Journal of Marine Science and Technology

Volume 24 | Issue 2 Article 25

EFFECTS OF DIETARY LIPID LEVELS ON GROWTH, SURVIVAL AND BODY FATTY ACID COMPOSITION OF GROUPER LARVAE, Epinephelus coioides AND Epinephelus lanceolatus

Jen-Hong Chu
Department of Aquaculture, National Taiwan Ocean University, Keelung County, Taiwan, R.O.C.

Shyn-Shin Sheen
Department of Aquaculture, National Taiwan Ocean University, Keelung County, Taiwan, R.O.C, shin@mail.ntou.edu.tw

Journal of Marine Science and Technology, Vol. 24, No. 2, pp. 311-318 (2016)
DOI: 10.6119/JMST-015-0512-2

EFFECTS OF DIETARY LIPID LEVELS ON GROWTH, SURVIVAL AND BODY FATTY ACID COMPOSITION OF GROUPER LARVAE, *Epinephelus coioides* AND *Epinephelus lanceolatus*

Jen-Hong Chu[1] and Shyn-Shin Sheen[1]

Key words: *Epinephelus coioides*, *Epinephelus lanceolatus*, lipid, weight gain.

ABSTRACT

Two feeding experiments were conducted to study the effects of dietary lipid levels on the growth, muscle fatty acid profile and survival of 0.14 g *Epinephelus coioides* and 0.09 g *Epinephelus lanceolatus* larvae. Five semi-purified diets included 0%, 5%, 10%, 15% and 20% lipid were formulated. Each diet was randomly assigned to three replicate groups of grouper larvae. The final weight, weight gain percentage and survival of two grouper species fed diet without supplemented lipid were significantly lower than those of larvae fed treatment diets. *E. coioides* had lower weight gain percentage than *E. lanceolatus*. The weight gain percentages of two grouper species fed diets included 5% to 20% lipid showed no significant difference, respectively. Based on the broken-line regression analysis of weight gain percentage of *E. coioides* and *E. lanceolatus*, the optimum dietary lipid requirements were 95 and 75 g lipid /kg diet, respectively.

I. INTRODUCTION

Grouper are among the most popular and important species cultured in the Asia-Pacific region (Boonyaratpalin, 1997; Liao et al., 2001). This high-value grouper species is increasingly becoming a popular cultured species in many parts of the world including Taiwan, Indonesia, Malaysia, the Philippines, Thailand, Hong Kong and Mainland China (Miao and Tang, 2002). Grouper are cultured either in floating net cages or earthen ponds (Pudadera et al., 1999).

Paper submitted 01/12/15; revised 03/19/15; accepted 05/12/15. *Author for correspondence: Shyn-Shin Sheen (e-mail: shin@mail.ntou.edu.tw).*
Department of Aquaculture, National Taiwan Ocean University, Keelung County, Taiwan, R.O.C.

Grouper culture is widespread in the Asian region; however, its development is constrained by the limited available fingerlings. Most countries, except Taiwan, rely on the wild-caught fry and fingerlings for stocking (Rimmer, 2004). The highly demand for wild seeds has led to capture of large amount of seed with relatively less investment in time and effort. The inadequate supply of seed is further aggravated by the lack of appropriate handling techniques during collection, transport and storage (Nagasawa and Cruz-Lacierda, 2004). Therefore, a major constraint of grouper production is due to the heavy mortality during collection handling stress and diseases (Pudadera et al., 1999; Sadovy, 2000).

The mass grouper larvae production technology is already established, but seed production is still inconsistent and insufficient quantity to meet the highly demand of grow-out culture (Liao et al., 2001). The grouper culture in Taiwan can be divided into four stages: the first stage from eggs to 0.5 g larvae, the second stage from 0.5 g larvae to 7 g juveniles, the third stage from 7 g juveniles to adults, and the fourth stage from adult to brood stock culture (Liao et al., 2001; Pierre et al., 2008). Grouper larvae are small and fragile with small reserves of endogenous nutrition (yolk sac) and low initial feeding rates (Ordonio-Aguilar et al., 1995). At the first stage, insufficient nutrition supply and viral infection cause mass mortality. These adverse factors are considered to be a fundamental cause of the high mortality and delayed development during larval culture (Kohno et al., 1997). We usually feed 3 days after hatching (DAH) larvae with live prey, such as copepod nauplii and super small strain rotifer. However, the nutritional value of live prey cannot support sufficient nutrition requirement for fish larvae (Liao et al., 2001). Besides, the quality and quantity of live prey are variable. In addition, the live prey may carry viruses and bacteria which destroy larval cultivation. Therefore, the application of artificial diets to fish larvae is helpful and may avoid fish larvae from pathogen infection (Boonyaratpalin, 1997).

Lipids, providing energy and essential fatty acids, are

Table 1. Ingredient composition (percent dry weight) and proximate analysis of the experimental diets.

Ingredients	Dietary lipid (% dry weight)				
	0	5	10	15	20
Basal mix[a]	68.1	68.1	68.1	68.1	68.1
Lipid[b]	0	5	10	15	20
α-Starch	31.9	26.9	21.9	16.9	11.9
Analyzed composition (as fed)					
Moisture	10.99	10.61	10.01	9.59	8.74
Crude protein[c]	43.72	43.84	43.59	43.46	43.66
Crude fat[c]	0.52	5.39	10.06	15.01	20.34
Ash[c]	11.83	11.84	11.83	11.82	11.93
Crude fiber[c]	1.28	1.14	1.35	1.29	1.47
Gross energy(Kcal/100 g)	372.0	405.09	431.60	461.3	486.2

[a] Lipid-extracted fish meal 54%, lipid-extracted shrimp meal (Jian-Bao Foods Co., Ltd., Taiwan) 6.1%, β-glucan 1%, yeast (Sun Right foods Co., Ltd., Taiwan) 4%, mineral mix (Bernhart and Tomarelli, 1966 modified) 2%, vitamin mix (Chu et al., 2007 modified) (vitamin D_3 0.001%, vitamin A 0.06%, α-tocopheryl acetate 0.45%, vitamin K_3 0.4%, thiamine-HCl 0.5%, riboflavin 0.5%, calcium pantothenate1%, niacin 2%, biotin 0.4%, pyridoxine-HCl 0.06%, folic acid 0.15%, B_{12} 0.001%, inositol 20%, ascorbic-monophosphate-Mg 2.5%, choline chloride 40%, α-cellulose 31.978%) 1%.

[b] 75% fish oil, 24.8% soybean oil and 0.2% ethoxyquin.

[c] Expressed as percent dry weight.

important components in the diet for fish larvae to develop and grow. Fatty acids are important constituents of cell membranes, especially in brain and retina. They are also required during early life stages to assure normal visual and nervous development. When a dietary lipid is in inadequate supply, protein might be used as an energy source thereby reducing the utilization of protein for growth of animals. The protein sparing effect of dietary lipids has been reported for many fish species that are unable to utilize dietary carbohydrates efficiently (NRC, 1993; Vergara et al., 1996; Company et al., 1999; Kikuchi et al., 2000; Storebakken, 2002).

The lipid requirement of grouper may differ both in species and in the size of fish (Williams, 2009). Luo et al. (2005) indicated that 11 g *E. coioides* fed diet containing 53% crude protein and 10% lipid had the best growth. 4.4 g and 17 g *E. malabaricus* fed diets containing 9% and 12% lipid, respectively, had the optimal growth performance (Lin and Shiau, 2003; Tuan and Williams, 2007). For 150-400 g humpback grouper, *Cromileptes altivelis*, the optimal dietary lipid was 12% (Usman et al., 2005). The size of grouper mentioned above was large enough to digest the artificial diets. However, the 10-30 DAH grouper usually feed on live zooplankton which can be easily digested and absorbed by grouper.

The application of an artificial diet as the only dietary source to culture grouper larvae is innovative because they are usually fed with liver prey (Watanabe and Kiron, 1994). Moreover, this is the first study to feed grouper larvae with a compound diet. Indeed, 25 DAH grouper are generally fed with live prey, such as rotifer and *Artemia*. The replacement of live prey in larval feeding regime could lower the cost of larval production in hatcheries. Before compound diets can be available for larval grouper culture, the knowledge of nutritional requirements should be studied. Therefore, it is necessary to develop a suitable artificial diet for larval grouper. The present study investigated the effects of dietary lipid levels on the growth and survival of 0.14 g *E. coioides* and 0.09 g *E. lanceolatus* larvae.

II. MATERIAL AND METHODS

1. Experimental Diets

Five isonitrogenous diets were formulated with lipid levels ranging from 0 to 20% in 5% increments. Ingredient composition of the diets is provided in Table 1. Before formulation of the diets, lipid was extracted from fish meal and shrimp meal using hot ethanol (1:1, w/v) in three successive treatments to remove any endogenous lipid in fish meal and shrimp meal. Levels of alpha-starch were changed accordingly to different levels of dietary lipid. Dry ingredients of experimental diets were mechanically mixed to ensure homogeneity. Distilled water was added and the mixture blended thoroughly by hand until a consistency for extrusion was achieved. Diets were cold-extruded through a chopper (0.1 cm die diameter), and dried at 30°C to approximate 10% moisture content. The diets were then ground by Mortar Grinder RM 100 (Retsch, Germany) into 0.2 mm particles.

The experimental diets and muscle of grouper were analyzed for proximate composition based on AOAC (1984) methods. Crude protein was determined with a Kjeltec semi-autoanalyzer model 1007 (Tecator, Sweden). Crude lipid was determined by the chloroform-methanol (2:1, v/v) extraction method (Folch et al., 1957). Crude fiber was determined by acid and alkaline digestion using Fibertec system M1020 (Foss Tecator, Sweden). Ash and moisture were determined

by conventional methods using a muffle furnace and an oven, respectively. Gross energy of diets was determined by using bomb calorimeter (IKA calorimeter system, C2000 basic, German). The crude protein of the experimental diets ranged from 43.46% to 43.84% (dry weight).Crude lipid analyses indicated that the diets formulated to contain 0, 5, 10, 15, and 20% total lipid actually contained 0.52, 5.39, 10.06, 15.01 and 20.34%, respectively (Table 1).

2. Experimental Fish

Fertilized eggs of two species grouper, *Epinephelus coioides* and *Epinephelus lanceolatus* obtained from spontaneous spawning by cultured broodstock were hatched and reared to 25 days at a private hatchery farm (886 Apex Aquaculture Co., Ltd., Taiwan). One thousand 25 DAH grouper larvae were transported to laboratory at National Taiwan Ocean University, Taiwan by air, in tightly sealed bags, quarter filled with seawater and inflated with oxygen, enclosed in an insulated container. After one week acclimation at laboratory, 0.14 g *E. coioides* and 0.09 g *E. lanceolatus* were randomly distributed into 15 fiber reinforced plastics(FRP) aquaria (45 × 30 × 30 cm) containing 50 L seawater with 15 fish in each aquarium, respectively. Five experimental diets were randomly assigned to three replicate aquaria.

Grouper larvae were hand-fed to excess twice daily at 09:30 AM and 05:30 PM. The experiment was conducted in darkness except for feeding. Continuous aeration was provided to each aquarium through an air stone connected to a central air compressor. Water changed every day in the morning and afternoon approximate 30% to remove uneaten and fecal material. Water temperature ranged from 24 to 27°C during the course of study and was recorded daily. Mortalities were removed daily and counted. Wet weights of surviving individuals in each aquarium were determined (surface water removed) at initial and termination of the experiment and fish were fasted for 24 h before weighing. The duration of the experiment was 42 days. Growth factors including weight gain percentage, survival and hepatosomatic index (HSI) were calculated according to the following formulae:

$$\text{Weight gain percentage} = 100 \times (Wt - W0)/W0$$
$$\text{Survival} = 100 \times (Fi - Fd)/Fi$$
$$\text{HSI} = 100 \times (\text{liver weight/carcass weight})$$

where W0 = initial mean body weight (g), Wt = final mean body weight (g), Fi = initial fish number and Fd = number of dead fish.

3. Sample Collection and Analysis

At the termination of the experiment, all survival fish were sacrificed for muscle composition analysis. Diets and the muscle of survival grouper from each treatment were homogenized separately in chloroform/methanol (2:1, v/v) for 5 min to extract total lipid (Folch et al., 1957), and refluxed in 50% KOH for 40 min. The saponified lipids were then methylated by refluxing for 20 min in 2 ml of 14% borontrifluoride in methanol (BF3-MeOH) (Metcalfe and Schmitz, 1961) in preparation for fatty acid analysis by gas chromatography. Fatty acid methyl esters (FAME) were analyzed using a gas-liquid chromatography in a Trace GC 2000 instrument equipped with a flame ionization detector. The FAMEs were separated on a Restek's capillary column (30 m × 0.28 mm, 0.25 μm film thicknesses, Stabilwax) with isothermally at 208°C. Injection and detector temperature were maintained at 250°C and 200°C, respectively. Nitrogen was used as a carrier gas.

Fatty acids were identified by comparison with retention times of a reference standard (GLC-68A, Nu-Check-Prep) consisting of a mixture of saturated and unsaturated fatty acids. In addition, the peaks of chromatograms were compared with identified peaks from a sample of cod liver oil that served as a secondary reference standard.

4. Statistical Analysis

Data are presented as mean ± standard errors. Homogeneity of variance was tested by Levene's test, using arcsine-square root or logarithmic transformation when necessary. Mean weights, survival and data of fatty acid profiles associated with the dietary treatments were analyzed by one-way ANOVA at significant level of 0.05 using a Statistical Analysis System. Turkey's range test was used to identify significant differences between treatment means (Steel and Torrie, 1980).

III. RESULTS

Final weight, weight gain percentage and survival of two species of grouper larvae fed experimental diets for 42 days are shown in Table 2. Two species of grouper larvae fed diets supplemented with 5% to 20% lipid were not significantly different ($p > 0.05$) in weight gain percentage and significantly higher than those fed control diet. In comparison with growth performances between two species of grouper larvae fed treatment diets and under the same experimental condition, *E. lanceolatus* grew approximate four times faster than *E. coioides*.

The survival of *E. coioides* larvae fed the diet without supplemented lipid was 55.6% and significantly lower than that of larvae fed diets supplemented with 5 and 15% lipid (Table 2). The survival of *E. lanceolatus* larvae fed diets containing 10% to 15% significantly higher than that of larvae fed diets supplemented without and 5% lipid.

The HSI of two species of grouper larvae is shown in Table 3. HSI of *E. coioides* and *E. lanceolatus* ranged between 5.90% to 8.83% and 5.97% to 9.83%, respectively. The HSI of *E. coioides* larvae fed diets supplemented with 5-15% lipid was significantly higher than that of larvae fed control diet. The HSI of *E. lanceolatus* larvae fed diet supplemented with 15% lipid was significantly higher than that of larvae fed diets supplemented with 0-10% lipid.

Fatty acid compositions of diets supplemented with different levels of lipid are presented in Table 4. The control diet

Table 2. Initial weight, final weight, weight gain percentage and survival of grouper larvae, (*E. coioides* and *E. lanceolatus*) fed diets containing different levels of lipid for 42 days.

	Dietary lipid (% dry weight)				
	0	5	10	15	20
Initial weight (g)					
E. coioides	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00
E. lanceolatus	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
Final weight (g)					
E. coioides	0.43 ± 0.12^{b}	0.71 ± 0.15^{a}	0.90 ± 0.25^{a}	0.73 ± 0.02^{a}	0.70 ± 0.05^{a}
E. lanceolatus	0.59 ± 0.01^{b}	1.53 ± 0.32^{a}	2.02 ± 0.28^{a}	1.51 ± 0.21^{a}	1.77 ± 0.36^{a}
Weight gain (%)					
E. coioides	206.85 ± 83.59^{b}	406.91 ± 103.88^{a}	542.11 ± 179.04^{a}	417.44 ± 10.79^{a}	397.46 ± 30.43^{a}
E. lanceolatus	559.26 ± 13.95^{b}	1602.45 ± 356.20^{a}	2152.17 ± 318.72^{a}	1580.91 ± 224.63^{a}	1867.58 ± 410.70^{a}
Survival (%)					
E. coioides	55.6 ± 3.9^{b}	75.6 ± 7.7^{a}	66.7 ± 6.7^{ab}	71.1 ± 10.2^{a}	64.4 ± 7.7^{ab}
E. lanceolatus	66.7 ± 6.7^{b}	66.7 ± 6.7^{b}	84.4 ± 3.9^{a}	80.0 ± 6.7^{a}	73.3 ± 6.7^{ab}

$^{a, b}$ Values in each row with different superscripts are significantly different ($p < 0.05$). Data are expressed as means ± S.D. (n = 3).

Table 3. Hepatosomatic index (%) of grouper larvae, (*E. coioides* and *E. lanceolatus*)fed diets containing different levels of lipid for 42 days.

Dietary lipid (%)	HSI	
	E. coioides	*E. lanceolatus*
0	5.90 ± 0.52^{b}	5.97 ± 0.68^{c}
5	8.83 ± 0.63^{a}	7.80 ± 0.91^{bc}
10	8.15 ± 1.66^{a}	7.67 ± 0.43^{bc}
15	8.35 ± 1.26^{a}	9.83 ± 1.66^{a}
20	7.76 ± 0.73^{ab}	8.59 ± 0.70^{ab}

$^{a, b, c}$Values in each column with different superscripts are significantly different ($p < 0.05$). Data are expressed as means ± S.D. (n = 3).

without lipid supplementation contained higher proportions of 20:2n-6 and 20:3n-6 and lower proportions of 20:5n-3 and 22:6n-3. The dietary levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased with increasing dietary lipid levels.

Fatty acid compositions of muscle of *E. coioides* are shown in Table 5. The percentages of 14:0, 20:0 and 22:0 level of the muscle of *E. coioides* fed diet without lipid supplementation were significantly higher than those fed other treatment diet. The percentages of 20:5n-3, 22:5n-3 and 22:6n-3 of the muscle of *E. coioides* fed diet without lipid supplementation were significantly lower than those fed other treatment diets. The percentage of 20:4n-6 of the muscle of *E. coioides* fed diets supplemented with 0-15% lipid were significantly higher than that of fish fed diet supplemented with 20% lipid.

Fatty acids compositions of muscle of *E. lanceolatus* are shown in Table 6. The percentages of 14:1, 16:1, 20:1, 22:1 and 20:4n-6 level of the muscle of *E. lanceolatus* fed diet

Table 4. Fatty acid composition (% of total fatty acid) of diets.

Fatty acids	Dietary lipid (%)				
	0	5	10	15	20
14:0	5.68	0.05	0.26	0.06	0.1
14:1	5.38	0.01	0.02	0.05	0.04
16:0	15.59	12.86	14.82	15.15	14.81
16:1	1.74	0.37	0.36	0.37	0.3
18:0	41.86	46.6	45.5	35.6	36.26
18:1	n.d.	0.15	0.28	0.35	0.55
18:2	6.11	0.4	0.54	0.36	0.37
18:3	4.54	1.9	1.97	2.23	2.21
20:0	n.d.	0.11	0.09	0.12	0.11
20:1	n.d.	0.13	0.18	0.11	0.14
20:2	6.01	0.31	0.24	0.53	0.48
20:3	0.89	1.06	1	1.32	1.23
20:3	2.61	1.72	1.5	2.01	2.17
20:4	n.d.	13.41	12.43	14.76	14.42
20:5n-3	n.d.	0.77	1.3	1.93	1.59
22:0	4.12	0.18	0.18	0.19	0.14
22:1	n.d.	0.9	1.22	0.45	0.31
22:5n-3	n.d.	5.42	4.56	6.29	6.39
22:6 n-3	5.47	13.65	13.58	18.15	18.37
Total SFA	67.25	59.8	60.85	51.12	51.42
MUFA	7.12	1.56	2.06	1.33	1.34
PUFA	20.16	5.39	5.25	6.45	6.46
n-3HUFA	5.47	19.84	19.44	26.37	26.35

n.d. = Not detected.
SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids.
PUFA: polyunsaturated fatty acids (2–3 unsaturated bonds).
HUFA: highly unsaturated fatty acids (≧4 unsaturated bonds).

Table 5. Fatty acid composition (% of total fatty acid) of muscle of *E. coioides*.

Fatty acids	Dietary lipid (%)				
	0	5	10	15	20
14:0	13.78 ± 0.09[a]	12.78 ± 0.15[b]	11.74 ± 0.05[c]	5.66 ± 0.15[e]	5.93 ± 0.07[d]
14:1	0.12 ± 0.03[a]	0.05 ± 0.01[b]	0.05 ± 0.01[b]	0.06 ± 0.02[b]	0.07 ± 0.00[b]
16:0	11.23 ± 0.13[d]	22.96 ± 1.06[a]	3.63 ± 0.37[e]	18.11 ± 0.50[c]	21.05 ± 0.24[b]
16:1	6.99 ± 1.46[c]	8.33 ± 0.06[b]	14.39 ± 0.63[a]	13.18 ± 0.17[a]	13.75 ± 0.32[a]
18:0	9.89 ± 0.28[c]	15.74 ± 0.85[a]	11.39 ± 0.45[b]	5.13 ± 0.17[e]	6.20 ± 0.05[d]
18:1	11.50 ± 2.45[c]	13.40 ± 0.28[c]	23.40 ± 0.50[a]	13.32 ± 2.06[c]	18.62 ± 0.13[b]
18:2	0.06 ± 0.01[c]	0.26 ± 0.02[b]	0.34 ± 0.02[b]	13.07 ± 0.23[a]	0.25 ± 0.02[b]
18:3	0.19 ± 0.05[c]	0.16 ± 0.05[c]	0.17 ± 0.02[c]	2.73 ± 1.13[a]	1.78 ± 0.02[b]
20:0	19.73 ± 1.80[a]	0.37 ± 0.02[c]	0.73 ± 0.01[c]	3.58 ± 1.41[b]	0.63 ± 0.04[c]
20:1	0.50 ± 0.07[c]	1.45 ± 0.04[a]	0.99 ± 0.09[b]	0.35 ± 0.08[d]	0.55 ± 0.01[c]
20:2	n.d.	0.43 ± 0.06[b]	0.28 ± 0.01[b]	3.59 ± 0.54[a]	0.35 ± 0.02[b]
20:3	n.d.	0.17 ± 0.07[a]	0.08 ± 0.03[b]	0.19 ± 0.05[a]	0.14 ± 0.01[ab]
20:3	n.d.	0.11 ± 0.03[b]	0.04 ± 0.01[b]	0.45 ± 0.04[a]	0.43 ± 0.12[a]
20:4	0.61 ± 0.05[a]	0.61 ± 0.12[a]	0.62 ± 0.07[a]	0.60 ± 0.02[a]	0.18 ± 0.13[b]
20:5n-3	0.18 ± 0.03[c]	0.43 ± 0.21[b]	0.67 ± 0.04[a]	0.54 ± 0.04[ab]	0.63 ± 0.02[a]
22:0	23.33 ± 1.63[a]	15.22 ± 1.66[b]	13.50 ± 0.21[b]	5.03 ± 0.71[d]	11.45 ± 0.49[c]
22:1	1.06 ± 0.12[c]	1.40 ± 0.07[b]	1.51 ± 0.25[b]	0.97 ± 0.11[c]	3.16 ± 0.03[a]
22:5n-3	0.13 ± 0.06[e]	4.48 ± 0.67[d]	12.32 ± 0.12[a]	10.04 ± 0.35[c]	11.52 ± 0.22[b]
22:6 n-3	0.69 ± 0.16[d]	1.64 ± 0.11[c]	4.14 ± 0.13[a]	3.40 ± 0.26[b]	3.30 ± 0.15[b]
SFA	77.96 ± 3.33[a]	67.07 ± 0.44[b]	40.98 ± 0.27[d]	37.51 ± 2.72[d]	45.26 ± 0.34[c]
MUFA	20.17 ± 3.69[d]	24.64 ± 0.31[c]	40.35 ± 0.22[a]	27.88 ± 1.77[c]	36.16 ± 0.43[b]
HUFA	1.61 ± 0.29[e]	7.15 ± 0.65[d]	17.75 ± 0.03[a]	14.58 ± 0.59[c]	15.64 ± 0.20[b]
n-3 HUFA	1.00 ± 0.24[e]	6.54 ± 0.53[d]	17.13 ± 0.05[a]	13.98 ± 0.56[c]	15.45 ± 0.15[b]

[a, b, c, d, e]Values in each row with different superscripts are significantly different ($p < 0.05$). Data are expressed as means ± S.D. (n = 3).

n.d.= Not detected. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. HUFA: highly unsaturated fatty acids (≧4 unsaturated bonds).

Table 6. Fatty acid composition (% of total fatty acid) of muscle of *E. lanceolatus*.

Fatty acids	Dietary lipid (%)				
	0	5	10	15	20
14:0	2.29 ± 0.06[c]	4.31 ± 0.08[b]	4.23 ± 0.17[b]	5.71 ± 0.15[a]	5.58 ± 0.03[a]
14:1	8.05 ± 0.33[a]	4.29 ± 0.02[b]	2.13 ± 0.14[c]	2.42 ± 0.38[c]	1.33 ± 0.05[d]
16:0	9.92 ± 0.41[d]	18.44 ± 0.30[c]	21.42 ± 0.96[a]	20.19 ± 0.31[b]	17.60 ± 0.09[c]
16:1	15.70 ± 0.33[a]	12.41 ± 0.10[b]	6.68 ± 0.26[d]	7.05 ± 0.73[d]	10.40 ± 0.06[c]
18:0	3.14 ± 0.19[d]	5.69 ± 0.27[bc]	9.94 ± 0.29[a]	5.87 ± 0.08[b]	5.48 ± 0.03[c]
18:1	22.16 ± 0.59[a]	23.45 ± 0.15[a]	22.35 ± 0.27[a]	15.94 ± 1.99[c]	19.66 ± 0.24[b]
18:2 n-6	4.44 ± 0.29[e]	10.08 ± 0.06[d]	11.90 ± 0.56[b]	11.04 ± 0.27[c]	13.98 ± 0.07[a]
18:3 n-3	1.43 ± 0.07[b]	1.05 ± 0.02[d]	1.18 ± 0.05[c]	1.52 ± 0.06[b]	1.69 ± 0.03[a]
20:0	0.16 ± 0.04[c]	0.10 ± 0.03[c]	0.74 ± 0.18[b]	1.80 ± 0.09[a]	1.97 ± 0.27[a]
20:1	12.48 ± 1.54[a]	0.24 ± 0.03[c]	2.70 ± 0.48[b]	3.12 ± 1.02[b]	0.90 ± 0.26[c]
20:2	n.d.	0.67 ± 0.08[a]	0.52 ± 0.03[b]	0.60 ± 0.01[ab]	0.56 ± 0.01[b]
20:3 n-6	0.15 ± 0.06[b]	0.23 ± 0.01[a]	0.18 ± 0.02[ab]	0.14 ± 0.01[b]	0.18 ± 0.01[b]
20:3 n-3	0.14 ± 0.05[d]	0.66 ± 0.01[bc]	0.59 ± 0.05[c]	0.78 ± 0.06[a]	0.69 ± 0.01[b]
20:4 n-6	1.34 ± 0.04[a]	0.55 ± 0.01[c]	0.48 ± 0.03[d]	0.57 ± 0.03[c]	0.81 ± 0.02[b]
20:5 n-3	n.d.	2.89 ± 0.15[c]	3.37 ± 0.15[b]	6.04 ± 0.38[a]	5.88 ± 0.19[a]
22:0	1.70 ± 0.21[c]	7.06 ± 0.30[a]	0.10 ± 0.03[e]	1.20 ± 0.12[d]	2.35 ± 0.07[b]
22:1	14.82 ± 0.90[a]	0.09 ± 0.04[c]	3.26 ± 0.36[b]	4.25 ± 1.71[b]	0.11 ± 0.02[c]
22:5 n-3	1.69 ± 0.22[c]	2.85 ± 0.51[b]	3.66 ± 0.58[ab]	4.17 ± 1.06[a]	4.30 ± 0.10[a]
22:6 n-3	0.40 ± 0.04[d]	4.96 ± 0.16[c]	4.54 ± 0.53[c]	7.61 ± 0.61[a]	6.52 ± 0.17[b]
SFA	17.20 ± 0.87[d]	35.60 ± 0.38[ab]	36.43 ± 1.18[a]	34.76 ± 0.37[b]	32.99 ± 0.26[c]
MUFA	73.20 ± 1.31[a]	40.48 ± 0.20[b]	37.13 ± 1.02[c]	32.79 ± 0.75[d]	32.40 ± 0.51[d]
HUFA	3.42 ± 0.28[c]	11.25 ± 0.59[b]	12.05 ± 0.84[b]	18.39 ± 0.14[a]	17.50 ± 0.40[a]
n-3 HUFA	2.09 ± 0.24[d]	10.70 ± 0.58[c]	11.57 ± 0.82[c]	17.82 ± 0.16[a]	16.70 ± 0.38[b]

[a, b, c, d, e]Values in each row with different superscripts are significantly different ($p < 0.05$). Data are expressed as means ± S.D. (n = 3).

n.d. = Not detected. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. HUFA: highly unsaturated fatty acids (≧ 4 unsaturated bonds).

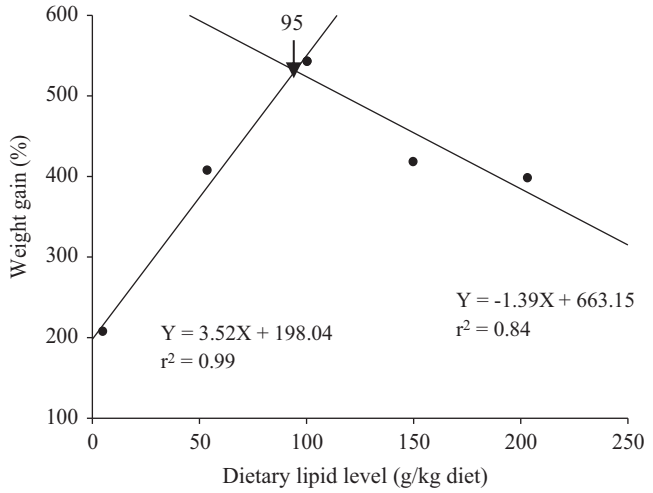

Fig. 1. Effect of dietary lipid level on weight gain of grouper larvae (*E. coioides*). The regression line that fit the dietary lipid requirement has a breakpoint at 95 g lipid /kg diet.

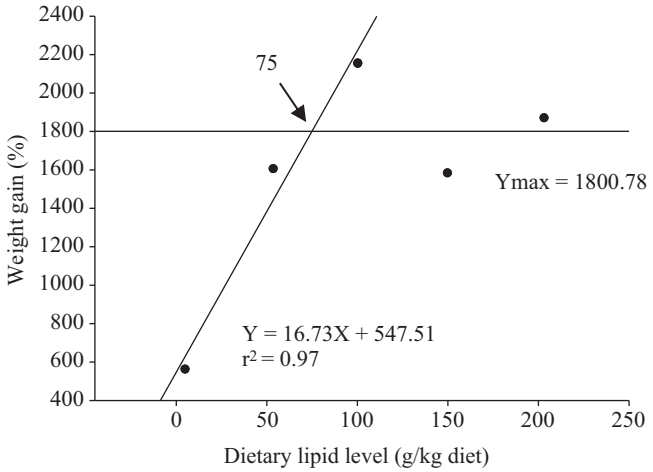

Fig. 2. Effect of dietary level of lipid on weight gain of grouper larvae (*E. lanceolatus*). The regression line that fit the dietary lipid requirement has a breakpoint at 75 g lipid /kg diet.

without lipid supplementation were significantly higher than those fed other treatment diets. The percentages of 18:0, 18:2n-6, 20:5n-3, 22:5n-3 and 22:6n-3 of the muscle of *E. lanceolatus* fed diets containing 5-20% lipid were significantly higher than those of larvae fed control diet.

The broken-line analyses based on the weight gain percentage for estimating the adequate requirements of dietary lipid for *E. coioides* and *E. lanceolatus* are shown in Figs. 1 and 2, respectively. The regression equations were Y = 3.52 X + 198.04 (r^2 = 0.99) and Y = -1.39 X + 663.15 (r^2 = 0.84) (Fig. 1). The broken point occurred at 95 g/Kg diet was estimated to provide the adequate level of dietary lipid for *E. coioides* larvae. The regression equations were Y = 16.73 X + 547.51 (r^2 = 0.97) and Ymax = 1800.78 (Fig. 2). The broken point occurred at 75 g/Kg diet was estimated to provide the adequate level of dietary lipid for *E. lanceolatus*.

IV. DISCUSSUON

The growth rates of fish normally decrease with increasing initial fish size (Bjornsson and Tryggvadottir, 1996). The 4.4 g *E. malabaricus* fed with diets supplemented 4-16% lipid for eight weeks and indicated that weight gain percentages of grouper ranged from 277 to 443% (Lin and Shiau, 2003). The 11 g *E. coioides* fed with diets supplemented 5-16% lipid for 56 days and indicated that weight gain percentages of grouper ranging from 94 to 120% (Luo et al., 2005). In the present study, weight gain percentages of 0.14 g *E. coioides* and 0.09 g *E. lanceolatus* larvae fed with treatment diets ranged from 397 to 542% and 1581 to 2152%, respectively. The smaller the fish are, the faster they grow.

The mass mortality of grouper larvae is attributed to the exogenous feeding, shock syndrome and cannibalism and survival of grouper larvae is generally low, usually below 10% (Rimmer, 2000). Lind et al. (2004) fed 19 days post-hatch *E. malabaricus* with *Artemia* sp. and artificial diet and indicated that survival of grouper larvae was lower than 20% for 30 days feeding period. Duray et al. (1997) fed 24 days post-hatch *E. suillus* with combination of *Chlorella* sp. *Brachionus* sp. and *Artemia* sp. and indicated that survival of grouper larvae was on average 16% for 36 days feeding period. In this study, two grouper larvae species were fed artificial diets and survival of *E. coioides* and *E. lanceolatus* was above 50%, respectively. Therefore, two grouper larval species fed experimental diets in this study overcome the exogenous feeding, shock syndrome and cannibalism to have above 50% survival.

Fish larvae need more dietary lipid as an energy source and fatty acids than both juvenile and adult fish (Ai et al., 2008). The 4.4 g *E. malabaricus* juvenile fed diet containing 9% lipid had the optimal growth (Lin and Shiau, 2003). However, 11 g *E. coioides* fed diet containing 10% lipid showed the optimal growth (Luo et al., 2005). In the present study, the optimal growth of *E. coioides* and *E. lanceolatus* were estimated by feeding them with diets containing 9.5 and 7.5% lipid, respectively. The rapid development of fish larvae needs more dietary lipids than juvenile fish.

Diets containing excessive lipid have the opposite effect on the weight gain of fish. Yoshii et al. (2010) fed 6.3 g *E. bruneus* with diet containing 27% lipid and indicated that weight gain of grouper was decreased. Lin and Shiau (2003) fed 4.4 g *E. malabaricus* diet containing 16% lipid showed a depressed growth. In our study, weight gain percentages of two grouper species fed diets included 5% to 20% lipid showed no significant difference. However, *E. coioides* larvae fed diets containing above 10% lipid showed the decreasing weight gain. This was in agreement with other species and suggested that the excessive lipid would reduce weight gain (Weatherup et al., 1997; Silverstein et al., 1999). Therefore, the reduced growth response of grouper to high levels of dietary lipid is probably due to inefficient utilization, particularly digestion and absorption of lipid for fish.

In the present study, HSI of two species of grouper larvae

increased with increasing dietary lipid level up to 15% and then decreased when dietary level beyond 15%. The lipid deposition may saturate in the liver when two species of grouper larvae were fed diet included up to 15% lipid. This observation is similar to *E. bruneus* (Yoshii et al., 2010) and Atlantic cod, *Gadus morhua* L. (Morais et al., 2001). However, HSI of grouper larvae in this study ranging from 5.9 to 9.8% was higher than that of 11 g *E. coioides* ranging from 2.15 to 2.97% (Luo et al., 2005).

At present, live preys are still the optional food in most situations for larval fish until the larvae are large enough to be maintained on artificial diets. However, the use of live preys to raise larval fish has their limits such as the essential fatty acids deficiency and pathogen carrier. The specific growth rate and survival of 35 DAH *E. fuscoguttatus* fed mysids, *Mesopodopsis orientalis* containing 13% DHA were higher than those of grouper fed *Artemia* containing without DHA (Eusebio et al., 2010). Larval yellowtail flounder, *Limanda ferruginea* fed rotifer enriched with DHA had higher growth rate and survival than those fish fed rotifer without enrichment (Copeman et al., 2002).The growth, survival and activity of the red sea bream, *Pagrus major* fed rotifer enriched with n-3 highly unsaturated fatty acid (n-3 HUFA) levels were effectively improved (Izquierdo et al., 1989). The growth and survival of *E. coioides* larvae fed live preys containing high level of lipid were higher than those of larvae fed low lipid diets (Su et al., 1997). In the present study, 25 DAH larvae fed artificial diets supplemented with 5-15% lipid containing 19.8 to 26.4% n-3 HUFA (20:5n-3 and 22:6n-3) showed higher growth performance than those fed control diet containing low level of n-3 HUFA. Therefore, larval fish need dietary lipid containing high level of n-3 HUFA for high survival and good growth rate.

The dietary n-3 HUFA (20:5n-3 + 22:6n-3) ranged from 0.03 to 5.36% when expressed as a percentage of the dry diet in this study. The n-3 HUFA requirements for humpback, *Cromileptes altivelis* and tiger grouper juvenile, *E. fuscoguttatus* have been reported to be 1 and 2.5% of diets, respectively (Suwirya et al., 2004a;b). Wu and Chen (2012) fed 11.3 g *E. malabaricus* with diets containing 1 or 2% linoleic acid or linolenic acid and showed that weight gain of grouper fed diets containing 0.57 to 1.24% n-3 HUFA can be improved. In general, a dietary 1-1.5% n-3 HUFA should satisfy the essential fatty acid requirements of juvenile grouper (Williams, 2009). In the present study, the diets containing 0.78 to 3.96% n-3 HUFA supported good growth of the grouper larvae. Therefore, the grouper larvae and juvenile required high dietary n-3 HUFA level for good growth and survival.

The fatty acid of muscle of two species of grouper larvae contained 11.5 to 23.5% oleic acid. Its contribution to the total monounsaturated fatty acid level ranged from 30 to 61%. The two species of grouper larvae fed the treatment diets containing low level of oleic acid had high levels of muscle oleic acid. This indicated that oleic acid may be biosynthesis from 18:0 by the grouper larvae and the level of muscle oleic acid was not affected by the dietary fatty acid composition. Therefore, it can be confirmed that the oleic acid of grouper larvae muscle can be biosynthesized from dietary shorter chain fatty acids.

Under the same experimental diets and conditions, the growth performances of *E. lanceolatus* grew faster than those of *E. coioides* in this study. From the physiological view point, growth hormone and insulin-like growth factors play important roles in the growth, development and metabolism of vertebrate. The growth hormone and two forms of insulin-like growth factors were identified in pituitary and liver of these two species and the concentrations of those factors of *E. lanceolatus* were higher than those of *E. coioides* (Dong et al., 2010). Therefore, it is obviously that *E. lanceolatus* grows faster than *E. coioides*.

V. CONCLUSION

The results in this study indicate that 9.5% and 7.5% of dietary lipid levels were required to optimize the growth for 0.14 g *E. coioides* and 0.09 g *E. lanceolatus*, respectively. The artificial diet can replace live prey for 0.14 g *E. coioides* and 0.09 g *E. lanceolatus*. The growth and survival of grouper larvae were influenced by n-3HUFA levels in artificial diets.

REFERENCES

AOAC (Association of Official Analytical Chemists) (1984). Official Methods of Analysis, 14th, edn. Washington, DC.

Ai, Q. H., J. Z. Zhao, K. S. Mai, W. Xu, B. P. Tan, H. M. Ma and Z. G. Liufu (2008). Optimal dietary lipid level for large yellow croaker (*Pseudosciaena crocea*) larvae. Aquaculture Nutrition 14, 515-522.

Bernhart, F. W. and R. M. Tomarelli (1966). A salt mixture supplying the National Research Council estimates of mineral requirements of the rat. Journal of Nutrition 89, 495-500.

Bjornsson, B. and S. V. Tryggvadottir (1996). Effects of size on optimal temperature for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 142, 33-42.

Boonyaratpalin, M. (1997). Nutrient requirements of marine food fish cultured in Southeast Asia. Aquaculture 151, 283-313.

Chu, J. H., S. M. Chen and C. H. Huang (2007). Effect of dietary iron concentrations on growth, hematological parameters, and lipid peroxidation of soft-shelled turtles, *Pelodiscus sinensis*. Aquaculture 269, 532-537.

Company, R., J. A. Calduch-Giner, J. Pérez-Sánchez and S. J. Kaushik (1999). Protein sparing effect of dietary lipids in common dentex (*Dentex dentex*): a comparative study with sea bass (*Dicentrarchu labrax*). Aquatic Living Resources 12, 23-30.

Copeman, L. A., C. C. Parrish, J. A. Brown and M. Harel (2002). Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture 210, 285-304.

Dong, H., L. Zeng, D. Duan, H. Zhang, Y. Wang, W. Li and H. Lin (2010). Growth hormone and two forms of insulin-like growth factors I in the giant grouper (*Epinephelus lanceolatus*): molecular cloning and characterization of tissue distribution. Fish Physiology and Biochemistry 36, 201-212.

Duray, M. N., C. B. Estudillo and L. G. Alpasan (1997). Larval rearing of the grouper *Epinephelus suillus* under laboratory conditions. Aquaculture 150, 63-76.

Eusebio, P. S., R. M. Coloso and R. S. J. Gapasin (2010). Nutritional evaluation of mysids *Mesopodopsis orientalis* (Crustacea:Mysida) as live food

for grouper *Epinephelus fuscoguttatus* larvae. Aquaculture 306, 289-294.
Folch, J., M. Lees and C. H. Sloane-Stanley (1957). A simple method for the isolation and purification of total lipids from animal tissue. The Journal of Biological Chemistry 226, 497-509.
Izquierdo, M. S., T. Watanabe, T. Takeuchi, T. Arakawa and C. Kitajima (1989). Requirement of larval red sea bream *Pagrus major* for essential fatty acids. Nippon Suisan Gakkaishi 55, 859-867.
Kikuchi, K., H. Sugita and T. Watanabe (2000). Effect of dietary protein and lipid levels on growth and body composition of Japanese flounder. Suisan Zoshoku 48, 537-543.
Kohno, H., R. S. Ordonio-Aguilar, A. Ohno and Y. Taki (1997). Why is grouper larval rearing difficult: an approach from the development of the feeding apparatus in early stage larvae of the grouper, *Epinephelus coioides*. Ichthyological Research 44, 267-274.
Liao, I. C., H. M. Su and E. Y. Chang (2001). Techniques in finfish larviculture in Taiwan. Aquaculture 200, 1-31.
Lin, Y. H. and S. Y. Shiau (2003). Dietary lipid requirement of grouper, *Epinephelus malabaricus*, and effects on immune responses. Aquaculture 225, 243-250.
Lind, C., R. Fotedar and S. M. Rana (2004). Growth, survival and morphometric measurements of malabar grouper (*Epinephelus malabaricus*) larvae when co-fed *Artemia* and an artificial diet. Pakistan Journal of Zoology 36, 89-101.
Luo, Z., Y. J. Liu, K. S. Mai, L. X. Tian, D. H. Liu, X. Y. Tan and H. Z. Lin (2005). Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper (*Epinephelus coioides*) juveniles fed isonitrogenous diets in floating netcages. Aquaculture International 13, 257-269.
Metcalfe, L. D. and A. A. Schmitz (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. Analytical Chemistry 33, 363-365.
Miao, S. and H. C. Tang (2002). Bioeconomic analysis of improving management productivity regarding grouper *Epinephelus malabaricus* farming in Taiwan. Aquaculture 211, 151-169.
Morais, S., J. G. Bell, D. A. Robertson, W. J. Roy and P. C. Morris (2001). Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilization, muscle composition and liver histology. Aquaculture 203, 101-119.
Nagasawa, K. and E. R. Cruz-Lacierda (2004). Diseases of Cultured Groupers- Introduction. In: Diseases of Cultured Groupers, edited by Nagasawa, K. and E. R. Cruz-Lacierda, Southeast Asian Fisheries Development Center, Aquaculture Department, Government of Japan Trust Fund, 1-2.
NRC (National Research Council) (1993). Nutrient Requirement of Fish. National Academy Press, Washington, DC.
Ordonio-Aguilar, R., H. Kohno, A. Ohno, M. Moteki and Y. Taki (1995). Development of grouper (*Epinephelus coioides*) larvae during changeover of energy sources. Journal of Tokyo University of Fisheries 82, 103-108.
Pierre, S., S. Gaillard, N. Prevot-D'Alvise, J. Aubert, O. Rostaing-Capaillon, D. Leung-Tack and J. P. Grillasca (2008). Grouper aquaculture: Asian success and Mediterranean. Aquatic Conservation: Marine and Freshwater Ecosystems18, 297-308.
Pudadera, B., H. L. H. A. Hamid and H. R. H. M. Yusof (1999). Grouper culture development in Brunei Darussalam. Report of the APEC/NACA Cooperative Grouper Aquaculture Workshop, Hat Yai, Bangkok, Thailand, 53-56.
Rimmer, M. A. (2000). Review of grouper hatchery technology. SPC Live Reef Fish Information Bulletin 7, 14-19.
Rimmer, M. A. (2004). Advances in Grouper Aquaculture-Introduction. In: Advances in Grouper Aquaculture, edited by Rimmer, M. A., S. McBride and K. C. Williams, ACIAR Monograph, Australian Centre for International Agriculture Research, Canberra, Australia, 1-5.
Sadovy, Y. (2000). Regional survey for fry/fingerling supply and current practices for grouper mariculture: evaluating current status and long-term prospects for grouper mariculture in South East Asia. Final report to the Collaboration APEC grouper research and development network, Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong, China.
Silverstein, J. T., K. S. Shearer, W. W. Dickhoff and E. M. Plisetskaya (1999). Regulation and nutrient intake and energy balance in salmon. Aquaculture 177, 161-169.
Steel, R. G. D. and J. H. Torrie (1980). Principles and Procedures of Statistics, 2nd edn, McGraw-Hill, New York.
Storebakken, T. (2002). Atlantic salmon, *Salmo salar*. In Nutrient requirements and feeding of finfish for aquaculture, edited by Webster, C. D. and C. E. Lim, CABI Publishing, New York, 79-102.
Su, H. M., M. S. Su and I. C. Liao (1997). Preliminary results of providing various combinations of live foods to grouper (*Epinephelus coioides*) larvae. Hydrobiologia 358, 301-304.
Suwirya, K., N. A. Giri and M. Marzuqi (2004a). Effect of dietary n-3 HUFA on growth of humpback grouper (*Cromileptes altivelis*) and tiger grouper (*Epinephelus fuscoguttatus*) juveniles. In: Advances in Grouper Aquaculture, edited by Rimmer, M. A., S. McBride and K. C. Williams, ACIAR Monograph, Australian Centre for International Agriculture Research, Canberra, Australia, 98-100.
Suwirya, K., N. A. Giri, M. Marzuqi and Trijoko (2004b). Utilization of dietary dextrin by juvenile humpback grouper (*Cromileptes altivelis*). In: Advances in Grouper Aquaculture, edited by Rimmer, M. A., S. McBride and K. C. Williams, ACIAR Monograph, Australian Centre for International Agriculture Research, Canberra, Australia, 107-109.
Tuan, L. A. and K. C. Williams (2007). Optimum dietary protein and lipid specifications for juvenile malabar grouper *Epinephelus malabaricus*. Aquaculture 267, 129-138.
Usman, R., A. Laining, T. Ahmad and K. C. Williams (2005). Optimum dietary protein and lipid specifications for grow-out hump back grouper *Cromileptes altivelis*. Aquaculture Research 36, 1285-1292.
Vergara, J. M., I. Robaina, M. Izquierdo and M. de La Higuera (1996). Protein sparing effect of lipids in diets for fingerlings of gilthead sea bream. Fisheries Science 62, 624-628.
Watanabe, T. and V. Kiron (1994). Prospects in larval fish dietetics. Aquaculture 124, 223-251.
Weatherup, R. N., K. J. McCracken, R. Foy, D. Ride, J. McKendry, R. J. Mairs and R. Hoey (1997). The effects of dietary fat content on performance and body composition of farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 151, 173-184.
Williams, K. C. (2009). A review of feeding practices and nutritional requirements of post larval groupers. Aquaculture 292, 141-152.
Wu, F. C. and H. Y. Chen (2012). Effects of dietary linolenic acid to linoleic acid ratio on growth, tissue fatty acid profile and immune response of the juvenile grouper *Epinephelus malabaricus*. Aquaculture 324-325, 111-117.
Yoshii, K., F. Takakuwa, H. P. Nguyen, T. Masumoto and H. Fukada (2010). Effect of dietary lipid level on growth performance and feed utilization of juvenile kelp grouper *Epinephelus bruneus*. Fisheries Science 76, 139-145.