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A Comparison of the Growth Performance and Body Composition of the Humpback Grouper, *Cromileptes altivelis* Fed on Farm-made Feeds, Commercial Feeds or Trash Fish

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ABSTRACT
Development of pelleted feed for cultured fish is an important aspect in aquaculture industry. Two farm-made feeds were formulated with a blend of alternative ingredients (poultry by-product meal and palm oil) in the place of marine fish meal and fish oil and fed to humpback grouper (*Cromileptes altivelis*) fingerlings (4.1±0.1 g) for 14 weeks. The performance of the poultry by-product-based farm-made feeds was compared with a local commercial marine fish feed, an imported commercial marine fish feed and with trash fish (*Sardinella* spp.). Growth performance of groupers fed the farm-made feeds was better than or comparable to fish fed the commercial feeds or trash fish. Feed Conversion Ratios (FCR) in fish fed pelleted feeds (1.3 to 2.4) were significantly better than the FCR of fish fed trash fish (5.0). The survival rate of fish fed trash fish (50%) was significantly lower compared to other fish groups which had a survival rate of more than 95%. Body proximate and fatty acid composition of the fish at the end of the feeding trial reflects the composition of the diet. The present study showed that not all marine fish feeds are of equal quality but when correctly formulated to meet the requirements of humpback grouper, pelleted feeds consistently give better performance compared to a diet of trash fish. Therefore, switching from trash fish to pelleted feeds is highly recommended for grouper farmers.

Key words: Grouper, farm-made feeds, poultry by-product meal, trash fish, commercial feeds

INTRODUCTION
Despite the rapid growth of marine finfish aquaculture in Asia, feeding with trash fish remains the method of choice in farming marine carnivorous fish such as groupers. The use of trash fish in marine aquaculture has always been associated with environmental degradation, over-exploitation of finite pelagic fish stocks and issues with pathogen transmission (Cho *et al.*, 1994; Tacon and Forster, 2003; Kim *et al.*, 2007). In addition, increasing price, shortage of supply, variable quality and poor feed conversion ratios of trash fish is used for feeding farmed grouper indicates it is not an economical feed input. Switching from feeding trash fish to pelleted feeds have been reported to reduce marine fish production costs and promising better fish survival, improved feed conversion and higher cash returns (Orachunwong *et al.*, 2005). In view of these issues and concerns, more

fish farmers are ready to make the switch from trash fish and to use pelleted feed for feeding their farmed marine fish. Development of cost-effective pelleted feed will greatly contribute to the success and sustainability of the thriving marine finfish aquaculture industry in Asia. Currently, there is very limited published information comparing the growth performance of farmed groupers fed pelleted feeds versus trash fish.

Fish meal and fish oil are still widely used as the main source of dietary protein and lipid, respectively, for most commercial marine fish pelleted feeds. The shortage in world production of fish meal and fish oil and increased demand has further increased fish meal and fish oil prices (Tacon *et al*., 2006). Therefore, it is critical to find suitable alternatives to fish meal and fish oil. The substitution of fish meal and fish oil by alternative protein and lipid sources to reduce the cost of feeding and to improve aquaculture sustainability has yielded good results (Rodriguez-Serna *et al*., 1996; El-Sayed, 1998; Ng, 2002). In carnivorous marine fish species, more success has been achieved when animal by-product sources are used as alternatives to fish meal compared to plant protein sources (Nengas *et al*., 1999; Pham *et al*., 2007). Research into the use of plant oil in feeds for marine fish has also shown encouraging results (Glencross *et al*., 2003; Raso and Anderson, 2003; Richard *et al*., 2006). The findings from our previous trials indicated that Poultry By-products Meal (PBM) and plant oil such as palm oil can be successfully used as alternative dietary protein and lipid sources, respectively, in feeds formulated for the humpback grouper (Shapawi *et al*., 2007, 2008). These and other findings obtained from our laboratory trials were used as the basis for the prototype feed formulation used in the present study. In this feeding trial, growth, feed utilization efficiency, survival, body indices and body composition of humpback grouper fed two different prototype farm-made feeds were compared with those fed established commercial marine fish feeds and with trash fish.

MATERIALS AND METHODS

Experimental feeds: Fish meal in both farm-made feeds (FarmFeed1 and FarmFeed 2) were replaced with PBM at a level not less than 50 and 75%, respectively, to supply the needed dietary protein. Added dietary lipid was in the form of Crude Palm Oil (CPO) and fish oil in FarmFeed 1 and only fish oil was used in FarmFeed 2. Tapioca starch was used as the carbohydrate source in both feeds. The exact ingredient formulation of these two prototype feeds as well as the two commercial feeds used (ComFeed 1 and ComFeed 2) are the proprietary of the respective companies/institutions. The proximate, amino acid and fatty acid composition of the experimental feeds as well as trash fish are presented in Table 1 and 2. The performance of the new test feeds was compared with a local commercial marine fish feed (ComFeed1), an imported commercial high-fat marine fish feed (ComFeed 2) and trash fish feed. According to the respective feed manufacturers, ComFeed 1 is a fish meal-based feed and ComFeed 2 consist of fish meal, plant protein and poultry proteins. Both commercial feeds have been used by local fish farmers to feed their farmed groupers. The trash fish used in the present study was the locally available sardine, *Sardinella* spp. and consisted of this one species throughout the feeding trial. Whole raw trash fish were minced using a meat mincer before feeding to the experimental humpback grouper fingerlings.

Fish and feeding: Weaned stock of humpback grouper, *Cromileptes altivelis*, fingerlings were obtained from a local hatchery. Fingerlings of mean initial body weight 4.1±0.1 g were randomly distributed into groups of 20 fish in fiberglass tanks (300 L) with a flow-through water system.

Each experimental feed was fed close to apparent satiation by hand twice a day to triplicate groups of fish. Feeds were given as much as the fish could eat within 30 min and the amount was recorded. Fish were individually weighed at the start and end of the feeding trial and bulk-weighed fortnightly. The feeding trial was conducted for 14 weeks. Muscle tissue from five fish per tank was dissected, pooled and stored frozen at -86°C for subsequent chemical analysis. The remaining fish were pooled and stored frozen for subsequent whole-body composition analysis. Samples of fish whole body, liver, muscle and experimental feeds were oven-dried and ground into powder before proximate analysis following methods described by AOAC (1990).

Amino acid analysis: Ingredient and feed samples were hydrolyzed in duplicate with 6 N HCl at 110°C for 24 h and derivatized with AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamite, Waters, Massachusetts, USA) before chromatographic separation using an AccQ*Tag™ reversed phase (3.9×150 mm) analytical column (Waters, Massachusetts, USA). The HPLC system and quantification of chromatographic peaks were previously described by Shapawi *et al.* (2007). Methionine and cystine were determined using the same method of acid hydrolysis after treatment with performic acid oxidation. Tryptophan was determined by a fluorescence detector (wavelength excitation 285 nm, emission 345 nm) using the reverse phase HPLC technique after sample preparation using alkaline digestion (Yust *et al.*, 2004).

Fatty acid analysis: Crude lipid extracts of muscle and liver were subjected to fatty acid analysis. The extracts were obtained using chloroform:methanol (1:1,v/v) following the method of Bligh and Dyer (1959). The lipid extract was then fractionated by a short column filled with silica gel 60 F254 (Merck, Darmstadt, Germany) with mesh size of 0.063-0.2 mm in a hexane:ethyl acetate solvent system (9:1, v/v). Methylation of the extract was carried out inside a reaction vessel for two hours using sodium methylate. The extract was purified using the silica gel column system before the fatty acid methyl esters were analyzed in a gas chromatograph (Shimadzu GC-2010, Shimadzu Corporation, Kyoto, Japan), equipped with a flame ionization detector and an auto injector. The esters were separated using a capillary column (60 m×0.25 mm ID; BPX70 column, SGE, Australia). Peaks were identified by comparing retention times to known standards (Supelco™ 37 Component FAME mix, Supelco Inc., Bellefonte, USA).

Calculations and statistical analysis: Growth performance, feed utilization efficiency and body indices were calculated as follows:

- % weight gain = percentage of initial body weight at the end of 14 weeks
- Specific Growth Rate (SGR) = [(ln final weight - ln initial weight)/days]×100
- Feed Conversion Ratio (FCR) = dry feed consumed (kg)/wet weight gain (kg)
- Condition Factor (CF) = [fish weight/(total length)^3]×100
- Hepatosomatic Index (HSI) = (liver weight/fish weight)×100
- Viserosomatic Index (VI) = (Viseral weight/body weight) ×100
- Intraperitoneal Fat (IPF) = (intraperitoneal fat/fish weight)×100

One-way Anova was used to compare the data on growth performance, feed utilization efficiency, survival, whole-body proximate composition and muscle fatty acid composition. Homogeneity of variances was tested with Levene's test and multiple comparisons among treatments were performed with a Tukey HSD post-hoc test. Significance level was set at $p<0.05$. Data expressed as ratios and percentages were subjected to arc-sine transformation prior to statistical analysis. The statistical package SPSS v.11.0 for Windows was used for all statistical analyses.

RESULTS

Nutrient composition of experimental feeds: Table 1 shows the proximate composition of the test pelleted feeds, commercial marine fish feeds and trash fish. In feeds FarmFeed1 and FarmFeed 2, the level of crude protein (497 and 503 g kg^{-1}, respectively) and crude lipid were similar (104-108 g kg^{-1}, respectively). Crude fiber, crude ash, nitrogen-free extract and dry matter in these feeds were 32 and 30 g kg^{-1}, 134 and 135 g kg^{-1}, 229 and 228 g kg^{-1} and 887 and 886 g kg^{-1}, respectively. Analyzed crude protein and crude lipid of the commercial feeds corresponded with the proximate composition printed on the feed bags by the manufacturers (ComFeed 1 contained 452 g kg^{-1} crude protein and 109 g kg^{-1} crude lipid and ComFeed 2 contained 516 g kg^{-1} crude protein and 75 g kg^{-1} crude lipid). The proximate composition of the trash fish used in the present study was 701 g kg^{-1} crude protein, 44 g kg^{-1} crude lipid and 151 g kg^{-1} crude ash on a dry matter basis. Dry matter content was 248 g kg^{-1}.

Methionine and lysine content of the pelleted feeds ranged from 2.3- 2.8 g/100 g amino acid and 5.3-5.8 g/100 g amino acid, respectively (Table 1). Methionine and lysine were 4.0 and

Table 1: Proximate and amino acid compositions of pelleted feeds and trash fish

Parameters	FarmFeed 1	FarmFeed 2	ComFeed 1	ComFeed 2	Trash fish
Proximate composition (g kg^{-1})					
Dry matter	887.0	886.0	915.0	917.0	248.0
Crude protein	497.0	503.0	452.0	516.0	701.0
Crude lipid	108.0	104.0	109.0	75.0	44.0
Ash	134.0	135.0	92.0	99.0	151.0
Crude fiber	32.0	30.0	26.0	39.0	ND
Nitrogen free extract	229.0	228.0	321.0	171.0	104.0
Amino acid composition (g/100 g amino acid)					
Aspartic acid	8.3	7.9	8.3	7.6	7.9
Serine	6.0	5.7	5.1	5.2	5.1
Glutamic acid	14.6	15.4	15.7	15.0	11.8
Glycine	10.1	11.5	7.6	8.1	6.3
Histidine	2.8	2.7	2.8	3.1	3.5
Arginine	8.9	8.9	7.4	9.4	8.4
Threonine	4.6	4.4	4.2	4.5	4.7
Alanine	5.2	5.6	5.6	5.3	5.6
Proline	6.8	5.8	5.5	5.9	4.5
Tyrosine	2.8	2.6	2.8	3.1	2.9
Valine	4.5	4.6	5.2	5.1	5.0
Methionine	2.7	2.4	2.8	2.3	4.0
Cystine	1.0	1.2	1.7	1.7	1.3
Isoleucine	3.9	4.0	4.6	4.6	4.7
Leucine	6.4	6.7	8.6	7.8	8.2
Phenylalanine	4.8	4.4	5.1	4.7	5.5
Tryptophan	1.3	0.8	1.3	1.1	1.9
Lysine	5.5	5.3	5.8	5.6	8.6

ND: Not detected

Table 2: Fatty acid composition of pelleted feeds and trash fish (% of total fatty acid)

Parameters	FarmFeed 1	FarmFeed 2	ComFeed 1	ComFeed 2	Trash fish
C12:0	0.1	0.10	0.30	0.2	0.8
C14:0	2.5	2.50	6.80	4.0	7.6
C14:1	0.1	0.10	0.10	0.1	0.3
C15:0	0.2	0.20	0.30	0.3	1.8
C15:1	0.0	0.00	0.10	0.1	0.2
C16:0	29.5	24.90	18.3	20.7	25.6
C16:1	4.9	7.10	6.10	6.4	4.0
C17:0	0.1	0.10	0.10	0.1	2.8
C17:1	0.2	0.20	1.00	0.8	0.6
C18:0	5.1	6.10	3.80	5.3	8.6
C18:1n9	32.7	34.00	15.3	24.7	8.7
C18:2n6	11.6	14.90	9.80	10.2	1.7
C18:3n6	0.1	0.20	0.20	0.2	0.1
C18:3n3	0.8	1.00	1.00	1.2	0.5
C20:0	0.2	0.20	0.30	0.3	0.9
C20:1n9	1.8	1.40	0.80	2.0	0.6
C20:2	0.2	0.20	0.10	0.1	0.3
C20:3n6	0.1	0.10	0.10	0.1	0.3
C20:4n6	0.1	0.10	0.10	0.1	0.1
C20:3n3	0.3	0.40	1.20	0.9	2.3
C20:5n3	2.0	2.20	20.9	11.9	6.5
C22:1n9	3.1	0.03	0.10	1.0	0.3
C24:0	0.1	0.02	0.50	1.0	0.9
C22:6n3	3.6	3.60	12.4	8.0	21.5
Tot saturates	38.3	34.30	30.7	32.4	49.5
Tot monoenes	43.0	42.90	23.5	35.0	15.3
Tot PUFA	18.6	22.80	45.8	32.5	35.2
Tot n-3	6.6	7.30	35.5	21.9	30.7
Tot n-6	11.8	15.20	10.2	10.5	2.4
n-3/n-6	0.6	0.50	3.50	2.1	13.0

8.6 g/100 g amino acid in trash fish. It was observed that the relative levels of glycine in test feeds FarmFeed 1 and FarmFeed 2 were higher (10.1 and 11.5 g/100 g amino acid, respectively) than the level in other feeds (6.3 to 8.1 g/100 g amino acid). Both commercial feeds contained slightly higher level of cystine (1.7 g/100 g amino acid) than other feed groups (1.0 to 1.3 g/100 g amino acid) (Table 1). In general, on a dry matter basis, the profile of amino acid (recalculated based on g amino acid 100/g tissue) in trash fish was superior to other feed groups (eg., methionine and lysine in trash fish were 2.8 and 5.9 g amino acid 100/g feed compared to 1.1 to 1.4 and 2.6 to 2.8 g amino acid 100/g feeds, respectively, in other feeds).

The fatty acid composition of experimental feeds is presented in Table 2. The difference between FarmFeed1 and FarmFeed 2 was mainly with the higher total saturates and lower PUFA levels of FarmFeed1 (38.3 and 18.6%, respectively) compared to FarmFeed2 (34.3 and 22.8%, respectively). This was due to the higher level of palmitic acid (C16:0) in FarmFeed 1 as a result of added crude palm oil. Higher levels of total n-3 fatty acids were observed in the commercial feeds and trash fish than in the FarmFeed 1 and FarmFeed 2 test feeds. In contrast, feeds FarmFeed 1 and FarmFeed 2 contained slightly higher total n-6 fatty acids. The trash fish used in the present study was characterized by a relatively high level of docosahaexanoic acid, C22:6n3.

Growth, survival and feed utilization efficiency: Final fish weight, % weight gain and SGR of fish fed feed FarmFeed 2 were significantly higher ($p<0.05$) than fish fed other feeds (Table 3). No significant difference ($p>0.05$) was observed in the growth performance of fish fed the FarmFeed 1 feed or ComFeed 2. Similarly, the final weight and % weight gain of fish fed feeds ComFeed 1 or trash fish were not significantly different ($p>0.05$). Specific growth rate was the highest in fish feed FarmFeed 2 (2.0% d^{-1}), followed by ComFeed 2 and FarmFeed 1, trash fish and ComFeed 1. Except in the trash fish group, survival rate was high in the present study (95-100%) and was not influenced by the dietary treatment among the fish fed the various pelleted feeds. Survival of fish fed trash fish at the end of trial was significantly lower at 50%, about half of the survival rate observed in fish fed pelleted feeds. Fish fed trash fish was observed to have fin rot and lesions. Total feed intake (dry matter basis) was significantly higher in fish fed trash fish compared to fish fed the various pelleted feeds. No. significant difference was observed in total feed intake among fish fed pelleted feeds. Dry matter FCR ranged from 1.3 to 2.4 in fish fed pelleted feeds. These values were not significantly different among fish fed the FarmFeed 2, ComFeed 2 or FarmFeed 1 feeds. However, FCR of fish fed ComFeed 1 was significantly lower compared to fish fed FarmFeed 2, but not significantly different to fish fed FarmFeed 1 or ComFeed 2. Feeding fish with trash fish resulted in significantly poorer FCR of 5.0 (Table 3).

Body indices and proximate composition: Fish condition factor was not affected by the dietary treatments (Table 4). The HSI of fish fed the commercial feeds was significantly higher than in other fish groups ($p<0.05$). VSI was the lowest in humpback grouper fed trash fish. VSI was similar

Table 3: Growth performance, feed utilization and survival rate of humpback grouper fingerlings fed pelleted feeds or trash fish

Parameters	FarmFeed 1	FarmFeed 2	ComFeed 1	ComFeed 2	Trash fish
Initial weight (g)	4.1±0.1	4.1±0.1	4.2±0.1	4.1±0.1	4.2±0.1
Final weight (g)	22.2±0.6^{b}	29.5±1.8^{a}	12.6±0.5^{c}	23.0±.4^{b}	16.6±1.1^{c}
Weight gain (%)	440.0±20.8^{b}	619.1±33.5^{a}	204.7±10.5^{c}	454.6±34.9^{b}	295.6±20.1^{c}
SGR (%d^{-1})	1.7±0.04^{b}	2.0±0.05^{a}	1.1±0.04^{d}	1.7±0.06^{b}	1.4±0.05^{c}
Total feed intake (g dry matter $fish^{-1}$)	29.6±0.7^{a}	34.0±1.2^{a}	20.8±2.6^{a}	25.7±2.2^{a}	62.9±8.9^{b}
FCR	1.6±0.03^{ab}	1.3±0.04^{a}	2.4±0.2^{b}	1.4±0.1^{ab}	5.0±0.4^{c}
Survival rate (%)	95.0±2.9^{a}	100.0±0.0^{a}	95.0±0.0^{a}	97.0±1.7^{a}	50.0±5.8^{b}

Values with different superscript within row are significantly different ($p<0.05$)

Table 4: Body indices and whole body composition of humpback grouper fed pelleted feeds or trash fish

Parameters	FarmFeed 1	FarmFeed 2	ComFeed 1	ComFeed 2	Trash fish
Body indices					
Condition factor	1.2±0.9	1.1±3.2	1.1±0.9	1.4±2.4	1.2±1.9
Hepatosomatic index	2.0±0.2a	1.9±0.2a	3.7±0.4b	3.5±0.4b	1.5±0.3a
Viscerosomatic index	7.1±0.3ab	7.2±0.2ab	8.5±0.7b	10.0±0.5b	5.1±1.0a
Intraperitoneal fat	0.6±0.06a	0.8±0.2a	0.6±0.2a	1.5±0.1b	0.1±0.01a
Whole body composition (g kg^{-1} wet weight basis)					
Moisture	705.0±0.3a	706.0±0.3a	715.0±1.4ab	702.0±0.1a	740.0±0.9b
Crude protein	176.0±0.2	176.0±0.5	170.0±0.2	167.0±0.2	171.0±0.7
Crude lipid	62.0±0.4ab	64.0±0.3ab	54.0±0.3b	76.0±0.25a	31.0±0.8c
Ash	52.0±0.3	49.0±0.1	49.0±0.2	45.0±0.1	52.0±0.2

Values with different superscript within row are significantly different ($p<0.05$)

Table 5: Muscle fatty acid composition (% total fatty acids) of humpback grouper fed pelleted feeds or trash fish

Parameters	FarmFeed 1	FarmFeed 2	ComFeed 1	ComFeed 2	Trash fish	SEM
C14:0	2.4	1.9	3.8	3.4	3.4	0.3
C15:0	0.2	0.2	0.3	0.3	0.5	0.1
C16:0	22.8^{b}	20.3^{ab}	18.5^{a}	19.7^{ab}	19.0^{a}	0.6
C16:1	4.4	5.3	4.6	5.7	4.1	0.3
C17:0	0.1^{a}	0.2^{a}	0.4^{ab}	0.4^{ab}	0.8^{b}	0.1
C17:1	0.3^{a}	0.2^{a}	0.5^{a}	0.7^{b}	0.5^{ab}	0.1
C18:0	6.4	6.8	5.6	6.5	7.0	0.4
C18:1n9	27.4^{c}	30.7^{c}	20.5^{ab}	24.8^{bc}	16.3^{a}	0.9
C18:2n6	11.7^{bc}	14.0^{c}	13.4^{bc}	10.7^{b}	5.0^{a}	0.4
C18:3n3	0.9	0.9	1.1	1.2	0.8	0.1
C20:1n9	1.4^{a}	1.3^{a}	1.1^{a}	1.6^{a}	2.6^{b}	0.1
C20:3n3	1.3	1.5	1.2	1.1	1.6	0.1
C20:5n3	4.1^{b}	2.9^{b}	8.7^{d}	8.3^{d}	5.9^{c}	0.2
C22:1n9	1.5^{c}	1.0^{b}	0.9^{b}	0.1^{ab}	2.2^{d}	0.1
C24:0	1.0	1.1	1.9	1.8	1.5	0.1
C24:1n9	0.4	0.3	0.4	0.3	0.7	0.1
C22:6n3	11.7^{bc}	9.0^{a}	15.3^{c}	11.2^{b}	26.0^{d}	0.4
Tot saturates	34.3	31.2	31.1	32.9	33.1	0.8
Tot monoenes	35.5^{b}	38.9^{b}	28.1^{a}	33.9^{b}	26.5^{a}	0.7
Tot PUFA	30.3^{ab}	29.2^{a}	40.2^{c}	33.1^{b}	40.2^{c}	0.5
Tot n-3	17.9^{b}	14.4^{a}	26.2^{d}	21.8^{c}	34.3^{e}	0.4
Tot n-6	12.0^{bc}	14.4^{c}	13.7^{bc}	11.1^{b}	5.7^{c}	0.1
n-3/n-6	1.5^{ab}	1.0^{a}	1.9^{b}	2.0^{b}	6.1^{c}	0.1

SEM: Standard errors mean, Values with different superscript within row are significantly different ($p<0.05$)

in fish groups fed the pelleted feed. However, there was a trend for increased VSI in fish offered the commercial feeds. IPF of fish given ComFeed2 was significantly higher than that of fish fed other feeds.

The proximate composition of fish fed different types of feeds is shown in Table 4. Crude protein and ash were not affected by the dietary treatments. Except for the fish fed ComFeed 1, moisture content of fish fed trash fish was significantly higher than other fish groups. Whole-body crude lipid was the highest in fish fed ComFeed 2, followed by fish fed FarmFeed 2, FarmFeed 1, ComFeed 1 and trash fish.

Muscle fatty acid composition: In general, the fatty acid composition of fish muscle reflected the fatty acid composition of experimental feeds (Table 5), with the possible exception of total saturated fatty acids which was less dependent on dietary levels. It was observed that muscle total monoenes and total PUFA of fish fed ComFeed 1 (a fish meal-based feed) or trash fish were similar. The muscle total monoenes levels of fish fed ComFeed 1 or trash fish (28.1 and 26.5%, respectively) were significantly lower than the levels found in fish fed other three different feeds (33.9 to 38.9%). Both fish groups fed ComFeed1 or trash fish (40.2%) had also similar level of muscle total PUFA and these values were significantly higher than that found in fish fed other feeds (29.2 to 33.1%). Fish fed the two commercial feeds contained higher EPA (C20:5n-3) than in their muscle lipids compared to fish on the other diets. Muscle DHA (C20:6n-3) levels was highest in humpback groupers fed trash fish. This is consistent with the higher level of DHA found in trash fish. Reflecting the fatty acid profile of the trash fish, total n-3 and n-6 were the highest and lowest, respectively, in the muscle lipids of groupers on the trash fish diet. It was observed that muscle of fish fed FarmFeed 2 had a balanced proportion of total n-3 and n-6, giving a n-3/n-6 ratio of 1. Higher levels of palmitic acid (C16:0) was observed in fish fed FarmFeed1 compared to other groups as a result of palm oil addition in the feed.

DISCUSSION

The performance of the prototype humpback grouper feeds, FarmFeed 1 and FarmFeed 2 was similar to or better than the imported commercial marine fish feed, ComFeed 2. Both feeds, FarmFeed 1 and FarmFeed 2, gave better growth performance compared to fish fed the local commercial marine fish starter feed (ComFeed 1) or the monospecies trash fish. In case of humpback groupers fed FarmFeed 2, growth was significantly higher than those given FarmFeed 1 or ComFeed 2, three times higher than the growth of fish fed ComFeed1 and double the growth of fish on the trash fish diet. FarmFeed 2 performed better than FarmFeed 1 even though the later contained a higher fish meal inclusion level. However, it is important to note that FarmFeed 1 contained some portion of crude palm oil which made the feed contain higher total saturates and lower PUFA compared to FarmFeed 2. The difference in growth between fish fed the FarmFeed 1 and FarmFeed 2 test feeds could be due to differences in the dietary fatty acid profile since diet proximate and amino acid compositions are similar. The present study also confirmed the nutritional value of PBM-based feeds for carnivorous marine fish as previously reported in present study with humpback grouper (Shapawi *et al*., 2007) and in other fish species (Nengas *et al*., 1999; Takagi *et al*., 2000; Gaylord and Rawles, 2005).

The imported commercial feed, ComFeed 2, contained some portion of PBM and had a similar level of crude protein as that found in FarmFeed 1 and FarmFeed 2. However, this feed contained a higher level of fat at 170 g kg^{-1}. The performance of ComFeed 2 in the present study is consistent with the findings by Williams *et al*. (2004) which reported reduced appetite and no growth benefit when dietary lipid level was increased above 150 g kg^{-1} in the feed of humpback grouper. In addition, inclusion of high dietary lipid level in the feed will not only increase the feed cost, but it will also makes the feed more susceptible to rancidity, especially when marine lipids are used (Sargent *et al*., 2002). The poorer performance of ComFeed 1 was unexpected as it is a fish meal-based feed. However, it should be pointed out that the crude protein content of the feed was lower than that of FarmFeed 1, FarmFeed 2 and ComFeed 2. The lower protein content of the feed probably contributed partly to the lower growth of the fish. It was also visually observed that the palatability of the feed was lower in this group of fish even though there was no significant difference in terms of measured feed intake was detected among the fish fed the various pelleted feeds. Nevertheless, fish fed ComFeed 1 gave the lowest feed intake of all dietary groups. It is known that ComFeed 1 was specifically formulated for seabass (*Lates calcarifer*) but currently marketed as a generic marine fish feed. The results of the present study provided evidence that feeds formulated for one marine species are not always successful when used for another species. In contrast, ComFeed 2, performed better probably due to its higher nutrient specifications which made it more suitable as a grouper feed compared to ComFeed 1.

The amino acid composition of the two test feeds was comparable to the amino acid composition of the commercial feeds. Little is known about the quantitative amino acid requirement for grouper species. However, methionine and lysine were reported as the limiting amino acids in PBM-based feeds (Shapawi *et al*., 2007; Gaylord and Rawles, 2005). The methionine level of feed FarmFeed 2 was 2.4 g/100 g amino acid and higher level of 2.7 g/100 g amino acid was observed in FarmFeed 1, mainly due to higher fish meal inclusion level in this feed compared to feed FarmFeed 2. Lysine level in feeds FarmFeed 1 and FarmFeed 2 (5.5 and 5.3 g/100 g amino acid, respectively) were comparable to the levels in commercial feeds (5.6-5.8 g/100 g amino acid, respectively). Apparently, the amino acid levels found in FarmFeed1 and FarmFeed 2 were able to support good growth of grouper fingerlings and provided good palatability to these feed. As expected, higher level of methionine and lysine were observed in trash fish (4.0 and 8.6 g/100 g amino acid, respectively) compared to the pelleted feeds on a dry weight basis. However, it should be noted that more than 70% of the weight of trash fish is made up of water.

The trash fish used in the present study contained high levels of protein (701 g kg^{-1} and DHA (21.5% of total fatty acid), similar to the trash fish profile (710 g kg^{-1} protein and 23.2% DHA) reported by Navas *et al.* (1998). Nevertheless, humpback groupers fed trash fish had poor FCR of 5.0 compared to fish fed the pelleted feeds which showed a FCR ranging from 1.3 to 2.4 which were significantly better. Despite the high nutritional content of trash fish, poor FCR values observed in trash fish feeding is common in marine fish culture, with dry matter FCRs typically ranging from 5 to 10 (Rimmer, 2004; Orachunwong *et al.*, 2005). The poor FCR of groupers fed trash fish is due mainly to losses of feed material during feeding. Unlike pelleted feed, trash fish break up into small pieces when eaten. Consequently as much as 30 to 50% of the trash fish fed to fish can be lost during feeding process (Sim *et al.*, 2005). Feed loss in marine fish culture sites are associated with localized pollution and may facilitate the transmission of parasites and diseases (Cho *et al.*, 1994; Sim *et al.*, 2005). In the present study, the survival rate of fish fed trash fish was lower than that of other fish groups which were fed pelleted feeds. The humpback groupers showed symptoms of fin rot and skin lesions towards the end of the experimental period. This probably contributed to the high mortality of the fish. Fin rot and lesions could be the result of secondary bacterial infection. In the present study, trash fish was prepared by blending the whole-fish including head and gut content to form a homogenous dough before being hand fed to the humpback grouper fingerlings. There is a possibility of bacteria originating from the gut contents of the trash fish that contributed towards the infection. Bacterial infection as a result of trash fish consumption is common in marine fish culture which depends on trash fish as a source of feeding (Muroga, 2001; Kim *et al.*, 2007). In addition, thiaminase, an enzyme which can destroy Vitamin B_1, might be present in the gut of trash fish and this could contribute to the slow growth of farmed marine fish fed trash fish. Thus, removing the gut content of trash fish might be able to minimize some of the problems related to disease outbreaks and potential vitamin deficiencies in grouper farming.

Higher hepato and viscero-somatic indices were observed in fish fed the commercial feeds. HSI is related to the nutritional state of fish and may directly relate to energy requirements for growth. Poor growth of fish fed ComFeed 1 as a result of lower protein content and palatability of the feed probably had contributed to a higher value of HSI of in this treatment. The IPF value for fish fed ComFeed 2 was significantly higher than other fish groups. The high dietary lipid level found in ComFeed 2 caused increased lipid deposition in the liver and the peri-visceral cavity which may have negative impacts on fillet quality and shelf-life of whole fish. The high deposition of body fat indicated that the dietary lipid level in ComFeed 2 was in excess and was not utilized by the fish for energy. Crude protein content of fish was not affected by the various dietary treatments. This agrees with our previous findings whereby feed compositions were observed to have relatively little effect on the whole-body protein of humpback groupers (Shapawi *et al.*, 2007, 2008). Feeding fish with trash fish resulted in higher body moisture and the lowest whole-body lipid content compared to fish fed the pelleted feeds.

The muscle fatty acid profile of humpback grouper in the present study showed a very strong influence from the dietary fatty acid profile with the possible exception of saturated fatty acids. Muscle total saturates were not significantly affected by dietary treatments. The minimal impact of feeds on saturated fatty acids in fish tissues was also observed in our previous study (Shapawi *et al.*, 2008) and by other researchers (Greene and Selivonchick, 1990; Ng *et al.*, 2001). Similar to our earlier findings, the DHA levels in muscle lipids of humpback groupers was higher compared to EPA levels. The suggested optimal dietary n-HUFA for humpback grouper was reported to be 1.5-2.0% of the feeds (Suwirya and Giri, 2005). The good growth of fish fed FarmFeed 1 and FarmFeed 2 indicated that the essential fatty acid requirement of the humpback grouper was met. It is also interesting to note that both prototype feeds contained lower levels of total PUFA and n-3 fatty acids compared to the levels in other dietary treatments. Even though the beneficial effects of n-3 PUFA for human health is well recorded, elevated levels of n-3 PUFAs in fish might not be beneficial, as high levels of PUFAs are reported to caused oxidative stress (Greene and Selivonchick, 1990).

CONCLUSION

In Southeast Asia, most grouper farmers do not own many cages and these high-value groupers are usually stocked at low densities in these cages. Using on-site farm made feeds as opposed to commercially manufactured feeds is a viable alternative for these farmers. The feed formulation of the prototype grouper feeds used in the present study can be used by traditional grouper farmers to reduce the feed costs. In conclusion, the findings of the present study will contribute towards reducing the marine fish farmers' dependency on trash fish as the main feed input and further contributes towards the sustainability of the thriving grouper aquaculture industry.

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