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RESEARCH ARTICLE

Effect of dietary protein on the growth of mullet, *Chelon labrosus*, reared in sea cages

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Abstract. The purpose of the study was to obtain preliminary data on the effect of dietary protein on the growth of the thick-lipped grey mullet (*Chelon labrosus*) juveniles reared in net sea-cages. The juveniles (6.2 ± 0.61 g) were obtained from the wild and were stocked into net sea-cages at a stocking rate of 100 juveniles per cubic meter and fed with experimental diets including 25, 30, or 35% crude protein for 87 days. The highest mean weight and specific growth rate (SGR) were 39.5 g and 2.13% day^{-1}, respectively, at protein levels of 30%. The diet also resulted in the most efficient feed conversion ratio of 1.61.

Keywords: *Chelon labrosus,* dietary protein, growth, cage culture

Introduction

Mullet species are popular food fishes, and rearing them in aquaculture has recently attracted attention worldwide. The need to diversify marine aquaculture products in the Mediterranean has stimulated the farming of new species (Biandolino et al. 2010). Mullet species are considered to be potential candidates for the diversification of marine aquaculture since they have good biological characteristics, are in great demand, and obtain high market prices in Mediterranean countries. Mullet species are farmed primarily in brackish waters using extensive techniques in lagoons and coastal areas and with various culture methods with additional feeding and fertilization practiced in some countries (Cataudella et al. 2005). There are also reports of culturing mullet species in freshwater and brackish water ponds (Barman et al. 2005, Biswas et al. 2012). The salinity tolerance of mullet species, which has been reported previously, permits good growth in a variety of ecosystems and is evidence of their potential in various aquaculture systems (Hotos and Vlahos 1998).

Mugil cephalus, *Chelon labrosus*, *Liza aurata*, *Liza saliens* and *Liza ramada* are the most commonly farmed mullet species in Mediterranean countries, which are the largest producers of various mullet species (El-Sayed and El-Ghobashy 2011). Because of wild-caught fish pressure on environment, in recent years increasing attention has been focused on research concerning hatchery-produced seeds and larval rearing of mullet species in the region (Cataudella et al. 1988, Khemis et al. 2006, El-Sayed and El-Ghobashy 2011). On the other hand, not enough information is available regarding feed utilization or growth performance of mullets species fed artificial feeds. Previous studies have mostly investigated the ecology and feeding habits of wild mullet species. Similar to most of other

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mugilids, the thick-lipped grey mullet has been described as an omnivorous, planktivorous fish that feed on detritus, benthic organisms, and algae (Brusle 1981). Therefore, it seems that natural sources and low cost feeds can be used to meet the nutritional requirement of this species. Accordingly, research on mullet feeding have focused on evaluating different protein sources and how they influence growth and feed utilization in mullet species (Wassef et al. 2001, Belal 2004, Luzzana et al. 2005). In one previous study, it was demonstrated that the use of algae-based diets in *C. labrosus* has limitations with respect to growth performance and feed utilization (Davies et al. 1997). The main function of food is to meet fish energy requirements for maintenance, growth, and reproduction. For *C. labrosus* to grow at optimal rates, adequate quantities of energy must be provided, and lipids are thought to be a more effective source of non-protein energy than are carbohydrates (Ojaveer et al. 1996). Protein requirements of some mugilids have also been studied (Papaparaskeva-Papoutsoglou and Alexis 1986, El-Sayed 1991, Lin et al. 1998, Carvalho et al. 2010). In addition, several fish feeds (e.g., for sea bream, trout, carp) have been tested (Höner et al. 1989, Cardona and Castello 1995, Ağirağac and Kalma 1999), but the results were not as encouraging as predicted.

No commercial diets are available for mullet species in many countries. The availability of efficient feed is a certainly a bottleneck in enhancing the production of these species in fish culture, and it is necessary to determine the nutritional requirement of species under culture conditions in oder to formulate well-balanced, low cost, environmentally-friendly diets. Additionally, advancing mullet aquaculture depends on the development of intensive-farming techniques, which are currently also limited for these species. Environmental issues and potential conflict of interests among sectors in coastal zones are also considered important driving forces in open-sea farming (Burbridge et al. 2001). Little information is available on the growth of mullet species in sea-cages, and diets containing various levels of protein have yet to be studied under natural conditions. The present study was conducted to investigate adaptive responses and evaluate the effects of dietary protein on the growth performance of *C. labrosus* reared in cages.

Materials and methods

Juvenile mullet were obtained the wild in Sìğacik-Seferihisar Estuary (Turkey). The fish were stocked into fiberglass tanks at the Hatchery Unit of the Faculty of Fisheries, University of Ege (Izmir, Turkey) for acclimation to artificial feeding and rearing conditions. The fish were anesthetized with 2-phenoxyethanol (0.2 ppt) and were weighed (6.2 ± 0.61 g mean body weight) before being stocked into sea-cages (Urla-Izmir). Twelve net sea-cages (1.5 x 1 x 1 m; depth x length x width) with water volumes of 1 m^3 each and 10 mm mesh size were suspended from floating platforms. The net sea-cages were covered with protective net to keep the fish from escaping by jumping out and to protect them from bird predation. Stocking density was 100 fish per sea-cage and growth was compared using formulated, floating diets containing 25, 30, and 35% protein (Table 1) in triplicate. Thirty fish per replicate were sampled and weighed at two-week intervals throughout the feeding trial. The fish were fed four times daily to apparent satiation, and consumption was recorded during the experimental period. Mortalities, water temperature, and dissolved oxygen in the sea-cages were recorded daily. Salinity and pH were measured weekly.

Diet preparation and chemical analysis

Three isocaloric (19.6 ± 0.2 MJ kg^{-1}) diets containing 25, 30, or 35% crude protein were formulated according to published nutrients requirements of mullet (Table 1). Locally produced fish meal (FM), soybean meal, and corn meal were used as the protein sources. The dietary ingredients were blended, moistened, and cold pelleted with a laboratory feed mill. The pellets were then dried at 40°C for 24 hours and stored at -20°C until delivery. Experimental diet

Table 1
Formulation and composition of the test diets

	Dietary protein level (%)			
Ingredients (%)	25	30	35	Carp feed
Fish meal	15	20	30	
Soybean meal	25	30	30	
Wheat meal	30	25	18	
Corn meal	20	15	12	
Vitamin premix[a]	1.7	1.7	1.7	
Mineral premix[b]	1	1	1	
Cod-liver oil	7	7	7	
Lignosulfonate	0.3	0.3	0.3	
Proximate composition				
Dry matter	91.35	91.95	91.55	91.33
Crude protein	25.53	30.13	35.58	36.45
Crude lipid	12.03	12.08	12.38	10.3
Ash	8.8	9.22	10.05	9.4
Crude fibre	2.96	3.09	2.99	5.3
NFE	50.68	45.48	39.00	38.55
GE (MJ kg^{-1})	19.49	19.71	20.01	19.32
P:E (g protein MJ kg^{-1}GE)	13.10	15.28	17.78	18.87

[a] Per kg premix: vitamins A: 4000000 IU, D_3: 480000 IU, K_3: 2.4 g, B_1: 4 g, B_2: 6 g, B_6: 4 g, B_{12}: 10 mg, Ca-panthothenate: 10 g; D-biotin: 100 mg; folic acid: 1.2 g; inositol: 60 g and 40 g niacin, vitamin E and C
[b] Per kg premix: 23.75 g Mn, 75 g Zn, 5 g Cu, 2 g Co, 2.75 g I, 100 mg Se, 200 g Mg

dry matter, crude ash, crude protein, and crude lipid contents were determined by 24-h drying at 105°C, 12-h combustion at 525°C, the Kjeldahl method (N×6.25), and petroleum ether extraction (Horwitz et al. 2000). The nitrogen-free extract (NFE) was calculated by subtracting the sum of crude ash, crude protein, and crude lipid from 100. The energy content of the test diets was estimated using conversion factors of 39.5, 23.7, and 17.2 kJ g^{-1} for fat, protein, and carbohydrate, respectively (Brett and Groves 1979). The suitability of commercially available carp feed was also tested to compare it with the experimental diets. The results were expressed as mean ± SD and analyzed with one-way ANOVA to test the effect of the dietary protein levels. Post hoc analysis was conducted using Duncan's new multiple range test with the statistical significance set at the 0.05 probability level to compare mean values among treatments. Additionally, the optimal dietary protein level was determined with the broken-line model method. Percentage data were normalized using arcsin transformation before analysis. All statistical analysis was performed using SPSS Statistics version 20.0 (IBM, New York).

Results

The fish were well adapted to the net-cages, accepted the diets regardless of dietary protein content, and no mortality was recorded under natural conditions after stocking into the sea-cages or for the further two-week period. Recorded water temperatures, dissolved oxygen, salinity, and pH were all within ranges suitable for fish culture (Fig. 1). There were no significant differences in the water quality parameters among the levels in the sea-cages or the ambient environment. Final mean weights and specific growth rates (SGR) were higher for the fish fed with 30% dietary protein (Table 2). No significant ($P > 0.05$) effect of increasing dietary protein level from 30 to 35% was observed on fish growth (Table 2), whereas the fish fed the commercial carp feed (36.5% protein) exhibited a markedly reduced growth rate

Table 2
Performance of fish fed diets with varied protein levels (mean ± SD)

	Dietary protein level (%)			
Parameters	25	30	35	Carp feed
Initial body weight (g)	6.2±0.5[a]	6.2±0.5[a]	6.2±0.6[a]	6.3±0.7[a]
Final weight (g)	29.1±1.0[a]	39.5±1.9[b]	35.1±1.6[bc]	33.7±1.1[c]
SGR (% day^{-1})	1.77±0.04[a]	2.13±0.05[b]	1.99±0.05[bc]	1.94±0.04[c]
FCR	2.10±0.09[a]	1.61±0.07[b]	1.78±0.06[bc]	1.84±0.04[c]
PER	2.39±0.07[a]	2.46±0.09[a]	1.92±0.05[b]	1.83±0.05[b]
Survival (%)	96.0±0.6[a]	98.3±0.3[b]	98.3±0.7[b]	98.0±0.0[b]
Biomass (kg)	2789.8±106.4[a]	3887.0±187.3[b]	3454.5±163.7[bc]	3307.5±101.1[c]

Means in rows with different superscripts are significantly different (α=0.05). SGR (% day^{-1}): 100 (Ln final weight – Ln initial weight)/days. FCR: dry feed intake (g)/weight gain (g). PER: weight gain (g)/protein intake (g)

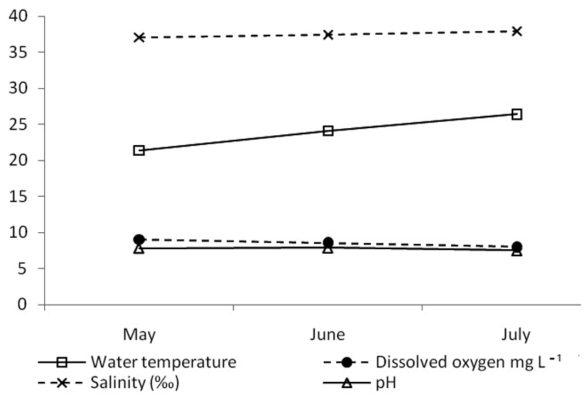

Figure 1. Water quality parameters during the feeding trial.

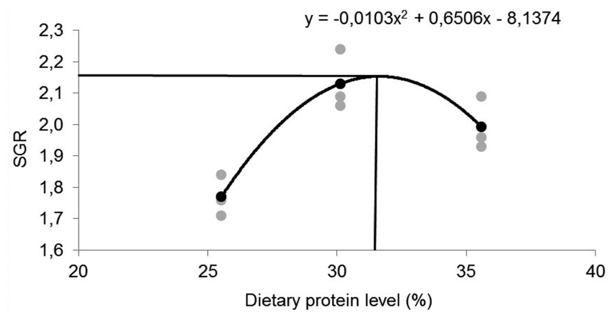

Figure 2. Second-order polynomial fitting of SGR to dietary protein level.

(P < 0.05). Regression analysis indicated that the maximum SGR occurred at a 31.6% protein level (Fig. 2).

Mortalities in groups usually occurred following handling and can be attributed to manipulation stress. However, fish mortality in the 25% dietary protein group was recorded mostly during the last period of the experiment and not following handling. This could also be caused by manipulation injuries or could be attributed to some extent to other, unknown stress factors.

Biomass was similar until day 51, but this diverged thereafter. The effect of dietary protein on final yield in the sea-cage culture of thick-lipped grey mullet was highly significant (P < 0.05) when the fish were fed 30% dietary protein. Fish growth was depressed when dietary protein was decreased below or increased above this protein level. Lower feed conversion (1.61) ratios were detected in the fish fed the diet containing 30% dietary protein, and on either side of this level, feed conversion ratio (FCR) deteriorated with either increased or decreased protein levels. Protein efficiency ratio (PER) ranged from 1.85 to 2.46 and was negatively correlated with dietary protein.

Discussion

Comparisons of studies are complicated by experimental variations including those regarding fish species, fish sizes and ages, protein quality, diet formulations, culture systems, duration, salinity, and stocking density and also by mullet diet variation depending on species, age, location, and season (Brusle 1981). In a previous study, the growth rates of *L. macrolepis* and *M. cephalus* fed 40% protein diets ranged from 0.1 to 0.3 g day^{-1} in cages in brackish water and in a power plant cooling (Jones and Strawn 1982, 1983); whereas the growth of fish was 0.4 g day^{-1} in our study. This outcome was attributable to salinity, since growth and feed utilization have been found to increase, and feed conversion ratios to decrease, with increasing salinity (up to 36‰) in mullet (El-Sayed 1991). Nevertheless, in tanks (30‰ seawater), Baker et al. (1998) reported an SGR of 1.51% day^{-1} for thick-lipped grey mullet (11-30 g body weight) fed a 38% protein diet.

Davies et al. (1997) reports higher SGR (3% day^{-1}) when thick-lipped grey mullet were fed a diet with 47% protein containing 68% fish meal in a seawater (30-33‰) aquarium system, whereas partial replacement of the fishmeal (33%) with seaweed meal (56%) resulted in decreased growth (2.4% day^{-1}). The amount of fish meal in the study cited above was still higher and the fish were smaller (1-9 g), which could be, in part, the explanation of some higher SGR when compared to the present study. In fact, growth (1.2% day^{-1}) was not improved by the addition of 85% fish meal to the diet (38-59% protein) of thick lipped grey mullet (15.2-41.6 g) in seawater tanks (Ojaveer et al. 1996). Wassef et al. (2001) obtained comparable growth rates (2.1-2.4% day^{-1}) to those of the present study with a 41% protein diet (fishmeal = 20-35%) in *M. cephalus* (6.4 g) reared in fixed cages in a pond (salinity, 8‰), but the stocking rate was six times less than that in the preset study. The mullet species seemed to respond differently individually to the same vegetable proteins (Wassef et al. 2001, Luzzana et al. 2005, El-Dahhar et al. 2013), and the different amino acid profiles of vegetable proteins could partially explain these differences. The results of the present study indicate that the diet containing 20% fishmeal and 30% soybean seems to be the most economical and appropriate than other diets for rearing fish in sea-cage culture as profitability depends mainly on the cost of feed production, feed conversion, and fish growth and survival rates. Our results are also in broad agreement with those of other authors who found that dietary protein levels between 28-35% induced optimum growth and feed utilization in mullet species under laboratory conditions (Lin et al. 1998, Argyropoulou et al. 1992, Yoshimatsu et al. 1992). Additionally, these early studies clearly recognized that there is a trend toward decreasing dietary protein requirements with age. Dietary protein levels of 30-40% were determined to be optimal for growth and feed conversion in the production of *L. ramada*, *M. platanus*, and *L. macrolepis* (up to 1.5 g) at different salinities ranging from 0 to 36‰ (El-Sayed et al. 1991, Carvalho et al. 2010). On the other hand, optimal dietary protein requirement of *M. capito* juveniles up to 10 g ranged from 24 to 36% under full seawater conditions (Papaparaskeva-Papoutsoglou and Alexis 1986). The present study revealed that the growth of thick-lipped grey mullet (40 g body weight) fed with a 30% protein diet was comparable to that of fish fed with higher protein diets in sea-cages.

The type of culture method (raceway, cage, open ponds, etc.) can also affect optimum protein intake of fish species because nutrient requirements determined for certain nutrients under optimum conditions increase when fish are exposed to environmental conditions such as poor water quality, waves, rain, stress, pathogens, etc. (Izquierdo and Robaina 2000). Since extreme variations were not observed during the experimental period, rearing environment could be another promising factor affecting growth production. Also, unlike most of previous experiments conducted indoors (e.g., in aquariums or tanks), our study was undertaken in net sea-cages where a portion of the feed delivered could have been lost (even if it was minimized) because of currents. FCR, which was calculated using the amount of feed offered rather than the actual intake, therefore, should be lower if exact feed intake data is to be obtained. In the present study, the feed efficiency reached a plateau with a protein level of 30-35%. Such trends have been reported previously for mullet species (Papaparaskeva-Papoutsoglou and Alexis 1986, El-Sayed 1991, Carvalho et al. 2010). The utilization of dietary protein for fish is linked with the balance between dietary protein and non-protein energy; therefore, excess protein in the diet is catabolized as an energy source (Carvalho et al. 2010), or it can depress growth because of higher energy requirements (Siddiqui and Khan 2009). Although our 35% protein diet had a slightly higher protein to carbohydrate ratio, which Ojaveer et al. (1996) also reports to promote fast growth in thick-lipped grey mullet, growth rates were not significantly different between the 30 and 35% dietary protein groups. Digestibility and energy content had variable effects on fish performance (Trung et al. 2011). In our study, growth depression was observed in the fish fed carp feed (36.5% protein). This might be explained by the composition of the carp feed, which contained high levels of indigestible fibre and/or low levels of dietary lipid concentration. Dietary lipids can be considered to be a better source of non-protein energy than carbohydrates in mullet species and in other fish species (Jones and Strawn 1983, Argyropoulou et al. 1992). The FCR in the present study was superior to those reported previously (1.8-4.9) for mullet species (Jones and Strawn 1983, Papaparaskeva-Papoutsoglou and Alexis 1986, Davies and Brown 1997, Barman et al. 2005). Moreover, FCR increased in the fish group fed carp feed, but it was still considerably better than those observed in previous studies on mullet species fed carp, trout, or seabream feeds in sea-cages, ponds, and recirculating aquaculture systems (Höner et al. 1989, Sanchez et al. 1993, Cardona and Castello 1995, Ağirağac and Kalma 1999). In the present study, the availability of natural plankton food in the sea-cages could also explain the favorably lower FCR. Further investigation is required to better understand the effects of mullet feeding on plankton depending on stocking density in sea-cages. The variations could also be attributed to water temperature, which affects feed consumption (Soderberg 1992, Watanabe et al. 2001), and was in the range (20-32°C) for the optimum growth of mullet species (Marin and Dodson 2000, El-Dahhar et al. 2013) in the present study. PER was the highest when the dietary protein requirement was met and appeared to decrease with increasing dietary protein content at the same energy level. Similar trends are reported for mullet and other species (El-Sayed 1991, Carvalho et al. 2010). The present findings also indicated a considerable reduction in PER with carp feed. An increase in the dietary energy level at lower dietary protein levels improves the efficiency of protein utilization and retention (Ali et al. 2008). Therefore, this reduction in carp feed probably results from the low lipid content or the types of vegetable proteins, as is mentioned above. However, the observed PER values were considerably higher than those reported previously (1.16-0.93) for *C. labrosus* and *M. cephalus* (Davies and Brown 1997, Wassef et al. 2001).

In conclusion, it is increasingly necessary to diversify aquaculture products with a focus on developing open sea and sustainable aquaculture in the face of threats from pollution risks and limited freshwater sources. The current findings indicate that 30% dietary protein can be utilized in the sea-cage farming of mullet species without any pathological symptoms or mortality. Further studies should be conducted to investigate the effect of diet quality and feed utilization when rearing market-sized mullet in cages at high stocking densities.

Author contributions. M.A. conceived the study and designed with O.Ö., who supervised the research work. M.A. performed the experiment, collected and analyzed the measurements, and wrote the paper.

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