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Effects of dietary lipid levels on growth performance and feed utilization in juvenile Black Sea turbot (*Psetta maxima*) with reference to nitrogen excretion.

Fatma Burcu Harmantepe[1*], Murat Yigit[2], Gaye Dogan[3], Zafer Karsli[3], Umut Yigit[2], Orhan Uyan[4,5]

[1] *Cukurova University, Yumurtalik Vocational School, Fisheries Program, Yumurtalik, Adana, Turkey*

[2] *Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology, Department of Aquaculture, Terzioglu Campus, Canakkale, Turkey*

[3] *Sinop University, Faculty of Fisheries, Department of Aquaculture, Sinop, Turkey*

[4] *Kagoshima University, Faculty of Fisheries, Laboratory of Aquatic Animal Nutrition, Kagoshima-shi, Japan*

[5] *MY Grup, Aquaculture Research & Development Group, B.Cekmece, İstanbul, Turkey*

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ABSTRACT

In the present study, iso-nitrogenous test diets were formulated to contain two different levels of lipid (9% and 12%) with dietary energy ranging from 19 kJ/g to 20 kJ/g diet. White fish meal was the main protein source of the diet and the protein level was 55% in all diets offered to triplicate groups of juvenile Black Sea turbot (mean initial weight of 24.5±0.3 g) for a period of 70 days. Specific growth rates were significantly better ($P < 0.05$) in high lipid diet group compared to the groups fed low lipid diets. Feed conversion rates (FCR) were influenced by the lipid levels and the best FCR was obtained in fish given the diet with high lipid content. Similarly, protein efficiency was also significantly better ($P < 0.05$) in fish fed diets with high lipid level. The better utilization of protein in the diet with high lipid content resulted in a significantly higher ($P < 0.05$) nitrogen retention as percent of intake when compared to the other group with lower lipid level. The amount of nitrogen loss into the water environment was significantly reduced ($P < 0.05$) to the same extent in the group fed diets with high lipid level. The lipid retention values calculated based on the intake rates were also better in the high lipid diets compared to the other group with low lipid level, however the difference was not significant ($P > 0.05$) among the experimental groups. The results in the present study indicate that the use of high lipid level with a protein to energy ratio of 27 mg/kJ (12% lipid, 55% protein, 20 kJ/g gross energy) performed best. Increasing the dietary lipid to 20 kJ/g diet has reduced the total nitrogen excretion from Black Sea turbot by 23%.

Introduction

Turbot is of great aquacultural interest in Europe (Person-LeRuyet, 1993) and its production has been expanded over the last years (Sæther and Jobling, 2001). Compared to other fish species commonly cultured in European aquaculture industry, turbot usually doubles its market prize distinguishing itself from other aquaculture species. The Black Sea turbot however is a new candidate for the aquaculture industry and the development of suitable feeds for use in intensive turbot culture is one of the main and important issues affecting product quality, fish health, and overall success of the farming activity. Due to the over dependence of the aqua-feed industry on wild fish stocks, the use of fish meal sources is an important issue that needs to be controlled in fish diets to achieve the

*Corresponding author
E-mail address: burcukaraali@hotmail.com (F.B. Harmantepe)
Tel:+: +90 505 90 505 359 82 74 fax: +90 322 671 26 74

best profit from the diet used. Using a diet with too high protein level, the excess will be catabolized to provide energy for fish growth, which might then lower the protein efficiency rate of fish (Adron et al. 1976). In contrast, feeding the fish with a diet containing a too high energy level more than necessary, might decrease feed consumption and growth of fish can be reduced (Lovell, 1998). Besides the high production costs due to inadequate diet formulations, the deterioration of environment derived by uneaten or wasted feed and ammonia nitrogen excretion through gills and kidneys may also be a result of inadequate feeds (Yigit et al. 2002). The reduction of ammonia nitrogen excretion from fish can achieved by increasing dietary protein retention, which can be accomplished by adjusting the protein to energy ratio in the diet with an optimal balance (Watanabe et al. 1987). Several published papers on protein or lipid levels in turbot diets are available; however the nutritional information for the Black Sea turbot still needs to be investigated. Hence the aim of the present study was to evaluate the effects of two lipid levels in the diet on growth performance, feed utilization, protein utilization and body composition in Black Sea turbot.

Material and methods

Preparation of test diets

Two experimental diets were formulated to contain lipid levels of 12% and 9%, both with a protein level of 55%. White fish meal (WFM) was the main protein source for both diets prepared with high lipid (HL) and low lipid (LL) levels. WFM is a product containing oil less than 6% obtained from white fish or white fish waste such as filleting offal (FAO, 2001). White fish meal used in the present study derived from Alaska Cod meal. Ingredients, chemical composition and the amino acid profiles of the diets are given in Table 1. In a study where ammonia nitrogen excretion rates were used as index for the protein quality assessments of anchovy, goby and whiting, Yigit et al. (2003) reported better protein quality for whiting compared to anchovy in diets for Black Sea turbot. Hence WFM was used as protein source in this study. Total n-3 HUFA contents were calculated according to the equation given below as described by Yigit et al. (2006) and ranged from 2.35% for the LL diet to 3.1% for the HL diet (Table 1). The nutrient composition and amino acid profiles of white fish meal, soybean meal and turbot are given in Table 2.

Total n-3 HUFA in diet, g/kg = (total fish oil in diet, g/kg) x (% n-3 HUFA in fish oil used).

During feed production, all dry ingredients were sieved through a mesh of 500 μm, and thoroughly mixed with fish oil, then tap water was added to produce a homogeny dough, which was then pelleted with a meat grinder. After the pelleting process, experimental diets were dried at 60°C in a constant temperature oven until a constant weight was obtained and finally stored at –25°C until the start of the experiment.

Table 1. Ingredient composition and proximate analysis of the experimental diets.

	Experimental diets		Turbot requirements
	HL diet	LL diet	
Ingredients (%)			
White Fish meal[a]	71.0	71.0	
Soybean meal	14.0	14.0	
Corn meal	1.5	4.0	
Dextrin	2.0	2.0	
Alfa starch	2.0	2.0	
Fish oil[b]	6.5	4.0	
Binder[c]	1.0	1.0	
Vitamin mix[d]	1.0	1.0	
Mineral mix[e]	1.0	1.0	
Proximate composition (g/kg dry diet)			
Moisture	12.9	13.8	
Crude protein	55.3	55.8	
Crude lipid	12.1	9.5	
Crude fiber	1.0	1.1	
Crude ash	16.4	16.7	
Nitrogen-free extracts[f]	15.2	16.9	
Gross energy (kJ/g diet)[g]	20.4	19.8	
Protein:energy (mg/kJ)	27.1	28.2	
Crude fat (g/kg)	5.5	5.5	
Fat from white fish meal	3.91	3.91	
Total fish oil in diet	10.41	7.91	
n-3 HUFA in fish oil[h]	29.76	29.76	
Total n-3 HUFA(g/kg diet)	3.10	2.35	0.6[i] – 0.8[j]
Amino acid content (%)[k]			
Arginine	4.06	4.06	2.66
Lysine	4.92	4.92	2.77
Histidine	1.28	1.28	0.83
Isoleucine	2.61	2.61	1.44
Leucine	4.41	4.41	2.55
Valine	2.96	2.96	1.61
Methionin+Cystine	2.29	2.29	1.49
Phenylalanine	2.42	2.42	-
Threonin	2.46	2.46	1.61
Tryotophan	1.38	1.38	0.33

[a] Cod meal (Alaska)
[b] Anchovy oil (Black Sea)
[c] Binder (0.985%); Berda,Rosch Co., Germany
[d] Vitamin mix
[e] Mineral mix
[f] Nitrogen free extracts=100-(crude protein + crude lipid+crude fiber+crude ash)
[g] Gross energy is calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, 17 kJ/g NFE.
[h] According to Güner et al. (1998)
[i] According to Gatesoupe et al. (1977)
[j] According to Léger et al. (1979)
[k] Essantial amino acid contents based on data in Table 2.

Table 2. Proximate analyses of white fish meal, soybean meal and turbot.

	White Fish Meal	Soybean Meal	Turbot*
Proximate analysis (%)			
Moisture	6.3	10.5	
Protein	66.1	41.4	
Lipid	5.4	11.9	
Ash	21.5	4.9	
Fiber	-	4.6	
Indispensible amino acids (%)			
Arginine	5.00	3.67	4.8
Lysine	6.32	3.09	5.0
Histidine	1.56	1.22	1.5
Isoleucine	3.25	2.14	2.6
Leucine	5.50	3.64	4.6
Valine	3.66	2.55	2.9
Methionin+Cystine	2.94	1.43	2.7
Phenylalan.+Tyro.	3.16	1.25	-
Threonin	3.09	1.89	2.9
Tryptophan	N/A	N/A	-
Dispensible amino acids (%)			
Aspartic acid	7.01	-	-
Serine	1.88	-	-
Glutamic acid	8.31	-	-
Proline	2.81	-	-
Glycine	2.46	-	-
Alanine	3	-	-
Tyrosine	2.31	-	-
Taurine	0.31	-	-

*Data on amino acid contents of turbot from Kaushik (1998).

Experimental fish and rearing conditions

Hatchery reared Black Sea turbot (*Psetta maxima*) were obtained from Japan International Cooperation Agency (JICA) and the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey and transported to the Marine Aquaculture Facility of Faculty of Fisheries, Sinop University (formerly Ondokuz Mayıs University) in Sinop, Turkey. Turbot were maintained on a commercial diet (55% crude protein, 16% crude lipid, 9% nitrogen free extracts, 21 kJ gross energy/g feed, 26.19 mg protein / kJ) for an adaptation period of 4 weeks before the start of the feeding trial. Experimental fish with similar size (mean initial weight 24.5±0.3 g) were randomly distributed among 12 identical 60-l rectangular polypropylene tanks filled with 50-l water (10 fish per tank with three replicates per treatment). In an indoor flow-through system sea water (17 ppt salinity) was supplied at a flow rate of 1.5 l/min. Water temperature averaged 19.64 ± 3.26°C and pH ranged 7.5-8. Fish were exposed to a natural light regime (Sinop, 42°01'18.09"N-35°08'36.98"E) and hand fed twice daily at 8:30 and 16:30 until satiation during a period of 70 days. After each feeding, fecal matter and uneaten feed were removed. Uneaten feed was deducted from feed offered for an accurate calculation of feed eaten by fish.

Sampling and analytical methods

Prior to sampling, fish were fasted for 24 h and then anaesthetized by immersion in a MS-222 bath (100 mg/L). At the beginning of the trial, 10 fish from an initial pool of the initial batch were removed, anaesthetized at high dose level and stored at -25°C for subsequent analysis of body composition. At the end of the trial, 5 fish per tank (15 per diet) were removed the same way as mentioned above and stored at -25°C for subsequent analysis of body composition.

For determination of the proximate composition of the experimental diets and freeze-dried whole body of fish, procedures according to AOAC (1984) guidelines were followed: Moisture by weight loss after 24 h in an oven at 105°C; crude ash by incineration in a muffle furnace at 550°C for 24 h; crude protein (% Nx6.25) by the Kjeldahl method after acid digestion; lipids by ethyl ether extraction in a Soxhlet System. Amino acid analyses of WFM and SBM were performed in the Laboratory of Aquatic Animal Nutrition at Kagoshima University, Faculty of Fisheries, Kagoshima-Japan.

Statistical analyses

Results were analyzed by two-way analysis of variance (ANOVA) using SPSS for Windows, Version 10.0 for significant differences among treatments means. Duncan's multiple range test (Duncan, 1955) was used to compare differences among individual means. Probability values less than 0.05 were considered significant.

Results

Specific growth rate (SGR) in fish fed with HL diets were significantly better ($P < 0.05$) than those of the LL diets. Daily feed intake (DFI) of fish increased with the increase of dietary lipid level and the DFI in the HL group was significantly higher ($P < 0.05$) than that of the LL group. Feed conversion rate (FCR) were influenced by the lipid levels. FCR decreased when dietary lipid level increased. Similar to the FCRs, Protein efficiency rate (PER) was also significantly better ($P < 0.05$) in fish fed the HL diet compared to the LL one (Table 3). No mortality was observed during the experiment and survival in all groups was 100%.

Total nitrogen loss per body weight gain was significantly ($P < 0.05$) lower in fish fed the HL diet, compared to the value recorded for the LL group. Nitrogen retention per body weight gain was slightly higher in the HL group, but not significantly different ($P > 0.05$) compared to the LL group. Nitrogen Retention per Intake, however, was significantly higher ($P < 0.05$) in the HL group compared to the fish fed the LL diet. Total lipid loss per body weight gain was not significantly ($P > 0.05$) different among test diets. The value of lipid retention per body weight gain was found significantly different ($P < 0.05$) with a higher value for the experimental group fed the HL diets. Overall, the retention values calculated based on the intake rates were better in fish fed HL diets compared to those fed LL diets (Table 4).

Whole body moisture of fish fed on the HL diet was significantly lower (*P < 0.05*) compared to the experimental group fed the LL diet. Conversely to whole body moisture, crude lipid of fish was lowest in fish fed the LL diet compared to the HL groups (*P < 0.05*). The whole body protein of fish were not affected by the dietary lipid levels. Significantly higher (*P < 0.05*) ash contents were recorded in fish fed diets with LL levels (Table 5).

Table 3. Growth performance and feed utilization of Black Sea turbot juveniles fed the experimental diets.

	Experimental diets	
	HL diet	LL diet
Initial weight (g)	24.5 ± 2.9	24.6 ± 2.8
Final weight (g)	66.9 ± 5.7[b]	57.7 ± 6.7[a]
Specific growth rate (SGR, %)	1.39 ± 0.06[b]	1.18 ± 0.03[a]
Daily Feed Intake (FI, g/fish)	0.51 ± 0.03[b]	0.46 ± 0.01[a]
Feed Conversion Rate (FCR)	0.84 ± 0.02[a]	0.98 ± 0.04[b]
Protein Efficiency Rate (PER)	2.14 ± 0.05[b]	1.83 ± 0.07[a]
Survival (%)	100	100

SGR = [ln (final weight) – ln (initial weight) / 70days] x 100
DFI = (dry feed intake/number of fish) / 70days
FCR = dry feed intake (g) / weight gain (g)
PER = weight gain (g) / protein intake (g)

Table 4. Nitrogen and lipid budget per unit body weight gain and nutrient retention in Black Sea turbot juveniles fed diets containing different lipid levels for a period of 70 days.

	Experimental diets	
	HL diet	LL diet
(g N/kg BW gain)		
Nitrogen Intake[1]	74.7 ± 1.81[b]	87.7 ± 3.12[a]
Nitrogen Retention[2]	30.1 ± 0.65[a]	29.7 ± 0.45[a]
Nitrogen Loss[3]	44.6 ± 2.45[b]	57.9 ± 3.38[a]
(g L/kg BW gain)		
Lipid Intake	102.1 ± 2.47[c]	93.3 ± 3.33[d]
Lipid Retention	53.8 ± 1.02[c]	47.9 ± 0.91[d]
Lipid Loss	48.4 ± 2.99[c]	45.4 ± 3.33[c]
(% of intake)[4]		
Nitrogen Retention per Intake	40.3 ± 1.82[b]	33.9 ± 1.56[a]
Lipid Retention per Intake	52.7 ± 1.89[b]	51.4 ± 1.99[b]

[1] Nitrogen or lipid intake= {(feed intake(g/fish) x nitrogen or lipid concentration in diet (%) / 100) / (mean body weight gain (g))} x 1000.
[2] Nitrogen or lipid retention= {final mean body weight (g) x final whole body nitrogen or lipid concentration (%) / 100) - (initial mean body weight (g) x initial whole body nitrogen or lipid concentration (%)/100)} / (mean body weight gain (g)) x1000.
[3] Nitrogen or lipid loss= nitrogen or lipid intake (g/kg body weight gain) - nitrogen or lipid retention (g/kg body weight gain).
[4] Retention per intake in percent= nitrogen or lipid retention (g/kg body weight gain) x 100)/ nitrogen or lipid intake (g/kg body weight gain).

Discussion

Compared to the numerous studies conducted on the nutritional requirements of the Atlantic turbot, there are only few studies on diets for the Black Sea turbot, which is a new candidate for the aquaculture industry. A variety of study on protein requirement of turbot has been reported and results are fairly variable. Best growth performance for Turbot of 47 g with a diet containing 55% protein was reported by Cho et al. (2005) while a lower dietary protein requirement level of 49% was reported for 89 g turbot by Lee et al. (2003). Devesa (1994) found that 45-50% protein levels meet the requirements for turbot. In contrast, Caceres-Martinez et al. (1984) reported a higher level of protein requirement of 69% for turbot of 10 g. It can be estimated that the difference between these studies are due to the difference in feed ingredients and protein quality, feeding rate, fish size, and environmental conditions such as water temperature or other. Due to the similarity of the size of the experimental fish used in the present study and those used in the study conducted by Cho et al. (2005), 55% protein level (iso-nitrogenous diet) was also used in the present study.

Black Sea turbot juveniles fed on the experimental diets with a 12% lipid produced a better growth performance than those fed on a lower lipid diet of 9%. The significant increase in growth performance of fish fed the high lipid diet compared to the low lipid diet used in this study, is in agreement with previous studies showing that high lipid diets produced better growth than low lipid ones in marine fish species such as Atlantic halibut (Helland and Grisdale-Helland 1998), gilthead seabream (Company et al. 1999), and Atlantic turbot (Cho et al. 2005). Fish fed the diet with high lipid content showed higher FCR and PER compared to those fed the low lipid diet. By increasing the dietary lipid from 9% to 12%, it is likely that the proportion of dietary protein used for catabolic processes decreased with the use of higher levels of lipid in the diet, which might be attributed to the smaller amount of share of the protein intake for maintenance. Therefore, it can be concluded that the lower nitrogen loss in fish fed diets with higher lipid levels was noted due to lower protein catabolism in the present study. Similar to the rates of nitrogen retention, lipid retention was also improved with the use of higher lipid diet. As a result, lower nitrogen excretion from fish may lead to cost-effective fish culture with environment friendly diets, which in long term might support the sustainability of the aquaculture industry.

In the present study, PER (2.14) found for the best performing group fed on HL diet was similar to the PER values reported by Lee et al. (2003) and Cho et al. (2005) for Atlantic turbot (2.18; 2.01). Martins et al. (2007) and Árnason et al. (2009) also found similar results for PERs in Atlantic halibut (2.50; 2.07). PERs found in the present study are in the range of values (1.82-2.49) reported for the

Table 5. Whole body chemical composition and conditional factor of Black Sea turbot juveniles fed diets containing different lipid levels for a period of 70 days (dry basis, except for moisture).

	Initial	Final	
		HL diet	LL diet
Moisture (%)	79.98	75.03 ± 0.44^{b}	75.84 ± 0.40^{c}
Crude protein (%)	67.79	67.57 ± 0.20^{b}	68.07 ± 0.27^{b}
Crude lipid (%)	3.89	14.79 ± 0.17^{c}	12.75 ± 0.14^{a}
Crude ash (%)	28.26	17.57 ± 0.06^{a}	19.09 ± 0.15^{b}
Condition factor	1.27 ± 0.08	1.42 ± 0.07^{a}	1.38 ± 0.06^{a}

Black Sea turbot by Turker et al. (2005), Yigit et al. (2006), Ergun et al. (2008a,b), and Yigit et al. (2010). Compared to the findings in the present study, lower levels of PERs were reported for European sea bass (1.38-1.48) fed similar dietary lipid levels (Boujard et al. 2004) and for Atlantic turbot (1.63) (Peres and Oliva-Teles, 2008). The differences in fish sizes or feed types used in the studies may result in variations of findings between studies with similar conditions and fish species.

The rates of feed conversion and specific growth followed the same trend as the protein efficiency rates in the present study. Depending on the fact that the SGR, FCR, and PER improved with the increase of the lipid level in the experimental diets, it is likely to note the well-known protein sparing effect of lipids, that was also reported in earlier studies on Rainbow trout (Yigit et al. 2002), Eurasian perch (Mathis et al. 2003) and Atlantic turbot (Cho et al. 2005). Growth performance, feed utilization, and nitrogen budget found for the Black Sea turbot in the present study are within the range of previous reports on the Atlantic turbot (Burel et al. 2000; Day and Plascencia Gonzalez, 2000; Fournier et al. 2003, 2004) and also the Black Sea turbot nutrition (Turker et al. 2005; Hasimoglu et al. 2007; Yigit et al. 2006, 2007; Ergun et al. 2008a,b; Yigit et al. 2010).

In a previous study, Craig et al. (1995) reported higher lipid deposition in fish fed on diets with higher energy contents, which has been pointed out as an indication of a poor nutrient utilization of fish (Craig et al. 1999). An increased lipid deposition in the fish body was recorded in the present study when fish fed the diets with higher lipid contents. This has also been reported in juvenile cobia by Craig et al. (2006). In addition, Yamamoto et al. (2000), and Wang et al. (2005) reported lower feed consumption in rainbow trout and juvenile cobia fed diets with high energy, however, this was not the case in the present study, where feed intake values significantly increased with the increase of dietary lipid levels.

According to the results in the present study, dietary lipid level of 12% with 55% crude protein content performed better than the lower lipid diet of 9% in terms of growth performance, feed utilization, protein efficiency, and nitrogen budget of juvenile Black Sea turbot. It is also interesting to see that the so called “protein sparing effect” is also acceptable for the Black Sea turbot. These findings are promising in terms of supporting the sustainable growth of turbot culture industry in the Southern European Seas with nutritionally balanced, environmentally sound and cost-effective diets. It is suggested that future investigations study lipid levels higher than 12% with a variation of dietary protein levels to clearly establish the optimum protein to energy ratio for this species as a new candidate for the aquaculture industry.

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