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THE USE OF GARLIC (*Allium sativum*) MEAL AS A NATURAL FEED SUPPLEMENT IN DIETS FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

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Abstract:

The incorporation of garlic meal (GM) in diets for European seabass juveniles were evaluated with a diet containing 43% protein and 17% lipid (gross energy 19kJ/g diet). Experimental diets with GM incorporation of 0, 2, 4, and 6% were fed to fish (10.60 ±0.16 g) until satiation for 60-days. Significant differences ($p<0.05$) were recorded for growth performance, with the highest rate in the 4% GM group, followed by the control group. Improved feed conversion (FCR) and protein efficiency rates (PER) were observed in the GM4 group compared to the other treatments. Nitrogen retention as a percent of intake was highest in the in GM4 group. Significantly higher values ($p<0.05$) were found for body protein and lipid, and lower values ($p<0.05$) for the hepatosomatic, viscerasomatic or mesenteric fat indexes in the GM4 group compared to the other treatments. Fish fed garlic supplemented diets showed lower saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), but higher polyunsaturated fatty acids (PUFA) compared to the control group with no garlic treatment. Results indicate that dietary GM inclusion of 4% can improve fish growth and nutrient utilization with an increase of fish muscle quality by elevated PUFA concentrations, and a reduction of total nitrogen excretion.

Keywords: Feed additives, Garlic meal, Growth performance, Nutrient utilization, Fatty acid profile

Introduction

The success in intensive aquaculture is basicly dependent on quality of feed and feeding strategies which are the most important factors influencing growth performance, feed utilization and body chemical composition of fish (Okumus and Mazlum, 2002). In intensive aquaculture, the aim is to gain maximum yields from water resources with providing artificial diets that thrives fish growth and gain maximum weight in the shortest time frame as possible (Bhosale et al., 2010). Recently, some valuable components stimulating the defense system and stress response of fish, the so-called immunostimulants, have been isolated from plants, animals or microorganisms (Sakai, 1999). The incorporation of immunostimulants in fish diets has been suggested as an effective method for the improvement of the activity of non-specific defense mechanisms increasing disease resistance (Dalmo and Seljelid, 1995) and for the control of biomass in aquaculture (Yokoyama et al., 2005). Probably known as one of the earliest medicinal plants (Farahi et al., 2010), the use of garlic in aquaculture became popular for providing protection against diseases or inducing fish feeds as a growth promoter. Garlic was used as a growth promoter in tilapia (Diab et al., 2002; Shalaby et al., 2006; Mesalhy et al., 2008; Soltan and El-Laithy, 2008; Metwally, 2009; Abdel-Hakim et al., 2010), in Asian seabass (Talpur and Ikhwanuddin, 2012), in sterlet sturgeon (Lee et al., 2014), and in Seabass fry (Saleh et al., 2015). To our knowledge, so far, no information is available on the effects of dietary garlic on growth performance, feed utilization and body chemical composition in juvenile European seabass, which is one of the main aquaculture species in the Mediterranean, where the Turkish and Greek aquaculture industries share a total production of 211.055 tons with a grand value of 1.293.082 USD for seabass together with seabream (FAO, 2012). During the last 10 years, aquaculture based fish production has doubled from around 41 million tons to over 90 million during with a market value of over 51 million USD in year 2000 and 144 million USD in 2012 (FAO, 2012). The increase of fish production worldwide triggers the demand for high quality diets improving fish growth and welfare.

The objective of this study was to evaluate the effects of dietary garlic meal as a natural feed supplement on growth performance, nutrient utilization, body biochemical composition, and nitrogen balance and muscle fatty acid profile of European seabass (*Dicentrarchus labrax*) juveniles.

Materials and Methods

Experimental fish, diets and culture conditions

European Seabass obtained from a commercial marine fish hatchery (Ida Gıda, Canakkale-Turkey) were transferred to the Marine Aquaculture Research and Development facilities of the Faculty of Marine Science and Technology at Canakkale Onsekiz Mart University. The experimental facility was a closed recirculation aquaculture system (RAS), run with mechanical and biological filtration, continuous air supply and water heaters. Water flow rate was 2.0 l/min (complete water turnover was about 2.4 times per hour). During the entire experimental period, temperature was 23.3 ±0.98ºC, dissolved oxygen 7.00 ±1.0 mgL^{-1}, salinity 24 ‰, pH 7.50 ±0.5, and Ammonia-N (NH_3-N) was 0.28 ±0.07 mgL^{-1}.

After transportation, the experimental fish were initially placed in indoor tanks (1.0 m^3) with a continuous seawater flow through system for an acclimatization period of 2 weeks. Thereafter, a total of 108 juveniles (mean initial weight of 10.60 ±0.16 g) out of the main stock were randomly distributed in to 12 identical shaped glass aquariums (9 fish per aquarium) with a water volume of 50 L, according to a triplicate design. Four different feed formulations were prepared and experimental diets were produced with garlic meal inclusion levels of 0, 2, 4 and 6%. The experimental group fed diets without garlic meal (0%) served as control. Chemical composition of the experiment diets is given in Table 1. Biochemical composition of the ingredients used in feed formulation and those for European seabass are shown in Table 2. Fish were fed until satiation twice a day (08:30 and 16:30), 6 days a week for a total period of 60 days. Satiation level was considered when sh no longer attacked food particles and refused feeding. During the trial a natural photoperiod regime was followed (N 40°04'29.98", E 26°21'35.60" - Canakkale, Turkey), and no additional light was applied.

Table 1. Feed ingredients and composition of the experimental diets

	Experimental Diets (%)				
Ingredient (g/100g)	Control	GM2	GM4	GM6	
Fish meal (FM)	57.50	57.30	57.10	56.90	
Soybean meal (SBM)	20.00	20.00	20.00	20.00	
Garlic meal (GM)	0.00	2.00	4.00	6.00	
Fish oil (FO)	10.60	10.61	10.61	10.62	
b-Corn starch	8.90	7.09	5.29	3.48	
Vit-Min Premix	3.00	3.00	3.00	3.00	
Analyzed biochemical composition (g/100g air dry basis)					
Moisture (%)	12.00	12.00	12.00	12.00	
Crude Protein (%)	43.30	42.80	43.20	43.10	
Crude Lipid (%)	16.69	17.56	17.09	16.64	
Crude Ash (%)	11.86	11.14	11.44	11.25	
Estimated nutrients					
NFE (%)	13.15	13.50	11.27	14.01	
GE (kJ/g diet)	19.05	19.33	18.86	19.13	
P/E (mg/kJ)	22.72	22.14	22.91	22.53	
PE/TE	0.54	0.52	0.54	0.53	
Amino acid composition of experimental diets (% dry matter)[a]					Seabass requirements
Arginine	3.05	3.05	3.05	3.05	1.80
Lysine	3.78	3.77	3.77	3.77	1.88
Histidine	1.26	1.26	1.27	1.27	0.63
Isoleucine	2.53	2.53	2.52	2.52	1.02
Leucine	3.93	3.93	3.94	3.94	1.68
Valine	2.70	2.70	2.70	2.70	1.13
Met+Cys	1.89	1.89	1.89	1.88	0.90
Phe+Tyr	3.98	3.98	3.98	3.98	1.02
Threonine	2.11	2.11	2.11	2.11	1.05
Triptophan	NA	NA	NA	NA	0.23
n-3 HUFA in experimental diet (%)					
Lipid in FM (%)	8.50	8.50	8.50	8.50	
Lipid from FM (%)	4.89	4.87	4.85	4.84	
Total FO in diet (%)	15.49	15.48	15.46	15.46	
n-3 HUFA in FO (%)[b]	29.76	29.76	29.76	29.76	
Total n-3 HUFA in diet (%)	4.61	4.61	4.60	4.60	
n-3 HUFA requirement (%)					0.7[c]

[a] Calculated according to values given in Table 2.
[b] Güner et al. (1998).
[c] Skalli and Robin (2004).
NFE (Nitrogen free extract) = 100 – (crude protein + crude lipid + crude ash)
GE= Gross energy, calculated according to energy fuels of 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g NFE.
P/E= mg Protein / kJ enerji ratio
PE/TE= Energy from protein to total energy ratio

Table 2. Biochemical composition of ingredients and European seabass used in the experiment

	European Seabass[a]	Fish Meal[b]	Soybean Meal[b]	Garlic Meal[c]
Analytical value (%)				
Moisture	8.0	11.0	10.0	
Crude Protein	66.0	46.3	6.5	
Crude Lipid	8.5	3.1	0.5	
Crude Ash	15.8	7.4	1.5	
Essencial amino acid (%)*				
Arginine	4.60	4.11	3.41	4.59
Lysine	4.80	5.49	3.10	4.48
Histidin	1.60	1.76	1.26	2.07
Isoleucine	2.60	3.38	2.92	2.26
Leucine	4.30	5.43	4.02	8.13
Valine	2.90	3.81	2.53	3.66
Methionine	N/A	2.16	0.72	0.78
Cystein	N/A	0.66	0.63	0.79
Met+Cys	2.30	2.82	1.35	1.57
Phenylalanine	N/A	3.03	2.45	3.89
Tyrosine	N/A	2.44	1.72	2.42
Phe+Tyr	2.60	5.47	4.17	6.31
Threonine	2.70	3.00	1.92	3.52
Tryptophan	0.60	0.82	0.68	N/A

[a] Kaushik (1998)
[b] Halver (1991)
[c] Aremu et al. (2011)
N/A = not available

Fish sampling and analytical methods

At the start of the experiment, fish samples (10 fish from an initial pool) were removed and anaesthetized at a high dose level and stored at -25 °C for subsequent analysis of fish body composition. At the end of the trial, 5 fish per tank (15 per diet) were removed following the same procedure as conducted for the initial samples and stored at -25 °C for subsequent analysis of final fish body composition. The proximate composition of the experimental diets and freeze-dried fish whole body proximate composition was determined following AOAC (2000) guidelines as follows: Moisture by weight loss after 24 h in an oven at 105 °C; crude ash by incineration in a muffle furnace at 550 °C for 24 h; crude protein (% Nx6.25) by the Kjeldahl method after acid digestion; lipids by ethyl ether extraction in a Soxhlet System. All laboratory analyses were performed in triplicate. Fatty acid was conducted using the Folch et al. (1957) method. After ethyl ether extraction of lipids in a Soxhlet System, fatty acids have been determined as ethyl esters, by Shimadzu capillary gas chromatograph equipped with flame ionization detecter (GC/FID) and cianopropyl-aryl HP-88 capillary column. For the esterization, the procedures of IUPAC (1987) were followed.

Statistical analyses

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

Results and Discussion

In the present study European seabass with initial mean weight of 10.60 ± 0.157 g were fed diets containing different levels of garlic meal for a period of 60 days. At the end of the trial, growth performance of fish showed significant ($p<0.05$) differences among dietary treatments. Best growth performance was obtained in fish fed diet with 4% garlic meal inclusion (GP4) with a final

mean weight of 25.15 ± 0.07 g, which was followed by the control diet with no garlic meal inclusion, showing a final weight of 24.27 ± 0.58 g. Relative growth rates (RGR) recorded during the study period showed a similar trend with the highest rate in the GM4 group fed 4% garlic meal diet, followed by the control group fed a diet without garlic meal inclusion, the GM2 and GM6, respectively. Spesific growth rate (SGR, %/day), which is the logarithmic expression of fish growth were similar to the RGR values. The highest SGR was recored in the GM4 group with 4% garlic meal inclusion (1.43 ± 0.03, %/day), followed by the control group (1.38 ± 0.07, %/day), GM2 (1.27 ± 0.08, %/day) and the GM6 (1.19 ± 0.03, %/day) groups, respectively (Table 3). This growth promotion effect of diets supplemented with garlic meal can be attributed to the improved feed efficiency, which is in agreement with the results in Nile tilapia (Diab et al., 2002; Shalaby et al., 2006; Mesalhy et al., 2008; Soltan and El-Laithy, 2008; Metwally, 2009; Abdel-Hakim et al., 2010), in Asian seabass (Talpur and Ikhwanuddin, 2012), in sterlet sturgeon (Lee et al., 2014), and in Seabass fry (Saleh et al., 2015), where the incorporation of different levels of garlic increased final weights and specific growth rates of fish. Soltan and El-Laithy (2008) reported that the incorporation of 1% garlic into diets improved survival rate of Nile tilapia. Similarly, Abdel-Hakim et al. (2010) found better achievements of dietary garlic on growth performance and feed utilization with low levels of garlic inclusion at 0.5 % level in tilapia. Better growth effects were found with higher incorporation levels of garlic meal in diets for Nile tilapia by Shalaby et al. (2006), who tested garlic incorporation levels from 10 g/kg to 40 g/kg diet, and recommended the incorporation of 3% dietary garlic for an increased growth, reduction of total bacteria, and improvement of fish health and welfare. Similarly, Lee et al. (2014) suggested that dietary garlic powder incorporation of about 3% could positively affect growth performance and protein retention in fingerling sterlet sturgeon. Farahi et al. (2010) used different levels (1%, 2% and 3%) of galic meal in rainbow trout diets and reported that the body protein was higher in the 3% galic group compared to the other experimental groups and that growth performance and fish health improved with the addition of galic meal in trout diets. Metwally (2009), used diets containing garlic in three different forms; natural garlic (40g/kg diet, 4%), garlic oil capsules (Strongus®, pure garlic oil capsules; 250 mg/kg diet) and garlic powder tablets (32 g/kg diet, 3.2%), and reported that the dietary addition of garlic in any form can promote growth rate, decrease mortality and increase the antioxidant activity in fish. Mabrouk (2011) tested dietary garlic and onion inclusion levels of 4 % and 6 %, respectively and a 10 % mixture of garlic and onion (4% garlic - 6% onion) in diets for Nile tilapia, and reported that the addition of 10% mixture of garlic and onion significantly increased growth performance and feed utilization rather than solitary addition. To our knowledge so far, the only one study dealed with dietary garlic inclusion in European seabass feeds is the one reported by Saleh et al. (2015), who tested garlic incorporation levels of 10, 20, and 30 g/kg diet, and recommended 3% dietary garlic for the best growth, improved fish health and welfare. However, the highest garlic incorporation level tested by Saleh et al. (2015) was 3%, so based on their report it is not possible to comment on higher levels of garlic additon in the diet for seabass. A dietary garlic incorportaiton of 4% gave better results in terms of growth performance and feed utilization in the present study. Furthermore, Saleh et al. (2015) investigated the dietary garlic incorporation for seabass fry with an initial weight of 0.4 g, while in the present study larger size of seabass with an initial weight of 10 g were used.

In general, our results are in agreement or comparable with previous findings in terms of better growth performace in fish fed diets with 4% garlic incorporation, and the results from the present study and those of earlier ones revealed that garlic incorporation in fish diets improved growth preformance, feed utilization, fish health and welfare. The discrepencies between the results of the present study and some of the previous ones regarding the effects of dietary garlic on growth performance of fish, feed utilization or body composition can be attributed to the differences in fish species or fish size, environmental conditions such as water temperature and salinity, type or level of the additives accomponying the main ingredients in diet formulation, or type of the garlic source used in the feeds, fish physiology or a combination of these factors together.

Table 3. Weight gain and feed utilization of juvenile European seabass fed diets with different garlic meal inclusion levels for a period of 60 days.

	Experimental diets			
	Control	GM2	GM4	GM6
Initial weight (g)	10.61±0.225^{a}	10.56±0.191^{a}	10.64±0.148^{a}	10.58±0.147^{a}
Final weight (g)	24.27±0.577^{c}	22.67±0.674^{b}	25.15±0.067^{c}	21.57±0.524^{a}
RGR (%)	128.9±10.07^{bc}	114.8±10.24^{ab}	136.4±3.56^{c}	103.9±3.93^{a}
SGR (%/day)	1.38 ± 0.074^{bc}	1.27 ± 0.079^{ab}	1.43 ± 0.025^{c}	1.19 ± 0.032^{a}
FI (%/day)	1.77 ± 0.101^{b}	1.67 ± 0.008^{b}	1.70 ± 0.083^{b}	1.54 ± 0.029^{a}
FCR	1.36 ± 0.074^{a}	1.38 ± 0.082^{a}	1.26 ± 0.042^{a}	1.35 ± 0.052^{a}
PER	1.75 ± 0.098^{a}	1.70 ± 0.103^{a}	1.82 ± 0.061^{a}	1.72 ± 0.067^{a}
DFI (g/fish)	0.271±0.016^{c}	0.244±0.003^{b}	0.268±0.012^{c}	0.218±0.007^{a}
DPI (g/fish)	0.130±0.008^{c}	0.119±0.001^{b}	0.133±0.006^{c}	0.107±0.003^{a}
DEI (kJ/fish)	1.28 ± 0.08^{bc}	1.18 ± 0.01^{b}	1.32 ± 0.06^{c}	1.03 ± 0.03^{a}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level. (One-way ANOVA and Duncan's multiple range test, P<0.05). (W1 = initial weight, W2 = final weight, t2-t1 = feding days)
GM (Garlic meal)
RGR (relative growth rate, %) = (W2 – W1 / W1) x 100
SGR (specific growth rate, % growth/day) = ((lnW2 - lnW1) / (t2-t1)) x 100
FI (feed intake, percent of biomass per day, %/day) = (total feed offered / ((W1 + W2) / 2) / gün) × 100
FCR (feed conversion rate) = feed intake (g) / weight gain (g)
PER (protein efficiency rate) = (weight gain (g) / protein intake (g)
DFI (daily feed intake, g/fish) = (feed intake (g) / number of fish) / day
DPI (daily protein intake, g/fish) = (feed intake x crude protein in diet / 100) / day
DEI (daily energy intake, kJ/fish) = (feed intake x energy in diet / 100) / day

Interestingly, Mesalhy et al. (2008) found that the period of feeding has also affected the results. In their study, they reported that Nile tilapia fed with 10 or 20 g/kg garlic incorported diets for two months or 20 g/kg diet for one months showed significant increase in the final body weights compared to the control diet group. El-Nawawy (1991) reported that the growth promoting effect of garlic is due to the increase of glucose inflow into the tissues. It is also reported that the sulfur compounds in garlic, the active antimicrobial agents can improve the immune system, stimulating growth of the animal (El-Afify, 1997). Dietary additives which have immunostimulant effects can increase serum lysozyme activity, either because of the increase of phagocytes secreting lysozyme, or due to the increase of the amount of lysozyme synthesized per cell (Engstad et al., 1992). The lysozyme activity is mainly affected by the type of immunostimulants incorporated in the diets, and the increase of lysozyme induced by the addition of immunostimulants in diets has been reported in several fish species (Lapatra et al., 1998; Paulsen et al., 2003). Higher lysozyme has been reported in fish fed garlic supplemented diets compared to those fed diets without garlic addition (Sahu et al., 2007; Ndong and Fall, 2011). The improvements in growth performance induced by garlic inclusion to the diet may due to its antimicrobial, antioxidant, and antihypertensive characteristics (Konjufca et al., 1997; Sivam, 2001; Ibrahim et al., 2004). Block (1992) and Amagase and Milner (1993) suggested that these functions can be attributed to the bioactive components of garlic such as allin, allicin and diallylsulphides containing organosulphur compounds, particularly to thiosulfinates. Allicin in garlic promotes the performance of the intestinal flora according to Khalil et al. (2001), who indicated that the digestion is improved and the utilization of energy is enhancing, which can explain the improved growth of fish fed garlic supplemented diets.

Essential amino acid profile calculations of diet ingredients used in the present study indicate that the amino acid composition of garlic meal show quite similarities to those of the fish meal. It is well known that the incorporation of alternative feed ingredients or additives may influence the amino acid imbalance of the diet, hence linking to a reduced growth performance or decreased feed consumption. The dietary incorporation of plant sources in fish feed is mostly limited upto a

certain percent especially in carnivorous fish species, due to the lack of some essential amino acids in their composition. However, amino acid composition of garlic meal used in the present study was very similar to that of the fish meal source. Some of the essential amino acids in garlic meal were even higher then the fish meal amino acid levels, with the exception of methionine compared to fish meal. This provides important indications that garlic meal can be used in combination with other plant protein sources such as soybean meal which is considered as a strong and promising alternative protein source for fish diets, but lacking in methionine or lysine, which are the most limiting amino acids for soybean protein sources (Ergün et al., 2008a,b; Yigit et al., 2010). Results from an earlier study (Mabrouk, 2011) support this hypothesis with the work on Nile tilapia, where 50% of fish meal was replaced with soybean meal and the diet was incorporated with galic and onion meal at different levels. A diet combination of 50% fish meal and 50% soybean meal was enriched with a 10% mixture of garlic and onion, and as a result Mabrouk (2011) reported an improved growth performance and feed utilization in Nile tilapia.

Nitrogen retention as a percent of nitrogen intake was highest, while the nitrogen excretion as a percent of intake was lowest in the GM4 group compared to the other treatments (Table 4).

It is well known that the incorporation of plant feedstuffs in fish diet at an excess level may increase nitrogen excretion, lowering the retention rate of nitrogen (Burel et al., 2000; Fournier et al., 2004; Ergün et al., 2008a,b; Yigit et al., 2010). In the present study, eventhough no significance was found, the nitrogen retention as a percent of intake in the 4% garlic meal diet was higher then the control group with no garlic meal addition. When the garlic meal inclusion level increased to 6%, however, the nitrogen excretion as a percent of intake significantly decreased to a level below the control group, showing that the supplement of garlic should not exeed the 4 % level. In contrast to nitrogen retention rates, the nitrogen excretion as a percent of nitrogen intake showed a slight decline with the increase of dietary garlic meal and was recorded lowest in the 4% garlic diet, whereas again over this level the excretion rate increased to a level over the control group. Based on these tendencies of nitrogen excretion or retention rates, it might be interesting to see the long-term effect of dietary garlic meal on nitrogenous end-products.

Initial and final body moisture of experimental fish was found around 80% and did not significantly differ ($p>0.05$) among the experimental groups. Final fish body protein increased to over 46% in all treatment groups over the initial body protein of 42% at the end of the 60 days feeding trial. Highest protein content was found as 49.3 ±0.67 % in the fish fed diets with 4% garlic meal inclusion (GM4), which was followed by the GM2 (48.9 ±0.46 %) and the control diet (47.7 ±0.40 %), respectively. The lowest body protein was found in fish fed the 6% garlic meal inclusion diet (GM6) with a value of 46.9 ±0.75 %. Different then the fish body protein contents, the body lipids did not differ significantly ($p>0.05$) among diet treatments. However, compared to the initial values, body protein tended to decline, but not significantly except the GP4 group with 4 % garlic meal inclusion. Ash content in fish body showed a decline over a 60 days feeding period compared to the initial value, and these differences were recorded as significant ($p<0.05$). Nitrogen free extracts were lowest in fish fed diets with 4% garlic meal inclusion while the highest gross energy level was recorded again in the 4% garlic meal diet group and the control group. The gross energy level in the 4% garlic meal diet and the control diet group were significantly higher ($p<0.05$) then the initial values (Table 5).

The finding concerning the significant increase recorded in the body protein content of fish fed garlic meal diets at all inclusion levels, could be possibly explained by the increase in muscle free amino-acid contents that can lead to the enhanced protein synthesis. Similarly, increase in fish body protein levels were reported in rainbow trout (Gabor et al., 2010) and in seabass fry (Saleh et al., 2015), when fed on diets supplemented with 3% garlic.

Table 4. Nitrogen budget of juvenile European seabass fed diets with different levels of garlic until satiation for a period of 60 days

	Experimental diets			
	Control	GM2	GM4	GM6
N budget (mg g^{-1} production)				
N intake (NI)	91.7 ± 5.0[a]	94.4 ± 5.7[a]	88.1 ± 2.9[a]	93.3 ± 3.6[a]
Total N retention	16.7 ± 1.5[a]	17.7 ± 1.6[a]	17.4 ± 0.2[a]	16.1 ± 1.4[a]
Total N retention (%, NI)	18.2 ± 1.3[ab]	18.7 ± 0.6[ab]	19.7 ± 0.4[b]	17.2 ± 1.3[a]
Total N excretion	75.0 ± 4.3[a]	76.8 ± 4.1[a]	70.8 ± 2.7[a]	77.3 ± 3.0[a]
Total N excretion (%, NI)	81.8 ± 1.3[ab]	81.3 ± 0.6[ab]	80.3 ± 0.4[a]	82.8 ± 1.3[b]

Values with different superscripts (means±standart deviation, triplicate groups) are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, $p<0.05$).
GM (Garlic meal)
N intake (mg/g production) = (DPI x day / 6.25) / (W2 – W1)
N retention (mg/g production) = (total g protein remained in fish body / 6.25) / (W2–W1)
N excretion (mg/g production) = (N intake (g) – N retention in fish body (g)) / (W2 – W1)

Table 5. Body composition of juvenile European seabass fed diets with different levels of garlic until satiation for a period of 60 days.

		Experimental diets			
	Initial	Control	GM2	GM4	GM6
Moisture (%)	80.0±0.26[a]	79.9±0.74[a]	79.9±1.15[a]	80.1±1.01[a]	80.3±1.45[a]
Crude Protein (%)	42.4±1.05[a]	47.7±0.40[bc]	48.9±0.46[cd]	49.3±0.67[d]	46.9±0.75[b]
Crude Lipid (%)	25.1±2.15[a]	24.7±1.75[a]	21.7±1.64[a]	25.3±2.50[a]	23.1±3.73[a]
Crude Ash (%)	20.0±0.12[d]	14.5±0.02[b]	13.5±0.58[a]	15.9±0.28[c]	15.5±1.06[bc]
NFE (%)	12.5±2.53[ab]	13.0±2.12[ab]	15.9±0.94[b]	9.51±3.19[a]	14.5±3.89[ab]
GE (kJ)	22.1±0.48[a]	23.3±0.41[b]	22.9±0.41[ab]	23.3±0.57[b]	22.7±0.78[ab]

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
NFE (Nitrogen free extract) = 100 – (crude protein + crude lipid + crude ash)
GE= Gross energy, calculated according to energy fuels of 23.6 kJ/g protein, 39.5 kJ/g lipid, 17.2 kJ/g NFE.

Eventhough no significant differences (p>0.05) were observed, higher levels of total saturated fatty acids (SFA) such as Palmitoleik acid (PA, 16:1), Oleic acid (OA, 18:1n-9), Miristoleic acid (MA, 14:1) and Nervonic acid (NA, 24:1) were found in the initial fish and the control group fed garlic incorporated diets. The only exception among the SFAs was observed for Gadoleic acid (GA, 20:1n-9), which showed the lowest level (4.758 ±0.02) in the initial fish body, and presented an increase with dietary garlic meal inclusion levels with the highest value of 6.978 ±0.23 in fish fed the 4% garlic meal diet (p<0.05). Similar to the SFAs, the total monounsaturated fatty acids (MUFA) in the initial fish body samples, Linoleic acid (LA, 18:2n-6) was highest (12.77 ±0.26) in the initial fish body, while LA content in fish tissues showed a decline with the increase of dietary garlic level and the lowest value (7.99 ±3.38) was recorded for the GM4 group (p<0.05). α-Linoleic acid (α-LA, 18:3n-6) however was highest (0.152 ±0.006) in the control and lowest (0.128 ±0.004) in the GM6 group (p>0.05). Among the total MUFA, Arachidonic acid (AA, 20:4n-6) in fish body was highest in the initial fish samples, and showed a decline with the increase of dietary garlic meal in the experimental groups. Compared to the SFAs and MUFAs, the total polyunsaturated fatty acids (PUFA, n3/n6) showed a converse trend, with lower levels of PUFA in the initial fish samples or the control group and higher levels for fish fed with garlic incorporated diets. Among the PUFAs, Eicosatrienoic acid (EA, 20:3n-6) in the

final fish body samples from the GM4 treatment was significantly ($p<0.05$) higher then the values found for the initial fish, and those for the GM2 or GM6 groups, but no significant difference ($p>0.05$) was found between the 4% garlic meal and the control diet groups. Ecosapentanoic acid (EPA, 20:5 n-3) followed the same trends with EA values, with a significantly higher ($p<0.05$) value in the experimental group fed the 4% garlic diet. Docosahekzanoic acid (DHA, 22:6 n-3) in the fish muscle tissues were highest in the initial fish samples and the GM4 group, however there was no significant difference ($p>0.05$) among the experimental treatments in general for the DHA. Fatty acid composition in the muscle tissues of seabass juveniles fed experimental diets with different levels of garlic meal is given in Table 6.

The n-3 HUFA such as EPA (20:5n-3), DHA (22:5n-6) or the n-6 HUFA such as Arachidonic acid (AA, 20:4n-6) are indispansible for fish health and welfare as well as a proper growth performance. PUFA such as DHA is indispensable for breeding performance and a high growth and survival rate of larvae (Fernandez-Palacios et al., 1997). Several beneficial effects of polyunsaturated fatty acids (PUFA, n3/n6) have been reported in human health (Li et al., 2008; Buckley and Howe, 2009; Arab-Tehrany et al., 2012; Howe and Buckley, 2014; Yessoufou et al., 2015). In earlier reports, it has been observed that the incorporation of 3% fermented garlic powder in diets for laying hens increased the PUFA:SFA ratio in the egg yolk compared to laying hens fed the control diets or the diets with lower garlic incorporation (Ao et al., 2010). Similarly, Lee et al. (2012) reported that juvenile sterlet sturgeon fed diets with garlic showed lower SFA and MUFA, but higher PUFA compared to the initial fish or those fed the control diet without garlic treatment. The findings in the present study are in accordance with previous reports, indicating that dietary garlic meal may improve unsaturated fatty acid concentrations in fish body by accumulating EPA (20:5n-3) and DHA (22:5n-6) in the tissues. However, this observation found in the present study, in terms of decreasing SFAs and increasing PUFAs in European seabass with dietary garlic incorporation could not be compared with other studies, since there are no reports regarding the relation between dietary garlic and PUFA:SFA ratio in seabass, to our knowledge so far.

Hepatosomatic indexes (HSI) of the initial and the final fish samples were highest in the experimental groups fed with 2% (GM2) and 6% (GM6) garlic meal diets, while significantly ($p<0.05$) lower rates of HSI were found in the control and the 4% (GM4) garlic meal treatments. Significantly lower ($p<0.05$) viscerasomatic indexes (VSI) were also found for fish fed the control and the 4% garlic meal inclusion diet (GM4) compared to the GM2 and GM6 treatments. Similar findings were also recorded for the lipid accumulations around the internal organs, the so called mesenteric fat index (MFI), with significantly lower ($p>0.05$) values for the control and the GM4 groups compared to the GM2 and GM6 treatment groups (Table 7).

In animal nutrition studies, the hepatosomatic index (HSI) is used as an indicator for the energy reserve status of the animal. Since the liver is a target for the metabolism in the fish body, the hepatosomatic index is an effective biomarker for the detection of hazardous effects derived from environmental factors (Pait and Nelson 2003). The HSI in the present study was significantly lower in fish fed diets with 4 % garlic compared to the other garlic inclusion leves. However, the lowest HSI recorded in the 4% garlic group was not significantly different then the control group without garlic meal addition. Our results are in partial agreement with the findings of Abdel-Hakim et al. (2010) who reported that there are slight differences in HSI in fish fed the garlic meal diet however the diffeneces between the garlic treatment groups and the control group were insignificant. Shalaby et al. (2006) found that supplementing garlic meal in Nile tilapia diets at increasing levels from 1 to 4 %, did not affect the HSI in percent. In contrast, Metwally (2009) who used different forms of garlic in diets for Nile tilapia fingerlings (natural garlic 40 g/kg diet, garlic oil capsules 250 mg/kg diet, and garlic powder 32g/kg diet), reported that HSI in all experimental diets with different forms of garlic decreased significantly. Similarly, Lee et al. (2014) also presented significantly lower HSI in sturgeon fed diets containing garlic powder than that of fish group fed diets without garlic inclusion.

Table 6. Fatty acid composition (%) in muscle tissues of juvenile European seabass fed diets with different levels of garlic meal until satiation for a period of 60 days.

			Experimental diets			
		Initial	Control	GM2	GM4	GM6
ΣSFA (Total saturated fatty acid)						
16:1	PA	7.116 ± 0.66^{a}	6.994 ± 0.85^{a}	6.895 ± 0.15^{a}	6.674 ± 0.67^{a}	6.910 ± 0.02^{a}
18:1 (n-9)	OA	28.26 ± 0.44^{a}	30.65 ± 1.45^{a}	30.46 ± 0.06^{a}	30.39 ± 2.13^{a}	30.42 ± 0.11^{a}
14:1	MA	0.0388±0.003^{a}	0.0424±0.011^{a}	0.0369±0.000^{a}	0.0349±0.002^{a}	0.040±0.001^{a}
20:1 (n-9)	GA	4.758 ± 0.02^{a}	5.206 ± 1.15^{ab}	6.421 ± 0.23^{bc}	6.978 ± 0.23^{c}	6.640 ± 0.06^{c}
24:1	NA	0.0388±0.001^{b}	0.0296±0.003^{a}	0.0366±0.001^{b}	0.0369±0.000^{b}	0.036±0.001^{b}
ΣMUFA (Total mono unsaturated fatty acid)						
18:2 (n-6)	LA	12.77 ± 0.26^{b}	10.83 ± 1.03^{ab}	10.21 ± 0.13^{ab}	7.99 ± 3.38^{a}	10.34±0.20^{ab}
18:3 (n-6)	α-LA	0.149 ± 0.009^{b}	0.152 ± 0.006^{b}	0.140 ± 0.001^{ab}	0.144 ± 0.001^{b}	0.128±0.004^{a}
20:4 (n-6)	AA	0.619 ± 0.025^{b}	0.425 ± 0.054^{a}	0.439 ± 0.011^{a}	0.442 ± 0.013^{a}	0.423±0.021^{a}
ΣPUFA (Total polyunsaturated fatty acid, n3/n6)						
20:3(n-6)	EA	0.156 ± 0.004^{a}	0.158 ± 0.002^{ab}	0.155 ± 0.002^{a}	0.169 ± 0.009^{b}	0.152±0.002^{a}
20:5 (n-3)	EPA	5.127 ± 0.199^{a}	5.687 ± 0.123^{b}	5.249 ± 0.031^{a}	5.728 ± 0.152^{b}	4.967±0.099^{a}
22:6 (n-3)	DHA	12.81 ± 0.51^{a}	11.99 ± 1.31^{a}	11.89 ± 0.01^{a}	12.37 ± 0.14^{a}	11.52 ± 0.02^{a}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
GM (Garlic meal), PA (Palmitoleik acid, 16:1), OA (Oleic acid, 18:1 n-9), MA (Miristoleic acid, 14:1), GA (Gadoleic acid, 20:1 n-9), NA (Nervonic acid, 24:1), LA (Linoleic acid, 18:2 n-6), α LA (α-Linoleic acid, 18:3 n-6), EA (Eicosatrienoic acid, 20:3 n-3+n-6), AA (Arachidonic acid, 20:4 n-6), EPA (Ecosapentanoic acid, 20:5 n-3), DHA (Docosahekzanoic acid, 22:6 n-3).

Table 7. Body morphological indices of juvenile European seabass fed diets with different levels of garlic meal until satiation for a period of 60 days.

	Experimental diets			
	Control	GM2	GM4	GM6
HSI	0.99±0.29^{ab}	1.46±0.41^{c}	0.93±0.25^{a}	1.27±0.26^{bc}
VSI	8.30±1.07^{a}	9.34±1.72^{ab}	8.43±1.63^{a}	10.2±1.26^{b}
MFI	1.64±0.72^{a}	4.16±1.30^{b}	1.93±0.82^{a}	3.22±1.39^{b}

Values (means±standart deviation, triplicate groups) with different superscipts are statistically different at 0.05 level (One-way ANOVA and Duncan's multiple range test, P<0.05).
HSI= Hepatosomatic index, VSI= Viscerasomatic index, MFI= Mesenteric fat index, GM= Garlic meal

Conclusion

The results obtained in the present study demonstrated that garlic meal as a natural feed additive represents alternative solutions to induce aquafeeds as a growth promoter. It might be concluded that the dietary garlic inclusion levels affect growth performance, feed utilization and body protein content of European seabass at ongrowing stage. Furthermore, a lowering effect on nitrogen excretion rate was also recorded when garlic meal was incorporated in diets for seabass at ongrowing stage. Based on the tendency of a lowered nitrogen excretion or enhanced retention rates found in the present study, it might be interesting to search the long-term effect of dietary garlic on nitrogenous end-products and fish growth. Additionally, dietary garlic meal improved unsaturated fatty acid concentrations by accumulating EPA (20:5n-3) and DHA (22:5n-6) in the tissues of seabass juveniles, as a result of lowered SFAs and MUFAs, but increased PUFAs in fish fed garlic supplemented diets. The suggested dietary garlic for seabass juveniles in the present study was 4% (40 g/kg) for a positive influence on growth performance and nutrient utilization. Further studies are encouraged to focus on the total economic cost and benefit analysis for

the use of garlic in large scale aquaculture operations.

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