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Effects of Long-Term Feeding with Dried Microalgae Added Microdiets on Growth and Fatty Acid Composition of Gilthead Sea Bream (*Sparus aurata* L., 1758)

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Abstract

The aim of the present study was to investigate the effects of replacing fish oil by different microalgae products on the growth performance and fatty acid composition of gilthead sea bream (0.02±0.00 g) during 90 days of post larvae and weaning period. Four diets were evaluated: control diet was exclusively based on fishoil; SBAE diet included the commercial product SBAE®, which consists of four microalgae: *Tetraselmis suecica, Isochrysis sp., Nannochloropsis oculata* and *Phaeodactylum tricornutum;* Algamac diet included the commercial product Algamac 3050, which consists solely of the microalgae *Schizocthytrium sp.*, and Mix diet that consisted of a mixture of both commercial products in equal amounts, SBAE® and Algamac®. At the end of the feeding trial, the highest weight gain was obtained in Control (1.34±0.00 g) and Mix (1.34±0.03 g) groups (P<0.05). The highest survival rates were found in Control and Mix diet groups, whereas the lowest was found in SBAE diet group. Experimental diets showed significant effects on the fatty acid composition of larvae. The total long-chain n-3 polyunsaturated fatty acid (LC-PUFA), eicosapentaenoic acid and docosahexaenoic acid levels were increased with inclusion of microalgae products. Based on the results of growth performance and fatty acid composition, fish oil substitution by a blend of equal amounts of microalgae products SBAE® and Algamac® 3050 seems to be promising in weaning diets for gilthead sea bream larvae.

Keywords: Gilthead sea bream, nutrition, microalgae, growth performance, survival, essential fatty acids.

Kurutulmuş Mikroalg İçeren Mikrodiyetler ile Uzun Süreli Beslenmenin, Çipura (*Sparus aurata*, L.,1758)' larda Büyüme ve Yağ Asidi Kompozisyonuna Etkileri

Özet

Bu çalışmanın amacı, çipura (0,02±0,00 g) post larval ve ön büyütme döneminde 90 gün süresince balık yağı yerine farklı mikroalg ürünleri kullanılmasının, büyüme performansı ve yağ asidi kompozisyonuna olan etkilerinin incelenmesidir. Kontrol yeminde yağ kaynağı olarak sadece balık yağı kullanılmıştır; *Tetraselmis suecica, Isochrysis sp., Nannochloropsis oculata* ve *Phaeodactylum tricornutum* olmak üzere 4 mikroalg türünden oluşan SBAE® ticari ürünü içeren SBAE yemi; sadece mikroalg *Schizocthytrium sp.*'den oluşan ticari Algamac 3050 ürünü içeren Algamac yemi ve her iki ticari ürünün eşit oranlarda karışımını içeren Mix yem olmak üzere toplam dört farklı deney yemi kullanılmıştır. Besleme deneyi sonunda, en yüksek ağırlık kazanımı Kontrol (1,34±0,00 g) ve Mix deney (1,34±0,03 g) gruplarında gözlenmiştir (P<0,05). En yüksek hayatta kalma oranları da Kontrol ve Mix gruplarında bulunurken, en düşük değer SBAE grubunda görülmüştür. Deneysel yemler, yavruların yağ asidi kompozisyonları üzerinde etkili olmuştur. Toplam uzun zincirli n-3 çok doymamış yağ asitleri (LC-PUFA), eikosapentaenoik asit ve dokosahekzaenoik asit seviyeleri, mikroalg ürünlerinin kullanılmasıyla artış göstermiştir. Büyüme performansı ve yağ asidi kompozisyonu sonuçlarına dayanarak, balık yağı yerine eşit oranlarda SBAE ve Algamac 3050 mikroalg ürünlerinin kullanılmasının çipura yavrularının ön büyütme yemlerinde olumlu sonuçlar verdiği neticesine varılmıştır.

Anahtar Kelimeler: Çipura, besleme, mikroalg, büyüme performansı, yaşama oranı, esansiyel yağ asitleri.

Introduction

Total global production of capture fisheries and aquaculture is estimated to reach 172 million tons by 2021 driven by increased demand for fish consumption. In the next 10 years, aquaculture production will probably overcome the production from capture fisheries by around 33% (FAO, 2012). In order to supply that requirement, larvae and juvenile production must be succesfully managed and increased in number. Succesful marine fish production mainly depend on broodstock

management, larval husbandry and nutritional quality of feed used in larval and juvenile feeding protocol (Izquierdo *et al.*, 2001; Infante and Cahu, 2010).

Importance of long-chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) is described by several studies in larval fish nutrition (Tocher and Harvie, 1988; Bell *et al.*, 1995; Sargent *et al.*, 1997; Tocher and Ghioni, 1999; Bell *et al.*, 2002; Izquierdo *et al.*, 2005). These LC-PUFA occurance in live prey and early weaning diets plays vital role for nerve system development, growth performance, survival and quality of larvae and juvenile marine fish (Watanabe *et al.*, 1989; Bessonart *et al.*, 1999; Liu *et al.*, 2002; Van Anholt *et al*., 2004; Ganga *et al.*, 2005; Izquierdo and Koven, 2010; Eryalçın *et al*., 2013). Because of the limited ability of elongation and desaturation of short chain fatty acids in marine fish, LC-PUFA should be readily presented in their feeds (Sargent *et al.*, 1997; Koven *et al.*, 2001; Sargent *et al.*, 2002).

Fish oil is the main LC-PUFA source presented in commercial marine fish feeds. Because of increasing demand for fish oil by developing aquaculture industry, its availability is becoming limited which leads its price to be increased (Tacon and Metian, 2008). To promote sustainable aquaculture production, fish oil must be partially or completely replaced by alternative oils in fish diets. Therefore, investigations of alternative oils in weaning diets have been given high priority lately. In this context, there are two types of alternative oil resources currently studied: terrestial plant oils and microorganism based oils (Pickova and Morkore, 2007; Turchini *et al.*, 2009). Replacement of fish oil by terrestial plant oils such as linseed oil (Wassef *et al.*, 2009), sunflower oil (Merida *et al.*, 2010), palm oil (Fountoulaki *et al.*, 2009), soybean oil (Yıldız and Şener, 2004; Peng *et al.*, 2008), cottonseed oil (Güler and Yıldız, 2011; Eroldoğan *et al*., 2012), rapeseed oil (Mourente and Bell, 2006) and canola oil (Huang *et al.*, 2007) have been evaluated in marine fish nutrition. Unlike marine microorganism oil sources, terrestial plant oils lack of n-3 LC-PUFA and several negative effects may occur on survival, growth performance, flesh quality and taste parameters of fish fed these diets, including gilthead sea bream (Montero *et al.*, 2008). Marine microorganism produced oil such as microalgae oil were successfully used as lipid sources substituting by fish oil in fish feeds without any hazardous effects (Ganuza *et al.*, 2008; Eryalçın *et al.*, 2013).

These microorganisms are primary producers in marine food chain and highly rich in n-3 LC-PUFA, protein, vitamins and pigments. Recently, several studies have been focused on n-3 LC-PUFA production from microalgae (Kyle and Gladue, 1991; Kyle *et al.*, 1992; de Swaaf, 2003; Pulz and Gross, 2004; Ratledge, 2004; Spolaore *et al.*, 2006; Ganuza and Izquierdo, 2007; Castillo *et al.*, 2009) and its effectiveness on partial or complete replacement of fish oil in marine fish feeds (Mustafa *et al.*, 1995; Navarro and Sarasquete, 1998; Miller *et al.*, 2007; Atalah *et al.*, 2007; Ganuza *et al.*, 2008; Eryalçın *et al.*, 2013). Microorganism produced n-3 LC-PUFA oils in marine feeds should be obtained by not only one species but also more than one microorganism species, in order to sustain better n-3 LC-PUFA composition and to create a wide range of fatty acid composition (Harel *et al.*, 2002). Several microalgae were investigated as alternative LC-PUFA sources and nowadays, these products are commercially available (Ratledge, 2001; de Swaaf, 2003; Ganuza and Izquierdo, 2007).

Commercial microalgae products are still being developed in order to yield better quality and high content of essential nutrients, and they are considered promising alternative ingredients in feeds of larval and juvenile marine fish (Qiao *et al.*, 2014). Microalgae production at commercial scale is highly required in aquaculture industry as well as in the newly developing biodiesel technology. However microalgae production at industrial volume is limited by both difficulties in culture procedures and elevated production costs (Lewis *et al.*, 1999; Hemaiswarya *et al.*, 2011; Martins *et al.*, 2013).

In our study, two types of microalgae products were evaluated for larval weaning and juvenile nutrition of gilthead sea bream. SBAE (*Tetraselmis suecica, Isochrysis sp., Nannochloropsis oculata, Phaeodactylum tricornutum*) product that is based on four phototrophic microalgae and the Algamac 3050, which is based on heterotrophically cultured microalgae species *Schizochytrium sp.* So far, there are no studies that tested microalgae inclusion for long feeding period of juvenile stages of marine fish larvae.

The aim of this study was to investigate the effectiveness of SBAE product as EPA source and Algamac product as DHA source in larvae diets and test their individual/combined suitability to substitute fish oilin larvae and juvenile nutrition of gilthead sea bream during 90 days.

Material and Methods

Experimental Conditions and Fish Husbandry

Gilthead seabream larvae were obtained from Kılıç Deniz Aquculture Company. Larvae (0.83±0.54 cm total length, mean±SD; 0.02±0.00 g body weight) were previously fed rotifers (*Brachinus plicatilis*) enriched with docosahexaenoic acid (DHA) Protein Selco® (INVE, Dendermond, Belgium) until 26 dah and they were randomly distributed in 12 experimental tanks at a density of 2100 larvae tank^{-1}. Experimental diets were tested in triplicate. The first two days of the experiment, larvae were fed with non-enriched rotifers twice per day at 12:00 and 16:00.

Rotifer abundance was kept at 400.000 ind/tank in order to obtain a final density of 2 ind/ml, in rearing tanks. All tanks (250-L fiberglass cylinder tanks with conical bottom and painted a light grey colour) were supplied with filtered seawater (36 mg L^{-1} salinity) at an increasing rate of 0.4–1.0 L min^{-1} to assure good water quality during the experiment. Water was continuously aerated (125 $ml.min^{-1}$) supplying 6.0±1.0 ppm dissolved oxygen. Average water temperature and pH along the trial were 21.0±0.2 °C and 7.6±0.1, respectively. Photoperiod was kept at 12 h light: 12 h dark by fluorescent daylights and the light intensity was 1700 lux (digital Lux Tester YF-1065; Powertech Rentals, Osborne Park City, Australia) at the water surface. The tanks were siphoned daily in order to remove uneaten feeds and feces. Larval growth was determined by measuring the total length of 10 larvae from each tank with binocular microscope every 15 days as from the beginning of the experiment. Dry weight measurement were carried out by precision balance. Survival rate was calculated by counting all live larvae in each tank at the end of the experiment.

Experimental Diets

Four experimental microdiets (pellet size <250 μm, 250-500 μm, 750 μm) with different microalgae products were formulated (Table 1). A control diet (C diet) included anchovy oil and anchovy meal; SBAE diet contained solely SBAE product (*Tetraselmis suecica, Isochrysis sp., Nannochloropsis oculata, Phaeodactylum tricornutum*) to completely substitute the anchovy oil; Algamac diet contained Algamac 3050 product(*Schizochytrium sp.*) to increase the total dietary DHA level and the final diet (Mix diet) contained both dried microalgae products, SBAE and Algamac 3050 to completely substitute the fish oil with high levels of both dietary EPA and DHA. The dietary ingredients were mixed in a mortar and then gelatin dissolved in water (at 80 °C) was added. The pelleted products were dried in an oven at 40°C for 24 h. The pellets were ground and sieved to achieve a particle size of 250, 400 and 750 μm to proper feed growing fish during the experiment. Larvae of all experimental groups were fed with experimental diets in a ratio of 5% of body weight (of each tank) day^{-1}. During the first 45 days the feeding rate was 2.0-2.5 g day^{-1}/tank, and then 5.0 g day^{-1}/tank at the last 45 days of the experiment. Feeds were manually given to each tank every 45 minutes during light periods.

Proximate Analysis

At the end of the feeding trial, all fish in the tanks and microdiets were collected in plastic bags as triplicate and kept in -80°C for chemical analysis. Moisture (AOAC, 1998a), crude protein (AOAC, 1998b) and crude lipid (Folch *et al.*, 1957) contents of larvae and experimental diets were analyzed (Table 2). Fatty acid methyl esters were obtained by transmethylation of crude lipids as described by IUPAC (1987) separated by GLC, quantified by FID (SUPELCO, 18919).

Growth Parameters

Specific growth rate (SGR) was calculated as SGR (% day^{-1}) =100 x ($\ln(W_f / W_i)$) x d^{-1} where W_f and W_i are the final and initial weights (g) and d is the number of days (90 days) of experiment. Total feed efficiency ratio (FER) was calculated as FER (g g^{-1}) = total weight gain (g)/FC (g). The condition factor (CF) was calculated as (CF) = 100 x (body weight)/body length $(cm)^3$ (Ricker, 1979).

Table 1. Composition of the experimental diets

	Control	SBAE	ALGAMAC	MIX
Ingredients (g 100 g^{-1} of diet)				
Fish meal [1]	75.3	70.3	69.1	68.0
Algamac 3050 ® [2]	-	-	10.7	5.9
SBAE ® [3]	-	11	-	5.9
Fish oil [4]	6	-	-	-
Jelatine	3	3	3	3
Soy Lecithin	2	2	2	2
Olive oil	-	-	1.5	1.5
α-tocopherol	0.2	0.2	0.2	0.2
Vit mix [6]	6	6	6	6
Min mix [7]	4.5	4.5	4.5	4.5
Attractants [8]	3	3	3	3

[1]Fish meal (Anchovy) (Trabzon, Turkey).
[2]Algamac 3050, included dried *Schizochytrium* sp.
[3]SBAE, included dried *Tetraselmis suecica*, *Isochrysis sp.*, *Nannochloropsis oculata*, *Phaeodactylum tricornutum* microalgae.
[4]Fish oil (Trabzon, Turkey).
[6]Vit mix (NRC, 1993)
[7]Min mix (NRC, 1993)
[8]Attractants (mg 100g diet $^{-1}$); Inosin-5-Monofosfat (500), Betain (660), L-Serin (170), L-Tirosin (170), DL-Alenin (500), L-Fenilalanin (250), L-Valin (250)

Table 2. Proximate composition of the experimental diets

	Diets			
	Control	SBAE	ALGAMAC	MIX
Dry Matter	92.19±0.18	90.69±0.68	97.32±0.22	89.72±0.14
Crude Protein	58.51±0.99	61.72±0.40	57.20±1.12	58.73±0.90
Crude Lipid	12.20±0.36	9.95±0.10	9.16±0.04	11.88±0.07
Crude Ash	21.20±0.16	21.77±0.30	20.06±0.13	20.68±0.13
Crude Fibre	2.49±0.24	1.13±0.34	2.14±0.11	3.13±0.04
A.E.M.	4.81±0.67	5.81±0.36	13.77±0.84	8.32±0.69
Total Energy (Kkal/kg)	445.85±8.11	433.31±1.44	393.36±6.11	444.00±4.34
Metabolized Energy (Kkal/kg)	321.76±4.83	307.76±0.61	303.07±2.84	316.62±1.61

1 Data are reported as mean ± SD (n = 3).

Fatty Acid Methyl Esters Preparation and Quantification

Fatty acid methyl esters were obtained by transmethylation of crude lipid as described by Christie (1982) with 1% sulphuric acid in methanol. The reaction was conducted in dark conditions under nitrogen atmosphere for 16 h at 50ºC. Afterwards, fatty acid methyl esters were extracted with hexane: diethyl ether (1:1 v/v) and purified by adsorption chromatography on NH_2 Sep-pack cartridges (Waters S.A., Massachusetts, USA) as described by Christie (1982). Fatty acid methyl esters were separated by GLC (GC-14A, Shimadzu, Tokyo, Japan) in a Supercolvax-10-fused silica capillary column (length: 30 m; internal diameter: 0.32 mm; Supelco, Bellefonte, USA) using helium as a carrier gas. Column temperature was 180°C for the first 10 min, increasing to 215°C at a rate of 2.5°C/min and then held at 215°C for 10 min, following the conditions described in Izquierdo *et al.* (1990). Fatty acid methyl esters were quantified by FID and identified by comparison with external standards and well-characterized fish oils (EPA 28, Nippai, Ltd Tokyo, Japan). Fatty acid composition of total lipids (% dry weight) in experimental diets is given in Table 3.

Statistical Analysis

All data were normal and homoscedastic (Shapiro-Wilk's test was used for normality and Levene's tests), not requiring any transformation and were treated using one-way analysis of variance (ANOVA). Means were compared by Tukey tests ($P<0.05$) using SPSS software (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA).

Results

Body dry weight gain was significantly ($P<0.05$) different among experimental groups after 60 days of experiment. At the end of the experiment, fish fed Control and Mix diets had a significantly ($P<0.05$) higher dry body weight gain than other groups (Figure 1).Total length of experimental fish did not differ significantly among the groups (Figure 2) ($P>0.05$). Final survival rates were not statistically different among the groups (31%-38%), despite a level lower survival in fish fed SBAE diet (31%) (Figure 3).

At the end of the study, significant ($P<0.05$) differences were observed in protein and lipid composition of fish fed different experimental diets. Fish fed Mix diet showed higher protein level than other groups. Lipid content was found significantly ($P<0.05$) higher in control group than SBAE, Algamac and Mix group fish. Moisture content was not significantly ($P>0.05$) different among the groups. Ash content was significantly ($P<0.05$) higher in all microalgae included groups than control group (Table 4).

Specific growth rates were found significantly higher in fish fed control and Mix diet than fish fed SBAE and Algamac diet ($P<0.05$). Feed efficiency ratio values ranged from 0.93 to 1.13 and were significantly higher in fish fed control and Mix diet than those fed SBAE and Algamac diet ($P<0.05$). Protein effiency ration values were found significantly higher in Mix group larvae than other groups ($P<0.05$). Condition factor values were significantly higher in control group fish ($P<0.05$) (Table 5).

Fatty acid composition of Algamac and Mix diet showed reduced total n-6 PUFA levels ($P<0.05$) and SBAE and Mix diet showed greater total n-3 LC-PUFA contents (Table 3). Algamac and Control diet represented the highest concentration of saturated fatty acids, respectively. The dietary fatty acids were reflected to fatty acid compositions of experimental fish (Table 6). The palmitic acid (16:0) content of fish fed SBAE and Algamac diets was significantly ($P<0.05$) higher than other groups. The total monounsaturated fatty acids levels were found significantly ($P<0.05$) lower in fish fed Mix diet. SBAE group showed significantly ($P<0.05$) high er oleic acid (18:1n-9) content compared to other groups. Linoleic (18:2n-6) acid level decreased in all experimental fish compared to initial levels. The amount of total n-3 LC-PUFA differed significantly ($P<0.05$) among the dietary treatments. Fish fed SBAE and Mix diet showed significantly ($P<0.05$) higher level of both total n-3 LC-PUFA and n-3 PUFA. The highest proportion of DHA:EPA was detected in Algamac (5.77) and Mix (4.82) groups, respectively.

Table 3. Fatty acid composition of the experimental diets

Fatty Acid[1] (%)	Control	SBAE	ALGAMAC	MIX
12:0	n.d.	n.d.	n.d.	0.13
14:0	5.77	5.83	11.87	7.70
14:1	0.12	0.08	n.d.	0.04
15:0	0.71	0.33	n.d.	0.34
16:0	19.96	17.16	30.46	19.11
16:1	5.55	5.95	3.52	2.83
17:0	1.09	0.35	n.d.	0.24
18:0	4.07	3.26	3.03	2.04
18:1n-9	13.63	9.19	22.32	14.16
18:2n-6	5.34	6.79	2.32	3.28
18:3n-6	0.16	0.14	n.d.	0.15
18:3n-3	1.22	1.22	0.17	0.53
20:0	0.37	0.18	0.01	0.22
20:1n-9	0.43	0.35	n.d.	0.22
20:4n-6	0.64	1.11	n.d.	0.59
20:3n-3	0.13	0.07	n.d.	0.17
20:3n-6	0.20	0.14	n.d.	0.19
20:5n-3	11.46	16.84	3.61	7.50
20:5n-6	1.48	2.26	n.d.	1.02
22:6n-3	16.22	18.55	16.53	25.78
∑ Saturates[2]	32.32^{b}	27.42^{c}	45.89^{a}	29.92^{c}
∑ Monounsaturated[3]	20.72^{b}	16.43^{c}	31.16^{a}	17.72^{c}
∑ (n-6) LC-PUFA	7.83^{b}	10.38^{a}	2.32^{d}	5.23^{c}
∑ (n-3) LC-PUFA	29.04^{c}	36.70^{a}	20.31^{d}	33.99^{b}
∑ (n-3) LC-HUFA	27.82	35.47	20.14	33.46
DHA/EPA	1.41	1.10	4.57	3.43

[1]Data are reported as mean ± SD (n=3). Means with different superscript letter in a row are significantly different (P<0.05)
[2]Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0, and 24:0,
[3]Includes 14:1, 15:1, and 17:1.
n.d.: not detected

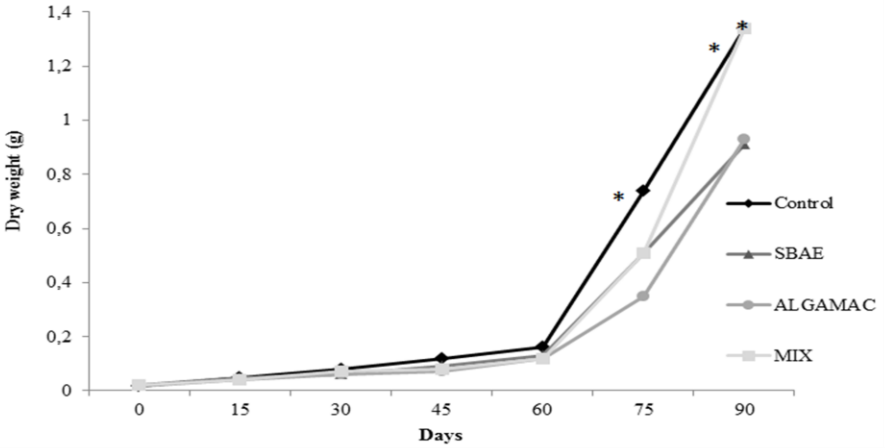

Figure 1. Dry weight (g) of gilthead seabream larvae fed diets with different lipid sources.

Discussion

Dietary fish oil replacement by terrestrial plant oils (Bell *et al.*, 2003; Bransden *et al.*, 2003; Şener *et al.*, 2006; Huang *et al.*, 2007; Piedecausa *et al.*, 2007; Fountoulaki *et al.*, 2009; Bell *et al.*, 2010) and alternative microorganism oils have been extensively studied in marine fish feeds (Miller *et al.*, 2007; Atalah *et al.*, 2007; Eryalçın *et al.*, 2013; Qiao *et al.*, 2014; Sprague *et al.*, 2015). Total fish oil substition by vegetable oils in sharpsnout sea bream (*Diplodus puntazzo*) and red sea bream (*Pagrus major*) did not negatively affect growth of those fish, suggesting that the respective essential fatty acids requirements were met by dietary lipid source (Glencross *et al.*, 2003; Piedecausa *et al.*, 2007). Conversely, total replacement of fish oil by terrestrial vegetable oils had been reported to reduce the essential n-3 LC-

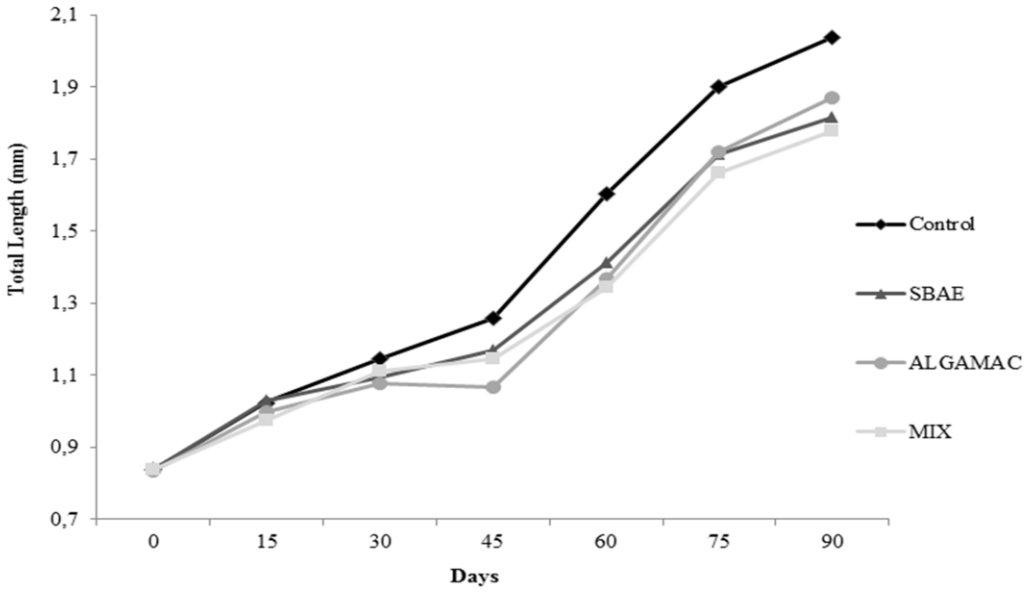

Figure 2. Total length (mm) of gilthead seabream larvae fed diets with different lipid sources.

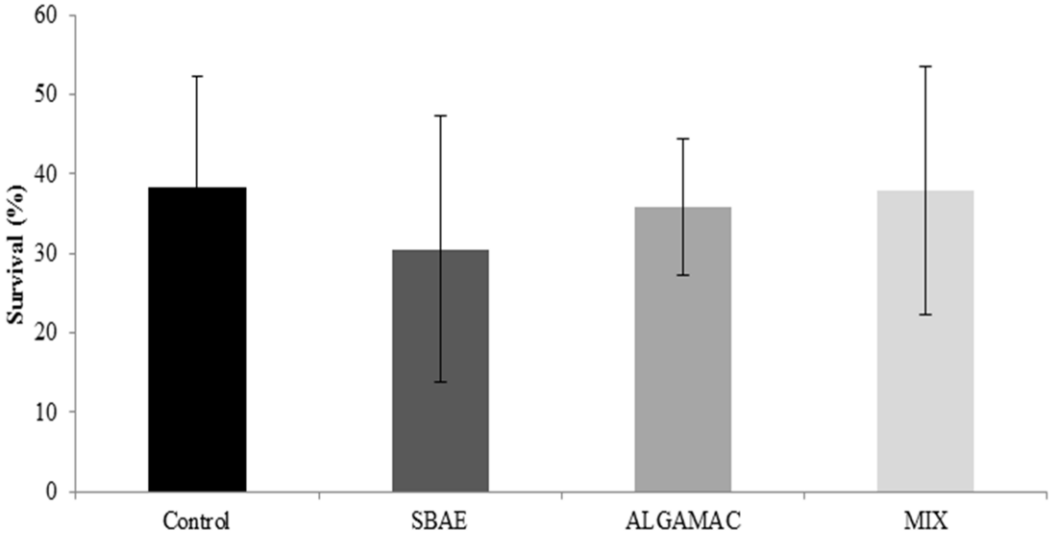

Figure 3. Final survival of gilthead seabream larvae fed diets with different lipid sources for 90 days.

PUFA level of fish fed these diets and could negatively affect health, flesh quality and growth of gilthead sea bream (Montero *et al.,* 2003; Izquierdo, 2005; Montero *et al.*, 2008; Cruz-Garcia *et al.,* 2011). The incongruent results obtained from different fish can be explained by specific essential fatty acid requirements of studied fish (Glencross *et al.,* 2009; Turchini *et al.,* 2009). In fact, except salmonids, most marine fish are not capable of synthesizing n-3 LC-PUFA because of their limited delta 5 and delta 6 desaturase enzymes expression by their genes (Sargent *et al.,* 2002). For that reason, n-3 LC-PUFA riched marine microorganism produced oils are gaining importance in aquaculture industry. In this study, total/partial substitution to fish oil by two different microalgae products was studied in post larval to juvenile stage of gilthead sea bream during 90 days of feeding. Among various experimented diets, the diet containing equal amounts of both products succesfully replaced fish oil. Therefore our results suggest that this mix diet satisfy the essential fatty acid requirements of gilthead sea bream.

Successful marine fish culture depends on the number of produced larval and weaning periods of fish. Survival and stress resistance of fish are important parameters to ensure good quality of fish production. Survival of larvae depends mainly on the nutritional quality of feeds and nursery conditions (Izquierdo and Koven, 2010). Gilthead sea bream larval survival can vary based on diets and

Table 4. Growth parameters of gilthead sea bream larvae at the end of the experiments

	Groups[1]			
Growth Performance	CONTROL	SBAE	ALGAMAC	MIX
Initial weight (g)	0.0184±0.00	0.0188±0.00	0.0181±0.00	0.0189±0.00
Final weight (g)	1.34±0.00^{a}	0.91±0.02^{b}	0.93±0.00^{b}	1.34±0.03^{a}
Specific growth rate[2]	4.79±0.53^{a}	4.28±0.45^{b}	4.38±0.49^{b}	4.72±0.39^{a}
Feed efficiency rate[3]	1.12±0.08^{a}	0.93±0.02^{b}	0.93±0.03^{b}	1.13±0.03^{a}
Protein efficiency rate[4]	2.08±0.33^{b}	1.66±0.22^{c}	1.77±0.25^{c}	2.58±0.29^{a}
Condition factor[5]	6.73±0.27^{a}	4.22±0.12^{c}	3.87±0.16^{d}	5.16±0.19^{b}

[1] Data are reported as mean ± SD. (n=10). Means with different superscript letter in a row are significantly different (P<0.05).
[2] SGR = specific growth rate = [(ln final weight-ln initial weight) /days]×100.
[3] FER = feed efficiency ratio = (fish weight gain /feed intake).
[4] PER= protein efficiency ratio = wet weight gain (g) / [(consumed feed during period (g) x protein content in feed) / 100]
[5] CF = condition factor = 100×[(body weight (g) /length (cm)].

Table 5. Whole body composition of larvae fed experimental diets (% dry weight)

	Diets[2]				
	Initial	Control	SBAE	ALGAMAC	Mix
Dry matter	19.76±0.34	21.81±0.57^{a}	21.41±0.34^{a}	21.06±0.32^{a}	21.58±0.54^{a}
Crude Protein	10.88±0.30	16.02±0.82^{c}	16.89±0.24^{bc}	18.06±0.53^{ab}	18.22±0.40^{a}
Crude Lipid	3.65±0.25	10.99±0.03^{a}	9.21±0.12^{c}	8.19±0.13^{d}	9.74±0.16^{b}
Crude Ash	1.52±0.14	3.11±0.10^{b}	3.57±0.32^{b}	3.77±0.10^{a}	3.90±0.06^{a}

Table 6. Fatty acid composition of gilthead seabream after feeding 90 days with diets containing different microalgal products

Total Lipids and Fatty Acids	Groups				
	Initial	Control	SBAE	ALGAMAC	MIX
Toplam Lipid	3.65±0.25	10.99±0.03	9.21±0.12	8.19±0.13	9.74±0.16
Fatty Acids (Percentage of total lipids %)					
14:0	n.d.	4.13±0.10^{b}	n.d.	4.50±0.06^{a}	3.45±0.01^{c}
16:0	16.05±0.13^{c}	18.17±1.24^{b}	20.50±0.80^{a}	20.21±0.18^{a}	16.18±0.08^{c}
16:1	1.04±0.08^{d}	5.59±0.05^{b}	n.d.	5.77±0.02^{a}	4.52±0.04^{c}
18:0	10.97±0.21^{a}	6.07±0.11^{c}	n.d.	6.59±0.07^{b}	5.88±0.12^{c}
18:1n-9	31.41±0.05^{b}	15.58±0.27^{d}	34.26±1.75^{a}	29.36±0.24^{c}	27.31±0.08^{c}
18:2n-6	7.45±0.37^{a}	3.05±0.04^{d}	6.23±0.58^{b}	4.90±0.04^{c}	5.82±0.16^{b}
18:3n-3	17.07±0.41	0.53±0.07	n.d.	n.d.	n.d.
20:0	n.d.	0.04±0.00^{b}	0.07±0.01^{a}	0.05±0.00^{b}	0.07±0.01^{a}
20:1n-9	1.84±0.00	n.d.	n.d.	n.d.	n.d.
20:4n-6	3.63±0.30	0.84±0.06	n.d.	n.d.	n.d.
20:5n-3	2.02±0.38^{d}	7.22±0.09^{a}	5.67±0.30^{b}	5.01±0.11^{c}	5.9±0.15^{b}
21:0	n.d.	0.97±0.11	n.d.	n.d.	n.d.
22:5n-6	n.d.	2.26±0.15^{b}	n.d.	1.86±0.20^{c}	3.22±0.04^{a}
22:6n-3	9.43±0.28^{c}	21.69±0.32^{b}	20.16±0.62^{b}	28.93±0.15^{a}	28.47±0.17^{a}
∑ Saturates[2]	27.02±0.34^{c}	29.36±1.12^{b}	23.22±0.81^{d}	31.35±0.02^{a}	25.6±0.20^{c}
∑ Monounsaturated[3]	33.38±1.38^{ab}	32.96±0.27^{ab}	34.26±1.75^{ab}	35.13±0.22^{a}	31.83±0.04^{b}
∑ (n-6) LC-PUFA	11.08±0.67^{a}	9.19±0.25^{b}	11.15±0.58^{a}	6.76±0.16^{c}	9.04±0.19^{b}
∑ (n-3) LC-PUFA	28.52±0.31^{c}	29.45±0.48^{b}	33.83±0.33^{a}	26.75±0.04^{d}	33.52±0.02^{a}
∑ (n-3) LC-HUFA	11.45±0.01^{d}	28.92±0.41^{b}	33.83±0.33^{a}	26.75±0.04^{c}	33.52±0.02^{a}
DHA/EPA	4.66±1.04^{b}	3.00±0.01^{d}	3.55±0.37^{c}	5.77±0.09^{a}	4.82±0.20^{b}

[1]Data are reported as mean ± SD (n=3). Means with different superscript letter in a row are significantly different (P<0.05)
[2]Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0, and 24:0
[3]Includes 14:1, 15:1, and 17:1.
n.d.: not detected

experimental conditions and reach up to 52-58% as obtained by Saleh *et al.* (2013). Evaluation of *Schizochytrium sp.* included Algamac products have previosuly showed higher weight gain and survival rate in gilthead seabream larvae (Robin and Vincent, 2003). In this study, acceptable results of survival for the larval to juvenile period were obtained for all experimental groups. While body weight gain was improved by the diet containing both products, total length was not affected by diets. On the other hand,

total length is mainly related to protein content of larvae, which was equally designed in our diets.

Substituting fish oil by vegetable oil resulted in SGR level that ranges between 1.44-1.75 in gilthead sea bream juveniles (Montero *et al.,* 2010). Dietary fish oil replacement by microalgae raw material gave SGR values between 1.3-1.5 in juvenile olive flounder (*Paralichthys olivaceus*) during 60 days of feeding (Qiao *et al.,* 2014). Because our feeding trial was started from very early age (26 dah), SGR value was found higher in our study in comparison to other studies. As for the FER level, our results are similar to those previously reported in the litterature. For example, Abdul-Kader *et al.* (2010) found FER level between 0.94 and 1.09 under similar conditions for red seabream fed algae containing diet as substitute to fish oil during 50 days feeding. In another trial, Atlantic salmon fed fish oil replaced by *Schizochytrium sp.* oil resulted in FER value between 0.53-0.66 in 40 g starting fish with 63 days of experiment. On the other hand, Li *et al.* (2009) found FER value between 0.67 and 0.69 in channel catfish juveniles fed dried algae *Schizochytrium sp.* in their diets. These findings were similar to our results. Those growth parameters data are hard to find in literature on larval and weaning period of gilthead sea bream, therefore data obtained from other species were used for comparison.

For gilthead sea bream, larvae need mainly more than 3% LC-PUFA with EPA (0.7-0.8%) and DHA (1.5%) on a dry basis at their larval period. Adequate proportion of LC-PUFA (DHA:EPA) is necessary for enhancing larval survival and it should be obtained as 1.5-2% in weaning diets (Salhi et al., 1997; Rodriguez *et al.*, 1997; Izquierdo and Fernandez-Palacios, 1997; Izquierdo *et al.*, 2000). In our study, these values were found between 1.10 (SBAE diet)-4.57 (Algamac diet) and DHA:EPA composition of microdiets was found similar to previously reported. DHA:EPA values of diets were reflected to our larval DHA:EPA ratio;this probably fulfilled their physological needs during fast growing period and resulted in enhanced growth performance of fish.

Different microalgae products have been studied for completely replacing fish oil in microdiets without any side effects on survival, growth and health of marine fish (Navarro and Sarasquete, 1998; Lazo *et al.*, 2000; Harel *et al.*, 2002; Miller *et al.*, 2007; Li *et al.*, 2009 Eryalcin *et al.*, 2013).The vital effect of EPA content on growth and survival was previously suggested by Ganuza *et al.* (2008) who concluded that DHA rich single cell microorganisms in microdiets cannot replace completely fish based products. This requirement of EPA sources in microdiets for gilthead seabream larvae was also shown in other studies (Atalah *et al.*, 2007; Eryalçın *et al.,* 2013). In this study, similar results to those obtained by the Control diet without any side effects on survival, growth, body and fatty acid composition of fish were obtained by the Mix diet, which contained both SBAE and Algamac products. The combination of microalgae strains can provide a wider range of fatty acids to obtain better dietary quality than by a single algae was previously suggested by Harel *et al.* (2002). In the present study, the replacement of fish oil by microalgae products not only sustained survival rates but also yielded high growth rates and positively effected total LC-PUFA level of fish. Algamac 3050 is produced from hetetrophically cultured and drum-dried algae *Schizochytriumsp.* and the second product, SBAE, is produced by the combination of four phototrophic microalgae; *Tetraselmis suecica*, *Isochrysis sp*., *Nannochloropsis oculata*, *Phaeodactylum tricornutum*. Algamac 3050 includes high level of DHA whereas SBAE product is rich in EPA as well as a wide range of LC-PUFAs originated from four different microalgae species. Therefore the combined usage of both products, SBAE and Algamac seem to be adequate as alternative feed ingredients to fish oil for larval to juvenile period of gilthead sea bream larvae.

In conclusion, high amounts of microalgae are necessary in order to sustain aquaculture industry and its production should be improved for cost effectiveness. New findings and innovative approaches in aquaculture sector should be introduced. Despite the efforts to obtain DHA production from heterotrophic marine organisms, the number of used species is very limited (Kyle, 1996; Ratledge, 2001; de Swaaf, 2003). Therefore, new marine based algae and process technology are needed for further research. However, these organisms can be a suitable alternative to fish oil use in aquafeeds, if production techniques for these organisms are improved to reduce their cost and increase their availability.

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