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Influence of Different Dietary Soybean Lecithin / Fish Oil Ratios on Growth Performance and Histology of Juvenile Grouper *Epinephelus coioides*

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Abstract: Four formulated microdiets supplemented with different soybean lecithin / fish oil (SL/FO) ratios: Diet 1 (0% /22%), Diet 2 (5% /14%), Diet 3 (10% /8%) and Diet 4 (15% /7%) were fed to 40-day-old juvenile grouper *Epinephelus coioides* after hatching. Each experimental diet was distributed in triplicate tanks. The survival, feed efficiency, growth performance and intestine histology of the grouper juveniles were monitored after a 40-day growth trial. Results showed that different dietary SL/FO ratios significantly affected the growth and survival ($P \leqslant 0.05$) of the grouper juveniles. The juveniles fed Diet 3 (10% /8%, SL/FO) and Diet 4 (15/7, SL/FO) gave significantly higher weight gain (WG) than those fed Diet 1 (0% /22%, SL/FO). The juveniles fed Diet1 (0% / 22%, SL/FO) had obviously lower whole body lipid content than those fed other diets ($P \leqslant 0.05$), but had significantly higher whole body protein content than those fed other diets ($P < 0.05$). For all treatments no significant differences were observed in feed efficiency (FE), hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF), specific growth rate (SGR), feed intake (FI), protein efficiency ratio (PER), and final body weight (FBW). Increase in dietary soybean lecithin/fish oil (SL/FO) ratio reduced lipid droplets in intestine enterocytes of grouper juvenile. It is concluded that the grouper juvenile microdiets should have a high dietary soybean lecithin/fish oil ratio (15% /7%).

Key words: *Epinephelus coioides* juvenile; soybean lecithin; fish oil; growth; survival
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During the past few years, much progress has been made in fish microdiets to replace live food, especially for shrimp and freshwater species, which can be fed on compound diets partly or even completely substituted for live prey as early as mouth opening [1-3]. Published studies concerning fish juvenile nutrition were mainly made to establish their requirements for lipids, protein and vitamins [4-6]. Dietary lipid as a source of energy is important for normal growth and development of fish juveniles. Fish oil as the main source of lipid is commonly used in fish microdiets due to its good palatability and highly PUFA content, and its use in fish diets is becoming limited

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because of its high price and unsteady supply.

Grouper *Epinephelus coioides* is one of the most important economic marine fish in China and is well favored by consumers due to its tender flesh, few bones, and good taste, which leads to a huge demand in market. Grouper aquaculture is expanded to meet the market demand, but the grouper feed should be more cost-effective.

The positive effects of soybean lecithin (SL) on larval fish growth have been demonstrated [7], and it has also been suggested that fish juveniles utilize dietary phospholipids more efficiently than neutral lipid [8-9]. Previous works have determined lecithin requirements using highly purified SL sources such as 95% pure phosphatidylcholine (PC) from *Penaeus vannamei* in purified diets [10], but it is difficult to use purified lecithin in microdiets because of its high cost. Studies on SL nutrition have already been reported in many species of marine fish juveniles such as sea bass [9], turbot [10] and sea bream [11], but there is still no information on juvenile *E. coioides*. This study is to investigate the influence of different dietary soybean lecithin/fish oil (SL/FO) ratios in the grouper microdiets on the growth performance, feed utilization and intestine histology of the grouper E. coioides juveniles.

1 Materials and methods

1.1 Experimental diets and diet preparation Four isonitrogenous diets (crude protein: 500 g · kg^{-1} dry matter) with different soy lecithin/fish oil ratios (0%/22%, 5%/14%, 10%/8%, 15%/7%) were prepared prior to the experiment. The composition and formulation of the experimental diets were listed in Tab. 1. All dry ingredients were well ground, weighed and mixed in a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd., Ottawa, Canada) for 30 mins, gradually added with fish oil and mixed constantly. The mixture was added slowly with water at 0.3—0.5 mL · g^{-1} of dry matter and blended until it became better-textured dough. The dough was screwed into noodle-like diets 1.0 mm in diameter using a twin-screw extruder (made in Institute of Chemical Engineering, South China University of Technology, Guangzhou, China). Then the diets were pelletized, sun-dried, sieved and stored at −20 ℃ before used for feeding.

Tab. 1 Formulation of experimental diets

Ingredient	Diet (g · kg^{-1}, dry diet)			
	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	560	560	560	560
Fish meal hydrolysate	140	140	140	140
Fish oil	150	100	50	0
Soy lecithin	0	50	100	150
Marine lecithin	30	30	30	30
Vitamin mixture[1]	40	40	40	40
Mineral mixture[2]	40	40	40	40
Betaine	10	10	10	10
Carboxymethyl cellulose	30	30	30	30
Proximate composition				
Dry matter /(g · kg^{-1})	951	914	968.2	930
Curd protein /(g · kg^{-1})	582.3	603.1	588.3	574.3
Curd lipid /(g · kg^{-1})	219.4	191.1	183.4	219.9
Crud ash /(g · kg^{-1})	165.4	160	160.7	165.5
NFE /(g · kg^{-1})	32.9	45.8	67.6	40.3
Gross energy (KJ · g^{-1})	25.25	24.53	23.89	25.08

Note: [1] Composition per kilogram of the vitamin mixture: choline chloride 60%, 333 g; vitamin A acetate, (500 000 UI · g^{-1}) 1 g; vitamin E (500 UI · g^{-1}) 20 g; vitamin B3 2 g, vitamin B5 4 g; vitamin B1 200 mg; vitamin B2 80%, 1 g; vitamin

B6 600 mg; vitamin C 35%, 28.6 g; vitamin B9 80%, 250 mg; vitamin concentrate B12 (10 g · kg^{-1}), 0.2 g; biotin, 1.5 g; vitamin K3 51%, 3.92 g; meso-inositol 60 g; cellulose, 543.3 g

[2] Composition per kilogram of the mineral mixture: 90 g KCl, 40 mg KIO_3, 500 g; $CaHPO_4 \cdot 2H_2O$, 40 g; NaCl, 3 g; $CuSO_4 \cdot 5H_2O$, 4 g; $ZnSO_4 \cdot 7H_2O$, 20 mg; $CoSO_4 \cdot 7H_2O$, 20 g; $FeSO_4 \cdot 7H_2O$, 3 g; $MnSO_4 \cdot H_2O$, 215 g; $CaCO_3$, 124 g; $MgSO_4 \cdot 7H_2O$, and 1 g NaF

1.2 Fish and feeding trial *E. coioides* eggs collected from a commercial farm (Huiwen Fish Farm, Wenchang, China) were hatched and cultured to juvenile size by feeding live foods in an earthen pond. At 35 DAH, they were transferred to indoor culture systems where they were acclimated to experimental conditions and fed on the formulated diets for 5 days. After acclimation, fish (initial body weight: 0.45 g) were pooled and randomly distributed into twelve 150 L fiberglass tanks to form groups of 50 fish for rearing. The feeding trial lasted 40 days. Low-pressure electrical blowers provided aeration via air stones, and dissolved oxygen (DO) levels were maintained at or near to saturation. Filtered water was supplied at a flow rate of 2.2 L · min^{-1} to each rearing tank, and water temperature was measured daily and maintained at (27 ± 1) ℃. Dissolved oxygen (> 7.03 mg · L^{-1}), pH (8.05—8.23), salinity (30.16—35.23 g · L^{-1}), and total ammonia (< 0.11 mg N · L^{-1}) were measured weekly using methods described in the work of Allan et al [12]. Experimental fish were subjected to a 12 L∶12 D photoperiod using fluorescent lighting during the trial period.

Four experimental diets were randomly assigned within the 12-tank system with each dietary treatment being given to three tanks. Fish were hand-fed on the prescribed diets to satiation three times daily at 800 h, 1 200 h and 1 700 h until pellets were first seen to sink to the bottom of the tank and feed intake was recorded daily.

1.3 Sampling At the end of the trial, fish were deprived of food for 16 h prior to sampling, and then the same protocol of slaughter was followed for each tank after the fish were euthanized (MS-222 at 10 mg · L^{-1}). Thirteen fish from each tank were randomly collected for proximate analysis, 5 for analysis of whole body composition, and 8 were dissected to separate and weigh the viscera, liver and mesenteric fat before the individual whole body was weighed up. White muscles were also dissected from both sides of the fillets without skin.

1.4 Analytic methods Proximate composition of diets and chemical composition of whole body, liver and muscle homogenates were determined following standard methods [13]. Crude protein (N × 6.25) was determined by the Kjeldahl method after an acid digestion using an auto Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was determined by ether-extraction using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Dry matter was analyzed by oven-drying at 105 ℃ for 24 h to constant weight. Crude ash was incinerated at 550 ℃ in a muffle furnace for 24 h.

Samples for histological analysis were fixed in formaldehyde solution for 24—48 h at 25 ℃ and then handled by increasing concentrations of ethanol (70%, 80%, 90%, 95%, 100% ×2, 15 min each), 50% ethanol + 50% xylene (30 min), 100% xylene (until the sample became transparent), paraffin wax (58 – 60 ℃, 2 h). All samples were embedded in paraffin wax and cut into 6μm slices which were then stained with hematoxylin and eosin: Put the sample sections into 100% xylene, 2 changes (8 hours and 5 min separately); 100% ethanol, 2 changes (5 min); 95% ethanol, 2 changes (5 min); 85% ethanol (3 min); 75% ethanol (2 min); water (1 min); hematoxylin solution; water; chlorhydric acid and ammonia water (15 sec); water (5 min); pure water (2 min); 75% ethanol (2 min); 85% ethanol (2 min); 0.5% eosin (mixed in 90% ethanol, 3min); 95% ethanol (5 min); 100% ethanol, 2 changes (5 min each); xylene, 2 changes (30 min and 5 min separately); and mount the sections onto slides.

Changes in foregut histology of grouper juveniles were examined under a light microscopy (OLYMPUS BX 41).

1.5 Calculations Condition factor (CF): 100 × (live weight, g) /(body length, cm)^3. Intraperitoneal fat (IPF) ratio: IPF weight × 100/body weight. Nitrogen-free extract (NFE) (including fibre): calculated by difference (100-crude protein – crude lipid – crude ash). Gross energy (KJ · g^{-1}): calculated based on

23.6 kJ·g^{-1} protein, 39.5 KJ·g^{-1} lipid, and 17.2 KJ·g^{-1} nitrogen free extract [14].

Weight gain (WG): 100 × (final mean weight-initial mean weight) /initial mean weight. Specific growth rate (SGR) (% day^{-1}): 100 × (ln (final mean weight) − ln (initial mean weight)) /days. Feed efficiency (FE): (final mean body weight-initial mean body weight) /feed intake (FI). Protein efficiency ratio (PER): g weight gain /g protein fed.

1.6 Statistic analysis All data were presented as means ± SD and subjected to one-way analysis of variance (ANOVA) ($n=3$) to test the effects of experimental diets using the software of the SPSS (version 11.0) for windows. Duncan's multiple range test was used to resolve the differences among treatment means [15]. Differences among means were considered significant at $P \leqslant 0.05$.

2 Results

2.1 Growth performance Results of growth performance and survival rate of grouper juveniles fed the experimental diets were presented in Tab. 2. Survival rate was above 30% at the end of the feeding trial for all groups. Weight gain was significantly lower in Diet 1 than in Diet 3 or Diet 4 ($P \leqslant 0.05$). Final body weight (FBW), weight gain and SGR in all treatments varied in the order of Diet 4 > Diet 3 > Diet 2 > Diet 1. There were no significant differences in FBW, SGR and FI ($P > 0.05$).

Tab. 2 Survival rate, initial body weight, final body weight, weight gain, specific growth rate, and feed intake of *E. coioides* juveniles fed the diets supplemented with different ratios of SL/FO[a] for 40 days

	Diet 1	Diet 2	Diet 3	Diet 4
Survival /%	31.25 ± 5.60c	45.83 ± 7.22bc	53.13 ± 3.26ab	52.08 ± 7.86a
Initial body weight/g	0.43 ± 0.049	0.45 ± 0.008	0.45 ± 0.006	0.45 ± 0.012
Final body weight/g	3.70 ± 0.47	3.94 ± 0.33	4.44 ± 0.90	4.92 ± 2.32
Weight gain/g	185.0 ± 0.46b	315.9 ± 5.18ab	449.6 ± 11.41a	588.3 ± 61.70a
Specific growth rate	5.07 ± 0.50	5.39 ± 0.17	5.69 ± 0.57	5.72 ± 1.37
Feed intake/g	6.44 ± 4.64	5.98 ± 1.06	6.11 ± 1.48	5.12 ± 1.63

Note: [a] Values are means ± SD of three replicates and values within the same row with different letters are significantly different ($P \leqslant 0.05$)

2.2 Morphological Indices Hepatosomatic index, viscerosomatic index and condition factor did not have significant differences in all treatments ($P > 0.05$). Intraperitoneal fat ratio was significantly lower in Diet 1 than in other diets ($P \leqslant 0.05$) (Tab. 3).

Tab. 3 Morphological indices of old *E. coioides* juveniles fed for 40 days the diets supplemented with different ratios of SL/FO[a]

	Diet 1	Diet 2	Diet 3	Diet 4
Viscerosomatic index	8.48 ± 1.11	9.71 ± 0.57	9.82 ± 1.01	8.53 ± 0.79
Hepatosomatic index	1.50 ± 0.23	1.75 ± 0.23	1.68 ± 0.36	1.30 ± 0.12
Intraperitoneal fat ratio/%	1.09 ± 0.22b	2.72 ± 0.74a	2.71 ± 0.33a	2.40 ± 0.85a
Condition factor	3.0 ± 0.25	2.67 ± 0.24	2.77 ± 0.2	2.74 ± 0.15

Note: [a] Values are means ± SD of three replicates and values within the same row with different letters are significantly different ($P \leqslant 0.05$)

2.3 Feed utilization and whole body compositions Feed efficiency, protein efficiency ratio and body moisture content had no significant differences among all the diets ($P > 0.05$) (Tab. 4), but had the same variation tendency: Diet 4 > Diet 3 > Diet 2 > Diet 1. Grouper juveniles fed Diet 1 had significantly lower whole body lipid content ($P \leqslant 0.05$), but significantly higher body protein content than those fed on other diets ($P \leqslant 0.05$).

Tab. 4　Body composition, feed efficiency and protein efficiency ratio of *E. coioides* juveniles fed the diets supplemented with different ratios of SL/FO[a]

	Diet no.			
	Diet 1	Diet 2	Diet 3	Diet 4
Body moisture/(g·kg^{-1})	683.1 ± 104.4	745.3 ± 20.5	768.1 ± 51.4	837.9 ± 20.6
Body lipid/(g·kg^{-1})	154.7 ± 12.8b	235.6 ± 36.2a	249.7 ± 42.9a	254.4 ± 31.9a
Body protein/(g·kg^{-1})	728.3 ± 25.5a	676.4 ± 21.4b	671.0 ± 12.6b	656.4 ± 13.1b
Feed efficiency/%	44.26 ± 12.00b	68.54 ± 2.63ab	69.40 ± 9.62ab	92.10 ± 5.23ab
Protein efficiency ratio/%	54 ± 0.29	82 ± 0.10	103 ± 0.10	164 ± 0.85

Note: [a] Values are means ± SD of three replicates and values within the same row with different letters are significantly different ($P \leqslant 0.05$)

2.4　Intestinal histology　In the anterior part of intestine, few lipid vacuoles were observed in the intestine among all the treatments (Fig. 1). The brush border of these juveniles covered mucosa of anterior intestinal sections and the height of the mucosal folds was not uniform. In contrast, a high incidence of lipid vacuoles was observed in the intestinal mucosa of the grouper juveniles fed on Diets 1, 2 and 3 (Fig. 1 a, b, c), and the degree of lipid accumulation in enterocytes was decreased with an increase in dietary SL level. Some enterocytes were mildly injured with evidence of vacuolization.

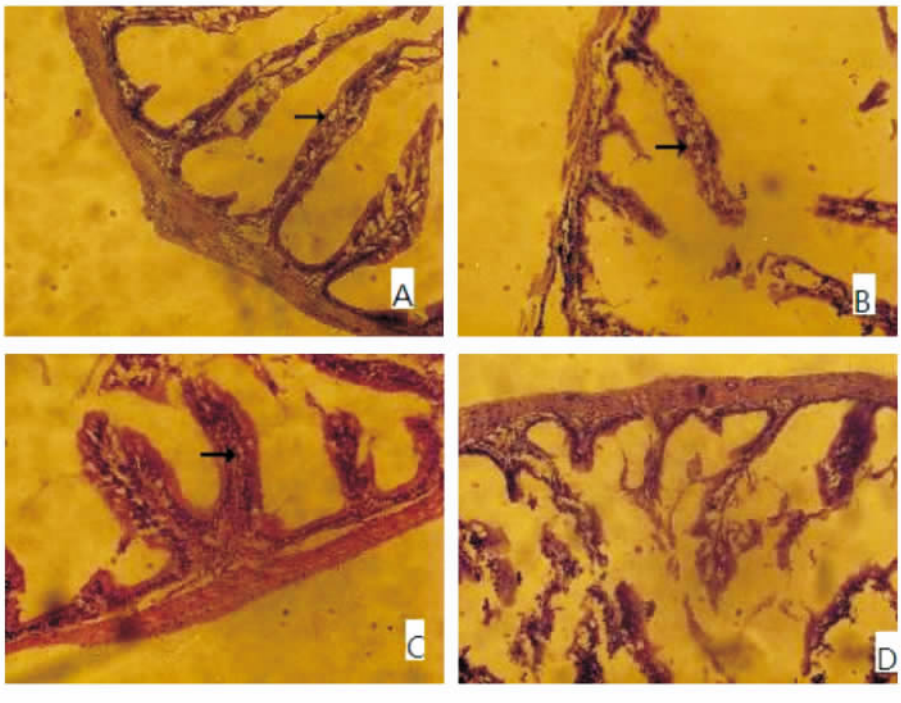

Fig. 1　Intestinal histology of *E. coioides* juvenile

A: Diet 1 ×40; B: Diet 2 ×40; C: Diet 3 ×40; D: Diet 4 ×40; Arrow: lipid vacuoles

3　Discussion

The present study shows that *E. coioides* juveniles responded well to all the microdiets under trial, and different levels of SL supplemented to microdiets significantly influenced the growth performance of grouper juveniles. Grouper juveniles fed Diet 4 exhibited the best growth performance, which is agreeable with previous reports that supplementation of SL in microdiets improved growth of certain species of fish juveniles, such as common carp, European sea bass and turbot [10,16-17]. Results obtained in this trial confirm that dietary SL promoted the growth of fish juveniles, which may be due to its positive role in the formation of new cell components during

the development of fish juveniles [17-18].

The data on the whole body lipid and IPF ratios showed that the diets supplemented with SL increased lipid accumulations in grouper juvenile body. Previous works also have reported that the SL was more efficiently absorbed than neutral lipids as a source of EFA for fish juveniles of which digestive system is not fully developed [19-20]. The intestinal histology also indicated that the dietary SL deficiency resulted in an accumulation of fat droplets in the enterocytes of the anterior intestine. The degree of lipid accumulation in enterocytes tended to decrease with decreasing dietary oil supplementation level and increasing SL supplementation. Fontagné et al [21] suggested a specific function of SL for the synthesis and secretion of chylomicrons or very low density lipoproteins (VLDL). They found that common carp juveniles fed on the diets deficient of polar lipid had an accumulation of fat droplets in the enterocytes of the anterior intestine, and similar result was also found in this study. The lipid droplets in intestine may result from a low ability of lipid transport. OLSEN et al [22] reported that larval codfish had a requirement for polar lipid to provide energy and EFA since it has limited digestibility of neutral lipids. In European sea bass, CAHU et al [3] also suggested that SL was better utilized by fish juveniles than neutral lipid. As reported above, SL is more easily digested by fish juveniles than the mixed oils, which indicated that the beneficial effects of SL on growth of juveniles could not be attributed to the dietary UFA content [16-17,23]. It has been reported that the SL supplemented to the diet as the sole lipid source could meet the requirement of fish juvenile growth in spite of its lack of DHA and EPA [24].

In this experiment, the significantly high WG, FE and survival rate in Diet 4 showed that the SL supplement in microdiets was feasible for feeding fish juveniles based on the results in European sea bass [25], in which it was demonstrated that dietary SL could improve digestion and absorption of nutrients. The low body protein content in the SL-based groups may be attributed to the limited absorbing capability of the intestines of fish juveniles [19-20], and it still needs to be further studied in the future. There were no significant differences in FI, VSI, HSI and SGR values among different groups, indicating that different dietary SL levels may have little influence on fish juvenile's feed intake and morphological indices, which agreed with the result reported by ALIYU-PAIKO et al [26].

In conclusion, *E. coioides* juveniles can be fed with the formulated diets at early developmental stages and showed good performance, and the diets supplemented with a dietary soybean lecithin / fish oil ratio of 15% /7% promoted juvenile growth. But further research needs to be done to select the best ratio of soybean lecithin / fish oil ratio.

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饲料中大豆卵磷脂/鱼油比例对斜带石斑鱼稚鱼生长发育及肠道组织的影响

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摘 要: 为探讨饲料中不同大豆卵磷脂/鱼油比例对斜带石斑鱼稚鱼成活率、生长行为、饲料利用以及肠道组织学的影响,本实验设计了 4 种添加不同大豆卵磷脂/鱼油比率的微颗粒饲料: 0% /22% (饲料 1),5% /14% (饲料 2),10% /8% (饲料 3) 以及 15% /7% (饲料 4),饲喂斜带石斑鱼稚鱼。每种实验饲料均设 3 个平行。试验持续 40 d。结果表明,饲料中不同大豆卵磷脂/鱼油比例对斜带石斑鱼稚鱼生长以及成活率有显著影响($P \leqslant 0.05$)。饲料 3 和饲料 4 的增重分别为: (449.6 ± 11.4) g 和(588.3 ± 61.7) g,显著高于饲料 1 的数值(185.0 ± 2.5) ($P \leqslant 0.05$)。饲料 1 鱼体脂肪含量为: (154.7 ± 12.8) g·kg^{-1},显著低于其他饲料组($P \leqslant 0.05$),但其鱼体蛋白含量为(728.3 ± 25.5) g·kg^{-1},显著高于其他饲料组。饲料效率、肥满度、特定生长率、摄食量和蛋白质效率以及终末体重中,4 种饲料之间无显著性差异。随饲料卵磷脂/鱼油比例升高,石斑鱼稚鱼肠道脂滴生成数量出现下降,且在饲料 4 中未观察到肠道脂滴。研究表明斜带石斑鱼稚鱼生长发育阶段需要较高的饲料卵磷脂/鱼油比例(如: 15% / 7%)。

关键词: 斜带石斑鱼稚鱼; 大豆卵磷脂; 鱼油; 生长; 成活率

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