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Effects of Hazelnut Meal Levels on Growth Performance, Feed Utilization and Digestibility in Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In this study, the effects of hazelnut meal (HM) levels in juvenile rainbow trout (*Oncorhynchus mykiss*) diets on growth, feed consumption, and digestibility were investigated. Four experimental diets containing a gradient of HM (0%, 15%, 30% and 45% respectively) were prepared to feed two replicate groups of trout (initial body weight of 37.89±0.02 g) twice daily to apparent satiation for 60 days. The highest weight gain rate (WGR) and specific growth rate (SGR) were obtained from the fish fed with Diet 1 and Diet 2 and it was determined that the increase of HM level in the diet up to 30% did not affect feed conversion ratio (FCR) and protein efficiency ratio (PER). The lowest WGR, SGR and PER were obtained from the fish fed with the diet containing 45% HM. The apparent digestibility coefficients (ADC) of protein (90.93-91.76%) and lipid (96.78-98.33%) were at high with no significant difference among treatment (P>0.05). The ADC of dry matter decreased with increasing HM in diets. These results indicated that HM can be utilized by 15% in diet for juvenil rainbow trout, without adverse effect on growth performance.

Keywords: rainbow trout, fish meal, hazelnut meal, growth, digestibility.

Fındık Küspesi Seviyelerinin Gökkuşağı Alabalığı Yavrularında (*Oncorhynchus mykiss*) Büyüme Performansı, Yemden Yararlanma ve Sindirilebilirlik Üzerine Etkileri

Özet

Bu çalışmada, fındık küspesi (FK) seviyeleri farklı yemlerin Gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularında büyüme, yemden yararlanma ve sindirilebilirlik üzerine etkileri araştırılmıştır. Başlangıç ağırlıkları 37,89±0,02 g olan balıklar, artan oranlarda FK içerecek şekilde hazırlanan dört deneme yemi (sırasıyla %0, %15, %30, %45) ile 60 gün boyunca günde iki kez görünür doygunluk sınırına kadar yemlenmiştir. En yüksek canlı ağırlık artışı (CAA) ve spesifik büyüme oranı (SBO) 1. Yem ve 2. Yem ile beslenen balıklardan elde edilirken rasyondaki FK oranının %30'a kadar yükselmesinin yem değerlendirme sayısını (YDS) ve protein değerlendirme randımanını (PDR) etkilemediği saptanmıştır. En düşük CAA, SBO ve PDR %45 oranında FK içeren yemle beslenen balıklardan elde edilmiştir. Protein (%90,93-91,76) ve yağın (%96,78-98,33) sindirilme oranları yüksek oranda bulunmuş ancak gruplar arasında önemli bir fark bulunmamıştır (P>0,05). Kuru madde sindirim oranı ise yemlerdeki FK'nın artışına bağlı olarak azalmıştır. Bu sonuçlar, Gökkuşağı alabalığı yavrularının yemlerinde FK'nın büyüme performansı üzerinde olumsuz bir etki yapmaksızın %15 oranına kadar kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Gökkuşağı alabalığı, balık unu, fındık küspesi, büyüme, sindirilebilirlik.

Introduction

Feed expenses are among the most important factors affecting aquaculture. When formulating a cost effective diet, the cost of the ingredients as well as their digestibility efficiencies to meet the nutrient requirements in fish species, should be considered (Yiğit and Ustaoğlu, 2003).

Fish meal (FM) is considered as the most desirable animal protein ingredient in aquaculture diets because of its high protein content, balanced amino acid profiles, high digestibility and palatability. However, in the last decade increasing demand, uncertain availability and high price with the expansion of aquaculture made it necessary to search for alternative animal or plant protein sources.

Alternative protein sources must be competitively priced relative to FM on a unit protein basis. They should not have a negative impact on fish performance (digestibility, growth, disease resistance, etc.) or product quality and must be commodities (i.e., traded internationally) (Hardy and Tacon, 2002).

Fish nutritionists have often tried to incorporate less expensive, more plentiful plant protein sources in aquatic diets for a long time. Considering the research for the substitution of FM in the diets of rainbow trout, numerous studies (Tacon *et al*., 1984; Hughes, 1991; Moyano *et al*., 1992; Pongmaneerat and Watanabe, 1992; Sanz *et al*., 1994; Morales *et al*., 1994; Gomes *et al*., 1995; Cheng and Hardy, 2002; Thiessen *et al*., 2003a; 2003b; Luo *et al*., 2006) have shown that it is possible to partially or even fully replace FM by plant protein sources, such as soybean meal, sunflower meal, lupin flour, corn gluten meal, cottonseed meal, canola meal, rapeseed meal and so forth.

The hazelnut meal (HM) resulting from the solvent extraction of hazelnut oil, is a valuable plant protein source. HM has a high protein level (approximate 40%), is low in fiber (approximate 8-10%), rich in amino acids like arginine, leucine and isoleucine (Akkılıç *et al*., 1982; Erener, 1991; Altop, 2006).

HM is being used in cattle and poultry feed as a feed ingredient, safely and effectively (Akkılıç *et al*., 1982; Erener, 1991; Sarıçiçek *et al*., 1995; Özer, 2002; Altop, 2006). To our knowledge, there is only one research about the effect of feeding this ingredient on rainbow trout performance and digestibility. Bilgin *et al.* (2007) in that study, examined the feasibility of using hazelnut meal as a partial replacement for soybean meal in the diet of rainbow trout. The objective of the present study was to determine the feasibility of using HM as an alternative protein source to FM in diets and to investigate the effects of experimental diets which contains different HM levels on growth performance, chemical composition and apparent nutrient digestibility of rainbow trout, *Oncorhynchus mykiss.*

Materials and Methods

Fish and Rearing Conditions

Rainbow trout obtained from the Ak Balık Ltd. (Samsun, Turkey) were transferred into the tanks 2 weeks before the growth trial. During the acclimatization period, the fish were fed twice daily with a commercial trout feed to visual satiation.

The feeding trial was carried out in the experimental facilities of Faculty of Fisheries, Ondokuz Mayis University (Sinop, Turkey) using 8 identical circular fiberglass tanks (330 L capacity), filled with a flow-through water system. Water temperature was maintained at 12.8±0.1°C. Twenty-five fish, having a mean initial body weight of 38 g, were randomly allocated to each tank after 1-day starvation. Two replicate groups of experimental fish were assigned to each dietary treatment. All rearing tanks were provided with continuous aeration and maintained under natural photoperiod. During the trial period, fish were hand-fed to apparent satiation twice (08:30 and 16:30) daily. Uneaten feed and faeces were removed from tanks daily using siphoning.

All experimental fish were individually weighed at the start of the experiment, after 15, 30, 45 days of trial and at the end of the experiment (day 60). Before weighing, fish were deprived of feed for 1 day. At the beginning and end of the experiment, samples of fish were killed with an overdose of benzocaine and used for chemical analysis. An initial group of 10 fish was randomly selected from stock tanks, killed and stored at -25°C until analysis of body composition. Five fish from each tank were also sampled at the end of the experiment for calculation of nutrient retention rates.

Diet Preparation

Four experimental diets were formulated to contain different levels of HM. The control diet (Diet 1) contained no HM and FM was the main protein source. HM was used at 15% (Diet 2), 30% (Diet 3) and 45% (Diet 4) in other experimental diets. All diets were formulated to be isonitrogenous. The formulation and the nutrient composition of the experimental diets are shown in Table 1. The proximate composition and amino acid composition of fish meal, hazelnut meal and corn meal are given in Table 2.

The dry ingredients sieved through a 500 µm mesh. All ingredients were thoroughly mixed with fish oil, and water was added to produce stiff dough. The dough was then pelleted using a meat grinder with a 3mm holes die. After pelletting, all diets were dried at 60°C in a constant temperature oven individually bagged and stored in a cool location (–25°C) until used. For digestibility measurements, 0.5% chromic oxide was added (with reduced amount of wheat flour) as an inert marker in each diet.

Digestibility Measurements

Apparent digestibility was measured at the end of the experiment. Fish within each tank were fed the with experimental diets containing a marker, chromic oxide, for 21 days. On the last 14 days of the feeding period, faecal samples from tanks were collected by the siphoning method between $09:^{00}$ and $15:^{00}$ hours every day. Pooled faeces from each treatment group were homogenized and then stored at -20°C until analysis. Apparent digestibility coefficents (ADC) of experimental diets were calculated as follows:

ADC(%) = 100–[100x(%Cr_2O_3 in Diet /% Cr_2O_3 in Faeces) (%Nutrient in Faeces / % Nutrient in Diet)]

Table 1. Formulation and nutrient composition of the experimental diets

Ingredient (%)	Experimental Diets (Hazelnut meal levels)			
	1 (0%)	2 (15%)	3 (30%)	4 (45%)
Fish meal (Anchovy)	56.0	47.0	38.5	29.5
Hazelnut meal (HM)	-	15.0	30.0	45.0
Corn meal	28.4	21.6	14.6	8.1
Wheat flour	5.0	5.0	5.0	5.0
Fish oil (Anchovy)	10.2	11.0	11.5	12.0
Vitamin- mineral mix[1]	0.4	0.4	0.4	0.4
Proximate composition				
Moisture (%)	5.4	10.4	4.5	10.3
Crude protein (% Dry matter)	45.4	45.7	44.9	44.9
Crude lipid (% Dry matter)	25.0	25.5	25.2	25.6
Crude ash (% Dry matter)	6.5	6.4	6.7	6.5
Essential and non-essential amino acids (% Dry matter)[2]				
Threonine	2.01	1.81	1.64	1.44
Valine	2.29	2.12	1.95	1.75
Methionine	1.33	1.14	0.96	0.76
Isoleucine	2.00	2.08	2.19	2.29
Leucine	3.56	3.37	3.22	3.05
Phenylalanine	1.86	1.73	1.62	1.49
Lysine	3.61	3.17	2.77	2.33
Histidine	1.49	1.40	1.33	1.25
Arginine	2.60	2.85	3.12	3.37
Serine	1.87	1.79	1.74	1.67
Proline	1.79	1.60	1.43	1.24
Glycine	2.17	2.01	1.88	1.72
Tyrosine	1.38	1.17	0.98	0.84

[1] Vit-Min Mix: Vit. Mix (in diet g/kg); tocopherol (E), 0.2; menadione $NaHSO_3$-$3H_2O$ (K_3), 0.01; thiamine (B_1), 0.015; riboflavin (B_2), 0.025; niacin (nicotinic acid), 0.2; Ca panthothenate, 0.024; pyridoxine-HCl (B_6), 0.02; cyanocobalamine (B_{12}), 0.00002; folic acid, 0.008; vitamin C, 0.21; inositol, 0.2; d-biotine, 0.0005; (in diet IU/kg) vit-A, 12.500; vitamin D_3, 2.500. Min. Mix (in diet g/kg); manganese, 0.02; zinc, 0.075; copper, 0.005; cobalt, 0.005; iodine, 0.003; selenium, 0.0003. Vit-min mixture was supplied by Sibal A.S. (Sinop, Turkey).
[2] Essential and non-essential amino acid contents calculated from data Table 2.

Table 2. Proximate and amino acid composition of fish meal, hazelnut meal and corn meal

Proximate analysis(%)	Fish meal	Hazelnut meal	Corn meal
Dry matter	91.47	87.32	85.74
Protein	70.64	44.83	7.07
Lipid	7.56	3.36	5.54
Ash	9.23	6.68	14.25
Essential and non-essential amino acids (%)[1]			
Methionine	2.29	0.15	0.18
Threonine	3.43	0.89	0.33
Valine	3.91	1.26	0.40
Isoleucine	3.41	2.82	0.30
Leucine	5.80	2.77	1.07
Phenylalanine	3.12	1.21	0.40
Histidine	2.55	1.07	0.20
Lysine	6.32	0.99	0.24
Arginine	4.42	4.53	0.43
Serine	3.15	1.58	0.40
Proline	2.79	0.80	0.80
Glycine	3.69	1.36	0.35
Tyrosine	2.31	0.15	0.30

[1] Data on amino acid contents of fish meal and corn meal from Karaali (2005), of hazelnut meal from from Erener (1991)

Analytical Methods

Proximate analysis of ingredients, diets and fishes were made following the usual procedures (AOAC, 1984): dry matter, after drying in an oven at 105°C for 24 h; crude protein, by the Kjeldahl method after acid digestion; crude fat, by ethyl ether excraction in a Soxhlet System; ash, by combustion at 550°C in a muffle furnace for 12 h. The chromic oxide in diets and faeces contents were measured using a spectrophotometry procedure involving perchloric acid digestion (Furukawa and Tsukahara, 1966).

Calculations

Calculations of growth and feed utilization parameters were conducted according to Cho and Kaushik (1985).

Statistical Analysis

All data were subjected to one-way analysis of variance procedure and the differences among means were compared by Duncan's New Multiple Range Test (P<0.05).

Results

There was no mortality observed during the entire period of the experiment. The growth performance and feed efficiency values are shown in Table 3. In this study, fish growth decreased in relation to the levels of HM. A significant reduction in the weight gain rate (WGR) of fish in groups 3 and 4 was observed compared with fish fed Diets 1 and 2. Differences in specific growth rate (SGR) were similar to the differences in WGR. No significant difference was observed for feed intake (FI) and protein intake (PI) between the fish fed with Diet 1 and Diet 2 (P>0.05). However, rainbow trout fed with Diet 3 and Diet 4 showed a significantly lower FI and PI than the fed Diet 1. Meanwhile, FI and PI of Diet 4 were significantly lower than those of Diet 2. The feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish fed Diet 1 were significantly (P<0.05) higher than that of fish fed with Diet 4. Body composition of rainbow trout fed on experimental diets was given in Table 4. No significant difference was found in crude protein, crude lipid and moisture content among the dietary treatments

The data on ADC are presented in Table 5. The highest ADC of dry matter was in fish fed with Diet 1, followed by fish fed with Diets 2, 4 and 3, which contained 15%, 45% and 30% HM. Decreasing ADC of dry matter values was recorded with increasing levels of hazelnut meal in fish diets. There were no significant differences between ADC of protein and lipid values of the different diets.

Discussion

In the present study, HM level in fish feed is an effectual factor on the WGR and SGR of rainbow trout and increased the level of HM in fish feed causing decreased growth. WGR was observed to be similar in fishes which were fed with Diet 1 and Diet 2, but WGR of fishes fed with Diet 3 and Diet 4 decreased due to the increased level of HM level in

Table 3. Growth performance and feed efficiency of rainbow trout fed on experimental diets for 60 days

	Experimental diets (Hazelnut meal levels)			
Parameters	1 (0%)	2 (15%)	3 (30%)	4 (45%)
Initial body weight (g)	37.90±0.08	37.88±0.06	37,90±0,03	37.88±0.06
Final body weight (g)	100.95±2.70^{a}	96.22±2.35^{a}	83.89±3.21^{b}	74.53±2.00^{b}
Weight gain rate (%)	155.50±7.64^{a}	154.00±5.82^{a}	112.50±8.29^{b}	92.76±0.09^{c}
Specific growth rate (%)[1]	1.56±0.05^{a}	1.55±0.04^{a}	1.25±0.06^{b}	1.09±0.00^{c}
Feed intake (g/fish)	60.76±5.16^{a}	54.43±0.05^{ab}	49.07±3.23^{bc}	45.22±2.66^{c}
Protein intake (g/fish)[2]	27.59±2.34^{a}	24.91±0.02^{ab}	22.03±1.45^{bc}	20.33±1.20^{c}
Feed conversion ratio[3]	0.96±0.04^{a}	0.93±0.04^{a}	1.04±0.01^{ab}	1.23±0.15^{b}
Protein efficiency ratio (%)[4]	2.28±0.10^{a}	2.34±0.09^{a}	2.13±0.03^{ab}	1.81±0.22^{b}
Survival rate (%)	96^{a}	100^{a}	96^{a}	98^{a}

Values are mean ± standard deviation. Values in the same row with different superscripts are significantly different (P<0.05).
[1] Specific growth rate (SGR) = [(ln final wet wt-ln initial wet wt)/days] x 100
[3]Protein intake (PI) = feed intake x crude protein in diet / 100
[4]Feed conversion ratio (FCR) = feed fed/ body weight gain
[4]Protein efficiency ratio (PER) = wet weight gain / protein intake x 100

Table 4. Body composition of rainbow trout fed on experimental diets

		Experimental diets (Hazelnut meal levels)			
Parameters	Initial	1 (0%)	2 (15%)	3 (30%)	4 (45%)
Moisture (%) (wet weight)	75.79	76.08±1.40^{a}	75.91±0.55^{a}	74.49±0.35^{a}	77.06±0.59^{a}
Crude protein (%)	18.49	18.26±0.78^{a}	17.78±1.10^{a}	18.42±0.02^{a}	17.39±0.46^{a}
Crude lipid (%)	4.89	4.13±0.15^{a}	5.91±1.45^{a}	6.42±0.62^{a}	5.36±0.29^{a}
Crude ash (%)	1.40	1.41±0.07^{a}	1.47±0.19^{a}	2.16±0.35^{b}	2.17±0.07^{b}

Values are mean ± standard deviation. Values in the same row with different superscripts are significantly different (P<0.05).

Table 5. Apparent digestibility coefficients (ADC) of experimental diets

Experimental diets (HM levels)	Apparent Digestibility Coefficients (%)		
	Dry matter	Protein	Lipid
1 (0%)	83.57±0.14 [a]	91.76±0.11 [a]	98.33±0.26 [a]
2 (15%)	80.20±0.06 [b]	91.24±0.00 [a]	97.56±0.49 [a]
3 (30%)	77.49±0.04 [c]	91.24±0.26 [a]	97.18±0.24 [a]
4 (45%)	79.91±0.24 [d]	90.93±0.40 [a]	96.78±0.46 [a]

Values are mean ± standard deviation. Values in the same column with different superscripts are significantly different (P<0.05).

fish feed. In relation to this study, Çakmak and Özdemir (2002) indicated that corn gluten could be successfully used in rainbow trout diet up to 15%, without decreasing growth rate, but a corn gluten level of 30% causes WGR to decrease. Cheng and Hardy (2002) reported that cottonseed meal could be used at 10% inclusion rate in trout diets. According to Luo *et al.* (2006), this level could be as high as 30%. On the contrary to these results, Gomes *et al.* (1995) stated that plant protein sources like lupin seed meal, bean meal, pea seed meal, corn gluten, full-fat soybean and rape seed meal mixture could be utilized in rainbow trout diet up to 66%, to replace of FM. Considering that each and every plant protein source has different nutrient qualities, combining adequate doses of 6 different plant protein sources and using high levels of the combination in fish diets does not cause any negative effects on growth. The most probable reason of the said observation is the use of a combination of different protein sources that make up for each other`s lack of qualities instead of relying on one plant protein source in fish diets. In the sole study about HM use in fish diet, Bilgin *et al.* (2007) stated that HM could replace up to 20 % of the soybean meal in rainbow trout diets.

It is possible to concieve that increased dietary HM level with decreased WGR and SGR has got something to do with decrease to FI amounts. Compared FI values, it is seen that FI of fishes that were fed with Diet 2 and Diet 4 was significantly different (P>0.05). Thus, we could reach the conclusion that decreased WGR and SGR have got nothing to do with decrease in FI but directly related to the increased dietary HM and due to poor amino acid balance that is caused by that.

In this study, it was determined that a HM level of 30% in fish feed did not effect FCR but increasing HM level into 45% caused FCR to decrease. Cheng and Hardy (2002) stated that cottonseed meal level of 10% in fish diet did not affect FCR but raising the level to 15%-20% decreases it. Çakmak and Özdemir (2002) reported that corn gluten level of 15% did not have a negative effect on FCR but raising the corn gluten level in the fish diet to 30% negatively effects FCR. As far as FCR is concerned, it could be said that using HM in trout diets is a better option compared to using cottonseed meal or corn gluten.

The lowest PER value was obtained from fishes that were fed with Diet 4. The fishes that were fed with Diet 4 had the lowest WGR and PER values, because of the increased levels of HM. The poor amino acid balance that comes with excessive use of HM in fish feed augments deamination of aminoacids and the energy which otherwise would be used for growth is instead used for deamination and ammonia excretion which emerges from deamination process. The PER values in this study were lower than PER of Bilgin *et al.* (2007). It is possible to say that this difference has got something to do with dissimilarity of protein levels, varying amounts of FM and the different kinds of protein sources used in the fish feeds of the said study.

ADC of dry matter decreased due to increased plant protein sources used in fish diet. That happens most likely due to the increased fibre levels which rise with the HM. The findings of Uyan (2004), who studied on another carnivor fish (*Paralichthys olivaceus*) stated that increased fibre causes ADC of dry matter to decrease. The decrease of ADC of dry matter was thought to be due to the size of HM particules. This result was in unison with the result of Çakmak and Özdemir (2002) in rainbow trout.

As a conclusion of this study, it is suggested that HM is a good source of nutrients and can safely be used up to 15% as an ingredient in diets for rainbow trout without any adverse effect on growth, survival, FCR, PER and body composition. Supplementation of the diets with the limiting amino acids (arginine and lysine) or attractants may allow a higher usage level and this deserves to be further research.

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