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Effects of *Ulva* Meal on Growth Performance of Gilthead Seabream (*Sparus aurata*) at Different Levels of Dietary Lipid

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Abstract

A nutrition trial was carried out to investigate the effects of inclusion of *Ulva* meal at different dietary lipid levels on growth performance, feed utilization and body composition of juvenile gilthead seabream, *Sparus aurata*. In this study, gilthead seabream with an average weight of 8.5±0.01 g were fed experimental diets for 7 weeks. The Eight isonitrogenous diets (crude protein 50%) were designed to provide 13% (13), 16% (16), 19% (19) and 22% (22) crude lipid with or without 4% inclusion of Ulva meal (13U, 16U, 19U and 22U). Final mean weight and specific growth rate (SGR) of gilthead seabream fed 22L-U were higher than that of 13L and 13L-U groups. The mean feed conversion ratio varied from 0.92 (22L-U) and 1.33 (13L and 13L-U). The present study shows that dietary low level (4%) Ulva meal at all dietary lipid levels could be used in juvenile gilthead seabream without causing any adverse effects on growth and feed utilization.

Keywords: Ulva meal, lipid, growth performance, gilthead seabream.

Farklı Seviyelerde Yağ İçeren Ulva Yeminden Hazırlanan Diyetin Çipura (*Sparus aurata*)'nın Büyüme Performansına Etkileri

Özet

Bu araştırmada, farklı yem yağ seviyesinde yeme eklenen *Ulva* ununun yavru çipuraların (*Sparus aurata*)'un büyüme performansı, yem kullanımı ve vücut kompozisyonu üzerine etkileri çalışılmıştır. Çalışmada, yaklaşık 8,5±0,01 gr ağırlığında olan çipura balıkları 7 hafta boyunca deneme yemleri ile beslenmişlerdir. Sekiz adet izonitrojenik deneme yem, farklı yağ oranlarına (%13 (13L), %16 (16L), %19 (19L) ve %22 (22L)) ulva unu ilave edilmemiş (%0) ve ilave edilmiş olarak (%4) hazırlanmıştır. 22L-U yem grubu ile beslenen çipuraların son ortalama vücut ağırlığı ve spesifik büyüme oranı 13L ve 13L-U gruplarına göre önemli derecede yüksek bulunmuştur ($P<0,05$). Deneme gruplarının yem dönüşüm oranı 0,92 (22L-U) ile 1,33 (13L ve 13L-U) arasında değişim göstermiştir. Bu çalışmanın sonuçları, tüm farklı yağ düzeylerinde yeme düşük oranda (%4) Ulva unu ilavesinin yavru çipuraların büyüme ve yem kullanımı olumsuz yönde etkilemeden kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Ulva unu, lipit, büyüme performansı, çipura.

Introduction

Gilthead sea bream, *Sparus aurata* are the one of the most important and successfully farmed Mediterranean fish species. The total global production volume of reared gilthead sea bream has increased with two folds over the past decade, from 82,152 tons in 2001 to 154,821 tons in 2011 (FAO, 2013). As a result, sea bream has been described as the most important aquaculture species of the twenty-first century. It is essential to reduce the cost of production for the future progress of sea bream culture. Feed cost represent more than half of the total production cost in sea bream and any changes in the cost of feed or feed ingredients will have a relatively huge impact on the total cost production. Therefore, it is critical to preserve the price of finfish diet as low as possible whereas aquafeed provide indispensable nutrients to fish for maximum growth and minimum environmental impacts (Glencross *et al.*, 2007; Tacon and Metian, 2008).

Gilthead sea bream do not appear to use high dietary lipid rates as efficiently as rainbow trout and salmon. Lipids are one of the important nutrients for carnivorous finfish (Sargent *et al.*, 2002). Dietary lipid is a source of energy for metabolism, indispensable fatty acids, and fat soluble vitamins for finfish and to reduce undesirable environmental effects from fish farm effluents (Watanabe, 1982). However, the increase of dietary lipid levels can alter body lipid composition, flesh quality and final consumer's acceptance. Thus, optimal dietary lipid level is important for growth and final product quality. Recently, some feedstuffs and feed additives used to enhance lipid metabolism to decrease body lipid and improve carcass quality (Nakagawa and Montgomery, 2007).

Some dietary macroalge meals are improved the growth, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality of various fish species (Ergün *et al.*, 2009; Güroy *et al.*, 2011, 2013). Inclusion of dietary low level *Ulva* meal has been found to enhance growth performance and lipid deposition for several fish species including rainbow trout *Oncorhynchus mykiss* (Güroy *et al.*, 2011) and tilapia *Oreochromis niloticus* (Güroy *et al.*, 2007; Azaza *et al.*, 2008; Ergün *et al.*, 2009). *Ulva* is a good source of protein, pigments, minerals and vitamins, especially rich in vitamin C (Ortiz *et al.*, 2006; Garcia-Casal *et al.*, 2007). Vitamin C promotes lipid metabolism thus, may reduce carcass lipid and increase protein levels (Ji *et al.*, 2003). To the authors' knowledge there is no literature to date concerning the use of *Ulva* meal in diets at different lipid levels for gilthead seabream. Therefore, the aim of the present study was to evaluate the effects of *Ulva* meal at different dietary lipid levels on the growth response and body composition of gilthead seabream, *Sparus aurata*.

Materials and Methods

Diet Formulations

Ulva rigida was freshly collected from the near-shore waters of Armutlu, Yalova-Turkey. Algal samples were thoroughly washed with sea water, dried at 40ºC for 48 h, and fine-milled with a laboratory blender to produce raw *Ulva* meal. The experiment was a 2 × 4 factorial with two dietary *Ulva* meal concentrations (0 and 4%) and four dietary lipid concentrations (13, 16, 19 and 22%). Eight experimental diets were prepared, and 13L, 16L, 19L and 22L diets were formulated to contain 13%, 16%, 19% and 22% crude lipid without *Ulva* meal supplementation. In the supplementary group, 4% *Ulva* meal were added to formulate 13L-U, 16L-U, 19L-U and 22L-U diets with 13%, 16%, 19% and 22% crude lipid.

All diets were formulated to contain 49.9% (50%) crude protein. The formulation and chemical composition of the experimental diets is displayed in Table 1. Dietary ingredients were mixed in a food mixer (Dirmak Food Equipment, Turkey, model no: IBT-22) with water (around 50°C) until a soft slightly moist consistency was achieved. This was then cold press extruded (PTM P6 extruder, Yalova, Turkey) to produce a 2 mm pellet. The moist pellets were then fan-dried and stored frozen at - 20 °C until use.

Experimental Facility And Fish

The nutrition trial was carried out at the

Table 1 Formulation and proximate composition of experimental diets (%)

	13L	13L-U	16L	16L-U	19L	19L-U	22L	22L-U
Fish meal[1]	62.0	62.0	62.0	62.0	62.0	62.0	62.0	62.0
Ulva meal[2]		4.0		4.0		4.0		4.0
Corn gluten[3]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Starch[3]	11.0	11.0	8.0	8.0	5.0	5.0	2.0	2.0
Wheat feed[4]	9.0	5.0	9.0	5.0	9.0	5.0	9.0	5.0
Fish oil[5]	6.0	6.0	9.0	9.0	12.0	12.0	15.0	15.0
Vitamin mix[6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[7]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Cholin chloride[8]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Proximate Composition								
Crude Protein (%)	49.79	49.69	49.78	49.67	49.76	49.66	49.75	49.64
Crude Lipid (%)	12.97	12.92	15.95	15.90	18.93	18.89	21.92	21.87
Crude Ash (%)	9.10	9.07	9.08	9.05	9.06	9.03	9.04	9.01
Crude Fiber (%)	0.75	0.67	0.70	0.62	0.65	0.57	0.60	0.52
Nitrogen Free Extract (%)	25.38	21.27	22.48	18.38	19.59	15.48	16.69	12.58
Gross Energy (MJ/Kg)	21.29	20.54	21.97	21.22	22.64	21.89	23.32	22.57

[1] Anchovy fishmeal. Agromey Feed Mill Company, İzmir, Turkey.
[2] *Ulva rigida* was freshly collected from the near-shore waters of Armutlu, Yalova-Turkey.
[3] Cargill, İstanbul, Turkey.
[4] Agromey Feed Mill Company, İzmir, Turkey.
[5] Anchovy fish oil. Agromey Feed Mill Company, İzmir, Turkey.
[6] Per g mixture: vitamin A: 342 IU; vitamin D3: 329 IU; vitamin E: 0.0274 IU; vitamin K3: 5.48 mg; vitamin B1: 2.05 mg; vitamin B2: 3.42 mg; vitamin B3: 20.5 mg; vitamin B5: 5.48 mg; vitamin B6: 2.05 mg; vitamin B12: 2.74 mg; vitamin C: 24.0 mg, biotin: 0.411 mg; folic acid: 0.685 Agromey Feed Mill Company, İzmir, Turkey.
[7] Per g mixture: mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Agromey Feed Mill Company, İzmir, Turkey.
[8] DSA Agrifood Products, Ankara, Turkey

Beymelek Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. Fish were obtained from a base population of juvenile grown in the institute's hatchery. Before commencement of the experiment; the fish were acclimatized to experimental conditions for two weeks. During the adaption period, fish were fed a sinking extruded commercial sea bass diet with 46% protein and 19% lipid (Bioaqua, Çamlı Yem, İzmir, Turkey). At the start of the trial, fish weighing 8.50±0.01 g were randomly distributed into twenty four 200L square fiberglass tanks at 20 fish per tank.

Water parameters such as temperature, dissolved oxygen and pH were monitored daily using the OxyGuard Handy Gamma DO meter (DK-3460, Birkerod, Denmark; Deviation 9) and the Testo pH meter (Testo, Vic., Australia), respectively. Fish were subjected to the natural photoperiod June to August at 36°15'.160"N latitude and 30°2'.397'°W longitude, and ranged from 14 h light: 10 h dark to 13 h light: 11 h dark. (36º16'N - 30º03'E) Throughout the study, an 8-L/min flow of sand filtered sea water was supplied to each tank to ensure suitable water conditions. Water parameters were pH 7.85±0.01, salinity 35.1±0.11 ppt, temperature 26.50±1.23°C, dissolved oxygen 5.80±0.48 ppm and ammonium level between 0.01 and 0.03 mg l^{-1}. Fish were weighed in bulk biweekly after suspending feeding for 1 day. Fish were fed near satiation by hand at 08:00 and 16:00 hours for 7 weeks. Feed was carefully administered by dropping a few pellets until the feeding activity ceased.

Sampling and Proximate Composition

Six fish were randomly taken from the initial pool of fish at the beginning of the experiment and two fish from each tank (six fish per treatment) were sampled at the end of the trial to determine whole body proximate analysis. Samples were kept frozen at -20°C until analysis.

At the end of the feeding trial, all fish were starved for 48 h to ensure that the digestive tract was devoid of feed. Analysis of crude protein, moisture, fiber and ash in the whole body of fish and the diets was performed according to standard AOAC (2000) procedures. Dietary and whole body lipids were extracted according to the procedure of Soxhlet methods. Nitrogen-free extract (NFE) was calculated by taking the sum values for crude protein, lipid, ash and crude fiber and then subtracting this value from 100. The gross energy content of the diets and fish was calculated using the conversion factors of 23.7 kJ g^{-1} for protein, 39.5 kJg^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate (Brett and Groves, 1979).

Fish Performance and Somatic Indices

Fish growth performance and nutrient utilization were calculated according to the following formulae:

Feed Conversion Ratio (FCR) = FI/WG
Specific Growth Rate (SGR) (%) = 100((ln FBW−ln IBW)/T)
Protein Efficiency Ratio (PER) = WG/PI
Net Protein Utilisation (NPU) (%) = 100(PG/PI)

Six fish were randomly taken from the initial pool at the beginning of the experiment and two fish from each tank (six fish per treatment) were randomly sampled at the end of the trial to determine somatic indices.

The somatic indices were calculated according to the following formulae:

Condition Factor (CF) = $100(FBW/(SL^3))$
Viscerosomatic (VSI) (%) = 100(VW/ FBW)

where FBW is the final body weight (g), IBW is initial body weight (g), FI = feed intake (g)

WG = weight gain (g), T = time (days), PI = dietary protein intake (g), PG = protein gain (g), SL = standard length (cm) and VW = viscera weight (g)

Statistical Analysis

All data were subjected to a two-way analysis of variance (ANOVA), considering dietary lipid level and *Ulva* meal level as variables. All means were compared by the Duncan multiple range test using the statistical software package Statgraphics 7.0 (Manugistics Incorporated, Rockville, MD, USA) (Zar, 2001). All percentage data and ratios were arcsine transformed before being subjected to the analysis. The results were treated statistically significant at the 5% level.

Results

The growth performance and nutrient utilization parameters for juvenile gilthead sea bream fed the experimental diets are shown in Table 2. No mortalities for all treatments were observed throughout trial. Final mean weight (FMW) ranged from 27.12 g (13L) to 28.77 g (22L-U) and was significantly higher in fish fed 50/22U than the 50/13 and 50/13U diets. FMW, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly ($P<0.05$) affected by dietary lipid levels (Table 2). The 22L-U diet displayed significantly lower FCR compared with fish fed 13L and 13L-U diets ($P<0.05$). Overall, best improvements in growth parameters measured were found in high lipid dietary groups. No significant differences were found in the net protein utilization (NPU) of gilthead sea bream fed the various experimental diets. Also, all growth parameters were not affected by dietary lipid concentrations and *Ulva* meal × dietary lipid interactions ($P>0.05$).

Data on biometric parameters of fish fed the

Table 2. Growth performance of gilthead seabream after 7 weeks of feeding on experimental diets (n=3)

	Dietary Treatments								ANOVA (P- Value)		
	13L	13L-U	16L	16L-U	19L	19L-U	22L	22L-U	Lipid	Ulva	LxU
IMW (g)	8.50± 0.02	8.51± 0.03	8.54± 0.02	8.46± 0.02	8.51± 0.01	8.51± 0.03	8.51± 0.01	8.51± 0.01	0.9331	0.4046	0.1571
FMW (g)	27.12± 0.28^{a}	27.27± 0.18^{a}	28.01± 0.27^{ab}	28.34± 0.48^{ab}	28.17± 0.23^{ab}	28.49± 0.37^{ab}	28.22± 0.66^{ab}	28.77± 0.72^{b}	0.0402	0.2914	0.9768
SGR	2.37± 0.02^{a}	2.38± 0.02^{a}	2.42± 0.02^{ab}	2.47± 0.03^{ab}	2.44± 0.01^{ab}	2.47± 0.03^{ab}	2.45± 0.05^{ab}	2.49± 0.05^{b}	0.0385	0.2183	0.9496
FCR	1.02± 0.02^{b}	1.02± 0.01^{b}	0.98± 0.01^{ab}	0.96± 0.02^{ab}	0.97± 0.01^{ab}	0.96± 0.02^{ab}	0.97± 0.03^{ab}	0.94± 0.03^{b}	0.0299	0.2742	0.9428
PER	1.96± 0.03^{a}	1.98± 0.02^{a}	2.05± 0.03^{ab}	2.10± 0.05^{ab}	2.08± 0.02^{ab}	2.11± 0.04^{ab}	2.08± 0.07^{ab}	2.14± 0.08^{b}	0.0372	0.2359	0.9578
NPU (%)	40.24± 1.14	36.19± 1.59	37.97± 2.50	36.47± 1.10	40.44± 2.03	37.49± 0.99	36.73± 2.87	35.55± 0.94	0.4423	0.0734	0.8413

IMW: Initial mean weight (g), FMW: Final mean weight (g), SGR: Specific growth rate (SGR;% day−1), FCR: Feed conversion ratio (FCR), PER: Protein efficiency ratio, NPU: Net protein utilization.

Table 3. Biological indices of gilthead seabream after 7 weeks of feeding on experimental diets (n=6)

	Dietary Treatments								ANOVA (P-Value)		
	13L	13L-U	16L	16L-U	19L	19L-U	22L	22L-U	Lipid	Ulva	LxU
CF	1.57± 0.09^{a}	1.56± 0.04^{a}	1.64± 0.05^{ab}	1.55± 0.05^{a}	1.69± 0.01^{ab}	1.67± 0.03^{ab}	1.66± 0.06^{ab}	1.78± 0.05^{b}	0.0313	0.9789	0.2676
VSI	4.41± 0.48^{bc}	3.51± 0.06^{a}	4.29± 0.17^{bc}	4.01± 0.12^{ab}	4.42± 0.09^{bc}	4.07± 0.12^{ab}	4.75± 0.02^{c}	4.51± 0.09^{bc}	0.0249	0.0693	0.0439

experimental diets are displayed in Table 3. Condition factor (CF) was significantly (P<0.05) affected by dietary lipid level (Table 3), while viscerosomatic (VSI) index significantly (P <0.05) influenced by dietary lipid and *Ulva* meal levels (Table 3). The CF of seabream fed the 22L-U was significantly higher than those fed 13L, 13L-U and 16L-U diets. The VSI tended to increase as dietary lipid levels increased and the highest levels were found in fish fed 22L (P <0.05). Fish fed 4% *Ulva* meal showed a decreased VSI compared with fish fed non-*Ulva* supplemented diets, irrespective of dietary lipid level.

The whole body proximate compositions of the gilthead seabream fed the experimental diets are given in Table 4. Moisture and ash contents of body were significantly (P <0.05) affected by dietary lipid levels. Protein content of body was influenced (P<0.05) by all treatments. Fish fed *Ulva* supplemented diets showed a decreased protein content of body compared with fish fed non-*Ulva* supplemented diets, irrespective of dietary lipid level. Lipid content of body was not significantly affected (P>0.05) by lipid levels, *Ulva* meal levels or its interaction.

Discussion

Macroalgae meal such as *Ulva*, *Ascophyllum*, *Porphyra*, *Sargassum*, *Gracilaria* and *Laminaria* has been used as a feed additive in aquafeed throughout the world. Dietary inclusion of 4% *Ulva* meal was used in our study could be used without significant negative effects on the growth performance, nutrient utilization, and body composition. Previous researches have declared that a low level dietary inclusion of *Ulva* meal (3–5 %) enhanced growth, feed utilization, immune response, and lipid mobilization in numerous finfish species such as gilthead seabream (Wassef *et al.*, 2005), tilapia (Ergün *et al.*, 2009), rainbow trout (Güroy *et al.*, 2011), mugil *Mugil cephalus* (Wassef *et al.*, 2001), black sea bream *Acanthopagrus schlegeli* (Nakagawa *et al.*, 1993) and snakehead *Channa striatus* (Hassan and Hashim, 1995). Other studies have shown that a 5% or 10% inclusion of dietary *Ulva* meal had no negative effects on the growth performance of juvenile European seabass *Dicentrarchus labrax* (Valente *et al.*, 2006), tilapia (Güroy *et al.*, 2007), and rainbow trout, *Oncorhynchus mykiss* of different sizes (Güroy *et al.*, 2013; Dantagnan *et al.*, 2009; Soler-Vila *et al.*, 2009).

Results of this study suggest that dietary low-level inclusion of *Ulva* meal can enhance growth performance of sea bass fed high-lipid diets. The observed weight gain double relative to the initial body weight over a 7 weeks period is quite acceptable for this species under the rearing conditions. The overall growth performance achieved in the current study (SGR from 2.37 to 2.49%/day) was higher than those establish by Emre *et al.* (2008) in juvenile gilthead seabream. The SGR of fish in the nutrition trial was comparable with results reported previously when juvenile sea bream were fed diets (Chatzifotis *et al.*, 2009).

The effects of dietary lipid levels on growth performance and nutrient utilization depend on fish species, fish size, dietary protein-to-lipid ratio, or capability of fish to use carbohydrates as an energy source, dietary protein and energy levels and feeding

Table 4. Body composition of gilthead seabream after 7 weeks of feeding on experimental diets (n=6)

	Dietary Treatments								ANOVA (P- Value)		
	13L	13L-U	16L	16L-U	19L	19L-U	22L	22L-U	Lipid	Ulva	LxU
Moisture	71.23± 0.83[b]	70.79± ±0.80 [b]	71.16± 0.38 [b]	71.39± 0.54[b]	69.59± 0.37 [ab]	69.79± 0.66[ab]	69.43± 0.26[ab]	69.06± 0.96[a]	0.0153	0.8348	0.9247
Protein	18.48± 0.51[c]	16.94± 0.46 [abc]	17.13± 0.70[abc]	16.38± 0.3 [ab]	16.68± 0.52[abc]	17.83± 0.67[bc]	16.55± 0.67[abc]	15.87± 0.67[a]	0.0967	0.2822	0.1654
Lipid	5.09± 0.71	5.58± 0.67	5.81± 0.50	6.21± 0.64	6.81± 0.36	6.13± 0.91	6.78± 0.80	6.57± 0.45	0.2141	0.9991	0.7873
Ash	5.13± 0.40 [b]	4.73± 0.20 [ab]	4.77± 0.07 [ab]	4.97± 0.10 [ab]	4.38± 0.14 [a]	4.33± 0.24 [a]	4.55± 0.14 [ab]	4.47± 0.14 [a]	0.0478	0.5963	0.6219

time (Sargent *et al.*, 2002; Kusku *et al.*, 2011). The optimum dietary lipid level may vary with species and size of fish. For instance, Peres and Oliva-Teles (1999) reported that increasing dietary lipid levels in sea bass did not promote fish performance, while Dias *et al.* (2003) declared that evidenced a growth increase with dietary lipids. In the nutrition trial, significant differences were observed in growth performance of juvenile gilthead sea bream with the dietary lipid levels from 13 up to 22%. Compared with lower lipid diet (13%), fish fed higher lipid level (22%) displayed improved weight gain, SGR, PER, and FCR. Similarly, Pereira *et al.* (1987) stated a beneficial effect of an increased dietary lipid level on growth performance of gilthead seabream and feed utilization as in sea bass (Metailler *et al.*, 1981). The supplementation of dietary *Ulva* meal resulted in greater benefits when fed with higher-lipid diets. In fact, higher lipid-*Ulva*-fed fish showed the best performance in all parameters measured.

Dietary lipid levels in fish feed is an essential source of energy, essential fatty acids and some fat-soluble vitamin (Sargent *et al.*, 2002). However, excessive dietary lipid can lead to reduced nutrient utilization resulting in poor fish performance. In the present study, body lipid was not significantly affected (P>0.05) by lipid levels, *Ulva* meal levels or its interaction. The effects of dietary algal meals on body lipid levels can be inconsistent; whereas some reports suggest a raise of body lipids (Mustafa *et al.*, 1995; Diler *et al.*, 2007; Ergün *et al.*, 2009), others suggest a decrease (Güroy *et al.*, 2007; Azaza *et al.*, 2008).

The present study shows that dietary low level (4%) *Ulva* meal could be used in juvenile gilthead seabream without causing any adverse effects on growth and feed utilization. Dietary *Ulva* meal at higher lipid levels clearly offers much potential for inclusion in diets for gilthead seabream and this preliminary study provides a basis for more extensive investigations.

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