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

Yuyu Wang1,2, Mingzhi Li3,4, Gang Lin4, Xiaohua Guo5, Aihua Sun5, Hao Dong6, Qinghui Ai1, * and Kangsen Mai1, *

1The Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture) and Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, P.R. China
2College of Marine Engineering, Rizhao Polytechnic, Rizhao 276826, China
3College of agriculture, Ludong University, Yantai Shandong 264025, P.R. China
4Alltech, 3031 Catnip Hill Pike Road, Nicholasville, Kentucky 40356, USA
5Shandong Meijia Group Co. Ltd., Rizhao 276800, China

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 and S10 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 diets S0 and S15 (P<0.05). Fish fed diets S5, S10 and S15 had significantly 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 *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...
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 most 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 derived products 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 ºC 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 meal¹                                       | 550  | 550  | 550  | 550  |
| Shrimp meal¹                                     | 100  | 100  | 100  | 100  |
| Squid meal¹                                      | 50   | 50   | 50   | 50   |
| Bear Yeast meal¹                                 | 30   | 30   | 30   | 30   |
| Mussel meal¹                                     | 50   | 35   | 25   | 15   |
| Schizochytrium meal²                             | 0    | 50   | 100  | 150  |
| Fish oil¹                                       | 64   | 44   | 24   | 5    |
| Wheat flour¹                                     | 63   | 48   | 28   | 7    |
| Sodium alginate                                 | 10   | 10   | 10   | 10   |
| Vitamin premix¹                                  | 10   | 10   | 10   | 10   |
| Mineral premix¹                                  | 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 |

¹Those ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. (Qingdao China). ²Schizochytrium meal was supplied by Alltech Inc., Kentucky, USA. ³Vitamin 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

**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 ºC until used.
been enriched with *Chlorella*, yeast and refined fish oil to increase EPA and DHA contents. The *Chlorella* (8–10×10⁴ 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 (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, and the water temperature was 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 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 CaCl₂ solution addition, 1 mL homogenate was diverted for intestinal enzyme assays. After addition of 0.1 M CaCl₂, 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.

### 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 CaCl₂ solution addition, 1 mL homogenate was diverted for intestinal enzyme assays. After addition of 0.1 M CaCl₂, 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 (Agilent 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

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

| Fatty acids | Schizochytrium meal, 10 g kg⁻¹ of total fatty acids | Diet no. (Schizochytrium meal level, 10 g kg⁻¹ of total fatty acids) |
|-------------|---------------------------------------------------|---------------------------------------------------------------|
| SFA         | 87.04                                             | S0 55 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                                          |
| ΣSFA        | 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                             |
| ΣMUFA       | 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                                      |
| Σn-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                                          |
| Σn-6 PUFA   | 1.7                                               | 14.19 12.90 12.18 11.60                                      |
| ΣPUFA       | 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-un saturated fatty acids; n-3 PUFA: n-3 polyunsaturated fatty acids; n-6 PUFA: n-6 polyunsaturated fatty acids.
(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 muscle than fish fed diets with 0 and 150 g kg⁻¹ algae meal (P>0.05). 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 differences were observed among fish fed diets with 0 and 150 g kg⁻¹ algae meal (P>0.05). Specific activities of alkaline phosphatase (AKP) 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<sup>a</sup> | 0.48±0.02<sup>a</sup> | 0.44±0.01<sup>b</sup> | 0.42±0.02<sup>b</sup> |
| 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<sup>b</sup> | 25.90±0.31<sup>a</sup> | 25.44±0.23<sup>ab</sup> | 24.87±0.33<sup>a</sup> |

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<sup>a</sup> | 82.02±3.86<sup>b</sup> | 75.34±0.61<sup>a</sup> | 70.96±3.89<sup>b</sup> |
| Trypsin (I)/trypsin (P)<sup>b</sup> | PS                                      | 1.03±0.01<sup>a</sup> | 0.94±0.07<sup>b</sup> | 0.89±0.03<sup>b</sup> | 0.93±0.00<sup>b</sup> |
| 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 |

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

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<sup>-1</sup> 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 aquaculture diets 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<sup>-1</sup> algae meal was significantly lower than that of fish fed diet with 50 g kg<sup>-1</sup> 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 may be attributed to the depressed palatability (Coutinho *et al.*, 2006; Walker and Berlinsky, 2011; Jaume-Ceballos *et al.*, 2006). 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; Jaume-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 result 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; Ganzua 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 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 fishlet fatty acids 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±0.01² | 2.48±0.13³ | 2.43±0.31³ | 2.82±0.05³ |
| 16:0        | 22.24±0.12² | 22.56±0.89³ | 25.32±0.22³ | 27.96±0.89³ |
| 18:0        | 7.60±0.00³ | 8.33±0.17³ | 8.30±0.29³ | 7.10±0.12³ |
| 20:0        | 0.31±0.00³ | 0.30±0.01³ | 0.31±0.02³ | 0.26±0.01³ |
| ∑SFA        | 33.39±0.17³ | 33.66±0.83³ | 36.36±1.20³ | 38.14±1.19³ |
| 16:1n-7     | 4.15±0.02³ | 2.82±0.04³ | 2.46±0.24³ | 2.61±0.26³ |
| 18:1n-9     | 15.09±0.03³ | 11.47±0.25³ | 10.57±1.12³ | 10.12±1.16³ |
| ∑MUFA       | 19.24±0.05³ | 14.29±0.29³ | 13.03±0.88³ | 12.73±1.42³ |
| 18:3n-3     | 1.03±0.02³ | 0.73±0.07³ | 0.71±0.02³ | 0.67±0.04³ |
| 20:5n-3 (EPA)| 2.86±0.12³ | 2.26±0.09³ | 1.73±0.13³ | 1.26±0.23³ |
| 22:6n-3 (DHA)| 9.64±0.56³ | 13.15±0.26³ | 15.31±0.33³ | 10.98±0.34³ |
| ∑n-3 PUFA   | 13.52±1.00³ | 16.14±0.60³ | 17.75±0.68³ | 12.91±0.75³ |
| 18:2n-6     | 11.72±0.10³ | 9.69±0.19³ | 9.87±0.37³ | 9.72±0.41³ |
| 20:4n-6     | 1.18±0.14³ | 1.11±0.02³ | 1.14±0.11³ | 0.70±0.04³ |
| ∑n-6 PUFA   | 12.90±0.35³ | 10.79±0.30³ | 11.01±0.36³ | 10.42±0.64³ |
| ∑PUFA       | 26.42±1.35³ | 26.93±0.30³ | 28.76±1.03³ | 23.33±1.12³ |
| n-3 PUFA    | 1.05±0.04³ | 1.50±0.07³ | 1.61±0.01³ | 1.24±0.10³ |
| DHA/ EPA     | 3.37±0.05³ | 5.82±0.12³ | 8.88±0.46³ | 8.97±1.39³ |
| 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 x100); enterocytes height and microvilli height were analyzed in a higher magnification of objective lens of microscope (magnification x200).

**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 (Pères 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, Vizzaino 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) (Qiao et al., 2015) 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 PUFA 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 PUFA 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 PUFA 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). Further 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 study showed 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.

**REFERENCES**

Arney, B., Liu, W., Forster, I., McKinley, R.S., and Pearce, C.M., 2015. Feasibility of dietary substitution of live microalgae with spray-dried Schizochytrium sp. or Spirulina in the hatchery culture of juveniles of the Pacific geoduck clam (Panopea generosa). *Aquaculture, 444*: 117–133. 
https://doi.org/10.1016/j.aquaculture.2015.02.014

Atkinson, N., 2013. The potential of microalgal meals in compound feeds for aquaculture. *Int. Aqua. Feed.*, 16: 14-17.

Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C.M., and Izquierdo, M.S., 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparus aurata*
lairvae. Aquaculture, 264: 408–417. https://doi.org/10.1016/j.aquaculture.2006.10.024
Bessey, O.A., Lowry, O.H., and Brock, M.J., 1946. Rapid coloric method for determination of alkaline phosphatase in five cubic millimeters of serum. J. biol. Chem., 164: 321–329. https://doi.org/10.1016/S0021-9258(18)43072-4
Boglino, A., Darias, M.J., Ortiz-Delgado, J.B., Özcan, F., Estévez, A., Andree, K.B., Hontoria, F., Sarasquete, C., and Gisbert, E., 2012. Commercial products for Artemia enrichment affect growth performance, digestive system maturation, ossification and incidence of skeletal deformities in Senegalese sole (Solea senegalensis) larvae. Aquaculture, 324-325: 290–302. https://doi.org/10.1016/j.aquaculture.2011.11.018
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3
Buddle, J.R., and Bolton, J.R., 1992. The pathophysiology of diarrhoea in pigs. Pig News Inf., 13: 41N–45N.
Cahu, C.L., and Zambonino-Infante, J.L., 1994. Early maturation of the pancreatic and intestinal digestive functions in sea bass (Dicentrarchus labrax): effect of weaning with different protein sources. Fish Physiol. Biochem., 14: 431–437. https://doi.org/10.1007/BF00004343
Cahu, C.L., and Zambonino-Infante, J.L., 1995. Maturation of the intestinal motility and release of cholecystokinin in sea bass (Dicentrarchus labrax) larvae with a compound diet. Effect on digestive enzymes. Comp. Biochem. Physiol., 109A: 213–222. https://doi.org/10.1016/0300-9629(94)90123-6
Cahu, C.L., Zambonino-Infante, J.L., Quazuguel, P., and Le Gall, M.M., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass Dicentrarchus labrax larvae. Aquaculture, 171: 109–119. https://doi.org/10.1016/S0044-8486(98)00428-1
Carbone, S., Vignier, J., Chaintreul, M., Tocher, D.R., and Migaud, H., 2012. Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin Paracentrotus lividus throughout larval development. Aquaculture, 325: 250–258. https://doi.org/10.1016/j.aquaculture.2011.10.037
Carter, C.G., Bransden, M.P., Lewis, T.E., and Nichols, P.D., 2003. Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds. Mar. Biotechnol., 5: 480–492. https://doi.org/10.1071/sb0126-002-0096-8
Chien, Y.H., and Shiau, W.C., 2005. The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, Marsupenaeus japonicus Bate. J. exp. Mar. Biol. Ecol., 318: 201–211. https://doi.org/10.1016/j.jembe.2004.12.016
Coutinho, P., Rema, P., Otero, A., Pereira, O., and Fabregas, J., 2006. Use of biomass of the marine microalga Isochrysis galbana in the nutrition of goldfish (Carassius auratus) larvae as source of protein and vitamins. Aquac. Res., 37: 793–798. https://doi.org/10.1111/j.1365-2109.2006.01492.x
Crane, R.K., Boge, G., and Rigal, A., 1979. Isolation of brush border membranes in vesicular form from the intestinal spiral valve of the small dogfish Scyliorhinus canicula. Biochim. biophys. Acta, 554: 264–267. https://doi.org/10.1016/0005-2736(79)90024-5
Eryalçın, K.M., Ganuza, E., Atalah, E., and Hernández Cruz, M.C., 2015. Nannochloropsis gaditana and Cryptothecodinium cohnii, two microalgae as alternative sources of essential fatty acids in early weaning for gilthead seabream. Hidrobiológica, 25: 193-202.
Eryalçın, K.M., Roo, J., Saleh, R., Atalah, E., Benítez, T., Betancor, M., and Izquierdo, M., 2013. Fish oil replacement by different microalgal products in microdiets for early weaning of gilthead sea bream (Sparus aurata, L.). Aquac. Res., 44: 819-828. https://doi.org/10.1111/j.1365-2109.2012.03237.x
Fioramonti, J., Fargeas, M.J., Bertrand, V., Pradayrol, L., and Bueno, L., 1994. Induction of postprandial intestinal motility and release of cholecystokinin by polyamines in rats. Am. J. Physiol., 267: G960–G965. https://doi.org/10.1152/ajpgi.1994.267.6.G960
Ganuza, E., Benítez-Santana, T., Atalah, O., Vega-Orellana, E., Ganga, R., and Izquierdo, M.S., 2008. Cryptothecodinium cohnii and Schizochytrium sp. as potential substitutes to fisheries-derived oils from seabream (Sparus aurata) microalgal 131 microalgae 131 microalgal products. Aquaculture, 131: 131–131. https://doi.org/10.1016/j.aquaculture.2008.10.039
Gisbert, E., Giménez, G., Fernández, I., Kotzamanis, Y., and Estévez, A., 2009. Development of digestive enzymes in common dentex Dentex dentex during early ontogeny. Aquaculture, 287: 381–387. https://doi.org/10.1016/j.aquaculture.2008.10.039
Glencross, B.D., and Smith, D.M., 2001. Optimizing the essential fatty acids, eicosapentenoic and docosahexaenoic acid, in the diet of the prawn,
Penaeus monodon. *Aquac. Nutr.*, 7: 101–112. https://doi.org/10.1016/j.aquanutr.2014.12.002

Güroy, B., Şahin, İ., Mantoğlu, S., and Kayal, S., 2012. *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. *Aquac. Int.*, 20: 869–878. https://doi.org/10.1007/s10499-012-9512-x

Holm, H., Hanssen, L.E., Krogdahl, A., and Florholmen, J., 1988. High and low inhibitor soybean meals affect human duodenal proteinase activity differently: *In vivo* comparison with bovine serum albumin. *J. Nutr.*, 118: 515–520. https://doi.org/10.1093/jn/118.4.515

Jaime-Ceballos, B.J., Hernández-Llamas, A., García-Galano, T., and Villarreal, H., 2006. Substitution of Chaetoceros muelleri by *Spirulina platensis* meal in diets for *Liopsetenaeus schmitti* larvae. *Aquaculture*, 260: 215–220. https://doi.org/10.1016/j.aquaculture.2006.06.002

Ju, Z.Y., Deng, D.F., and Dominy, W., 2012. A defatted microalga (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fish meal in diets of Pacific white shrimp (*Liopsetenaeus vannamei*, Boone, 1931). *Aquaculture*, 354: 50–55. https://doi.org/10.1016/j.aquaculture.2012.04.028

Ju, Z.Y., Forster, I., and Dominy, W., 2009. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Liopsetenaeus vannamei*). *Aquaculture*, 292: 237–243. https://doi.org/10.1016/j.aquaculture.2009.04.040

Kolkovski, S., Tandler, A., and Izquierdo, M. S., 1997. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. *Aquaculture*, 148: 313–322. https://doi.org/10.1016/S0044-8486(96)01366-X

Kolkovski, S., Tandler, A., Kissil, G.W., and Gertler, A., 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. *Fish Physiol. Biochem.*, 12: 203–209. https://doi.org/10.1007/BF00004368

Kousoulaki, K., Østbye, T.K., Krasnov, A., Torgersen, J.S., Morkere, T., and Sweetman, J., 2015. Metabolism, health and fillet nutritional quality in Atlantic salmon (*Salmo salar*) fed diets containing n-3-rich microalgae. *J. Nutr. Sci.*, 4: 1-13. https://doi.org/10.1017/jns.2015.14

Lauff, M., and Hoffer, R., 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture*, 37: 335–346. https://doi.org/10.1016/0044-8486(84)90298-9

Lazo, J.P., Dinis, M.T., Holt, G.J., Faulk, C., and Arnold, C.R., 2000. Co-feeding microparticulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). *Aquaculture*, 188: 339–351. https://doi.org/10.1016/S0044-8486(00)00339-2

Lewis, T.E., Nichols, P.D., and McMeechin, T.A., 1999. The biotechnological potential of thraustochytrids. *Mar. Biotechnol.*, 1: 580–587. https://doi.org/10.1007/PL00011813

Li, M.H., Robinson, E.H., Tucker, C.S., Manning, B.B., and Kho, L., 2009. Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. *Aquaculture*, 292: 232–236. https://doi.org/10.1016/j.aquaculture.2009.04.033

Ma, H.M., Cahu, C., Zamponino, J., Yu, H.R., Duan, Q.Y., Le Gall, M., and Mai, K.S., 2005. Activities of selected digestive enzymes during larval development of large yellow croaker (*Pseudosciaena crocea*). *Aquaculture*, 245: 239–248. https://doi.org/10.1016/j.aquaculture.2004.11.032

Maroux, S., Louvard, D., and Baratti, J., 1973. The aminopeptidase from hog-intestinal brush border. *Biochim. biophys. Acta*, 321: 282–295. https://doi.org/10.1016/0005-2744(73)90083-1

Mata T.M., Martins A.A., and Caetano N.S., 2010. Substitution of marine microalgae for biodiesel production and other applications: A review. *Renew. Sust. Energy Rev.*, 14: 217–232. https://doi.org/10.1016/j.rser.2009.07.020

Miller, M.R., Nichols, P.D., and Carter, C.G., 2007. Replacement of fish oil with thraustochytrid *Schizochytrium* sp. L. oil in Atlantic salmon parr (*Salmo salar* L) diets. *Comp. Biochem. Physiol. A.*, 148: 382–392. https://doi.org/10.1016/j.cbpa.2007.05.018

Nakagawa, H., 2011. Quality control of cultured fish by feed supplements. *Bull. Fish Res. Agency*, 31: 51–54. https://doi.org/10.1007/978-90-481-8630-3_5

Navarro, J.C., McEvoy, L.A., Amat, F., and Sargent, J.R., 1995. Effects of diet on fatty acid composition of body zones in larvae of the sea bass *Dicentrarchus labrax*: A chemometric study. *Mar. Biol.*, 124: 177-183. https://doi.org/10.1007/BF00347121

Osborne, D.L., and Seidel, E.R., 1989. Microflora-derived polyamines modulate obstruction-induced colonic mucosal hypertrophy. *Am. J. Physiol. Gust. Liver Physiol.*, 256: G1049—G1057. https://doi.org/10.1152/ajpgi.1989.256.4.g1049
Online First Article

Shan, X., and Lin M., 2014. Effects of algae and Sargent, J.R., and Tacon, A.G.J., 1999. Development of Sukla, L.B., Pradhan, N., Panda, S., and Mishra, B.K., 2015. Environmental microbial biotechnology.  

In: Microalgae: Cultivation and application (eds. V. Aishvarya, J. Jena, N. Pradhan, P.K. Panda and L.B. Sukla). Springer International Publishing, Switzerland. pp. 289–311. https://doi.org/10.1007/978-3-319-19018-1_15

Vizcaíno, A.J., López, G., Sáez, M.I., Jiménez, J.A., Barros, A., Hidalgo, L., Camacho-Rodríguez, J., Martínez, T.F., Cerón-García, M.C., and Alarcón, F.J., 2014. Effects of the microalga Scenedesmus almeriensis as fishmeal alternative in diets for gilthead sea bream, Sparus aurata, juveniles. Aquaculture, 431: 34–43. https://doi.org/10.1016/j.aquaculture.2014.05.010

Walker, A.B., and Berlinksy, D.L., 2011. Effects of partial replacement of fishmeal protein by microalgae on growth, feed intake, and body composition of Atlantic cod. N. Am. J. Aquac., 73: 76–83.

Wang, Y.Y., Li, M.Z., Filer, K., Xue, Y., Ai, Q.H., and Mai, K.S., 2017. Evaluation of Schizochytrium fabae meal in microdiets of Pacific white shrimp (Litopenaeus vannamei). Aquac. Res., 48: 2328–2336. https://doi.org/10.1111/are.13068

Wild, G.E., Daly, A.S., and Sauriol, N., 1993. Effect of exogenously administered polyamine on the structural maturation and enzyme ontogeny of the postnatal rat intestine. Neonatology, 63: 246-257. https://doi.org/10.1159/000243938

Zambonino-Infante, J.L., and Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. Comp. Biochem. Physiol., 130C: 477–487. https://doi.org/10.1016/S1532-0456(01)00274-5

Zambonino-Infante, J.L., Cahu, C.L., Peres, A., Quazuguel, P., and Le Gall, M.M., 1996. Sea bass Dicentrarchus labrax fed different Artemia rations: growth, pancreas enzymatic response and development of digestive functions. Aquaculture, 139: 129–138. https://doi.org/10.1016/0044-8486(95)01149-8

Zuo, R.T., Ai, Q.H., and Mai, K.S., 2012. Effects of dietary n–3 highly unsaturated fatty acids on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (Larimichthys crocea) following natural infestation of parasites (Cryptocaryon iritans). Fish Shellf. Immunol., 32: 249–258. https://doi.org/10.1016/j.fsi.2011.11.005