth rate (0.59), protein efficiency rate (2.55) and food conversion ratio (0.9) were obtained in treatment 3(20% sesame seed).The lowest rate of weight gain (59±0.04g.), length gain (16.5±0.01cm), specific growth rate (0.46%), protein efficiency (1.72%) and food conversion ratio (1.3) was obtained in the control treatment. Results indicated presence of highly significant differences among the treatments ($p<0.01$). There was no significant difference between the composition of the carcasses of these fish fed with diet of 20 percent sesame and that of control treatment fish. These results demonstrated that sesame seed can be used as an alternative ingredient in its feeding diet instead of fish meal.

Keywords: Rainbow trout, Sesame seed, Alternative of fish meal

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Introduction

Rainbow trout (*O.mykiss)* as one of the most farming fish in most of breeding and farming centers of cold water fish, is well-known throughout the world. Rainbow trout feed on feeding diets which are based on several marine fish meal. Regarding the fact that fish meal is the main source of protein in fish food (Morales *et al.*, 1994), as well as the meal is palatable with high nutritional value in commercial foods of Salmonidae, it constitutes 25-65% of the fish diets (Takeshi, 1992).

However, fish meal is partially used in diets because of its highly expensive price (Lovell, 1989). Although plant protein sources have shortage of some essential amino acids, energy and minerals like phosphorous as compared to animal protein supplementary (Jackson, 1988), the former is used in fish diets to reduce the cost of production and avoid import of foreign fish food.

Of all plant protein sources, oil seeds are good alternative for fish meal among which concentrated protein soybean or soybean extracted protein powder are considered the best. However, concentrated soybean is expensive and the use of processed products of soybean such as soybean-assayed meal is limited due to anti-nutritional factors, high amount of fiber and unavailable carbohydrate (Brown *et al.*, 2008). Therefore, alternative proteins sources are required to replace bothfish meal and soybean meal as primitive protein sources for aquatic feeding diets (Reigh, 2008). Also, soybean meal is one of the most abundant oil seed meals which have been studied more than sesame seeds. Soybean meal and fish meal have the same rate of protein digestibility (Reinitz, 1980) and the digestibility of its essential amino acids ranged between78.6-96.7 percent. Major limiting amino acids in soybean protein are methionine and cysteine (Lovell, 1988). However, increased level of soybean in fish feeding diets decreases the soybean digestibility (Cuca and Sunde**,**1967). Although it has been reported that soybean meal has advantages when used as the primitive source of protein in rainbow trout diets (Oliva-Teles *et al*., 1994), as reportedby (Hepher, 1988), a considerable mortality rate or growth reduction in most fishes, including rainbow trout, occurswhen all ofthe fish meal is substituted by soybean meal (Takeshi, 1992). Gomes *et al*., (1995) reported a decreased growth rate when even 30 percent of fish meal was replaced by soybean meal in diets. However, comparison between feeding diets containing amino acids-supplemented soybean meal indicated that feeding diets containing soybean meal were similarwith fish meal but it could not completely replace fish meal (Hepher, 1988).

There is a limited access to soybean sources in many countries which has caused interest to evaluate novel protein sources such as available plant seeds.

The use of plant proteins in the food to provide all essential requirements of aquatic organisms as well as to develop their growth needs does not date back to a long period of time. Amongst them, sesame seed application is of great importance.

Farming, harvesting and oil extraction of sesame seed (*Sesamum indicum*) is wide-spread throughout the world. These products have more appropriate nutritional quality as compared to other oil seeds proteins including soybean and other traditional cereal seeds. And their potential as a source of nutritional protein in animal foods is well known. Sesame seed contain 45.5% protein, 50% oil, <15% saturated fatty acids like palmitic acid and arachidonic acid and 70% unsaturated fatty acids. Its unsaturated fatty acids include 45-55% oleic acid, 29-39% linoleic acid, 9% palmiticacid and 5% stearic acid (NRC, 1989).

Moreover, sesame seed contains ω_3 and ω_6 and is rich of vitamins like A, E, B, D, protein, lecithin; minerals such as copper, magnesium, zinc, calcium and phosphorous. Its calcium content is much higher than milk (FAO, 2012). In addition, this plant seed is iron-rich which helps the body to remove the exhaustion. The presence of an anti-oxidative substance, sesamol in, which is only found in sesame oil, increases the shelf life of the oil. Since, sesame oil has a high digestibility and is used in a combination with other oils to retard the degradation. Sesame contains amino acids of methionine, niacin, tryptophan and cysteine (FAO, 2012).

According to a previous research, sesame meal can be substituted for fish meal to an extent of less than 52% without needing to add supplementary amino acids in rainbow trout diets and no decline has been observed in fish growth when compared to the control treatment containing fish meal (Nang Thu *et al.*, 2010).

This study was aimed to investigate the nutritional value of sesame seed and its effects in growth enhancement of rainbow trout fingerlings (2g) when added in different concentrations to their feeding diets in order to replace fish meal without addition of amino acid supplements.

Material and Methods

The experiments were conducted in Breeding and Cultivation Centre of rainbow trout in Firoozkooh on 360 fish samples with an average weight of 2±0.02g. Fish samples were maintained in cement hexagonal pools with dimensions of 4×4 and 2 m depth. The pool was divided into four similar parts using a plastic net. Fish were kept for 10 days and fed with common commercial diets during adaptation period. Thereafter, fish samples were fed by the experimental diets in four treatments during a four-month period. Three replicates were used for each feeding treatment with 30 fish in each replicate. They were fed manually three times a day at 8:00,13:00 and 19:00.

Preparation of experimental diets

All diet materials were purchased from retailers. Obviously fish food factories will purchase materials in big quantities with lower prices compared with those we bought in low amounts from common shops. In this situation, producing fish diets will be cheaper than what we provided.

Sesame seed was replaced by fish meal in three different concentrations (10, 15, and 20%) along with a control diet. Completely randomized design with three replicates. Sesame seed was supplied from Halva Shekari Oghab factory and the remaining materials were supplied from Experiments were conducted in a Behparvar factory. Ingredients of diets are given in Table1. After grinding, sesame was mixed thoroughly with other ingredients.

Table 1: Ingredients of control and treatments(in percent)
Treatments with percent sesame replaced fish meal.

Ingredients	Control	10	15	20
Kilka fish meal	30	27	25.5	24
Amount of replacement of sesame seed meal by fish meal (%)	0	3	4.5	6
Wheat middling meal	17.5	17.5	17.5	17.5
Wheat meal	5	5	5	5
Soybean meal	25	25	25	25
Blood meal	10	10	10	10
Mineral premix	0.1	0.1	0.1	0.1
Vitamin premix	0.4	0.4	0.4	0.4
Colin chloride	0.17	0.17	0.17	0.17
Soy bean oil	10	10	10	10
Carboxymethyl cellulose	1.2	1.2	1.2	1.2

Fish biometry

Fish biometry was performed once every 15 days. The amount of daily feeding diets was based on weight gain. Total length and weight of the fish were measured on each biometry period, using biometry board and a digital scale with the accuracy of 0.1 g, respectively.

Calculation of growth parameters

The following equations were used to calculate the experimental results.

Specific Growth Rate(SGR)

Logarithmic mean weight on the beginning and end of the experimental period was calculated to obtain SGR index (Wiehlloughby, 1990).

$$\mathrm{SGR} = \frac{\mathrm{Lnw2} - \mathrm{Lnw1}}{\mathrm{T}} \times 100$$

Food Conversion Ratio (FCR)

Following equation was used to determine FCR index (Tacon, 1990)

$$\mathrm{FCR} = \frac{\text{Consumed food (g)}}{\text{weight gain (g)}}$$

Protein Efficiency Ratio (PER)

Following equation was applied to obtain PER index (Tacon, 1990).

$$PER = \frac{\text{Weight gain (g)}}{\text{taken protein (g)}}$$

Survival rate was calculated according to the following equation (Tacon, 1990).

$$SR = \frac{\text{No. of survived fish}}{\text{total fish number}} \times 100$$

Sampling procedure and analytical methods

At the end of the experiment, six fish samples were randomly selected from control treatment and the treatment containing 20% of sesame meal. Fish samples were killed and immediately frozen and transported to the research laboratory of Bashash Partolocated in Tehran. Preparation of the samples and analysis of amino acids by HPLC methods,lipid bySoxhlet, TVN and protein by Kjeldahl, ash and humidity by gravimetric method were conducted in the laboratory(AOAC, 1990). Physical and chemical analysis of the well water was conducted at Asa Laboratory located in Tehran.

Data analysis

Data analysis was performed using statistical software package of SPSS. Data were subjected to the analysis of variance (ANOVA). Mean comparisons was conducted by Duncan's new multiple range test. Data calculation and drawing of diagrams were done using software package of Excel.

Results

Results of physical and chemical analyses of well water during the experimental period are presented in Table (2).

Table 2: Results of physical and chemical analyses of well water during the experimental period.

Parameters	Value	Measurement unit
temperature	12.5	˚C
pH	7.71	-
oxygen	6	mg/l
electrical conductivity	850	mmhos/cm^2
turbidity	0.87	NTU
suspended material	Less than 1.0	mg/l
total soluble material	525	mg/l
alkalinity (to Phenolphthalein)	0.00	mg/l $CaCO_3$
total alkalinity/total acidity	300	mg/l $CaCO_3$
total hardness	342	mg/l $CaCO_3$
calcium	97.6	mg/l Ca
magnesium	23.52	mg/l Mg
chloride	46	mg/l Cl
sulphate	90	mg/l SO_4
sodium	60	mg/l Na
potassium	5	mg/l K
silica	10.4	mg/l SiO_2
ammonium (NH_3)	0.685	mg/l N
nitrate	2.69	mg/l N
nitrite	0.003	mg/l N

Results of fish growth

According to the obtained results, fish growth rate was higher in the treatment of 20% sesame seed compared to the control treatment. Mean weight of fish samples in the treatment of 10% reached from a primary weight of 2.81 (g) to a final weight of 60 (g); also there were a growth from 2.41 (g) to 63.5 (g) in the treatment of 15% and from 3.07 (g) to 74 (g) in the treatment of 20%. This ranged from a primary weight of 3 (g) to a final weight of 59 (g) in the control treatment. Results detected an asymmetrical weight gain in fish samples treated by different treatments during a 120-day period (Table 3).

Table 3: Mean values of fish growth during the experimental period.

experimental treatments	primary weight (g)	primary total length (cm)	final weight (g)	final total length (cm)
Control	3±0.04	5.6±0.01	59±0.04	16.5±0.01
10%	2.81±0.02	6±0.04	60±0.09	16.87±0.01
15%	2.41±0.02	5.37±0.01	63.5±0.01	17±0.04
20%	3.07±0.01	6±0.09	74±0.04	18.5±0.01

The obtained results include the comparisons of growth factors among treatments. At the end of the experiment, fish samples of treatment 20%, had highest specific growth rate, the lowest food conversion ratio and the highest protein efficiency ratio (Table 4). Analysis of this ratio revealed highly significant differences among various treatments ($p<0.01$).

Table 4: Mean values of SGR, food conversion ratio and protein efficiency ratio index in experimental treatments.

experimental treatments (percentage of fish meal replaced by sesame seed)	Specific Growth Rate(SGR)	Food Conversion Ratio(FCR)	Protein Efficiency Ratio(PER)
control	0.46	1.3	1.72
10%	0.47	1	2.34
15%	0.5	1	2.38
20%	0.59	0.9	2.55

Results of the analysis of variance showed that there were highly significant differences in SGR values of different treatments (p<0.01), while treatments of 15% and 20% had significant differences with other treatments. No significant difference was observed between the control and treatment of 10%(Fig. 1).

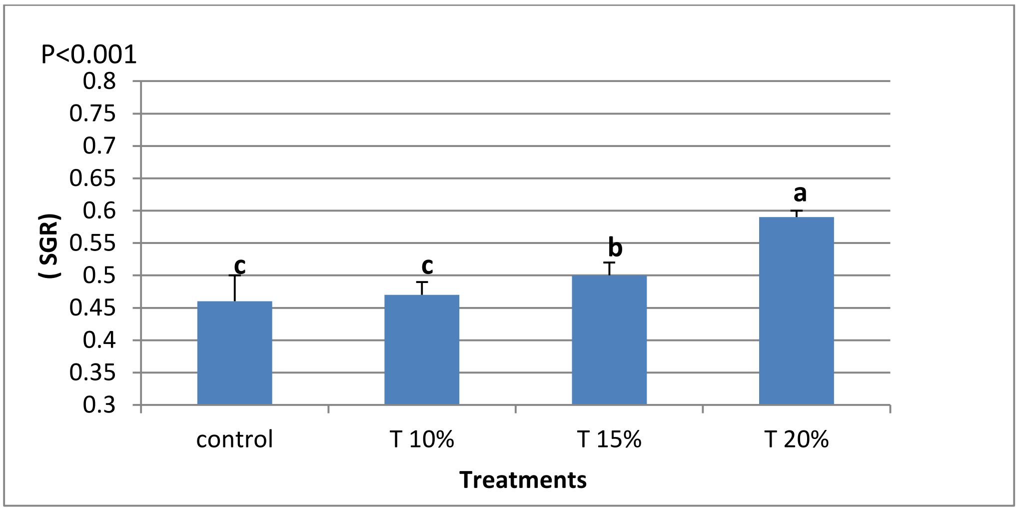

Figure 1: Results of one-way ANOVA and comparison of SGR in different treatments. Different letters on the top of the vertical bars indicate significant difference (*p*<0.05).

Results of the analysis of variance indicated significant differences in FCR values among various treatments (p<0.01) while the control and treatment of 20% had significant differences with other treatments. In addition, fish samples in treatments of 10% and 15% had similar FCR rates (Fig. 2).

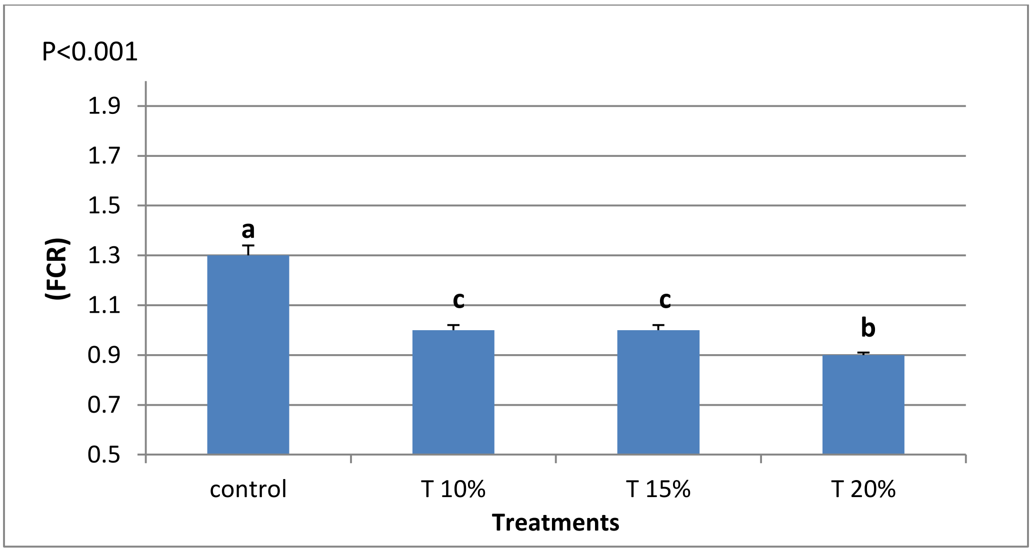

Figure 2: Results of one-way ANOVA and comparison of FCR in different treatments. Different letters on the top of the vertical bars indicate significant difference (*p*<0.05).

Results of analysis of variance showed significant differences in PER values of various treatments ($p<0.01$) while the control and treatment of 20% had significant differences with other treatments. Moreover, treatments of 10% and 15% revealed significant differences (Fig. 3).

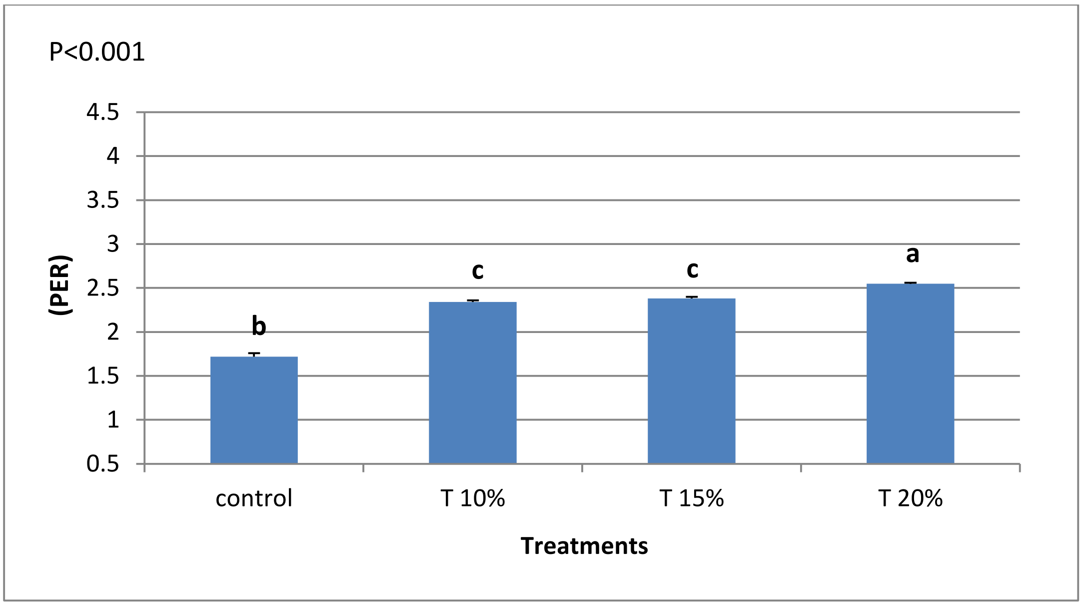

Figure 3: Results of one-way ANOVA and comparison of PER in different treatments. Different letters on the top of the vertical bars indicate significant difference ($p<0.05$).

Results of fish carcass composition

Result of carcass composition of control and 20 percent treatments are shown in Table 5.

Table 5: Results of fish carcass composition.

Carcass composition	control treatment	treatment of 20% sesame meal
Protein (%)	13.7	24.2
Humidity	71	74
Lipid (%)	9.4	7.4
TVN (mg/100g)	20.38	19.35
Peroxide value (meq/kg)	0	0
Ash (%)	1.2	1.7

Amino acid contents of control and 20 percent sesame instead of fish meal treatments are shown in Table 6.

Table 6:Amino acid content (mg/g of fish carcass) in carcass composition of cultured rainbow trout.

amino acid	control treatment	treatment of 20% sesame meal
aspartic acid	13.75	17.4
lutamic acid	23.78	26
serine	4.47	4.88
glutamine+histidine	2.28	2.25
arginine+threonine	4.4	10.87
glycine	24	8.9
alanine	10.57	11.68
tyrosine	7.29	6.4
methionine	15.28	15.28
valine	15.5	13.6
phenyl alanine	3.12	2.98
isoleucine	3.57	4.19
leucine	12.85	10.3
lysine	25	23.8
tryptophan	4.37	3

Economic study on feeding diets

Price calculation of each feeding diet(kg) was performed during the implementation of the research in an experimental scale based on the price of initial ingredients in the market. According to the previous researches, feeding diets of the fingerlings (per kg) in the control treatment was priced at 14,590 Rials/kg. For the treatment with the highest amount (%) of whole sesame, the price was estimated to be 15,590 Rials/kg(hand made diet).

Considering the price of feeding diets produced by fish food factories compared with low food conversion ratio of those diets containing sesame seeds, it is concluded that the present study has more economical benefits. Those fish which received more sesame seeds in their diets showed a considerable increase in their growth rate compared toother fish samples during the experiment. It seems that high factory production of food will not have too much difference with given handmade given price, considering changes in USD rate.

Discussion

According to the obtained results in present study, substituting a part of fish meal by sesame proteins led to the increased final weight gain, specific growth rate, protein efficiency ratio, as well as decreased food conversion ratio as compared to the control treatment. At the beginning of the experiment, all treatments had similar trend in terms of weight, while there were a significant differences between growth rates of control fish samples with others, feeding on diets containing different amounts of sesame meal. Sesame meal can be used as an alternative protein source in feeding diets of carnivorous fish at least in a half rate of fish meal protein (without amino acid supplementary) without any reduction in growth rate of rainbow trout fingerlings (Nang Thu *et al*., 2010). In addition, substituting a part of fish meal with sesame meal would result in a reduction in voluntary food take in common carp fingerlings, fingerlings of merigal fish (*Cirrhinus merigala*) and raho fish, *Labeorohita* (Hossain and Jauncey, 1990).

Adding sesame cake meal to the carp diet had lower palatability and propensity in their fingerlings and it resulted in reduction of food intake and decreased the growth rate. This discrepancy might be due to the species-specific differences and probably because of different source of whole sesame meal used in the studies (Hasan *et al*., 1997).

In present research, those fish fed with diets containing higher level of sesame seed, rather than other treatments, had more specific growth rate. Food conversion ratio is used as an evaluator index to determine fish ability in conversion of aken food ingredients, and fish treated by 20% sesame meal showed the lowest level. As demonstrated by Gomes *et al*., (1995), the substituting fish meal by protein sources to the level of 50%, caused no significant difference in FCR, while control diet indicated a significant difference in FCR with other feeding diets in this experiment.

Alternating sesame cake meal in feeding diets of tilapia fish to the level of

20%, decreased food expenditure without any harmful effect on fish growth rate (Ofojekwu and Kigbu, 2002). Regarding statistical analyses, the highest rate of protein efficiency was observed in fish samples treated with 20% whole sesame meal. At the end of the experiment, significant differences were detected amongst the experimental treatments and the control in protein efficiency ratio. This reduction in FCR and increased PAR in the treatment containing 20% of sesame meal in rainbow trout fish (2 g) might be implicated to the presence of lipid, unsaturated fatty acids, organic and minerals ingredients and vitamins in sesame seeds.

Sesame seed contains minerals like magnesium (0.46%), calcium (2.01%), phosphorous (1.36%), potassium (1.25%), iron (93 mg/kg), manganese (47.8 mg/kg), zinc (100 mg/kg), and sodium (0.04%)(NRC, 1993).

The vitamins include biotin (0.34 mg/kg), choline (1535 mg/kg), niacin (19 mg/kg), pantothenic acid (6 mg/kg), pyridoxine (12.46 mg/kg), riboflavin (3.4 mg/kg), and thiamine (2.8 mg/kg)(NRC, 1993).

Substituting fish meal by whole sesame meal in fish diets had no effect on fish survival because no dead fish was observed during the experiment. This might be attributed to the constant water flow in the farming pools, appropriate water quality and nutritional ingredients used in feeding diets.

Analyses of amino acids, protein, lipid, humidity, ash and TVN was performed in the control and treatment containing the highest amount of sesame meal in fish. According to the obtained results, the amount of protein, humidity and ash were higher in fish samples feeding on 20% whole sesame meal than the control, whereas lipid and TVN contents were higher in the control than treatment 3 which contained 20% sesame meal. The amounts of amino acids including aspartic acid, glutamic acid, serine, arginine, threonine, alanine, isoleucine in fish samples treated by 20% sesame meal were more than the control treatment. Moreover,metionine content was similar in both treatments. Amino acid contents of glutamine, histidine, glycine, tyrosine,valine,phenylalanine, lusine, lysine, and tryptophan were higher in the control than the treatment contained 20% of sesame meal. The higher contents of methionine and arginine in fish samples fed on 20% of sesame meal were directly related to the higher amount of these amino acids in sesame seeds (Gohl, 1975).

Feeding diets of rainbow trout and salmon fishes which lack essential amino acids including arginine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine will result in decrease of fish growth (NRC, 1993).

Non-essential amino acids includein galanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine are not necessary for the growth of salmon, rainbow trout and other fish (NRC, 1993). Based on feeding diet percentage, the required amount of amino acids for rainbow trout fish include arginine (1.45%), histidine (0.65%), isoleucine

(0.85%), leucine (1.35%), lysine (1.75%), methionine plus cysteine (0.95%), phenyl alanine plus tyrosine (1.75%), threonine (0.75%), tryptophan (0.2%) and valine (1.15%) (NRC, 1993). These amounts were lower in fish samples treated in present study.

Although no organoleptic assessment was conducted on the treatments, further analysis by our families on fish meat samples, showed that meat obtained from fish samples treated with 20% of sesame seed had remarkable quality and acceptability than other treatments, in particular, the control treatment.

The economic aspect of the present study is pertained to more rapid growth rate of the fish, and ultimately, more production in a farm scale. Furthermore, the price of sesame-containing diet (per kg) in an experimental scale was less than that of feeding diets produced by fish food factories, a fact which could be seen, even in a factory production.

There has been no research on substituting a part of animal protein by sesame seed protein in feeding diets of rainbow trout fingerlings. However, some information exists about the use of sesame meal. Until now, no survey was conducted on the substituting sesame seed meal in feeding diets of rainbow trout and other farming fish in order to improve the growth rate.

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