OPTIMUM LEVEL OF DIETARY PROTEIN AND LIPID FOR HYBRID GROUPER, BROWN-MARBLED GROUPER (*Epinephelus fuscoguttatus*) X GIANT GROUPER (*E. lanceolatus*)

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Abstract: A 12-weeks experiment was conducted to determine the optimum level of dietary protein and lipid for hybrid grouper, brown-marbled grouper (*Epinephelus fuscoguttatus*) x giant grouper (*E. lanceolatus*). The hybrid grouper juveniles (body weight: 6.6 ± 0.1 g) were fed twice a day with 12 experimental feeds containing different levels of protein (45, 50 and 55%) and lipid (8, 12, 16 and 20%). Results showed that the fish fed with a different combination of protein and lipid levels did not significantly ($p > 0.05$) affect the growth performances. An increasing trend in growth was observed when protein and lipid levels increased from 45 to 55% and 8 to 16%, respectively. Protein sparing effects by lipid can be observed when lipid increased to 16% at any protein levels; however, the growth of fish was decreased at 20% of lipid. The lipid retention efficiency, body lipid, VSI, ISI and IPF were increased correspondingly with the lipid level from 8 to 20%. No significant effect ($p > 0.05$) was observed on the ADC of protein and lipid. Based on the regression analysis, the present study suggests that 50% of dietary protein and 16.5% of dietary lipid are the optimum requirements to be provided in formulated feed for the hybrid grouper.

Keywords: Juveniles, protein retention efficiency, lipid retention efficiency.

Introduction

Hybrid grouper, brown-marbled grouper (*Epinephelus fuscoguttatus*) x giant grouper (*E. lanceolatus*) was first produced in the year 2006 by Universiti Malaysia Sabah (Ch'ng & Senoo, 2008). Since then, the hybrid grouper has become one of the most popular aquaculture fish among grouper production (Luin *et al*., 2013). However, feeding the fish with low-value fish is a common husbandry practice in Asia (Williams, 2009; Shapawi *et al*., 2014). The imbalanced nutrient requirement might occur and consequently, suppress the growth and hinder the production (NRC, 2011). Generally, carnivorous fish are known to utilize protein efficiently for energy compared to lipid and carbohydrate (Yong *et al*., 2015). Also, an improper combination of dietary protein and lipid levels may lead to nutritional disorders such as accumulation of excessive fat, reduced feed intake, suppression of growth and mortality (Lin & Shiau, 2003; Yong *et al*.,

2015). Thus, the development of nutritionally balanced practical feeds for this hybrid grouper is necessary.

Several studies on other groupers such as brown-marbled grouper, humpback grouper (*Cromileptes altivelis*), malabar grouper (*E. malabaricus*), orange-spotted grouper (*E. coioides*), red spotted grouper (*E. akaara*) and white grouper (*E. aeneus*) showed that the fish (below 250g) require high protein (45 – 55%) and moderate lipid (8 – 16%) level to achieve the optimum growth (Chen & Tsai, 1994; Luo *et al*., 2004; Lupatsch & Kissil, 2005; Usman *et al*., 2005; Tuan & Williams, 2007; Shapawi *et al*., 2014). Although protein requirement of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*) was reported to be similar with maternal fish, brown-marbled grouper and other groupers, contradicting results had been reported on the optimum level of dietary lipid for hybrid grouper (Jiang *et al*., 2015; Rahimnejad *et al*., 2015).

Jiang *et al*. (2015) recommended 7% of lipid level for the hybrid grouper while Rahimnejad *et al*. (2015) recommended double the lipid level at 14% for the same hybrid grouper. Therefore, a 3x4 factorial (3 levels of protein x 4 levels of lipid) experimental design was conducted to clarify the optimum level of dietary protein and lipid of the hybrid grouper in Sabah, Malaysia.

Materials and Methods

Experimental Feed Preparation

A 3 x 4 factorial design of 3 levels of protein (45, 50 and 55%) and 4 levels of lipid (8, 12, 16 and 20%) (Table 1) was prepared. Danish fish meal and wheat gluten meal were used as the source of protein while the industrial grade of fish oil was the main lipid source. The experimental feeds were prepared by mixing all of the ingredients until homogenous and added with 40% of water to form a moist dough. The moist dough was then made into pellet using a meat mincer (Orimas ®, TBS 200, Taiwan) with a 3mm diameter and rotation cutter. Then, the experimental feeds were dried in an oven (Ming-Li Electric MFG, BS-2210, China) at 40°C for 6 hours. The dried experimental feeds were then kept refrigerated at 4°C until use. The proximate composition of the experimental feeds is shown in Table 1.

Fish Rearing

The experimental fish was obtained from a private farm (Ocean Supplies Enterprise) in Tawau, Sabah, Malaysia. The experimental fish were acclimatized to the experimental condition and fed with commercial feed (45% crude protein and 8% crude lipid; Cargill Ltd., Malaysia). Groups of 20 fish (initial body weight: 6.6 ± 0.1 g) were randomly distributed into 100L cylindrical cage (height: 70 cm; diameter: 60 cm) and placed in two 10-tonne circular fibreglass tanks. A simple recirculation and filtration water system using coral rubbles as the filter was used throughout the feeding trial. About 40 to 50% of water was exchanged daily to maintain the water quality (temperature: 26.5-29.5°C; dissolved oxygen: 5.2-6.8 mgL-1; pH: 6.0-7.0; salinity: 28-31 ppt). Triplicate groups of the fish were fed with each diet twice daily at 0700 and 1500 until apparent satiation level for 84 days. The feed intake was recorded to determine the feed utilization. The body weight measurement of the fish was done fortnightly. At the end of the experiment, fish from each treatment (n=9) were sacrificed for determination of body indices, proximate composition and nutrient retention efficiency. The growth performances, survival, feed conversion ratio, nutrient retention efficiency and body indices were calculated as followed:

$$Weight\ gain\ (\%) = \frac{Final\ body\ weight\ (g) - Initial\ body\ weight\ (\ )}{initial\ body\ weight\ (g)} \times 100$$

$$SGR\ (\%\ day-1) = \frac{Ln\ Final\ body\ weight\ (g) - Ln\ Initial\ body\ weight\ (g)}{Time\ (days)} \times 100$$

$$Survival\ (\%) = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$$

$$Feed\ intake\ (g) = \frac{Total\ feed\ intake\ for\ 84\ days}{Total\ number\ of\ fish}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ intake\ (g)}{Wet\ weight\ gain\ (g)}$$

Table 1: Ingredients and proximate composition (g kg^{-1} dry weight basis) of experimental feeds fed to hybrid grouper juvenile

	Experimental Feed (Protein/Lipid)											
	45/8	45/12	45/16	45/20	50/8	50/12	50/16	50/20	55/8	55/12	55/16	55/20
Ingredients												
Fish meal[a]	496	496	496	496	567	567	567	567	638	638	638	638
Wheat gluten meal[b]	120	120	120	120	120	120	120	120	120	120	120	120
Fish oil	28	68	108	148	20	60	100	140	13	53	93	133
Starch[c]	286	246	206	166	233	193	153	113	169	129	89	49
CMC[d]	20	20	20	20	20	20	20	20	20	20	20	20
Vitamin premix[e]	20	20	20	20	15	15	15	15	15	15	15	15
Mineral premix[f]	20	20	20	20	15	15	15	15	15	15	15	15
Dicalcium phosphate[g]	10	10	10	10	10	10	10	10	10	10	10	10
Chromic oxide	5	5	5	5	5	5	5	5	5	5	5	5
Proximate Composition												
Crude protein	451.0	455.0	451.0	459.0	505.0	509.0	501.0	505.0	554.0	554.0	550.0	553.0
Crude lipid	83.0	120.0	164.0	204.0	85.0	126.0	163.0	205.0	81.0	120.0	163.0	201.0
Moisture	102.0	108.0	105.0	119.0	121.0	125.0	104.0	116.0	122.0	126.0	110.0	102.0
Ash	113.0	118.0	108.0	111.0	115.0	114.0	114.0	117.0	117.0	116.0	115.0	114.0
Energy (MJ kg^{-1})	18.2	18.8	19.8	20.4	18.1	18.9	20.5	20.8	17.8	18.7	20.2	21.3
Protein: Energy (g MJ^{-1})	24.8	24.2	22.8	22.5	27.9	26.9	24.5	24.3	31.1	29.6	27.2	26.0

Note: [a] Danish fish meal (Crude protein: 71%, crude lipid: 10%)
[b,c] Bake with Me Sdn. Bhd.
[d] Carboxylmethyl cellulose (CMC), Sigma
[e] Vitamin premix (Dexchem Industries Sdn. Bhd.), contains (g kg^{-1} dry weight basis): ascorbic acid 45g; inositol 5g; choline chloride 75g; niacin 4.5g; riboflavin 1g; pyridoxine HCl 1g; thiamine HCl 0.92g; dicalcium pantothenate 3g; retinyl acetate 0.6g; vitamin D3 0.08g; menadione 1.67g; dialpha tocopherol acetate 8g; d – Biotin 0.02g; folic acid 0.09g; vitamin B12 0.001g; cellulose
[f] Mineral premix (Dexchem Industries Sdn. Bhd.), contains (g kg^{-1} dry weight basis): calcium phosphate monobasic 270.98g; calcium lactate 327g; ferrous sulphate 25g; magnesium sulphate 132g; potassium chloride 50g; potassium iodide 0.15g; copper sulphate 0.785g; manganese oxide 0.8g; cobalt carbonate 1g; zinc oxide 3g; sodium selenite 0.011g; calcium carbonate 129.27g
[g] Dicalcium phosphate, Merck

$$Protein\ retention\ efficiency\ (PRE) = \frac{Final\ fish\ body\ protein - Initial\ fish\ body\ protein}{Total\ protein\ intake} \times 100$$

$$Lipid\ retention\ efficiency\ (LRE) = \frac{Final\ fish\ body\ lipid - Initial\ fish\ body\ lipid}{Total\ lipid\ intake} \times 100$$

$$Condition\ factor = \frac{Wet\ body\ weight\ (g)}{Standard\ length\ (cm)^3} \times 100$$

$$Hepatosomatic\ index\ (HSI) = \frac{Wet\ weight\ of\ liver\ (g)}{Wet\ body\ weight\ (g)} \times 100$$

$$Viscerosomatic\ index\ (VSI) = \frac{Wet\ weight\ of\ viscera\ (g)}{Wet\ body\ weight\ (g)} \times 100$$

$$Intestosomatic\ index\ (ISI) = \frac{Wet\ weight\ of\ intestine\ including\ pyloric\ cecae\ (g)}{Wet\ body\ weight\ (g)} \times 100$$

$$Intraperitoneal\ fat\ (IPF) = \frac{Wet\ weight\ of\ intraperitoneal\ fat\ (g)}{Wet\ body\ weight\ (g)} \times 100$$

Proximate Composition

Proximate composition was conducted on experimental feeds and fish samples. Crude protein was determined using Kjeltec-Protein Analyzer (Kjeltec™ 2300, Foss, Sweden) and crude lipid by petroleum ether (40-60°C boiling) extraction using Soxtec-Lipid Analyzer (Soxtec™ 2043, Foss, Sweden) (AOAC, 1999). Moisture was determined by oven-drying the sample at 105°C for 24 hours and the dried sample was then ignited in a muffle furnace at 550°C for 6 hours to determine ash content (AOAC, 1999). The energy of feeds and fish sample were analyzed directly using an automated oxygen automated bomb calorimeter (IKA®-WERKE, Germany).

Digestibility Analysis

After completion of the feeding trial, 20 fish from each treatment were pooled and transferred into a 12-fibreglass tank (volume: 100L) for faecal collection. The tanks were specifically designed with a sloping bottom to the central tank where drainage outlet was located and that was connected to a faeces collection column. The fish were fed with experimental feeds containing 0.5% of chromic oxide as an inert marker twice daily until apparent satiation level (0700 and 1500). The tanks were cleaned an hour after feeding to remove the uneaten feed and other detritus. The fresh faeces were collected from faecal collector after 3 hours of feeding. The collected faeces were rinsed with distilled water and tapped dry before keeping it in -80°C until analysis. Chromic oxide was determined using acid digestion method following Furukawa & Tsukahara (1966). The apparent digestibility coefficient (ADCs) of the experimental feeds were calculated as follow:

$$ADCs\ of\ nutrient = 100 \times \left[1 - \left(\frac{\%\ faeces\ nutrient}{\%\ dietary\ nutrient}\right) \times \left(\frac{\%\ dietary\ chromic\ oxide}{\%faeces\ chromic\ oxide}\right)\right]$$

Statistical Analysis

IBM Statistical Packages of Social Sciences Version 24.0 was used for statistical analysis. Data in percentage were arcsine transformed before the analysis. The quantitative data was then subjected to one-way analysis of variance (ANOVA) to determine the mean differences among the treatments at 0.05 significance level. The homogeneity of variance was tested using Levene's test and multiple comparisons among the treatments were presented using a Tukey's multiple range test. Effect of dietary protein, lipid and interaction of protein and lipid were performed by two-way ANOVA. A second-order polynomial regression analysis was conducted on weight gain and protein retention efficiency (PRE) of the fish to determine the suitable lipid level in the experimental feeds for each protein level (45, 50 and 55%).

Results

Growth Performances, Survival and Feed Utilization

Growth performances and survival of hybrid grouper juveniles fed with different levels of protein and lipid are shown in Table 2. Growth performance was not significantly affected ($p > 0.05$) by either dietary protein or lipid level. The

highest growth performance was found in the fish group fed with P55/L16 (55% protein and 16% lipid) while the lowest growth performance was found in the fish group fed with P45/L20. A second-order polynomial regression on weight gain showed a slightly increasing trend of the growth performances when the protein level increased from 45% to 55% regardless of the level of dietary lipid (Figure 1). This regession analysis also showed that the growth performance was increased when the lipid level increased from 8% to 16% and decreased at 20% (Figure 1). Survival of the fish ranged from 90.0 to 100.0%. Survival of the fish was significantly affected by dietary lipid. Fish group fed with P45/L8 ($90.0 \pm 5.0\%$) showed significantly ($p < 0.05$) lowest survival compared to the other fish group. However, it was not significant ($p > 0.05$) with fish fed with P55/L8 ($96.7 \pm 2.8\%$).

The total feed intake and feed conversion ratio (FCR) of hybrid grouper juveniles fed with the experimental feeds are presented in Table 3. The total feed intake was not significantly ($p > 0.05$) affected by either protein or lipid levels. However, the FCR was significantly ($p < 0.05$) affected by both protein and lipid levels. The FCR showed slightly lower values with the increased of protein and lipid levels.
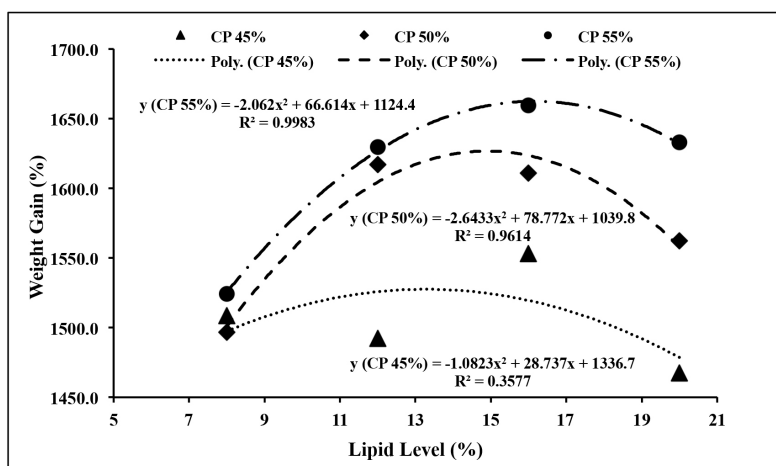

Figure 1: Relationship between lipid levels and weight gain (%) of hybrid grouper juvenile fed with different protein levels

Table 2: Growth performances and survival of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/ Lipid)	Parameter			
	Final Body Weight (FBW) (g)	**Weight Gain (WG) (%)**	**Specific Growth Rate (SGR) (% day-1)**	**Survival (%)**
One-way ANOVA				
45/8	106.8±7.8	1,508.6±144.1	2.8±0.1	90.0±5.0 [a]
45/12	104.6±8.9	1,492.1±125.4	2.8±0.1	98.3±2.9 [b]
45/16	110.6±11.3	1,553.1±144.8	2.9±0.1	100.0±0.0 [b]
45/20	101.9±10.8	1,467.4±145.5	2.8±0.1	100.0±0.0 [b]
50/8	105.1±12.2	1,496.5±153.0	2.8±0.1	100.0±0.0 [b]
50/12	113.6±11.2	1,617.1±178.1	2.9±0.1	98.3±2.9 [b]
50/16	111.7±13.4	1,610.7±169.6	2.9±0.1	98.3±2.9 [b]
50/20	110.7±4.4	1,562.1±68.0	2.9±0.0	100.0±0.0 [b]
55/8	106.7±13.3	1,524.3±229.8	2.8±0.1	96.7±2.9 [ab]
55/12	113.1±4.5	1,629.7±63.0	2.9±0.0	100.0±0.0 [b]
55/16	115.9±4.6	1,659.4±85.8	2.9±0.1	100.0±0.0 [b]
55/20	113.9±10.7	1,632.8±147.7	2.9±0.1	100.0±0.0 [b]
Two-way ANOVA (p value)				
Protein	0.29	0.22	0.17	0.06
Lipid	0.57	0.61	0.42	0.00
Interaction	0.93	0.98	0.91	0.00

Note: Mean (±SD) values with different superscripts within the column are significantly different ($p < 0.05$)

Nutrient Retention Efficiency

An almost similar trend with feed utilization was observed on the nutrient retention efficiency (Table 4). The protein retention efficiency (PRE) and lipid retention efficiency (LRE) were significantly ($p < 0.05$) affected by the protein and lipid levels. The PRE was lower in the low feed energy content (feed 45/8, 50/8 and 55/8) compared to other feeds. However, the value increased with the increasing level of lipid from 8% to 16% then decreased at 20% dietary lipid.

The highest PRE was observed in feed 50/16. Meanwhile, LRE increased with the increasing lipid level from 8% to 20% in the feeds. Higher LRE was observed at the highest lipid level at each of protein level. A second-order polynomial regression based on PRE for each protein levels showed that the optimum lipid level for hybrid grouper juveniles was estimated at 15%, 16.5% and 17% at 45%, 50% and 55% of protein, respectively (Figure 2). However, the highest performances observed at a combination of 16.5% of lipid and 50% of protein.

Table 3: Feed utilization of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/ Lipid)	Parameter	
	Total Feed Intake (g fish-1)	Feed Conversion Ratio (FCR)
One-way ANOVA		
45/8	131.5±2.2	1.32±0.09 [b]
45/12	119.0±7.2	1.22±0.04 [ab]
45/16	126.8±4.8	1.23±0.09 [ab]
45/20	114.3±8.9	1.20±0.06 [ab]
50/8	120.1±10.6	1.22±0.05 [ab]
50/12	120.7±8.4	1.13±0.05 [ab]
50/16	117.6±7.8	1.12±0.07 [ab]
50/20	114.9±2.6	1.11±0.03 [a]
55/8	116.0±8.1	1.16±0.07 [ab]
55/12	117.0±3.4	1.10±0.04 [a]
55/16	116.6±1.9	1.07±0.04 [a]
55/20	119.7±1.8	1.12±0.10 [a]
Two-way ANOVA (p value)		
Protein	0.10	0.00
Lipid	0.24	0.01
Interaction	0.15	0.94

Note: Mean (±SD) values with different superscripts within the column are significantly different ($p < 0.05$)

Body Proximate Composition

A result of whole-body proximate composition is shown in Table 5. The crude body protein and lipid were significantly ($p < 0.05$) affected by protein and lipid level while body moisture and ash were significantly ($p < 0.05$) affected by lipid level only. The body protein increased when the lipid level increased up to 16% then decreased to 20%. Meanwhile, the body lipid increased with increasing level of lipid from 8% to 20% in each level of protein. However, there was no definite trend observed for body moisture and ash for the fish fed with experimental feeds.

Body Indices

The present study showed that the condition factor and hepatosomatic index (HSI) of the fish were not significantly ($p > 0.05$) affected by the protein and lipid levels (Table 6). However, the viscerosomatic index (VSI), intestosomatic index (ISI) and intraperitoneal fat (IPF) were significantly ($p < 0.05$) affected by the experimental feeds. The increased of the dietary lipid from 8% to 20% has significantly increased the VSI, ISI and IPF.

Apparent Digestibility Coefficients (ADC)

The apparent digestibility coefficients (ADC) of protein and lipid in the present study are shown in Table 7. No significant effect ($p > 0.05$) was observed on the ADC of protein and lipid. The ADC of protein and lipid was ranged from 0.90 to 0.91 and from 0.92 to 0.95, respectively.

Table 4: Nutrient retention efficiency of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/ Lipid)	Parameter	
	Protein Retention Efficiency (PRE)	Lipid Retention Efficiency (LRE)
One-way ANOVA		
45/8	29.5±0.9 [bcd]	17.1±5.2 [a]
45/12	30.2±0.6 [cdef]	23.3±4.2 [bcd]
45/16	30.4±0.8 [def]	28.0±1.1 [def]
45/20	29.8±0.6 [bcde]	30.5±1.4 [ef]
50/8	27.0±0.7 [a]	19.6±1.9 [ab]
50/12	30.6±0.6 [def]	25.4±2.3 [cde]
50/16	31.6±0.3 [f]	30.8±2.6 [ef]
50/20	30.9±1.1 [ef]	33.5±1.8 [f]
55/8	26.4±0.6 [a]	21.1±2.0 [abc]
55/12	28.3±0.6 [b]	28.9±3.0 [def]
55/16	29.2±0.8 [bcd]	31.8±0.9 [f]
55/20	28.9±1.2 [bc]	32.4±2.2 [f]
Two-way ANOVA (p value)		
Protein	0.00	0.00
Lipid	0.00	0.00
Interaction	0.00	0.59

Note: Mean (±SD) values with different superscripts within the column are significantly different ($p < 0.05$)

Discussion

The present study indicates that feed formulated with different dietary protein (45, 50 and 55%) and lipid (8, 12, 16 and 20%) levels combination did not significantly affect the growth performances of hybrid grouper. However, based on the second-order polynomial regression analysis on PRE, 50% of dietary protein and 16.5% of dietary lipid are suggested to be the optimum level for hybrid grouper. In the previous study, the growth of hybrid grouper was not affected when the protein level in the feeds increased from 45 to 55% (Jiang *et al.*, 2015; Rahimnejad *et al.*, 2015). However, Jiang *et al.* (2015) and Rahimnejad *et al.* (2015)

demonstrated that providing protein below than 45% in the feed for hybrid grouper significantly reduced the fish growth. The finding of dietary protein for hybrid grouper in the present study was similar to reported dietary protein requirement for other grouper species such as maternal fish, brown-marbled grouper (50%) (Shapawi *et al.*,. 2014), malabar grouper (48%) (Chen & Tsai, 1994) and orange-spotted grouper (48%) (Luo *et al.*, 2004). In contrast, Nassau grouper (*E. striatus*) required higher protein levels (>55%) for optimum growth (Ellis *et al.*, 1996).

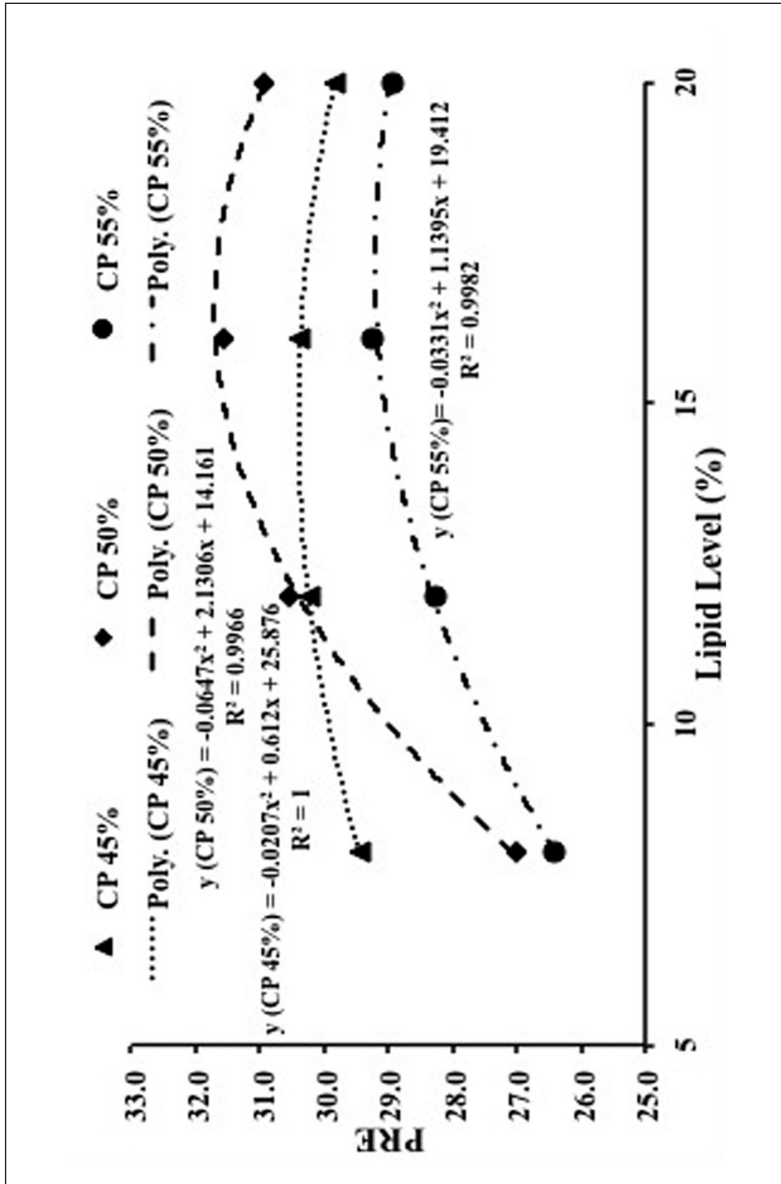The finding in the present study also revealed that dietary lipid increased from 8 to

Figure 2: Relationship between lipid levels and PRE of hybrid grouper juvenile fed with experimental feeds with different protein levels (A: 45%, B: 50%, C: 55%)

16% in fish feed resulted in improvement in weight gain, FCR and PRE. Also, providing dietary lipid at higher level reflects higher lipid retained (LRE, VSI and IPF) in the fish body. The findings showed that the hybrid grouper is utilizing lipid efficiently and able to store lipid in the body for further use. Therefore, better growth, feed utilization and nutrient retention in fish fed higher dietary lipid regardless of the dietary protein suggests that a protein-sparing effect by dietary lipid can be observed in the hybrid grouper. Similar to the present study, Rahimnejad *et al.* (2015) suggests that hybrid grouper required higher level (14%) of dietary

Table 5: Whole-body proximate composition (g/kg) of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/Lipid)	Parameter			
	Protein	Lipid	Moisture	Ash
One-way ANOVA				
45/8	161.4±5.0 [a]	19.1±8.1 [a]	707.3±1.4	52.3±5.4
45/12	165.5±3.1 [ab]	32.0±7.2 [b]	709.4±7.8	49.2±4.0
45/16	165.7±4.1 [ab]	65.1±5.8 [de]	689.9±15.9	46.9±5.4
45/20	162.8±3.1 [a]	67.4±5.4 [e]	695.7±14.7	47.7±3.3
50/8	162.3±0.7 [a]	16.5±3.6 [a]	726.1±19.4	50.3±4.3
50/12	173.6±3.4 [bc]	38.2±8.1 [bc]	701.0±3.0	52.0±2.1
50/16	175.1±1.8 [c]	37.7±5.2 [bc]	689.6±5.5	49.0±6.8
50/20	170.9±5.8 [bc]	54.5±12.4 [cde]	703.5±9.0	44.2±6.0
55/8	168.1±3.9 [b]	38.0±5.8 [bc]	705.7±17.3	52.3±1.1
55/12	170.3±3.7 [bc]	47.2±11.5 [bcd]	701.1±19.1	48.5±6.5
55/16	172.6±1.4 [bc]	51.3±11.3 [cde]	697.3±19.9	45.9±4.2
55/20	172.2±3.4 [bc]	51.2±10.2 [cde]	689.6±10.5	45.1±2.8
Two-way ANOVA (p value)				
Protein	0.03	0.00	0.59	0.55
Lipid	0.39	0.00	0.02	0.01
Interaction	0.00	0.00	0.47	0.45

Note: Mean (±SD) values with different superscripts within the column are significantly different ($p<0.05$)

lipid. However, only two levels of dietary lipid (7 and 14%) were tested on hybrid grouper by Rahimnejad *et al.* (2015). On the contradict, Jiang *et al.* (2015) suggest that 7% of dietary lipid improved the fish growth, increased protein efficiency ratio and reduced feed adiposity compared to higher levels (10 and 13%) of dietary lipid. The finding in the present study and Rahimnejad *et al.* (2015) are in agreement with the maternal fish brown-marbled grouper which showed a tendency to perform well as the dietary lipid increased from 8 to 16% (Shapawi *et al.*, 2014). Similar to the present study, Biswas *et al.* (2009) reported that higher PRE with increased dietary lipid from 9.2 to 17.9% in the feed for bluefin tuna reflected a better growth and feed utilization.

Generally, higher dietary lipid in fish feed leads to the suppression of fish appetite by negative feedback mechanism between fat adiposity and feed intake (Williams *et al.*, 2004; Usman *et al.*, 2005; Luo *et al.*, 2005; Tuan & Williams, 2007). However, the total feed intake of hybrid grouper in the present study was not significantly influenced by dietary protein and lipid. Similar to this study, the feed intake of Pacific bluefin tuna was not affected by varying dietary protein (53.3 to 72.8%) and lipid (9.2 to 27.0%) (Biswas *et al.*, 2009). On the other hand, the total feed intake of maternal fish brown-marbled grouper was significantly affected by the different level of dietary protein and lipid (Shapawi *et al.*, 2014). Higher feed intake was observed in the feeds with high protein (55%) compared to low protein (45%). However, the total feed intake of brown-marbled grouper decreased when dietary lipid increased to 16% at 45, 50 and 55% of dietary protein.

Table 6: Body indices of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/ Lipid)	Parameter				
	Condition Factor	Hepatosomatic Index (HSI)	Viscerosomatic Index (VSI)	Intestosomatic Index (ISI)	Intraperitoneal Fat (IPF)
One-way ANOVA					
45/8	2.96 ±0.36	1.43 ±0.26	9.89 ±0.72 [abc]	3.45 ±0.54 [ab]	1.56 ±0.36 [abc]
45/12	3.11 ±0.28	1.56 ±0.19	12.13 ±1.10 [cd]	4.21 ±0.53 [abc]	2.60 ±0.65 [bcd]
45/16	3.21 ±0.37	1.52 ±0.33	14.44 ±1.05 [d]	4.87 ±0.77 [bc]	4.51 ±1.02 [e]
45/20	3.17 ±0.28	1.41 ±0.35	14.24 ±1.51 [d]	4.90 ±0.83 [bc]	4.44 ±0.82 [e]
50/8	2.87 ±0.26	1.26 ±0.27	9.35 ±0.60 [ab]	3.07 ±0.51 [a]	1.28 ±0.35 [a]
50/12	2.81 ±0.21	1.15 ±0.37	9.39 ±1.22 [ab]	3.41 ±0.84 [ab]	1.18 ±0.38 [a]
50/16	3.07 ±0.33	1.54 ±0.59	12.39 ±1.18 [cd]	4.19 ±0.48 [abc]	3.15 ±0.93 [de]
50/20	3.22 ±0.37	1.48 ±0.33	14.00 ±1.42 [d]	5.03 ±1.09 [c]	3.82 ±1.03 [de]
55/8	3.01 ±0.31	1.16 ±0.34	8.78 ±1.71 [a]	2.95 ±0.85 [a]	1.23 ±0.75 [a]
55/12	2.90 ±0.14	1.04 ±0.36	9.06 ±1.71 [ab]	3.13 ±1.06 [a]	1.41 ±0.54 [ab]
55/16	2.90 ±0.10	1.34 ±0.40	11.89 ±0.73 [bcd]	3.88 ±0.97 [abc]	2.79 ±0.26 [cde]
55/20	2.96 ±0.25	1.06 ±0.25	12.11 ±1.13 [bcd]	4.02 ±0.50 [abc]	3.10 ±0.92 [de]
Two-way ANOVA (p value)					
Protein	0.05	0.01	0.00	0.00	0.00
Lipid	0.33	0.30	0.00	0.00	0.00
Interaction	0.44	0.52	0.21	0.66	0.33

Mean (±SD) values with different superscripts within the column are significantly different ($p<0.05$)

The condition factor and HSI of the hybrid grouper were not affected by dietary protein and lipid. Thus indicates that the hybrid grouper in good condition when fed by various levels of dietary protein and lipid in the present study. Meanwhile, the VSI, ISI and IPF values of hybrid grouper increased with increasing dietary lipid similar to previous study on brown-marbled grouper (Shapawi *et al.*, 2014), which indicates the excessive energy supplied retained in the body as fat (Li *et al.*, 2010; Jiang *et al.*, 2015). High fat deposition for a long term in the fish body may lead to a bad effect on health and reduces the quality of fish flesh (Regost *et al.*, 2001; Wang *et al.*, 2016). Providing high lipid in fish feed commonly related to the lowest

Table 7: Apparent digestibility coefficients (ADC) of hybrid grouper fed different protein and lipid levels combination for 84 days feeding trial

Experimental Feed (Protein/Lipid)	Parameter	
	ADC Protein	ADC Lipid
One-way ANOVA		
45/8	0.90±0.08	0.94±0.13
45/12	0.91±0.05	0.94±0.11
45/16	0.90±0.04	0.95±0.11
45/20	0.90±0.07	0.95±0.06
50/8	0.91±0.05	0.94±0.09
50/12	0.90±0.07	0.94±0.12
50/16	0.90±0.04	0.95±0.08
50/20	0.90±0.03	0.95±0.04
55/8	0.91±0.03	0.92±0.15
55/12	0.90±0.02	0.94±0.08
55/16	0.91±0.02	0.95±0.07
55/20	0.90±0.07	0.94±0.03
Two-way ANOVA (p value)		
Protein	0.67	0.16
Lipid	0.61	0.01
Interaction	0.54	0.96

Note: Mean (±SD) values with different superscripts within the column are significantly different ($p < 0.05$)

superoxide anions which can cause metabolic stress and decrease fish resistance towards disease (Cheng *et al.*, 2006). It has been reported that high fat deposition in the flesh of European seabass, *Dicentrarchus labrax* led to the limitation of collagen and muscle fibres which resulted in reducing their mechanical strength (Fasolato *et al.*, 2005). Changes in the flesh texture reflect on the reduction of the acceptance and market value of the fish (Torrisen *et al.*, 2001). Besides, it also led to lipid oxidation, which caused quality losses due to unpalatable flavour and odour, shortening the shelf life and losses of nutritional value in fish flesh (Secci & Parisi, 2016).

The apparent digestibility coefficient of dietary protein and lipid for all of the treatment were almost similar. This was probably caused by the digestible fish meal, wheat gluten meal and fish oil which used as a dietary protein and lipid source (Booth *et al.*, 2010). The better nutrient digestibility for all the treatment allowed higher nutrient uptake and reflected the growth performances of hybrid grouper.

Conclusion

The finding in the present study suggests that hybrid grouper can be fed with feed containing any levels of dietary protein (45, 50 or 55%) with optimum dietary lipid (15, 16.5 or 17%),

respectively. However, providing 50% of dietary protein with a combination of 16.5% dietary lipid in the feed is the most recommended for better utilization of nutrients.

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