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PROOF

The Effects of Diets containing Hazelnut Meal Supplemented with Synthetic Lysine and Methionine on Development of Rainbow Trout, *Oncorhynchus mykiss*

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Abstract

In the research, the effects of diets, including hazelnut meal supplemented with synthetic lysine and methionine, on development of rainbow trout were investigated. Six isonitrogenous and isocaloric diets were prepared. One of them was a fish meal based on control diet and the others were diets containing hazelnut meal (HM) with the increasing percentages as follows: 10 %, 20, 30, 40 and 50. In order to balance lysine and methionine of the diets as much as possible, synthetic lysine and methionine were added to each diet except the control diet which was based on the lysine and methionine content of the control diet. Rainbow trouts (114.16±0.03 g) were stocked in three replications for each group and fed with test diets twice a day until apparent satiation during 60 days. There were no significant differences among groups in terms of specific growth rate, feed conversion ratio, protein efficiency ratio and productive protein value. However, the best values were obtained from 40 % HM group ($P > 0.05$). The results showed that the protein digestibility was not affected from the HM ratio in the diet. Nevertheless, the total digestibility and lipid digestibility were decreased with respect to the increase of HM ratio in the diet to 50 % ($P < 0.05$). In conclusion, this research has shown that the HM supplemented with synthetic lysine and methionine could be best used at the rate of 40 % in rainbow trout diets.

Keywords: Hazelnut meal, rainbow trout, lysine, methionine, growth.

Sentetik Lisin ve Metionin İlave Edilmiş Fındık Küspesi İçeren Yemlerin Gökkuşağı Alabalığının (*Oncorhynchus mykiss*) Gelişimi Üzerine Etkisi

Özet

Araştırmada, balık ununa dayalı kontrol yemi ile artan oranlarda sırasıyla % 10, 20, 30, 40 ve % 50 oranlarında fındık küspesi içeren, izonitrojenik ve izokalorik altı farklı araştırma yemi hazırlanmıştır. Araştırma yemlerinin metiyonin ve lizin içeriklerini mümkün olduğunca dengelemek amacıyla kontrol yeminin lizin ve metiyonin içeriği esas alınarak, kontrol yemi dışındaki araştırma yemlerinin yapısına sentetik lizin ve metiyonin ilave edilmiştir. Ortalama canlı ağırlıkları 114.16±0.03 g olan gökkuşağı alabalıkları, her grup için 3 tekerrür olacak şekilde stoklanmış ve hazırlanan araştırma yemleriyle 60 gün boyunca günde iki kez doyuncaya kadar yemlenmişlerdir. Araştırma sonunda, spesifik büyüme oranı, oransal büyüme oranı, yem değerlendirme sayısı, protein değerlendirme randımanı ve prodüktif protein değeri bakımından gruplar arasında istatistiksel olarak önemli bir fark bulunamamış ancak en iyi değerler % 40 fındık küspesi içeren gruptan elde edilmiştir ($P > 0.05$). Protein sindirim oranının yemdeki fındık küspesi oranından etkilenmediği, toplam sindirim ve yağ sindirim oranının ise yemdeki fındık küspesi oranının % 50'ye çıkmasıyla önemli derecede azaldığı belirlenmiştir ($P < 0.05$). Bu araştırma; fındık küspesinin sentetik lizin ve metiyonin ilavesiyle, gökkuşağı alabalığı yeminde % 40 oranında kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Fındık küspesi, gökkuşağı alabalığı, lizin, metiyonin, büyüme

Introduction

Traditionally, fish meal (FM) has provided a major part of the protein source of formulated feeds because of its suitable protein quality (Vechklang *et al.* 2011). However, increasing costs, sustainability of pelagic fisheries and possible contaminants such as mercury, dioxins and PCBs underscore the importance of utilizing alternative protein sources as replacements for FM (Gatlin *et al.* 2007; Glencross *et al.* 2007). Therefore, reducing FM inclusion levels and replacing FM with cost-effective, widely

available and sustainable feedstuffs are considered essential for the future development of the aquaculture industry (Tacon *et al*. 2006; Gatlin *et al*. 2007).

In studies with different types of fish, researchers tested raw materials such as slaughter house byproducts, poultry byproducts, full fat soybean and soybean meal, canola meal, cottonseed meal, legumes, Poaceae species, wheat meal and gluten, corn meal and gluten, peanut, bean species, pea species, cowpea, meal, and also various protein concentrates, particularly of vegetable raw materials. These studies explored the physiological and histological influences of these raw materials on digestibility, growth and development, meat quality, and number of eggs. The research findings showed that these raw materials can be used; however, the type of raised fish, physiological state of the fish, substitution rate of alternative raw material used, nutrients of the raw material, limiting substances likely to be in the raw material, form of raw material handling, and the technology used in fish feed production are significant points in raw material use (Allan *et al*. 2000; Lee *et al.* 2006; Biswas *et al.* 2007; Olsen *et al*. 2007).

Turkey is the leading hazelnut producer and exporter in the world, and contributes to 70% of the global production and approximately 75% of global exports (Aktas *et al*. 2006).

Stable consumption and the absence of exportation lead to large amount of hazelnut stocks. Conservation of the hazelnuts leftover from the previous years when production was high and exportation was insufficient causes a significant problem. Hazelnuts can be stored only three years without decaying; after that period, they are sent to oil factories for oil production (Sipahioglu 1998; Nas *et al*. 2001). Furthermore, hazelnuts that are unsuitable in respect to shape and size and those that have been damaged during harvesting, transportation or due to mechanical cracks are also turned into oil.

The residual (Hazelnut meal) from oil production is widely used in hen rations in the feed industry. In addition, there are studies on the use of HM in mixed fish feed. Mixed fish feed with HM have been tested on trout (Dogan 2005; Bilgin *et al.* 2007), carp (Buyukcapar and Kamalak 2007), sea bream (Emre *et al.* 2008a); sea bass (Emre *et al*. 2008b) and turbot (Ergun *et al.* 2008) respectively. In the aforementioned studies, HM was compared to FM, or the ratio at which it can be substituted for soy meal or FM was investigated.

Despite the high protein content of HM, its protein quality is lower compared to soy meal and FM due to the insufficiency of certain amino acids (lysine and methionine). Lysine and methionine are the most crucial and limiting amino acids for protein synthesis (NRC 2011). Therefore, there is a need for studies to improve the protein quality of the HM. Composites are added amino acid in order to increase the quality of vegetable protein sources (Erener *et al.* 2003). To date, there has been no research on improving the protein quality of the HM used in trout feed by the addition of amino acids.

The main aim of our study was to determine the effects of using HM fortified with synthetic lysine and methionine on rainbow trout development.

Materials and Methods

Fish, Facilities and Experimental Procedure

The feeding trial was carried out at the Research Laboratory, Faculty of Fisheries and Aquaculture, Sinop University, Turkey. The rainbow trout were obtained from a commercial hatchery located in Samsun, Turkey. The trial was conducted in 330 L fiberglass tanks (filled with 300 L of water) in a flow through fresh water system where each tank was equipped with an inlet, outlet, and continuous aeration. The tanks were maintained under natural light/dark regime. A flow rate of 2.3 L min^{-1} was maintained throughout the experimental period. During the experimental period, the monitored water quality parameters (mean±S.E) were: water temperature 14.32±0.09°C; pH 8.18±0.01 and concentration of dissolved oxygen 8.43±0.18 mg L^{-1}.

Six diets were formulated using commercial ingredients in which the inclusion levels of HM were 0 %, 10, 20, 30, 40, 50 (diets coded HM_1, HM_2, HM_3, HM_4, HM_5, respectively). All diets contained 0.5 % chromic oxide as an indigestible marker for determination of nutrient digestibility. The control diet contained FM as the main crude protein source. Fish oil were supplied as lipid source, dextrin as carbohydrate or nitrogen free extract source and guar gum as the binding source. In order to balance lysine and methionine of the test diets as much as possible, synthetic L-lysine and DL-methionine were added to each test diet except the control diet which was based on the lysine and methionine content of the control diet. The formulation and chemical composition of the experimental diets are shown in Tables 1, and 2 respectively.

The ingredients were finely ground, weighed, mixed manually for 10 min and then transferred to a mixer for another 10-min mixing. DL-methionine and L-lysine was added to a preweighed premix and mixed until homogenous. Fish oil was then added to the mixer slowly while mixing was still continuing. All ingredients were mixed for another 10 min. Then, distilled water was added to the mixture-form dough. The wet dough was placed in a meat grinder with an appropriate diameter (3 mm) to prepare pellets. After pelletting, all diets were dried at 50°C in a constant temperature oven individually bagged and stored in a a deep freezer at -25 °C until used.

Table 1 Formulation and nutrient composition of the experimental diets (%)

HM level (%)	0	10	20	30	40	50
Groups	Control	HM_1	HM_2	HM_3	HM_4	HM_5
Ingredient (%)						
Fish meal	63	57	50	44	38	32
Hazelnut meal	-	10	20	30	40	50
Dextrin	22.95	18.21	14.21	9.42	4.50	0.14
Fish oil	13	13.30	13.75	14.10	14.50	14.80
Guar gum	0.20	0.20	0.20	0.20	0,2	0.20
Vitamin premix[1]	0.20	0.20	0.20	0.20	0,2	0.20
Mineral premix[2]	0.15	0.15	0.15	0.15	0,15	0.15
L - Lysine[3]	-	0.35	0.79	1.14	1.5	1.84
DL - Methionine[4]	-	0.09	0.20	0.29	0.38	0.47
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Proximate Composition (%)						
Dry matter	94.71±0.07	95.37±0.10	95.36±0.04	94.70±0.02	94.91±0.11	94.97±0.02
Crude protein	46.83±0.83	46.98±0.12	46.87±0.56	46.36±0.36	46.90±0.19	46.97±0.27
Crude lipid	18.17±0.02	18.76±0.02	18.97±0.21	18.90±0.20	18.35±0.16	18.57±0.25
Crude ash	8.20±0.04	7.98±0.02	7.98±0.01	7.86±0.01	7.80±0.03	7.85±0.03
Crude cellulose	0.44±0.01	1.28±0.02	2.11±0.40	2.95±0.28	3.79±0.24	4.62±0.13
Nitrogen free exract[5]	21.07±1.06	20.37±0.04	19.43±1.10	18.63±0.29	18.07±0.30	16.96±0.14
Total Energy (kJ g^{-1})[6]	21.85±0.34	21.99±0.06	21.89±0.10	21.61±0.24	21.42±0.05	21.33±0.10
Tannin (g kg^{-1})	2.32±0.08[a]	3.15±0.31[b]	4.74±0.28[c]	8.33±0.41[d]	8.06±0.21[d]	10.50±0.08[e]

[1]Vitamin premix (in diet mg or IU kg^{-1}); vitamin A, 31 250 IU; vitamin D_3, 6 250 IU; vitamin E, 500 mg; vitamin K_3, 25 mg; vitamin B_1, 37.5 mg; vitamin B_2, 62.5 mg; niacine, 500 mg; calcium pantothenat, 60 mg; vitamin B_6, 50 mg; vitamin B_{12}, 0.05 mg; folic acid, 20 mg; vitamin C, 525 mg; inositol, 500 mg; d-biotine, 1.25 mg.
[2]Mineral premix (in diet mg kg^{-1}); Mn, 100 mg; Zn, 375 mg; Cu, 25 mg; Co, 25 mg; I, 15 mg; Se, 1.5 mg
[3]Lysine; 78,8 % L – Lysine in kg
[4]Methionine; 99% DL – Methionine in kg
[5]Calculated by difference
[6]Gross Energy calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} nitrogen-free extract.

Table 2 Amino acid compositions of experimental diets

HM level (%)	0	10	20	30	40	50
Groups	Control	HM_1	HM_2	HM_3	HM_4	HM_5
Essential Amino Acids (g 100 g^{-1})						
Lysine	3.35	3.72	3.61	3.52	3.66	3.73
Methionine	1.57	1.60	1.60	1.58	1.60	1.58
Arginine	2.60	3.16	3.34	3.49	3.34	3.94
Phenylalanine	2.01	2.09	2.12	2.08	2.03	2.13
İsoleucine	2.05	2.06	2.07	1.89	1.77	1.85
Valine	2.37	2.38	2.32	2.17	1.98	2.08
Threonine	2.10	2.30	2.06	2.00	1.69	1.81
Histidine	1.26	1.49	1.28	1.34	1.07	1.35
Leucine	3.60	3.70	3.74	3.25	3.43	3.47
Tryptophan	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
Non-Essential Amino Asids (g 100 g^{-1})						
Serine	1.92	2.17	2.04	2.08	1.82	1.99
Proline	2.06	1.90	1.82	2.44	1.98	2.21
Gylcine	2.30	2.41	2.43	1.99	1.96	2.12
Alanin	2.84	2.90	2.94	2.58	2.38	2.48
Tyrocine	1.35	1.56	1.44	1.35	1.20	1.38
Cystine	0.35	0.40	0.33	0.31	0.25	0.30
Glutamic Acid	6.21	6.92	7.57	7.09	6.31	7.43
Aspartik Acid	4.51	4.78	4.95	4.40	3.89	4.00

*Method detection limit.

Feeding Protocol

At the beginning of the trial, fishes (average body weight, 114.16±0.03 g) were randomly stocked in previously prepared eighteen tanks at a stocking density of 20 fish per tank with triplicates per dietary treatment. All fish were fed the respective test diets satiation level by hand twice a day, for 60 days. All fish were weighed in bulk at 2 weeks interval to determine growth and check their health condition. . Collection of faeces samples were carried out for 14 days by siphoning using a pipe (1.5 cm diameter)

three hours after feeding. Uneaten diet was siphoned out
20 min after feeding. Pooled faeces from each treatment group were homogenized and then stored at -25°C until analysis.

Sample Collection and Biochemical Analysis

A pooled sample of 10 fish at the beginning was stored at −20 °C for whole body analysis. At the end of the experiment, all fish were fasted for 24 h prior to final sampling. All the fish were anesthetized with an overdose of benzocaine. Then the total number, individual body weight and length of fish from each tank were measured. A pooled sample of five fish from each replicate tank were randomly collected and stored at −20 °C for final whole body analysis. Samples of feed ingredients, diets, fishes and faecal matters were analyzed in replicate, using standard methods (AOAC 2000). The chromic oxide in diets and faeces contents were measured using a spectrophotometry procedure involving perchloric acid digestion (Furukawa and Tsukahara 1966). Measurement of total tannins was based on the Folin Denis (1912) method.

Statistical Analysis

All data for the two growth trials were analysed as a one-way ANOVA. When significant differences were detected, means were separated using Tukey test. Mean values were declared statistically different at $P<0.05$. Data were analysed using the "Minitab Release 15 for Windows" statistical software package.

Results

According to the present study, the ratio of HM in the feed was not a factor that had an effect on the growth of rainbow trout. Despite the significant difference in mean weight at the end of the trial between the control group and the HM_4 ($P<0.05$), the observed difference between the remaining groups was not significant ($P>0.05$), higher mean live weights in the groups fed with HM containing feed compared to the control group indicated that the addition of lysine and methionine amino acids had a positive effect. The growth performance and feed utilisation values are shown in Table 3. The feed intake (FI) of fish fed HM_4 was higher than that of fish fed with other experimental diets and no significant difference was found in FI among the dietary treatments ($P>0.05$). No significant difference was observed for protein productive value (PPV) between the fish fed with experimental diets ($P>0.05$). Body composition of rainbow trout fed on experimental diets was given in Table 4.

The apparent digestibility coefficients (ADCs) of fish fed the experimental diets are displayed in Table 5. Decreasing ADC of dry matter values was recorded with increasing levels of HM in fish diets. ADC of dry matter in the control groups was significantly higher than other experimental diet groups ($P<0.05$). During the study period, only one fish died in Control and HM_2 groups. Stress caused by these deaths were observed during weighing. According to these results, experimental diets had no significant effects on survival of experimental groups.

Discussion

There are various studies that aimed to determine the HM use ratio in fish feed. In the studies with sea bream and sea bass, Emre *et al.* (2008a) confirmed that growth performance and body composition are not influenced by HM use up to 40 %

Table 3 Growth performance and feed utilization of rainbow trout fed the experimental diets

HM level(%)	0	10	20	30	40	50
Groups	Control	HM_1	HM_2	HM_3	HM_4	HM_5
Parameters						
IBW	114.26±1.12^a	114.25±1.10^a	114.16±1.08^a	114.10±1.10^a	114.11±1.11^a	114.08±1.09^a
FBW	266.06±9.05^a	278.93±7.03^{ab}	294.60±6.95^{ab}	296.31±8.97^{ab}	304.28±6.50^b	285.00±6.41^{ab}
WG	2.53±0.10^a	2.74±0.19^a	3.00±0.31^a	3.03±0.12^a	3.16±0.13^a	2.84±0.24^a
SGR	1.40±0.03^a	1.48±0.06^a	1.57±0.11^a	1.58±0.04^a	1.63±0.04^a	1.52±0.08^a
FCR	1.09±0.04^a	1.02±0.09^a	1.02±0.05^a	1.09±0.02^a	1.06±0.05^a	1.10±0.04^a
FI	165.61±4.42^a	166.28±5.72^a	182.96±11.41^a	198.46±9.65^a	200.05±6.84^a	186.70±9.43^a
PER	1.96±0.09^a	2.12±0.18^a	2.09±0.10^a	1.98±0.04^a	2.03±0.09^a	1.94±0.07^a
PPV	41.85±2.02^a	39.83±3.20^a	37.33±3.26^a	35.26±40.59^a	40.59±0.69^a	40.82±1.74^a
SR	98.33±1.66^a	100^a	98.33±1.66^a	100^a	100^a	100^a

Values are mean ± standard error. Values in the same row with different superscripts are significantly different ($P<0.05$)
Initial body weight (IBW) (g), Final body weight (FBW) (g), Weight gain (WG) (g day^{-1}),
Specific growth rate (SGR) (% day^{-1}) =100×[Ln (FBW)−Ln (IBW)] / days
Feed conversion ratio (FCR) = Feed fed (g) / body weight gain (g), Feed intake (FI) (g fish^{-1}) = (Total feed consumption (g) / (number of fish).
Protein efficiency ratio (PER) = Weight gain (g) / total protein intake (g). Protein productive value (PPV) (%) =100×body wet protein gain (g) / protein intake (g). , Survival rate (SR) (%) =100×(final fish number / initial fish number)

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

HM level (%)	0	10	20	30	40	50
Groups	Control	HM_1	HM_2	HM_3	HM_4	HM_5
Proximate parameters (%)						
Dry matter	26.04±0.15[a]	25.15±0.12[b]	25.69±0.12[a]	26.15±0.29[a]	26.19±0.12[a]	26.19±0.22[a]
Crude protein	19.82±0.89[a]	19.44±0.30[a]	19.03±0.33[a]	19.10±0.07[a]	19.60±0.33[a]	20.20±0.36[a]
Crude lipid	4.97±0.21[ab]	4.40±0.15[a]	5.23±0.19[bc]	5.62±0.44[c]	5.28±0.24[c]	4.66±0.20[ab]
Crude ash	1.25±0.01[a]	1.29±0.01[ac]	1.43±0.01[b]	1.43±0.01[b]	1.32±0.02[c]	1.32±0.02[c]
T. Ener (kJ g^{-1})	6.64±0.01[a]	6.34±0.03[b]	6.57±0.02[c]	6.72±0.02[d]	6.71±0.01[d]	6.61±0.03[a]
Essential Amino Acids (g100 g^{-1})						
Lysine	1.91	1.99	1.54	1.63	1.63	1.52
Methionine	0.55	0.61	0.61	0.56	0.54	0.54
Arginine	1.22	1.27	1.19	1.17	1.19	1.16
Phenylalanine	0.88	0.90	0.79	0.78	0.79	0.75
Isoleucine	0.83	0.84	0.80	0.77	0.78	0.75
Valine	0.94	0.96	0.90	0.90	0.88	0.86
Threonine	0.93	0.93	0.82	0.84	0.88	0.79
Histidine	0.61	0.66	0.43	0.47	0.66	0.51
Leucine	1.56	1.59	1.49	1.47	1.46	1.43
Tryptophan	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
Non Essential Amino Acids (g 100 g^{-1})						
Serine	0.74	0.82	0.66	0.69	0.74	0.67
Proline	1.28	1.01	0.74	0.57	1.04	0.64
Glycine	0.76	0.86	0.84	0.77	0.82	0.80
Alanine	1.13	1.15	0.91	0.98	1.06	1.00
Tyrosine	0.71	0.73	0.54	0.61	0.70	0.59
Cystine	0.10	0.18	0.11	0.18	0.11	0.09
Glutamic Acid	2.54	2.66	2.11	1.87	2.38	2.16
Aspartic Acid	1.70	1.81	1.42	1.27	1.55	1.49

Values are mean ± standard error. Values in the same row with different superscripts are significantly different (P<0.05)
*Method detection limit

Table 5 Apparent digestibility coefficients (ADC) of experimental diets

HM level (%)	0	10	20	30	40	50
Groups	Control	HM_1	HM_2	HM_3	HM_4	HM_5
Apparent digestibility coefficients[1]						
Dry matterl	87.03±0.18[a]	85.02±0.13[b]	83.45±0.54[bc]	81.99±0.50[c]	79.91±0.43[d]	78.90±0.34[d]
Protein	91.89±0.18[a]	92.11±0.15[a]	91.94±0.31[a]	91.54±0.25[a]	91.59±0.16[a]	91.44±0.17[a]
Lipid	98.50±0.12[a]	98.32±0.13[a]	97.43±0.32[ab]	97.97±0.25[a]	98.09±0.24[a]	96.71±0.50[b]

Values are mean ± standard error. Values in the same row with different superscripts are significantly different (P<0.05)
[1]ADC(%) = 100–[100x(%Cr2O3 in Diet /% Cr2O3 in Faeces)x(%Nutrient in Faeces / % Nutrient in Diet)]

in sea bream feeds. Whereas Emre *et al.* (2008b) confirmed that growth performance and body composition are not influenced by HM use up to 30 % in sea bass feeds. On the other hand, Ergun *et al.* (2008) confirmed that HM can be used successfully in place of 20 % of soybean meal without causing growth failure in feeds of turbot. Compared to sea bream and sea bass feed, the fact that lower HM content can be used in turbot feed can be explained by the high protein demand of turbot and its more limited amino acid tolerance. On the other hand, Buyukcapar and Kamalak (2007) reported that HM could be substituted for 35 % (280 g kg^{-1}) of the FM protein in carp feed, which is an omnivorous species. When compared to the carnivorous species, carp is expected to have a greater ability to utilize vegetable protein resources, and the detection of these values for carp can be explained by the insufficient nutritional value (essential amino acid, energy, and protein/energy ratio coming from animal protein resources) of the feed used in these studies.

According to the studies on the use of HM in rainbow trout feed: Dogan (2005) reported that feed containing 15% HM could be used for optimal growth; Sevgili *et al.* (2009) reported that the addition of 30% HM into the feed did not cause any negative effect on growth performance; and Bulut *et al.* (2009) reported that the addition of 20% HM to the feed of trout raised in sea water had no negative effect on growth performance. The differences between these studies in fresh water and sea water can be explained by the nutrients of the feeds, the size of the fish, and different test environments. In all of the abovementioned studies, growth was depressed when the amount of HM in the feeds exceeded the specified rate. Depression of the growth can be explained by insufficient amino acid balance of HM as in the other vegetable raw materials, and the tannins in HM, which is an antinutritional substance.

When specific growth rate (SGR) results are examined, groups fed with feed containing HM had better SGR values compared to the control group, but the observed difference between the groups was not significant (P>0.05). The highest SGR was observed

in the group fed with feed containing 40 % HM, and the growth rate began to decrease as the HM content increased to 50 %. Despite the reduction, the observed values did not fall below the values of the control group or the group fed with feed containing 10 % HM. While the reduction can be attributed to

the decrease in the essential amino acid levels, only a small reduction in methionine level in feed containing 50 % HM suggests that the tannin content in the feed containing 50% HM may be the major factor (Table 1). The reduction in the SGR in the HM_5 group can be explained by the reduction in protein digestion, which is associated with the tannin amount. The general reduction in the essential amino acid composition of the fish meat (Table 4), of fish which were fed using feed containing 50% HM, supports this possibility.

While the HM content in the feed did not affect the feed consumption significantly, in the groups fed with feed containing HM was higher compared to the control group. To result from the addition of synthetic lysine and methionine to the HM containing feed. This observation is supported by the study by Mambrini *et al.* (1999), which reported that the addition of DL-methionine positively affected feed consumption in rainbow trout. The study by Nang Thu *et al.* (2007), which reported that the addition of lysine to feed with vegetable origin increased feed consumption, the study by Deng *et al.* (2011), which reported that the addition of lysine to feed significantly increased total feed consumption in carp. There was more growth in these groups with the increase in feed uptake.

Emre *et al.* (2008a), in their study with sea bream, Emre *et al.* (2008b), in their study with sea bass, and Bilgin *et al.* (2007), in their study with rainbow trout, confirmed that feed conversion ratio (FCR) was not influenced by the HM rate increase in the feed. Similarly, the present study indicated that FCR was not influenced by the HM rate increase up to 50%. On the contrary, Sevgili *et al.* (2009) stated that the FCR is influenced by the increasing the HM rate in the rainbow trout feeds and the FCR is better compared to the control group. The fact that Sevgili *et al.* (2009) found better FCR in the HM feeds can be explained by higher protein and energy amounts in the HM feeds compared to the feed given to the control group. Isonitrogenic and isocaloric feed were used in this study.

According to the results of the present study, there was no significant difference in protein efficiency ratio (PER) between the groups ($P>0.05$). Similarly, Emre *et al.* (2008b) stated that increasing levels of HM did not affect the PER (1.57-1.64) in sea bass. Ergun *et al.* (2008) confirmed that PER (1.82-2.24) of turbot worsened when the HM rate in the feed came up to 30 %; Büyükçapar and Kamalak (2007) confirmed that PER (1.7-2.1) of carp declined when the HM rate rose to 45 %. In these studies, PER is expected to decline with the increase in HM rate. These studies reported a reduction in growth in groups fed with feed containing 30 % and 45 % HM, and the reduction in growth is an indicator of the inability to utilize the protein within the feed. The differences between these studies can be explained by the amount and quality of the FM that the feed contained, the protein/energy ratio of the feed, and the differences in the protein quality of the HM used.

Bulut *et al.* (2009) confirmed that productive protein value (PPV) decreased as HM rate in the fish feed increased. However, according to the present study, the increase in HM rate in fish feed does not affect the PPV. The difference between the findings may result from the difference in the quality of FM and HM protein and the protein-energy ratio of the fish feeds.

According to the studies on the use of HM in fish feed, it is a common finding that dry matter digestibility decreased as the amount of HM in the fish feed increased, similar to the studies with vegetable raw materials. In the present study, when the values of food substance digestibility were evaluated, dry matter digestibility decreased as HM rate in the fish feed increased. This finding was higher compared to the study by Sevgili *et al.* (2009), which reported that the dry matter digestibility of feed prepared with HM did not exceed 75 %, and the study by Dogan (2005), which reported that the dry matter digestibility of feed prepared with HM ranged between 79-83 % in rainbow trout. The reduction in dry matter digestibility is believed to result from the increase in cellulose and tannin amounts in feed, which cannot be digested by fish. Cellulose cannot be digested by trout, and causes excretion of proteins, lipids, and carbohydrates from the system, whereas tannins decrease the digestion ratio of feed by forming complex compounds with essential minerals, proteins, and carbohydrates.

In this study, protein digestibility was significantly high in all groups, and the increase in HM rate in the fish feed did not negatively affect protein digestibility. Similarly, Dogan (2005) suggested that protein digestibility is not influenced by HM rate in fish feed, and the protein digestibility range from 90.93% to 91.76 %. Sevgili *et al.* (2009) suggested that fish feed consisting of increased HM do not affect protein digestibility negatively; however, digestion rate was below 85 %. They defined the reason for the low rate of digestion by the presence of tannins. Siddhuraju and Becker (2001) suggested that a high concentration of phenolic substances in fish feed reduces the protein digestibility and amino acid utilization by creating complex compounds such as phenol-protein or phenol-protein enzymes. In this study, the higher rate of protein digestion may result from waste collection method used. In the study of Sevgili *et al.* (2009), the stripping method was used for waste collection, whereas the siphonage method was used in this study. In the siphonage method, it is possible to collect proteins and amino acids that are

not fully digested and absorbed, therefore achieving a lower protein digestion rate. Furthermore, another reason for achieving a higher protein digestion rate is the compensation of cellulose- and tannin-induced losses with the addition of lysine and methionine amino acids. In addition, numerous factors including the water temperature used for breeding and fish size, which influence digestion, are effective.

Sevgili *et al.* (2009) determined a 95 % lipid digestibility and reported that the addition of 30 % HM to rainbow trout feed did not affect lipid digestion. Dogan (2005) reported that the lipid digestibility was not affect by the HM content of the feed, and the lipid digestibility ranged between 96.78-98.33 %. In this study, the lipid digestibility ranged between 96.71-98.50 %, and there was a decrease in the digestion rate when the HM content of the feed increased to 50 %. This reduction is believed to result from the high cellulose and tannin content of the feed given to the HM_5 group.

The feed fortified with synthetic lysine and methionine, and containing different levels of HM had no effect on the crude protein in fish body composition. This outcome is consistent with the findings by Dogan (2005) and Bulut *et al.* (2009), which reported that the level of HM in feed did not affect the crude protein of the body composition in rainbow trout. In this study, at the end of the trial the mean crude proteint of fish body composition ranged between 19.03□0.33 % and 20.20□0.36 %, and these results were higher compared to the protein contents in the aforementioned studies. This is believed to result primarily from the addition of synthetic amino acids to feed, and additionally, the difference in protein/energy ratios of the feed used in this study had an impact.

The mean crude lipid of fish body composition in groups fed with experimental feed ranged between 4.40±0.15% and 5.63±0.44%, the highest crude lipid was observed in the group fed with feed containing 30% HM, and there was a reduction in the crude lipid of fish body composition in the groups fed with feed containing 40% and 50 % HM. The values obtained in this study are similar to the results of Bulut *et al.* (2009), who determined 4.04-5.59 % crude lipid in fish body composition, while they were lower compared to the study by Dogan (2005), who found 4.13-6.42 % crude lipid in fish body composition. In the aforementioned studies, there was a visible reduction in the crude lipid of fish body composition corresponding to an increasing HM content of the feed, but the observed difference was not statistically significant ($P>0.05$). Similar to the present study, Buyukcapar and Kamalak (2007) also added synthetic methionine and lysine to the feed in their study, observed a reduction in crude lipid of fish body composition with increasing HM content, and reported that the difference between the groups was statistically significant ($P<0.05$). The reduction in crude lipid of fish body composition that were fed with feed containing HM can be explained by the suppression of lipid digestion and absorption by increased tannin and cellulose, and thus, the reduction in crude lipid retained in fish meat.

The ratio of HM in feeds affected the levels of methionine and lysine retained in the fish meat. The lowest lysine amount was observed in the HM_5 group, which was fed using feed containing the highest tannin content. When methionine amount were examined, the HM_3, HM_4 and HM_5 groups had lower methionine amounts compared to the other groups. The observation that HM_5 had the lowest lysine amount, and HM_3, HM_4, and HM_5 had the lowest methionine amount can be explained by the high tannin content and by extension, the suppression of methionine and lysine digestion. In light of these findings, it is possible to state that methionine is more sensitive to tannins compared to lysine.

Conclusion

According to the results, HM may be used up to 50%, but the ideal ratio for best growth performance was 40%. Considering the findings of the trial (e.g. digestion rates and amino acid levels in fish meat), it is clear that it would be possible to further increase the growth performance if the tannin levels in the HM are decreased.

In accordance with the current findings, the objective was met; however, further studies should be performed to propose alternative and more detailed results on HM use. There is a need to improve the methods that aim to reduce the tannin content of the HM, and HM processed with these methods should be reevaluated on fish species. It is estimated that better growth performances can be achieved by using HM with reduced tannin amount, lower levels of synthetic lysine and methionine can be added, and hence, the feed cost can be further lowered.

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