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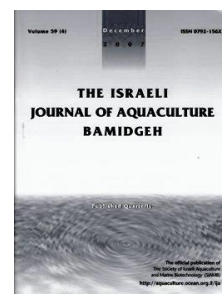
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The Effect of Dietary Lipid on the Growth Performance of Meagre (*Argyrosomus regius* Asso, 1801).

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Keywords: Meagre, *Argyrosomus regius*, Lipid, Development, Growth Performance

Abstract

The aim of the study was to investigate the effects of dietary lipid on the growth and feed utilization of Meagre (*Argyrosomus regius* Asso, 1801), taking into account their feeding behavior of collecting food from the bottom of the cages. The fish (141.07±0.5g, average weight ± SD; 22.18±0.53 cm, average total length± SD) were fed three isonitrogenous experimental diets (45% crude protein, dry matter) containing 16% (group A), 18% (group B), and 20% (group C) crude lipids for 570 days. The fish were stocked into 9 net cages (16 m diameter; 7 m deep) at a density of 16000 fish per cage with 2 replications. At the end of the experiment fish in the A, B, and C groups reached 1054.59±5.9, 1026.32±4.3, 955.31±2.3 mean live weight (g) and 45.78±1.6, 44.43±1.4, 43.88±1.4 mean total length (cm) respectively. FCR and CF values were 1.99, 2.07, 2.14 and 1.999, 1.131, 1.170 respectively for each group, at the end of the study. VSI, HIS, and GSI values were also calculated. Growth rate in fish from group A (fed the lowest lipid diet) was superior to the other dietary groups. Cross sections of their liver were checked and were found to have less lipidosis.

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Introduction

Associations such as World Health Organization (WHO) and the Food and Agriculture Organization (FAO) predict that the aquaculture sector has the highest potential of all production systems to provide food requirements in the future. The culture of alternative species to sea bass and gilthead sea bream which currently have been very important for Mediterranean aquaculture has expanded. Meagre (*Argyrosomus regius*) has become a preferred species due to its fast growth, good feed conversion ratio, high environmental tolerance traits and its moist and tasty low fat white meat (Quémèner, 2002; Chatzifotis et al. 2010).

Meagre (*A. regius*, Sciaenidae) is a carnivorous and semi-pelagic species that lives close to the bottom or near the surface. This fish migrates into lagoons for feeding and reproduction in summer and returns to deep waters in winter (Jiménez et al., 2005; Chatzifotis et al., 2011). Meagre can grow up to 2 meters long and weigh over 50kg. Meagre populations have been observed along the Mediterranean, the Sea of Marmara, along the west coasts of the Black sea, the Atlantic coasts of Europe, in the Red Sea, and in the Indian Ocean. This species can grow in temperatures of between 10°C-28°C, while optimal growth occurs between 19-26°C. In this temperature range it can reach a body weight of up to 1 kg at the end of the first year and up to 2 kg at the end of the second year (Duncan et al., 2008). Meagre aquaculture began in France in 1996 with fingerlings and in 1997 first commercial production occurred. Italy followed France in 2002 (FAO, 2005).

The nutritional requirements of Meagre are not well established and as a result sea bass and gilthead sea bream feeds are used in the aquaculture of this species (Roo et al., 2010; Chatzifotis et al. 2010; Velazco-Vargas et al., 2014). Meagre has low fat content (5% fat content of total body weight), while protein content is between 10-20% of total body weight (Poli et al., 2003, Fernandes, 2013). Studies suggest that protein levels of Meagre feed should be between 42-46% and lipid levels should not exceed 17% (Poli et al., 2003; Chatzifotis et al., 2010; Grigorakis et al., 2011; Martínez-Lorenz et al., 2011; Antonopoulou et al., 2014). Recommendations of 50% protein level and 17% lipid level for fish of 20-25 g live weight have been given (Chatzifotis et al., 2012). Lipids are required to spare the usage of proteins. However, when lipid levels in the feed exceed the optimum fat intake levels, growth decreases with a concomitant decrease in feed consumption and an increase in body fat content (Chatzifotis et al., 2010). Low dietary lipid tolerance for Meagre is in agreement with other findings (Antonopoulou et al. 2014).

In spite of improved understanding of the dietary requirements of Meagre, a diet specifically designed for this species is unavailable. The objective of our study was to develop a diet and nutrition protocols for this species. Apart from the effect of the above dietary regime on the Meagre growth performance, feed intake, liver fat retention, and market size (1000g) were also determined.

Materials and Methods

Experimental Diets. Three isonitrogenous extruded diets of 45% crude protein (CP) were formulated to provide three dietary lipid levels of 16 (group A), 18 (group B), and 20% (group C). Nutritional composition of the experimental diets is presented in Table 1. Extruder feeds were produced in 3,5,6,8, and 10 mm diameter by a commercial feed company (Abalioglu Feed Company). The fish were fed *ad libitum* twice and daily feed intake was recorded. All diets contained fish meal and fish oil, soybean by-products, cereals, vitamins, and minerals.

Table 1. Nutritional composition of experimental feeds.

Group	Humidity %	Protein %	Cellulose %	Lipid %	Ash %	Starch %
45/16 (A)	7,4	44,9	1,6	16,0	9,4	10,2
45/18 (B)	8,1	45,2	1,2	18,2	10,6	8,4
45/20 (C)	7,2	45	1,4	20,1	9,8	8,6

Experimental feeds were formulated using ingredients from 55% animal and 28% plant sources, 7-8% fish fat, 7-8% vitamin and mineral premixes, and 2% feed additive agents.

Fish and Experimental Settings. The study was carried out on a private farm located in Çandarlı-Denizköy, Izmir, near the Aegean Sea. Experiments were carried out on Meagre, supplied by that farm, with initial body weight (WW) of 141.07 ± 0.5 g, and 22.18 ± 0.53 cm mean total length. The fish were stocked in 9 net cages (16m diameter and 7-8m depth). Stocking density in each of the 9 cages was 16,000 fish/cage. The experiment was carried out with 3 repetitions per dietary treatment (A, B, and C). Experimental period was 19 months (570 days), from June 2013 until December 2014 until fish reached an average of 1000 ± 5 g (WW).

Sampling and Analysis. Initial biometric measurements (live weight-g and total length-cm) were performed using precision balance (± 0.01 g) and length (± 1 mm). These measurements were repeated once a month during the experimental period. For sampling, 200 fish from each group were measured after being anesthetized using phenoxy-ethanol (0.3ml/l). Mortalities and daily food consumption were recorded (see Table 2).

Temperature and soluble oxygen were measured 3 times a day at 2-3 meters depth; pH and salinity were measured weekly; nitrite, nitrate, and water phosphate content was measured monthly. At the end of the feeding trial the following indices were calculated: (De Silva and Anderson 1995; Korkut et al., 2007).

Condition factor (CF) = body weight (g) / (total length (cm))³.

Specific growth rate (SGR) = (ln(final body weight / initial body weight) / days of the experiment) × 100.

Feed conversion ratio (FCR) = feed consumed (g) / wet weight gain (g).

Hepatosomatic index (HSI) = (liver weight (g) / whole body weight (g)) × 100.

Viscerosomatic index (VSI) = (viscera weight (g) / whole body weight (g)) × 100

Gonadosomatic index (GSI) = (gonad weight (g) / whole body weight (g)) × 100

Histological Examination. About 50 fish were taken from each repetition (cage) on day 0, 360, and 570 for further postmortem examinations including paraffin block preparation. Other tissues samples were removed from fish immediately after their euthanization and preserved in neutral buffered formalin (10%) and Bouin's solution. They were processed according to routine techniques for microscopy. The fixed samples were gradually dehydrated and embedded in paraffin. Paraffin blocks were sectioned (Reichert-Rotary) at 6µm thickness and stained with Harris' hematoxylin-eosin (Luna, 1982). All slides were examined with an Olympus Microscope and photographed for the determination of lipid droplets in liver cells, and condition of pancreatic tissue.

Statistical Analyses. 200 fish were sampled from each cage during the experiment for biometric measurements. Randomly sampled fish were released back into their environment after the monthly measurements. Sampling error and confidence interval were 0.05 and 95% respectively. Data were analyzed by one-way Analysis of Variance (ANOVA) to test the effects of the three experimental diets on Meagre growth performance.

Results

Water temperature and soluble oxygen values varied seasonally from 11.9 in February to 26.2°C in August, and 5.38 to 7.96 mg/L, respectively throughout the experiment. The mean pH value was 7.37 ± 0.12 and the mean salinity was 35.68 ± 0.5 ppt during the entire study period. Mean ammonium (0.13 ± 0.08 mg/L), nitrate (0.1 ± 0.09 mg/L), and phosphate (0.006 ± 0.005 mg/L) were determined.

Significant differences in growth, feed utilization, and biometric parameters of fish were observed depending on the lipid level in the feed (Table 2). Fish in groups A and B fed with the 16-18% lipid diets showed similar growth rates and feed conversion rates. On the other hand, growth performance on group C was significantly different ($P < 0.05$) from the other dietary treatments. Total length and SGR ($P < 0.05$) were independent of

the dietary treatments. VSI values were significantly different between all groups. Groups A and B had similar HSI and GSI values, while the C diet group values were significantly different ($P < 0.05$).

	Group A	Group B	Group C
Initial L. weight (kg)	141.52±0.6	142.35±0.4	140.84±0.4
Final L. weight (kg)	1062.91±7.2 ^b	1027.38±6.8 ^b	948.62±3.6 ^a
Gained L. Weight(Kg)	921.39 ^b	885.03 ^{ab}	807.78 ^a
Initial Total Length (cm)	21.97±0.3	22.38±0.7	22.08±0.7
Final Total Length (cm)	45.18±1.7	45.78±1.8	43.98±1.6
Total Length Difference (cm)	23.21	23.4	21.32
Mortality %	8.85	8.59	8.86
FCR	1.99 ^a	2.07 ^a	2.15 ^b
SGR	0.328	0.321	0.309
VSI	6.89 ^a	9.04 ^c	8.69 ^b
HSI	2.07 ^a	2.26 ^a	2.61 ^b
GSI	1.04 ^a	1.07 ^a	1.29 ^b
CF	1.096 ^a	1.162 ^c	1.135 ^b

Table 2. Mean (\pm SEM) growth, feed utilization, and biometric parameters of Meagre fed the various diets. Values having different superscripts were significantly different ($P < 0.05$).

Group A fish, (low lipid diet), had reduced adiposity at the end of the experiment, compared with fish fed the other higher lipid diets (B,C groups). It was determined that at the beginning of the experiment all groups

would have the same, small diameter lipid droplets. Group A had smaller diameter lipid droplets on 360th and 570th days. Other groups had larger diameter oil droplets on the same days. As a result, lipid accumulation in the liver samples in group B and C were found to be more substantial than group A.

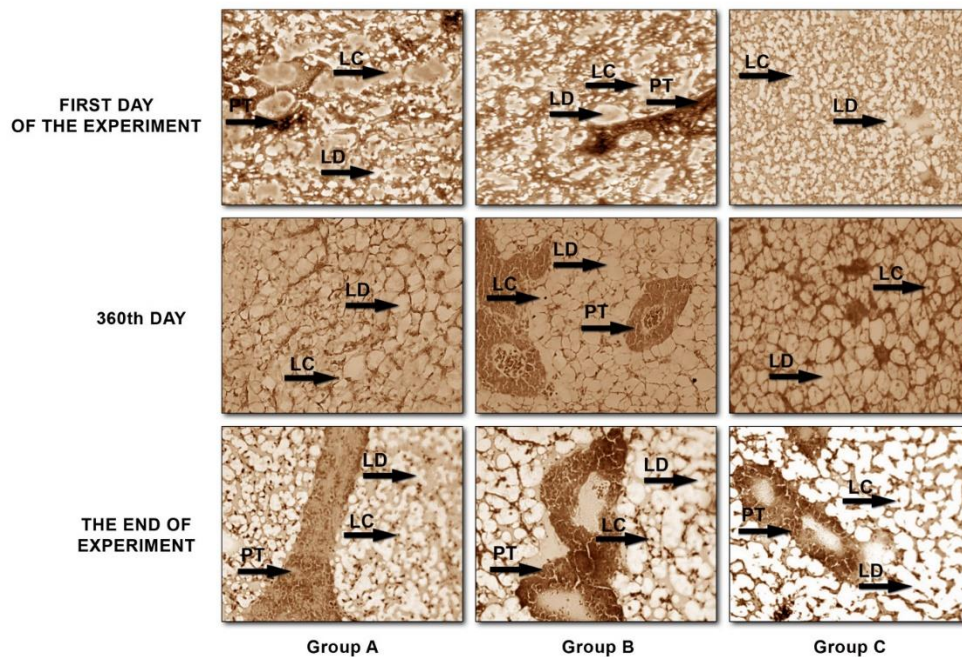


Figure 1. Liver fractions taken from the experimental groups on day 0, 360, and 570. Liver cells (LC), Lipid Droplets in Liver cells (LD), Pancreatic Tissue (PT).

Discussion

FAO (2005) recommends water temperature of 17-21°C for meager; when it is lower than 13-15 degrees, feeding activity of these fish significantly decreases. During our experiment water temperature was at the optimum level recommended by FAO 2005, except in January and February. Similarly, Quémener (2002) indicated that the most appropriate water temperatures for the growth of Meagre were 16-20°C. Average pH was 7.37 and average salinity was 0.3568‰ for the current study. Another study suggested that a mean pH value of 7.5 was optimum for the growth of Meagre (El-Shebly *et al.*,

(2007). Other water quality values obtained during our study were similarly appropriate for the aquaculture of this species. The concentration of NH₃-N under 0.05mg/L, NO₂⁻-N levels under 1.0 mg/L and nitrate (NO₃⁻-N) concentration under 10 mg/L, were suggested (Pillay and Kutty, 2005).

Initial average live weight of Meagre was 141.07±0.5g and they reached a marketable size of 1000±5g 19 months later. In the present experiment the SGR of the Meagre was 0.324%/day, which was lower than results from other studies. Meagre with initial weight of 178g reached 410g in 5 months (SGR 0.57 %/day) (Calderón et al., 1997); Meagre at an average initial weight of 95g reached 393g (SGR 1.2 %/day) at the end of 6 months after they were fed diet composed of 47% CP and 20% CL at 23 °C (Martínez-Lorens et al., 2011). Meagre reached 1850g (SGR 1.17%/day) from 110g initial weight in 8 months (Gracia et al. 2002). Growth rate SGR (2%/day) was found for Meagre with 23.5g initial weight compared to other studies when the fish were kept in tanks at 19°C (Chatzifotis et al., 2012). The latter study demonstrated that Meagre kept in cages grew significantly faster. Water temperatures change seasonally for cage experiments and feed conversion performance varies seasonally being generally higher in summer and lower in winter. Meagre in cages reached 821.5g in 15 months (SGR 0.92%/day) from the 12.8g initial weight (Piccolo et al. 2008). Similarly, in our study, fish reached marketable size in 19 months. This study was performed in tanks or cages of a commercial business. For this reason, the results obtained may vary according to the results gathered from those tanks or cages. The content of fish feed used in other studies should also be evaluated in this manner.

Lipid accumulated in the liver and visceral organs when the fish were fed a high lipid diet (Spisni et al., 1998). VSI and HSI values were checked in order to determine the impact of lipid levels used in this study. VSI (6.88, 9.09, and 8.33, respectively for the groups) was found significantly different for all treatment groups (A, B, and C). VSI values of 2.62, 2.53, and 2.78 respectively were calculated for Meagre weighing 230g and fed diets containing 13, 17, and 21% lipid in an experiment lasting 110 days. The lipid ratio in the feed did not affect retention values in the liver, although VSI values were different (Chatzifotis et al., 2010). Increase in cell numbers (Figure 1) and variation depending on the lipid degeneration were predominantly found in group C, and partly in group B at the end of histological investigations. VSI values have also supported this result. HSI values were close in groups A and B; the highest value was found in the group fed the 20% lipid diet. High lipid content in the feed increased HSI ratio in sea bass (Péres and Oliva-Teles, 1999).

In the present study, GSI values were similar in groups A and B, but higher in group C. In wildstock in natural conditions, male Meagre reach maturation when they are 45-62 cm long while females reach sexual maturity when they are 47-70 cm long (González-Quirós et al., 2011). In captivity, Meagre reach sexually maturation at earlier stages, (27cm for 2 year old males and 36cm for 3 year old females) in contrast to natural conditions (Mylonas et al. 2013). In our findings the total length difference of groups A, B, and C were: 23,21, 23,4, and 21,32 cm respectively. In an evaluation of monthly gonad development of Meagre it was found that nutrition affected gonad development (Gil et al., (2013). While nutritional conditions do affect the stage of maturity in Meagre the effect of dietary composition on gonadal maturity is still under-investigated.

Condition factor (CF) indicates healthy development of fish. This factor may vary yearly for fish according to their nutritional condition (Chatzifotis et al., 2010). This study presented variations in CF depending on the lipid levels in feed and there were significant differences between the groups. In other studies however there were no differences in CF in different groups (Poli et al., 2003; Piccolo et al., 2006; Chatzifotis et al., 2006)

Liver lipidosis in fish is commonly associated with high dietary lipid/energy that expresses itself in excessive triglyceride retention in vacuoles, and morphologic changes in the liver (Antonopoulou et al., 2014). These changes result in liver dysfunction, often with resulting mortality (Spisni et al., 1998). In this study, lipid droplets significantly increased (P<0.05) in group C (fed the highest, 20% lipid, diet). Lipidosis in aquaculture

is a result of high lipid content in aquafeeds (Spisni et al., 1998). Meagre are typified by reduced fat in their muscle tissue, thus they cannot tolerate high lipid ratio in feeds (Poli et al., 2003).

According to previous studies the suggested optimal protein level in feeds for Meagre is 42-46%, and lipid levels should not exceed 17% (Poli et al., 2003; Chatzifotis et al., 2011; Grigorakis et al., 2011; Martínez-Lorenz et al., 2011). According to the results of the current study, the best growth performance and food conversion ratio were found in group A, fed feed containing 45% protein level and 16% lipid level. This study complements knowledge obtained from growth experiments to appraise the nutritional status of Meagre.

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