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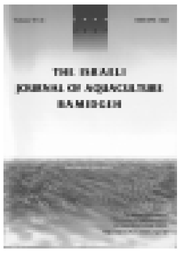

Effects of Replacement of Fishmeal with Hazelnut Meal on Growth Performance, Body Composition, and Nutrient Digestibility Coefficients in Rainbow Trout, *Oncorhynchus mykiss*

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Abstract

Hazelnut meal was evaluated as an alternative protein source to fishmeal in diets for rainbow trout, *Oncorhynchus mykiss* (initial wt 57.5±0.1 g). Hazelnut meal was incorporated in the diet at levels of 0% (control), 7.5%, 15%, 22.5%, and 30%. Diets were tested in triplicate for ten weeks. Higher levels of hazelnut meal produced better growth performance, that was significantly higher in fish fed 30% hazelnut meal than in the control ($p<0.05$). Feed utilization and protein efficiency ratios followed a similar trend while the level of hazelnut meal did not significantly affect whole body composition. Apparent digestibility of dry matter and energy of fish fed hazelnut meal at all levels was significantly lower than in fish fed the control ($p<0.05$). Crude and digestible protein, as well as energy retention, were significantly correlated with hazelnut meal level. Nevertheless, energy retention declined in fish fed 30% hazelnut meal. Based on growth performance and nutrient retention, the optimal level of hazelnut meal in diets for rainbow trout may be around 30%.

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Introduction

Fishmeal is a finite protein source for cultured fish that cannot be produced in sufficient quantities to sustain the current growth of aquaculture. Since the cost of feed represents the highest operational cost in aquaculture, partial replacement of fishmeal with cheaper plant proteins could reduce the dependence on this ingredient and associated costs (Hardy, 2006).

Hazelnut meal is obtained after oil extraction of hazelnuts. Turkey is the world's top producer, followed by Italy, Spain, and the USA (Kilic and Alkan, 2006). Hazelnut meal is a valuable protein source for layers (Ozen and Erener, 1992) and quail (Erener et al., 2003) and has recently become considered an efficient replacement of fishmeal and soybean meal in diets for rainbow trout, *Oncorhynchus mykiss* (Bilgin et al., 2007), common carp, *Cyprinus carpio* (Buyukcapar and Kamalak, 2007), European sea bass, *Dicentrarchus labrax* (Emre et al., 2008a), gilthead sea bream, *Sparus aurata* (Emre et al., 2008b), and Black Sea turbot, *Scophthalmus maeoticus* (Ergun et al., 2008). Based on these studies, up to 40% inclusion of hazelnut meal in diets has been suggested. However, information on this issue is insufficient and earlier studies did not examine the effects of different levels of hazelnut meal on nutrient digestibility.

Therefore, the present study examined the effects of partial replacement of fishmeal with hazelnut meal on growth, body composition, and nutrient digestibility coefficients in rainbow trout diets.

Materials and Methods

Fish and rearing conditions. The study was conducted at the Kepez Unit of the Mediterranean Fisheries Research Production and Training Institute in Antalya, Turkey. The experiment was conducted for 70 days using rainbow trout (avg initial wt 57.5±0.1 g) stocked in fifteen 200-l rectangular fiberglass tanks (three replicates of five treatments). Thirty fish were randomly allocated to each tank and acclimatized to experimental conditions by feeding a commercial diet (45% protein, 20% lipid) for two weeks before commencement of the trial. Water passed through a 1-mm filter and flowed at 10 l/min throughout the study. Water temperature (14.4±1.1°C), dissolved oxygen (8.2±0.4 mg/l), and pH (7.5±0.2) were monitored weekly with a YSI 58 DO Meter (Yellow Springs Instrument, Yellow Springs, OH, USA) and a pH meter (Expandable IonAnalyzer EA, Orion Research, Cambridge, MA, USA). Fish were maintained under a natural photoperiod of 10 light:14 dark hours throughout the study (November 12, 2005, through January 19, 2006).

Feed was offered twice a day at 09:00 and 15:30 at a daily rate of 2% of the body weight during first five weeks of the experiment and 1.5% of the body weight during last five weeks. The feed quantity was adjusted biweekly, based on total batch weighings. Fish remained in the tanks for an additional two weeks and were fed their respective diets twice a day to apparent satiety, to collect fecal samples. Feces were collected every three to four days by stripping fish anesthetized with 2-phenoxyethanol at a dose of 0.3 ml/l. The feces were immediately dried at 105°C and stored in vacuumed bags at 4°C until analysis.

Twenty fish at stocking and five randomly-chosen fish from each tank at the end of the experiment were taken for carcass analysis. Four fish from each tank were sacrificed to determine body measurements. For carcass analysis, whole fish were minced through a meat mincer, placed in plastic bags, and frozen at -20°C until analysis.

Experimental diets. Hazelnut meal and fishmeal were from the same sources as in studies on European sea bass and gilthead sea bream (Emre et al., 2008a,b). Diets were isonitrogenous and isocaloric (Table 1). Fishmeal was the only protein source in the control whereas experimental diets contained 7.5%, 15%, 22.5%, or 30% hazelnut meal, by weight. The ingredients were ground with a hammer mill (Kocamaz Machine, Model KT-20C, Izmir, Turkey), weighed at predetermined levels, and mixed through an experimental type horizontal mixer (Sahin Torna, Antalya, Turkey) for 5 min. The diets were pelleted using a pelleting machine with a 4-mm die and without steam, packed in plastic bags, and stored at ambient temperature until use. Chromic oxide was added to each diet as a marker to determine digestibility coefficients of nutrients.

Table 1. Ingredients and nutrient compositions of experimental diets.

	Diet (% hazelnut meal)					*Rainbow trout requirements*	
	Control	*7.5%*	*15%*	*22.5%*	*30%*	*% of dry diet*	*% of dietary protein*[1]
Ingredient (%)							
Fishmeal	68.18	63.18	58.18	53.18	48.18		
Hazelnut meal	0	7.5	15	22.5	30		
Fish oil	13.94	13.79	13.64	13.5	13.36		
Dextrin[2]	13.18	11.6	10	8.41	6.81		
Vitamin mix[2]	0.5	0.5	0.5	0.5	0.5		
Mineral mix[2]	0.2	0.2	0.2	0.2	0.2		
Cholin chloride[2]	0.15	0.15	0.15	0.15	0.15		
Pellet binder[2]	0.3	0.3	0.3	0.3	0.3		
Cellulose[3]	3.05	2.28	1.52	0.76	0		
Chromic oxide[4]	0.5	0.5	0.5	0.5	0.5		
Proximate composition (% of dry matter)							
Dry matter	91.97	92.31	92.05	92.03	92.03		
Crude protein	46.88	47.58	47.48	47.90	48.12		
Crude oil	22.26	20.20	20.04	19.94	19.46		
Crude ash	10.48	10.17	9.93	9.79	9.55		
Gross energy (MJ/kg)	19.74	19.71	19.73	19.78	19.81		
Essential amino acid composition, % of dry diet (% of dietary protein)[5]							
Arginine	3.2 (6.9)	3.4 (7.1)	3.5 (7.4)	3.6 (7.6)	3.8 (7.8)	2.0	5.0
Histidine	1.2 (2.5)	1.1 (2.4)	1.1 (2.4)	1.1 (2.3)	1.1 (2.3)	0.7	1.8
Isoleucine	2.4 (5.2)	2.4 (5.0)	2.3 (4.9)	2.3 (4.8)	2.3 (4.7)	0.8	2.0
Leucine	4.2 (8.9)	4.2 (8.8)	4.2 (8.8)	4.2 (8.8)	4.2 (8.8)	1.4	3.5
Lysine	3.3 (7.1)	3.2 (6.7)	3.1 (6.6)	3.0 (6.3)	2.9 (6.0)	1.8	4.5
Methionine+cystine	3.0 (6.4)	2.9 (6.0)	2.7 (5.8)	2.6 (5.5)	2.5 (5.2)	1.4	3.5
Phenylalanine+tyrosine	4.3 (9.2)	4.3 (9.1)	4.4 (9.2)	4.4 (9.2)	4.4 (9.2)	1.8	4.5
Threonine	2.0 (4.3)	2.0 (4.2)	2.0 (4.2)	2.0 (4.1)	2.0 (4.1)	0.8	2.0
Valine	2.4 (5.2)	2.4 (5.1)	2.4 (5.1)	2.4 (5.1)	2.5 (5.1)	1.3	3.2

[1] Hardy (2002)

[2] Emre et al. (2008a,b)

[3] Vitacel® Wheat Fibre, J.R.S. Rettenmaier & Soehne GmbH+Co., Rosenberg, Germany

[4] Alfa Aesar GmbH and Co. KG, Karlsruhe, Germany

[5] Calculated from data by Emre et al. (2008a,b)

Calculations. Growth and feed utilization parameters were calculated as follows: average weight gain = W_t - W_0, where W_t is the body weight of the fish on day t and W_0 is the body weight on day 0; specific growth rate (SGR %/day) = 100(ln W_t - ln W_0)/t, where t is the number of days; feed conversion ratio (FCR) = dry feed intake/wet wt gain; protein retention efficiency = wt gain/protein fed; condition factor = W_t/L^3, where L is the total fish length; viscero-somatic index (VSI) = 100(visceral wt/body wt); hepato-somatic index = 100(liver wt/body wt); and dressing percentage = 100(eviscerated body wt/body wt).

Apparent digestibility coefficients (ADC) of experimental diets were estimated as follows: ADC (dry matter %) = 100 - [100 x ($Cr_2O_{3feces}/Cr_2O_{3food}$)] and ADC (nutrient %) =100 - [100 x ($Cr_2O_{3food}/Cr_2O_{3feces}$) x ($nutrient_{feces}/nutrient_{food}$)]. Crude and digestible protein and energy retentions were determined using the following formula (Glencross et al., 2008): protein retention = [(P_t - P_i)/P_c] x 100, where P_t is the protein content of the fish at time t, P_i is the initial protein content of the fish, and P_c is the amount of protein consumed by the fish to time t. Energy and digestible nutrient retention were determined the same way, but with relevant criteria substituted instead of the protein criteria.

Proximate composition analyses. Proximate compositions of feedstuffs, diets, and fish were analyzed according to methods of the AOAC (1990): dry matter after drying in an oven at 104°C until constant weight, ash by incineration in a muffle furnace at 600°C for 2 h, crude protein (N x 6.25) by the Kjeldahl method after acid digestion, and lipid by petroleum ether extraction in a Soxhlet extractor. All analyses were conducted in duplicate. Chromic oxide concentration in feeds and fecal samples were determined according to Furukawa and Tsukahara (1966). Gross energy was calculated using conversion factors of 39.5, 23.7, and 17.2 MJ/kg for fat, protein, and carbohydrate, respectively (Young et al., 2005).

Statistical analysis. The experimental design was completely random, with five treatment levels (inclusion level of hazelnut meal) and three replicates (tanks per treatment). Normality and homogeneity were checked by Shapiro-Wilk W Test and Bartlett's test, respectively. All percentage values were arcsine transformed before analysis of variance. One-way analysis of variance (ANOVA), followed by Tukey *post hoc* test, was used to reveal the effects of treatments on the selected criteria. The level of significance level was $p<0.05$, unless otherwise stated. Crude and digestible nutrient retention were regressed against dietary hazelnut meal level.

Results

There was no mortality during the study due to the different treatments. Fish fed the 30% diet had significantly higher final weight, weight gain, and SGR, consumed significantly more feed, and had a significantly better FCR than those fed the control (Table 2). Protein retention efficiency, condition factor, hepato-somatic index, and dressing percentage were not affected by the inclusion level of hazelnut meal. Similarly, whole body composition did not differ significantly, regardless of the hazelnut meal level (Table 3).

Inclusion of hazelnut meal significantly reduced apparent digestibility of dry matter and energy, even at the lowest level, when compared to the control (Fig. 1). Hazelnut meal did not significantly alter the apparent digestibility of protein and lipid.

Both crude and digestible protein retention significantly correlated with the hazelnut meal level, reflecting an increase up to 25% hazelnut meal, a level-off around 30%, and a decline beyond this content (Figs. 2, 3). The correlation between energy retention and hazelnut meal level was highly significant and displayed more or less the same trend as protein retention.

Discussion

Utilization of plant feedstuffs in fish feeds has been viewed as an essential requirement for sustainable development of aquaculture because of their low cost and availability. To date, numerous studies have undertaken to replace fishmeal with plant protein sources with varying success

Table 2. Growth and feed utilization values of rainbow trout fed diets with different levels of hazelnut meal.

	Diet (% hazelnut meal)					
	Control	*7.5%*	*15%*	*22.5%*	*30%*	*Pooled SEM**
Initial wt (g/fish)	57.32	57.36	57.58	57.62	57.69	0.25
Final wt (g/fish)	196.25^{b}	208.26^{ab}	215.58^{ab}	215.61^{ab}	227.87^{a}	5.11
Avg wt gain (g/fish)	138.93^{b}	150.91^{ab}	158.02^{ab}	157.99^{ab}	170.18^{a}	5.07
SGR (%)	1.76^{b}	1.84^{ab}	1.89^{ab}	1.89^{ab}	1.96^{a}	0.03
Feed intake (g/fish)	129.14^{b}	134.04^{ab}	135.82^{ab}	136.26^{ab}	142.19^{a}	2.49
FCR	0.93^{a}	0.89^{ab}	0.86^{ab}	0.86^{ab}	0.84^{b}	0.02
Protein retention efficiency	2.29	2.37	2.45	2.42	2.49	0.05
Condition factor	1.36	1.41	1.37	1.38	1.37	0.03
Viscero-somatic index (%)	14.18	14.31	15.44	13.31	11.99	1.11
Hepato-somatic index (%)	1.35	1.24	1.29	1.22	1.11	0.06
Dressing percentage (%)	85.53	85.39	83.05	86.40	87.70	1.57

Means in a row with different superscripts are significantly different (p<0.05).
* Mean values (n = 3) and pooled standard error of mean (SEM) are presented for each variable.

Table 3. Whole body proximate composition of rainbow trout fed diets with different levels of hazelnut meal.

	Diet (% hazelnut meal)						
	Initial	*Control*	*7.5%*	*15%*	*22.5%*	*30%*	*Pooled SEM**
Moisture	67.14	69.28	67.96	67.10	67.93	67.68	0.55
Ash	2.52	2.14	2.30	2.17	2.31	2.20	0.05
Lipid	12.21	11.70	12.35	13.18	12.59	12.76	0.57
Protein	16.72	15.31	15.95	16.04	15.70	16.05	0.26

There were no significant differences among experimental treatments (p>0.05).
* Mean values (n = 3) and pooled standard error of mean (SEM) are presented for each variable.

(Gatlin III et al., 2007; Glencross et al., 2007). However, plant protein sources have some drawbacks compared with fishmeal such as limited protein and amino acids, namely lysine and methionine+cystine, and the presence of anti-nutritional factors that negatively affect growth performance and the health status of fish (Francis et al., 2001; Gatlin III et al., 2007). Nevertheless, in this study, none of the essential amino acids including methionine+cystine and lysine were

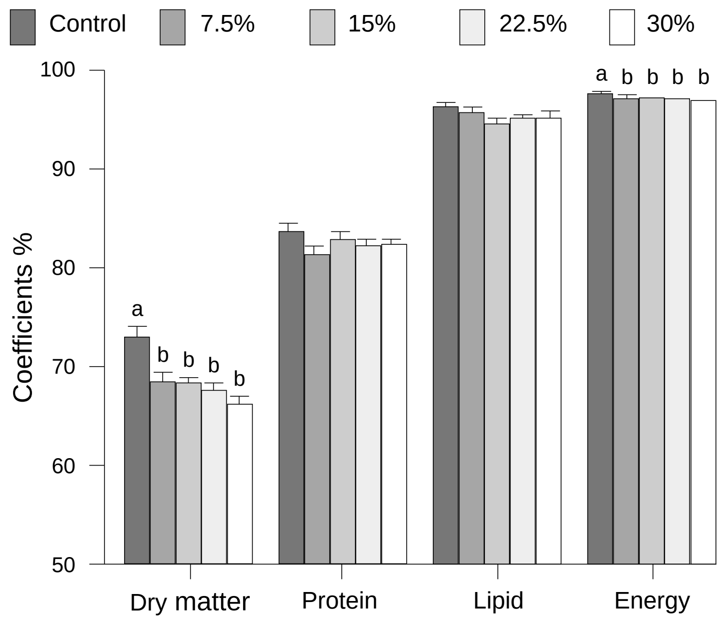

Fig. 1. Apparent digestibility coefficients (%) in rainbow trout fed diets containing different levels of hazelnut meal. Values within a group of bars with different letters are significantly different (p<0.05).

deficient in the test diets. Considering the amino acid requirements of rainbow trout, higher inclusion of hazelnut meal into rainbow trout diets seems to be possible.

The current study shows that partial replacement of fishmeal with hazelnut meal results in better growth performance. Indeed, growth and feed utilization variables increased as the inclusion of hazelnut meal in diets increased, and the 30% diet performed significantly better than the control that contained only fishmeal as a protein source. These findings support our previous findings in European sea bass and gilthead sea bream, indicating that 30% and 40% inclusion of hazelnut meal in diets, respectively, produced comparable growth as in fish fed a fishmeal-based diet (Emre et al., 2008a,b). Partial replacement of fishmeal with hazelnut meal in common carp diets showed that 35% of the fishmeal could be replaced by including 28% hazelnut meal without growth impairment (Buyukcapar and Kamalak, 2007).

There are studies regarding the potential of hazelnut meal to replace soybean meal in fish diets. For instance, a rainbow trout diet in which up to 30% of the soybean meal was replaced by hazelnut meal, in spite of being significantly lower in final weight than the control containing fishmeal and soybean meal, could totally replace soybean meal (Bilgin et al., 2007). On the other hand, replacement of over 20% of soybean meal by hazelnut meal resulted in poor growth in diets for common carp and Black Sea turbot (Buyukcapar and Kamalak, 2007; Ergun et al., 2008). Discrepancies among these findings may originate from differences in species and the sources and production method of the hazelnut meal and the fishmeal. Indeed, there is inherent variation in nutrient levels of hazelnut cultivars in Turkey (Ozdemir and Akinci, 2004; Koksal et al., 2006). Likewise, the protein level of hazelnut meal used in different studies fluctuated between 39% and 47% (Ozen and Erener, 1992; Erener at al., 2003; Bilgin et al., 2007;

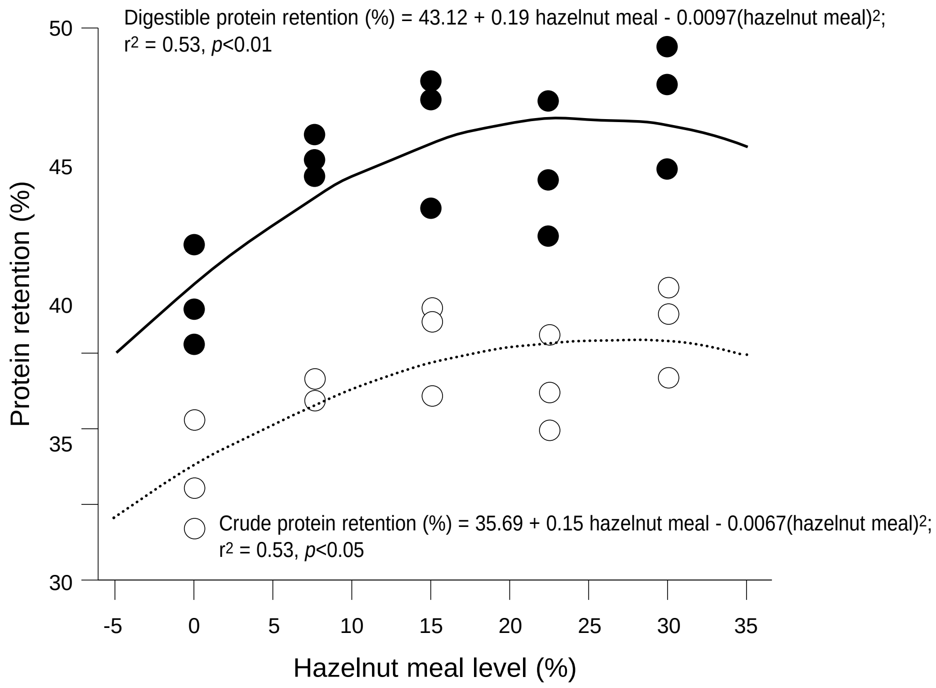

Fig. 2. Crude (○) and digestible (●) protein retention in fish fed diets containing graded levels of hazelnut meal.

Buyukcapar and Kamalak, 2007; Emre et al. 2008a,b; Ergun et al., 2008). Whatever the reason, all findings show that hazelnut meal is a valuable protein source.

Poor appetite is a general problem when fishmeal is replaced with plant protein sources (Gomez-Requeni et al., 2004; Gatlin III et al., 2007). Although consumption of diets including 30% hazelnut meal was low in rainbow trout (Bilgin et al., 2007), there were no palatability problems in common carp (Buyukcapar and Kamalak, 2007) or turbot (Ergun et al., 2008) fed diets containing hazelnut meal. These findings conform with our studies on European sea bass and gilthead sea bream, which did not refuse diets containing hazelnut meal (Emre et al., 2008a,b). In the present study, fish fed the 30% diet had a higher feed intake than the control, indicating that hazelnut meal is a palatable ingredient. However, we offered a fixed feed ration, and not an *ad libitum* or satiation level as suggested by Glencross et al. (2007) to reveal the palatability of an ingredient.

Apparent digestibility coefficients (ADC) provide evidence of the nutritional value of a diet ingredient. Generally, there is a close relationship between growth performance and ADC (Barrows et al., 2007). Thus, it is expected that high growth performance correlates with high nutrient digestibility. Interestingly, such a relationship was not observed in this study, as the ADC of dry matter and energy had inverse trends to those of growth and feed utilization performance. A similar inconsistency was found in rainbow trout, where there was a discrepancy between FCR and ADCs of dry matter and protein (Barrias and Oliva-Teles, 2000). Also, juvenile turbot fed increasing levels of a mixture of plant protein sources had lower growth performance but better ADCs of dry matter, nitrogen, and energy than those fed a diet based on fishmeal (Fournier et al., 2004). A comparison of nutrient digestibility, growth, and feed utilization in various selective-

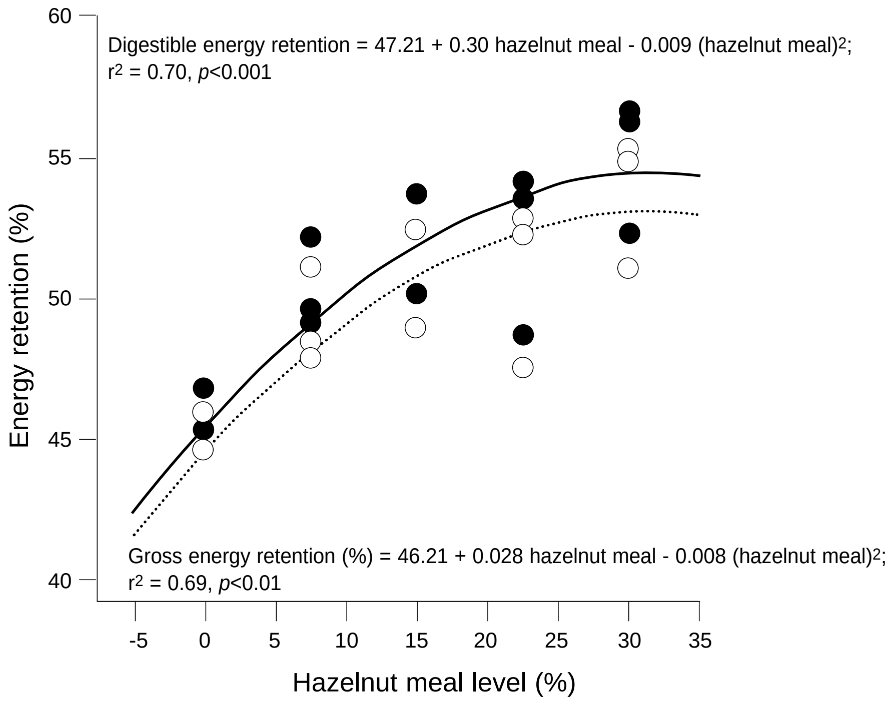

Fig. 3. Gross (○) and digestible energy retention (●) in fish fed diets containing graded levels of hazelnut meal. One replicate of the 15% diet was omitted.

ly-bred families of rainbow trout showed that protein digestibility highly correlates with growth and FCR in a consistent manner whereas lipid digestibility does not (Rasmussen and Jokumsen, 2009). Molina-Poveda and Morales (2004) and Niesar et al. (2004) obtained similar discrepancies in their studies. Therefore, we agree that high nutrient digestibility is a necessary but not sufficient prerequisite for high growth rates, as suggested by Arlinghaus and Niesar (2005). Challenging fish with diets containing restricted amounts of dietary protein may be a solution for this sort of dilemma (Glencross et al., 2008).

The ADCs of dry matter and protein in the present study were lower than those reported for fishmeal-based diets (Sugiura et al., 1998a,b; Weatherup and McCracken, 1998). This was probably due to the collection of feces by stripping, a method that generates lower values (Weatherup and McCracken, 1998; Vandenberg and de la Noue, 2001). However, the ADCs of lipid and energy in all treatments of this experiment were above 95%, suggesting that the lipid and energy in all the diets were highly digestible for rainbow trout, even if the ADC of energy was significantly lower in the hazelnut meal diets than in the control.

In the current study, final body composition was not significantly affected by the tested levels of hazelnut meal in the diets. This finding is compatible with those in European sea bass fed hazelnut meal up to 30% and gilthead sea bream fed up to 40% (Emre et al., 2008a,b). However, rainbow trout given a diet containing 30% hazelnut meal had higher moisture and ash, and lower protein, lipid, and energy contents than those fed a diet based on fishmeal and soybean meal (Bilgin et al., 2007). Incorporation of hazelnut meal into turbot diets above 20% reduced body protein but elevated moisture and crude lipid (Ergun et al., 2008).

In this study, the correlations of crude and digestible protein retention with dietary hazelnut meal level were significant, suggesting that as the hazelnut meal content increased up to 25%, more protein was involved in growth. However, the amount of protein used for energy and excreted as nitrogen rose at around 30% hazelnut meal inclusion (Fig. 2). In other words, the decline in protein retention suggest that including over 30% hazelnut meal may reduce the growth and feed utilization benefits. The findings from the present study are consistent with the finding that a high growth rate might not always be accompanied by high nitrogen uptake (Rasmussen and Jokumsen, 2009). The energy retention values followed a similar trend (Fig. 3), supporting the view that reduced nutrient retention may occur when hazelnut meal is included above 30%.

On the whole, the present study supports our previous findings that hazelnut meal is a valuable protein source (Emre at al. 2008a,b) and that incorporation of hazelnut meal up to 30% in rainbow trout diets can produce better performance than a control diet based on fishmeal as the only protein source. Based on the essential amino acid contents of the experimental diets, an inclusion level exceeding 30% seems possible. However, hazelnut meal above this level may reduce protein retention, leading to increased nitrogen excretion. Thus, the optimum hazelnut meal incorporation level may be around the highest level used in the current study (30%). Further studies should focus on inclusion levels of hazelnut meal in diets with limited protein contents and practical diets, as in Glencross et al. (2008), to better understand the nutritional value of hazelnut meal.

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