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Effects of Dietary Supplement of *Schizochytrium* Meal on Growth, Fatty Acid Profile and Activities of Digestive Enzymes in Turbot (*Scophthalmus maximus* L.) Larvae

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YYW and MZL performed the experiments, analyzed the data, wrote the manuscript. XHG, AHS and HD analyzed the samples and data. GL helped in preparation of the first draft of the manuscript. QHA and KSM conceived and designed the project and revised the manuscript.

Key words
***Schizochytrium* meal, Growth, Fatty acid, *Scophthalmus maximus* L.**

ABSTRACT

A feeding trial was conducted to investigate the effects of supplementation of *Schizochytrium* meal on growth, digestive enzymes activities and fatty acid composition of turbot (*Scophthalmus maximus* L.) larvae. Four isonitrogenous and isolipidic diets were formulated to contain 0 (S0, control diet), 50 (S5), 100 (S10) and 150 (S15) g kg^{-1} *Schizochytrium* meal. Fish (initial body weight, 0.06 g) were randomly allotted to 12 square fiberglass tanks. Fish were fed 5 times daily (7:00, 10:00, 14:00, 17:00 and 21:00) for 28 days. No significant differences were observed in survival and intestinal morphology among fish fed various levels of algae meal ($P>0.05$). Fish fed diet S5 had significantly higher final body weight than that of fish fed diet S15, and no significant differences were observed among fish fed diets S0, S5 and S10 ($P>0.05$). Trypsin in intestinal segments, specific activities of alkaline phosphatase (AKP) in intestine and purified brush border membrane (BBM) of intestine were significantly higher in fish fed diet S5 than that of fish fed diet S15 ($P<0.05$). Specific activities of leucine-aminopeptidase (LAP) in intestine and purified BBM of intestine was significantly higher in fish fed diet S10 than that of fish fed diet S15 ($P<0.05$). Fish fed diets S5 and S10 had significantly higher docosahexaenoic (DHA, 22:6n-3), n-3 PUFAs content and n-3/n-6 ratio in muscle than fish fed diets S0 and S15 ($P<0.05$). Fish fed diets S5, S10 and S15 had significant lower C18:3n-3, eicosapentaenoic (EPA, 20:5n-3), C18:2n-6, n-6 polyunsaturated fatty acids (PUFAs) content in muscle than fish fed the control diets ($P<0.05$). No significant differences were observed in intestinal morphology ($P>0.05$). In conclusion, 50-100 g kg^{-1} dry matter *Schizochytrium* meal in microdiets can improve growth performance and may be a valuable additive in microdiets of turbot larvae.

INTRODUCTION

A current major bottleneck in marine fish hatcheries is the dependence on live prey, such as rotifers and *Artemia* nauplii, which cannot be manipulated as desired (Lazo *et al*., 2000). In addition, live prey that used to feed marine fish larvae is lack of essential fatty acids. Moreover, the larvae had incomplete digestive tract and lack of digestive enzymes (Kolkovski *et al*., 1997). It is known that nutritional imbalances play a key role in morphogenesis and skeletogenesis at early stages and several dietary components have been identified that affect larval development (Boglino *et al*., 2012). Therefore, it would be ideal to produce nutritionally balanced microparticulate diets to replace zooplankton. However, ingestion rates of microparticulate diet are often lower than those of live prey during the first feeding, which may limit the availability of nutrients for proper growth and development (Lauff and Hoffer, 1984; Kolkovski *et al*., 1993).

Microalgae are prokaryotic (eg., Cyanobacteria) or eukaryotic (eg., green algae and diatoms) photosynthetic

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microorganisms that can grow rapidly and proliferate in a wide range of environmental conditions due to their unicellular or simple multicellular structure (Mata *et al*., 2010). Microalgae contain many valuable nutrients for aquafeeds, such as protein, essential amino acids, minerals, water-soluble vitamins, sterols and bioactive compounds (Ju *et al*., 2012; Atkinson, 2013). Microalgae are also rich in antioxidant pigments such as carotenoids, chlorophylls and phycobiliproteins, and had commonly been added in diets as pigmentation sources for shrimp, salmon and trout (Chien and Shiau, 2005; Güroy *et al*., 2012). Moreover, most microalgae are rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic (EPA, 20:5n-3), docosahexaenoic (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6). The beneficial effects of microalgae have been shown to be particularly important on survival, growth, feed utilization, immune responses, metamorphosis, appetites and early maturation of larval and juvenile finfish, crustacean and mollusk (Atkinson, 2013; Güroy *et al*., 2012; Ju *et al*., 2009, 2012; Patterson and Gatlin III, 2013; Shan and Lin, 2014; Vizcaíno *et al*., 2014) when these essential fatty acids are provided in sufficient amount or in adequate form in feed. Unfortunately, the use of algal meal to replace fish-based ingredients in aquatic feeds is challenged mostly because of the high cost of production and culture inefficiency.

Schizochytrium is a unicellular, heterotrophic thraustochytrid, which containing substantial amounts of lipid (~10–50%), and produce a high level of total lipids as DHA (30–70%) (Lewis *et al*., 1999; Arney *et al*., 2015). *Schizochytrium* can be used as a feed to effectively enriching both n-3 and n-6 LC-PUFAs contents of rotifers and *Artemia nauplii* prior to feeding to fish and shrimp larvae (Lewis *et al*., 1999; Li *et al*., 2009). Thraustochytrid-derived products could be used as promising alternatives, valuable additive or sustainable sources of LC-PUFAs in aquafeeds without detriment to growth of Atlantic salmon (*Salmo salar*) (Carter *et al*., 2003; Miller *et al*., 2007), white shrimp (*Litopenaeus vannamei*) (Patnaik *et al*., 2006; Ju *et al*., 2009) and sea bream (*Sparus aurata*) (Ganuza *et al*., 2008). *Schizochytrium* meal has never been investigated as an additive in turbot (*Scophthalmus maximus* L.) microdiets. Therefore, the aim of this study was to evaluate the effects of supplementation of *Schizochytrium* meal on survival, growth, digestive enzymes activities and fatty acid composition of turbot larvae.

MATERIALS AND METHODS

Experimental diets

The *Schizochytrium* meal (crude protein, 120.6 g kg^{-1}; crude lipid, 408.0 g kg^{-1}) was supplied by Alltech® Company (Nicholasville, Kentucky, USA). Four isonitrogenous and isolipidic diets were formulated to contain 0 (S0), 50 (S5), 100 (S10) and 150 (S15) g kg^{-1} dry matter of *Schizochytrium* meal. Ingredients and proximate composition of the experimental diets are presented in Table I and fatty acid composition of *Schizochytrium* meal and diets are shown in Table II. Microdiets were manufactured by micro-bonding technology as described by Wang *et al*. (2017). The dry pellets were ground into 150–250 μm and 250–380 μm particle sizes subsequently stored at −20 °C until used.

Table I. Ingredients and nutrients of the experimental diets.

Ingredient	Diet no. (*Schizochytrium* meal level, g kg^{-1} dry matter)			
	S0	S5	S10	S15
Fish meal[1]	550	550	550	550
Shrimp meal[1]	100	100	100	100
Squid meal[1]	50	50	50	50
Bear Yeast meal[1]	30	30	30	30
Mussel meal[1]	50	35	25	15
Schizochytrium meal[2]	0	50	100	150
Fish oil[1]	64	44	24	5
Wheat flour[1]	63	48	28	7
Sodium alginate	10	10	10	10
Vitamin premix[3]	10	10	10	10
Mineral premix[3]	15	15	15	15
Choline chloride	2	2	2	2
Antioxidant	0.5	0.5	0.5	0.5
Attractant	15	15	15	15
Lecithin	40	40	40	40
Sodium benzoate	0.5	0.5	0.5	0.5
Proximate analysis (% dry matter basis)				
Crude protein	534.2	527.2	530.9	536.5
Crude lipid	111.1	108.2	118.9	117.1

[1]Those ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. (Qingdao China). [2]*Schizochytrium* meal was supplied by Alltech Inc., Kentucky, USA. [3]Vitamin premix and Mineral premix were supplied by Qing Dao Master Bio-Tech, Co., Ltd. (Qingdao China).

Fish rearing

Fish larvae were obtained from a commercial hatchery (Shandong, China) and reared at Haiyang Yellow Sea Aquatic Product Co., Ltd (Yantai, Shandong, China). Larvae were fed with rotifers *Brachionus plicatilis* (5–10 ind./ml) from mouth opening (3 DAH) to 20 DAH, *Artemia nauplii* (0.1–0.2 ind./ml to 1–2 ind./ml) from 6 to 22 DAH, microparticulate diets (10.0–20.0 mg/fish/d) from 15 DAH to the end. Both the rotifers and *Artemia nauplii* had

been enriched with *Chlorella*, yeast and refined fish oil to increase EPA and DHA contents. The *Chlorella* ($8–10\times10^4$ cells/ml) was supplied in the rearing pool at the first 20 days. Larvae (23 days after hatching, initial body weight 0.06±0.00 g) were randomly distributed into 12 square fiberglass tanks (65×65×90 cm, water volume 200 L) with 800 fish each tank. Each diet was randomly assigned to triplicate tanks. The fish were hand fed five times daily (07:00, 10:00, 14:00, 17:00 and 21:00). During the rearing period, each tank was provided with continuous aeration to maintain the dissolved oxygen level above 6.5 mg L^{-1}, water temperature ranged from 18 to 20°C, pH from 6.8 to 7.2, ammonia-N was less than 0.1 mg L^{-1}, photoperiod was set at 12-h light and 12-h dark. Feeding behavior and mortality were monitored every day. The feeding trial lasted for 28 days.

Table II. Fatty acid composition of dried *Schizochytrium* meal and experimental diets.

Fatty acids	Schizochytrium	Diet no. (*Schizochytrium* meal level, 10 g kg^{-1} of total fatty acids)			
		S0	S5	S10	S15
14:0	6.43	5.68	5.05	4.91	3.60
16:0	35.43	23.66	26.73	33.18	40.59
18:0	1.43	4.21	3.47	3.10	2.91
20:0	0.19	0.41	0.33	0.28	0.23
∑SFA	43.48	33.97	35.58	41.46	47.33
16:1n-7	0.18	4.64	4.01	3.07	2.13
18:1n-9	—	13.68	10.93	9.00	8.25
∑MUFA	0.18	18.33	14.95	12.07	10.38
18:3n-3	0.66	1.96	1.80	1.48	1.30
20:5n-3 (EPA)	0.38	3.65	2.93	1.97	1.07
22:6n-3 (DHA)	40.64	7.78	10.41	13.57	15.55
∑n-3 PUFA	41.68	13.39	15.15	17.02	17.92
18:2n-6	0.53	13.69	12.32	11.53	10.88
20:4n-6	1.17	0.51	0.58	0.65	0.73
∑n-6 PUFA	1.7	14.19	12.90	12.18	11.60
∑PUFA	43.38	27.58	28.05	29.20	29.52
n-3/n-6	24.52	0.94	1.17	1.41	1.55
DHA/EPA	106.95	2.13	3.56	6.89	14.55
Total fatty acids	87.04	79.87	78.57	82.72	87.23

SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; n-3 PUFA: n-3 polyunsaturated fatty acids; n-6 PUFA: n-6 polyunsaturated fatty acids.

Sample collection

At the end of the feeding trial, the fish were fasted for 24 h before harvest. Total number and weight of fish in each tank were measured. Twenty fish were randomly collected from each tank to monitor final body weight and final body length. Fifty individuals were randomly collected from each tank, and then immediately stored at −80°C for enzyme activity assays. Twenty fish from each tank were collected and stored frozen (−20°C) for determination of fatty acid.

Activities of digestive enzyme analysis

Trypsin activity was assayed according to Holm *et al*. (1988). The fish were dissected to separate pancreatic and intestinal segments as described in Cahu and Zambonino-Infante (1994). Dissection was conducted on a glass plate maintained at 0°C. The dissected samples were weighed and homogenized in cold ultrapure water (tissue: water, 1:5). The homogenates were centrifuged at 3300 ×g at 4°C for 10 min, and then the supernatant was gently collected and frozen at −80°C for digestive enzyme activity analysis. Purified brush border membranes (BBM) from homogenate of intestinal segment were obtained according to a method described by Crane *et al*. (1979). Briefly, before $CaCl_2$ solution addition, 1 mL homogenate was diverted for intestinal enzyme assays. After addition of 0.1 M $CaCl_2$, the homogenates were centrifuged at 3300 ×g for 10 min in a centrifuge at 4°C. The supernatants were collected and stored frozen (−80°C) for digestive enzymes activities or protein content analysis. Trypsin activity was assayed according to Holm *et al*. (1988). Leucine-aminopeptidase (LAP) and alkaline phosphatase (AKP) were assayed both in intestinal segment and BBM according to Bessey *et al*. (1946) and Maroux *et al*. (1973), respectively. Protein concentration was determined according to Bradford (1976), and using bovine serum albumin (BSA; Sigma, Saint Louis, MO, USA) as a standard. All the enzyme activity assays were carried out in triplicate.

Fatty acid analysis

The fatty acid profiles were determined as described by Zuo *et al*. (2012) by using HP6890 gas chromatograph (Agilents Technologies Inc., Santa Clara, California, USA) with a fused silica capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector.

Histopathological observation

Distal intestine tissue of three fish per tank were cut and immersed in Bouin's fixative solution. After fixation for 24 h, the fixed intestine tissue samples were dehydrated in a graded series of ethyl alcohol, equilibrated in xylene and embedded in paraffin. Tissue sections (5 μm thickness) were cut from each sample and then stained with hematoxylin and eosin (H and E). The fold height

(HF), enterocyte height (HE) and microvillus height (HMV) were measured using a microscope equipped with a camera (E600, Nikon, Tokyo, Japan) and an image acquiring software (CellSens Standard, Olympus, Tokyo, Japan).

Statistical analysis

The data are presented as means ± S.D (n = 3). All data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was applied as a multiple sample comparison when significant differences was detected ($P<0.05$). All statistical analyses were carried out by using SAS 9.12 (Statistical Analysis System Institute, Cary, NC, USA) for Windows.

RESULTS

Growth performance

After the 28-day feeding trial, no significant differences were observed in survival and specific growth rate among fish fed diets with *Schizochytrium* meal ($P>0.05$). Fish fed the diet with 50 g kg^{-1} algae meal had significantly higher final body weight than that of fish fed diet with 150 g kg^{-1} algae meal, and no significant differences were observed among fish fed diets with 0, 50 and 100 g kg^{-1} algae meal ($P>0.05$). Final body length of fish fed diet with 50 g kg^{-1} algae meal was significantly higher than that of fish fed diets with 0 and 150 g kg^{-1} algae meal ($P<0.05$), but no significant differences were observed between fish fed diets with 50 and 100 g kg^{-1} algae meal ($P>0.05$) (Table III).

Specific activities of digestive enzyme

In this study, activity of trypsin in pancreatic segments, and activity of amylase and lipase in pancreatic and intestinal segments were not significantly affected by dietary *Schizochytrium* meal levels ($P>0.05$). Activity of trypsin in intestinal segments of fish fed diet with 50 g kg^{-1} algae meal was significantly higher than that of fish fed diet with 150 g kg^{-1} algae meal, and no significant difference was observed among fish fed diets with 0, 50 and 100 g kg^{-1} algae meal. Specific activities of alkaline phosphatase (AKP) in intestine and purified brush border membrane of intestine was significantly higher in fish fed diet with 50 g kg^{-1} algae meal than that of fish fed diet with 150 g kg^{-1} algae meal ($P<0.05$). Specific activities of leucine-aminopeptidase (LAP) in intestine and purified brush border membrane of intestine was significantly higher in the diet with 100 g kg^{-1} algae meal than that of fish fed diet with 150 g kg^{-1} algae meal ($P<0.05$). No significant differences were observed in AKP and LAP among fish fed diets with 0, 50 and 100 g kg^{-1} algae meal ($P>0.05$) (Table IV).

Fatty acid composition

The percentages of all the identified fatty acids in the muscle of fish fed with graded levels of algae meal are shown in Table V. Fish fed the 50 and 100 g kg^{-1} algae meal diets had significantly higher C18:0, C22:6n-3, n-3 PUFAs content and n-3/n-6 ratio in muscle than fish fed diets with 0 and 150 g kg^{-1} algae meal ($P<0.05$). Fish fed the control diet had significant higher C14:0, C16:1n-7, C18:1n-9, MUFA, C18:3n-3, C18:2n-6, n-6 PUFAs content in the muscle than fish fed the 50, 100 and 150 g kg^{-1} algae meal diets ($P<0.05$), however, no significant differences were observed in these fatty acids among fish fed 50, 100 and 150 g kg^{-1} algae meal diets ($P>0.05$). C16:0, SFA content and DHA/EPA ratio increased, while EPA decreased in muscle as dietary algae meal level increased; fish fed the 150 g kg^{-1} algae meal diet had significant lower C20:0, C20:4n-6 and PUFA content than fish fed the 0, 50 and 100 g kg^{-1} algae meal diets ($P<0.05$), however, no significant difference was observed among fish fed the 0, 50 and 100 g kg^{-1} algae meal diets ($P>0.05$).

Intestinal morphology

As shown in Table VI, there was an increase trend in HF, HE and HMV in fish fed diets with 5% and 10% *Schizochytrium* meal than 0 and 15% groups, but no significant differences were observed in HF, HE and HMV among fish fed different diets ($P>0.05$) (Fig. 1, Table VI).

Table III. Effect of dietary *Schizochytrium* meal levels on growth and survival of turbot (*Scophthalmus maximus* L.) larvae.

Diet no.	S0	S5	S10	S15
FBW (g)	0.44 ± 0.02^{ab}	0.48 ± 0.02^{a}	0.44 ± 0.01^{ab}	0.42 ± 0.02^{b}
SGR (%·day^{-1})	7.19±0.18	7.46±0.15	7.22±0.09	7.05±0.13
FBL (mm)	24.73 ± 0.38^{b}	25.90 ± 0.31^{a}	25.44 ± 0.23^{ab}	24.87 ± 0.33^{b}

Note: Data represent as means ± S.D; Values in the same row with different superscripts are significantly different ($P<0.05$). Specific growth rate (SGR, % day^{-1}) = (Ln FBW-Ln IBW) ×100/ experimental duration (d). IBW, Initial body weight; FBW, Final body weight; FBL, Final body length.

Table IV. Effects of dietary *Schizochytrium* meal levels on activities of digestive enzymes of turbot (*Scophthalmus maximus* L.) larvae.

Digestive enzymes		Diet no. (*Schizochytrium* meal level, %)			
		S0	S5	S10	S15
Trypsin(mU/ mg•protein)	PS	75.74±2.84	87.08±4.83	84.88±5.01	76.40±4.58
	IS	77.78±4.08^{ab}	82.02±3.86^{a}	75.34±0.61^{ab}	70.96±3.89^{b}
Trypsin (I)/trypsin (P)^{b}		1.03±0.01^{a}	0.94±0.07^{ab}	0.89±0.03^{b}	0.93±0.00^{ab}
Amylase (U/ mg•protein)	PS	0.61±0.04	0.57±0.03	0.63±0.03	0.62±0.05
	IS	0.60±0.03	0.52±0.03	0.59±0.01	0.59±0.02
Lipase (mU/ mg•protein)	PS	0.63±0.04	0.66±0.06	0.64±0.03	0.69±0.01
	IS	0.68±0.02	0.69±0.02	0.67±0.05	0.67±0.01
Specific activities of digestive enzymes in purified brush border membrane of intestine (U/ mg•protein)					
Leucine-aminopeptidase		227.89±3.53^{ab}	233.54±7.17^{ab}	239.53±9.23^{a}	214.01±7.80^{b}
Alkaline-phosphatase		375.39±17.55^{ab}	415.78±22.97^{a}	386.93±22.00^{ab}	343.12±19.71^{b}
Specific activities of digestive enzymes in intestine (U/ mg•protein)					
Leucine-aminopeptidase		84.86±3.87^{ab}	86.83±4.44^{ab}	88.46±2.29^{a}	78.95±1.90^{b}
Alkaline-phosphatase		95.50±3.85^{ab}	101.10±2.37^{a}	100.09±4.26^{ab}	91.12±2.29^{b}

Note: Data represent as means ± S.D; Values in the same row with different superscripts are significantly different (P<0.05). ^{a}PS, pancreatic segments; IS, intestinal segments; ^{b}Trypsin (I), trypsin of intestinal segment; trypsin (P): trypsin of pancreatic segment.

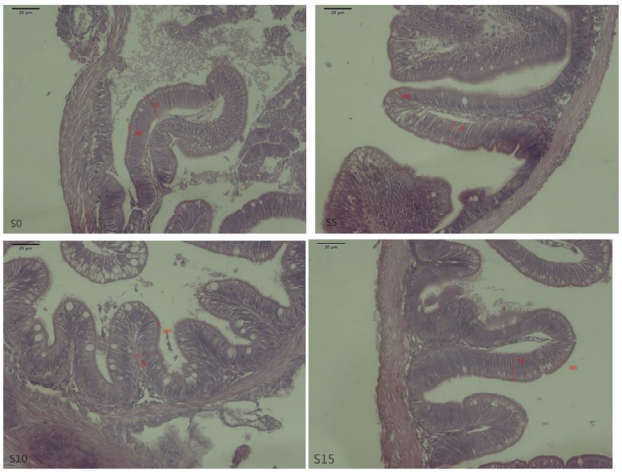

Fig. 1. Effect of dietary *Schizochytrium* meal levels on micromorphology of the intestine of turbot (*Scophthalmus maximus*) larvae.

DISCUSSION

Growth performance

This study was conducted to determine the feasibility of *Schizochytrium* meal use in microdiets for turbot larvae. The results indicate that 0-100 g kg^{-1} algae meal could be used as a promising additive in microdiets of turbot larvae. Many studies also indicated that the addition of dried algae meal to aquacfeed has a positive effect on growth and gut health than those fed diets without algae meal (Li *et al*., 2009; Ju *et al*., 2009; Güroy *et al*., 2012; Eryalçın *et al*., 2013, 2015; Kousoulaki *et al*., 2015).

In this study, growth of fish fed diet with 150 g kg^{-1} algae meal was significantly lower than that of fish fed diet with 50 g kg^{-1} algae meal. Similarly, the negative effects on growth and feed intake caused by high inclusion level or long-term utilization of microalgae have been reported for goldfish (*Carassius auratus*) (Coutinho *et al*., 2006), Atlantic cod (*Gadus morhua*) (Walker and Berlinsky 2011) and red drum (*Sciaenops ocellatus*) (Patterson and Gatlin III, 2013) and Atlantic salmon (Kousoulaki *et al*., 2015). The reduced growth of fish/shrimp maybe mainly attributed to the depressed palatability (Coutinho *et al*., 2006; Walker and Berlinsky, 2011; Ju *et al*., 2012). However, it is difficult to test if the high levels of algae meal affected the palatability and digestibility of fish larvae in this study. In addition, the lack of fatty acids and the lower digestibility may impair growth rate and development of fish larvae (Coutinho *et al*., 2006; Jaime-Ceballos *et al*., 2006; Kousoulaki *et al*., 2015).

The essential fatty acids, particularly DHA, are necessary for the normal growth, survival development of nervous system and sensory organs, behaviour of aquatic animals, particularly critical for marine fish larvae (Navarro *et al*., 1995; Sargent and Tacon, 1999; Carboni *et al*., 2012). Inadequate contents of essential fatty acids in diets may resulted in poor feeding, low survival and poor

growth, impaired predator behavior, skeletal deformities, abnormal pigmentation and immune-deficiency of marine fish larvae (Glencross and Smith, 2001; Benítez-Santana *et al*., 2007; Ganuza *et al*., 2008; Carboni *et al*., 2012). In present study, fatty acids analysis revealed that dietary DHA content and DHA/EPA ratio increased from 7.78% to 15.55% and 2.13 to 14.55, respectively, with increasing algae meal inclusion level from 0 to 15%. The imbalance n-3 LC- PUFAs may destroy the balance and structure of the cell membrane of fish larvae, subsequently affects the growth, behavior, quality and pigmentation of marine fish larvae (Reitan *et al*., 1993; Rainuzzo *et al*., 1994). Therefore, the imbalance DHA/EPA ratio may be one of the reasons responsible for decreased growth of fish or shrimp fed diets that contain high level of microalgae meal.

Table V. Effects of dietary *Schizochytrium* meal levels on fillet fatty acid composition of turbot (*Scophthalmus maximus* L.) larvae.

Fatty acids	Diet no. (*Schizochytrium* meal level, 10 g kg^{-1} of total fatty acids)			
	S0	S5	S10	S15
14:0	3.24±0.01^{a}	2.48±0.13^{b}	2.43±0.31^{b}	2.82±0.05^{ab}
16:0	22.24±0.12^{c}	22.56±0.89^{c}	25.32±0.22^{b}	27.96±0.89^{a}
18:0	7.60±0.00^{bc}	8.33±0.17^{a}	8.30±0.29^{ab}	7.10±0.12^{c}
20:0	0.31±0.00^{a}	0.30±0.01^{ab}	0.31±0.02^{a}	0.26±0.01^{b}
∑SFA	33.39±0.17^{b}	33.66±0.83^{b}	36.36±1.20^{a}	38.14±1.19^{a}
16:1n-7	4.15±0.02^{a}	2.82±0.04^{b}	2.46±0.24^{b}	2.61±0.26^{b}
18:1n-9	15.09±0.03^{a}	11.47±0.25^{b}	10.57±1.12^{b}	10.12±1.16^{b}
∑MUFA	19.24±0.05^{a}	14.29±0.29^{b}	13.03±0.88^{b}	12.73±1.42^{b}
18:3n-3	1.03±0.02^{a}	0.73±0.07^{b}	0.71±0.02^{b}	0.67±0.04^{b}
20:5n-3 (EPA)	2.86±0.12^{a}	2.26±0.09^{ab}	1.73±0.13^{bc}	1.26±0.23^{c}
22:6n-3 (DHA)	9.64±0.56^{c}	13.15±0.26^{b}	15.31±0.33^{a}	10.98±0.34^{c}
∑n-3 PUFA	13.52±1.00^{b}	16.14±0.60^{a}	17.75±0.68^{a}	12.91±0.75^{b}
18:2n-6	11.72±0.10^{a}	9.69±0.19^{b}	9.87±0.37^{b}	9.72±0.41^{b}
20:4n-6	1.18±0.14^{a}	1.11±0.02^{a}	1.14±0.11^{a}	0.70±0.04^{b}
∑n-6 PUFA	12.90±0.35^{a}	10.79±0.30^{b}	11.01±0.36^{b}	10.42±0.64^{b}
∑PUFA	26.42±1.35^{a}	26.93±0.30^{a}	28.76±1.03^{a}	23.33±0.12^{b}
n-3/n-6	1.05±0.04^{c}	1.50±0.07^{ab}	1.61±0.01^{a}	1.24±0.10^{bc}
DHA/EPA	3.37±0.05^{b}	5.82±0.12^{b}	8.88±0.46^{a}	8.97±1.39^{a}
Total fatty acids	79.05	74.88	78.15	74.20

Note: Data represent as means ± S.D; Values in the same row with different superscripts are significantly different (P<0.05). For abbreviation see Table II.

Table VI. Effect of dietary *Schizochytrium* meal levels on micromorphology of the intestine of turbot (*Scophthalmus maximus*) larvae.

Diet groups	S0	S5	S10	S15
HF (μm)	65.59±1.59	69.28±1.62	67.20±1.73	63.84±1.75
HE (μm)	19.19±0.83	21.26±0.76	19.16±0.77	18.89±0.93
HMV (μm)	1.96±0.05	2.11±0.07	2.07±0.07	1.93±0.05

Note: Values in the same row with different superscripts are significantly different (P<0.05). HF, fold height; HE, enterocyte height; HMV, microvillus height. Fold height was analyzed in a lower magnification of objective lens of microscope (magnification ×100); enterocytes height and microvilli height were analyzed in a higher magnification of objective lens of microscope (magnification ×200).

Specific activities of digestive

Marine fish larvae undergo major changes in morphology and functionality of their digestive tract during the first five weeks of life (Péres *et al*., 1997), and changes in enzymatic activities had been used as indicators for studying the effects of the dietary additives that might modulate the maturation process of the digestive tract (Gisbert *et al*., 2009). In this study, activity of trypsin in intestinal segments of fish fed diet with 50 g kg^{-1} algae meal was significantly higher than that of fish fed diet with 150 g kg^{-1} algae meal, similarly, the increased trypsin activity may improve growth or survival of sea bass larvae (Cahu and Zambonino-Infante, 1995); but no significant differences in activity of trypsin in the pancreatic segments, and activity of amylase and lipase in pancreatic and intestinal segments were observed among all treatments. Many compounds present in microalgae could potentially influence digestive enzyme activity in fish larvae. Fioramonti *et al*. (1994) pointed out that algae growth regulators, such as polyamides, can stimulate cholecystokinin release in rats, which mediates the release of pancreatic enzymes.

Brush border membrane (BBM) enzymes assays have been successfully used to determine the degree of the maturation process of the digestive function in intestine in fish larvae (Cahu and Zambonino Infante, 1995; Ma *et al*., 2005). Alkaline phosphatase (AKP) and leucine-aminopeptidase (LAP) are regarded as indicators for a well-differentiated intestinal BBM and have been found to exhibit high activities in fish larvae fed the optimal diets (Cahu *et al*., 1999; Zambonino-Infante and Cahu, 2001; Ma *et al*., 2005). In this study, no significant differences were observed in specific activities of AKP and LAP in intestine and purified BBM of intestine among fish fed diets with 0, 50 and 100 g kg^{-1} algae meal, but higher than that of fish fed diet with 150 g kg^{-1} algae meal. These results shown that algae meal did not cause negative effects on

both enzymatic activities at lower inclusion levels tested. Similarly, Vizcaíno *et al*. (2014) found that both AKP and LAP activities of gilthead sea bream tended to increased with increasing dietary *Scenedesmus almeriensis* level. An increase in specific activity of aminopeptidase has been related to maturation of the intestinal membrane and enhanced survival in fish (Cahu and Zambonino-Infante, 1995).

Fatty acid composition

The fatty acid profile of fish closely reflected the composition of diet (Boglino *et al*., 2012). In this study, fish fed diets with 50 and 100 g kg^{-1} algae meal had significantly higher DHA in muscle than fish fed diets with 0 and 150 g kg^{-1} algae meal, denoting the high nutritional value of the algae meal as an alternative source of DHA. Similar results have been reported for channel catfish (*Ictalurus punctatus*) (Li *et al*., 2009), olive flounder (*Paralichthys olivaceus*) (Qiao *et al*., 2014) and Atlantic salmon (Kousoulaki *et al*., 2015). The algae meal contains low level of EPA, the EPA levels in muscle decreased as dietary algae meal levels increased. The negative effects of fish fed algae meal based diets on EPA content in flesh has also been reported in Atlantic salmon (Carter *et al*., 2003; Miller *et al*., 2007) and seabream (Ganuza *et al*., 2008).

Fish fed diets with 50 and 100 g kg^{-1} algae meal had significantly higher n-3 PUFAs content and n-3/n-6 ratio in muscle than fish fed diets with 0 and 150 g kg^{-1} algae meal. Similarly, many researches had shown that feeding the algae meal increases n-3 PUFAs in fillet of Atlantic salmon (Miller *et al*., 2007; Kousoulaki *et al*., 2015) and channel catfish (Li *et al*., 2009), without adverse effects on flavor quality of fish product, which would benefit for humans. In contrast, no significant differences in muscle total n-3 PUFAs and n-3/n-6 ratio were observed in Atlantic cod (Walker and Berlinsky, 2011) and olive flounder (Qiao *et al*., 2014) when fish fed diets with various levels of algae meal. The disparate responses may be related to fish species, physiological state, algal products types and processing method.

Intestinal morphology

The intestinal morphology parameters can serve as an index to evaluate the functional structure of the intestine and its ability to maintain optimum nutrient absorption and digestive health (Buddle and Bolton, 1992). In the present study, although an increase trend in HF, HE and HMV were observed in fish fed diets with 5% and 10% *Schizochytrium* meal than 0 and 15% groups, no significant differences were observed in different groups. The HF, HE and HMV are important for intestinal function. The increased HF, HE and HMV, caused by *Schizochytrium* meal supplementation, may indicate an increase in the intestinal surface area and consequently increased nutrient absorption. This improvement in intestinal structure may be related to the active substances in *Schizochytrium* meal, such as spermine. Previous studies have established the positive effect of spermine on growth, pancreatic enzyme secretion, intestinal maturation and health of animals (Osborne and Seidel, 1989; Wild *et al*., 1993; Peres *et al*., 1997; Peulen *et al*., 2000). Futher study is warranted to investigate whether high levels of *Schizochytrium* meal would have a negative effect on intestinal maturation.

CONCLUSIONS

In conclusion, the results from the present has study shown that 50-100 g kg^{-1} *Schizochytrium* meal in microdiets can support better growth performance of turbot larvae. The use of this algae meal in turbot microdiets increased the n-3 PUFAs levels and n-3/n-6 ratios in the muscle, and therefore *Schizochytrium* meal could be used as a valuable additive in microdiets of turbot.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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