SECTION 3
GROW-OUT DIET DEVELOPMENT

Marine finfish cage farm in HaLong Bay, Vietnam. Fish farms in Southeast Asia are often family-run operations.

Nutritional research identified the major dietary requirements of groupers and enabled the development of appropriately formulated pelleted dry diets to replace fresh fishery bycatch ('trash' fish). Fish fed dry diets performed as well as, or better than, fish fed fishery bycatch. A high proportion of fishmeal (up to 80%) could be substituted by high quality meat meals, providing an option to decrease reliance on fishmeal. The use of dry diets rather than fishery bycatch provides a more efficient use of fishmeal resources, enables farmers to provide a nutritionally optimised and consistent feed source, and reduces pollution associated with feeding 'trash' fish.

Summary

The overall goal of the project's grow-out diet development research was to develop compounded pelleted grouper feeds as a more sustainable, lower-polluting and cost-effective alternative to the feeding of fresh fishery bycatch (or 'trash' fish) (Tacon and Forster 2003). The grouper species studied were humpback or polka dot grouper, *Cromileptes altivelis*, tiger or flowery grouper, *Epinephelus fuscoguttatus* and the gold spot or estuary cod, *Epinephelus coioides*. The research approach was to define the requirements of groupers for the key nutrients that largely determine the rate at which fish grow, determine the nutritive value of locally available marine and terrestrial feed ingredients and examine the extent to which high cost marine protein feed ingredients could be replaced using cheaper and more renewable terrestrial protein feed ingredients.

The crude protein (CP) requirement of humpback grouper and tiger grouper was met with diets that contained not less than 44% dry matter (DM) digestible CP (about 50% on an as-fed CP basis). Increasing the lipid content of the diet beyond about 9–10% did not improve fish growth rates but instead reduced the fish's appetite and resulted in higher rates of body fat deposition (Giri et al. 1999; Williams et al. 2004). Adding dietary lipid in the form of coconut oil as a rich source of medium chain fatty acids (C 10–14) resulted in an accelerated rate of lipid oxidation in humpback grouper compared with diets in which the lipid was provided as long chain (C 18+) fatty acids. However, this led to a profound depression of the fish's appetite and a profound decline in the fish's growth rate. Growth rate and survival of sea-caged humpback groupers were improved when diets were supplemented with up to 150 mg/kg of vitamin C as the heat-stable form of L-ascorbyl-2-monophosphate-Na-Ca (Laining et al. 2002). This benefit of vitamin C supplementation was most apparent following heavy flood rains, which caused a marked deterioration in water quality (increased turbidity and reduced dissolved oxygen content) around the cages. The dietary requirement for the essential omega-3 highly unsaturated fatty acids (n-3 HUFA) was examined for humpback grouper and tiger grouper. Increasing the supplementation rate up to 1–1.5% of the diet resulted in improved fish growth rates and better survival. In studies examining the capacity of humpback grouper to utilise different types of carbohydrate as energy sources, the best results were achieved using glucose, while starch and sucrose were the least effective (Usman 2002).

These nutrient requirement studies indicate that juvenile groupers require diets that are high in digestible CP (around 45%), moderately low in lipid (around 10%) and contain not less than 1.0%, and preferably 1.5%, of n-3 HUFA. The addition of at least 100 mg of a heat stable form of vitamin C per kg of diet is recommended and this should be increased to 150 mg/kg if stressful culture conditions are likely to occur.

The apparent digestibility of a comprehensive range of ingredients available in the Philippines and Indonesia was determined for gold spot grouper and humpback grouper respectively. The CP in both marine and terrestrial animal meals was well digested (above 76%) by both grouper species with the exception of oven-dried blood meal, which was poorly digested (55%) (Laining et al. 2003). The protein digestibility of plant products was more variable (from 43% to 100%) with high fibre meals such as rice bran and lucaena (ipil-ipil) meal being poorly digested. The DM digestibility of the meals was adversely affected by the amounts of ash and fibre they contained. A collation of the DM and CP apparent digestibility values of the tested ingredients is presented in Table 1.

In studies examining the ability of terrestrial protein meals to substitute for fishmeal in formulated feeds for juvenile gold spot grouper, a 4:1 combination of meat meal and ring-dried blood meal, respectively was able to replace up to 80% of fishmeal protein in the diet without adverse effects on growth, feed conversion or survival of the fish. Other terrestrial protein meals such as cowpea, corn gluten, lucaena (ipil-ipil) meal and soybean meal were less successful as fishmeal replacements. With humpback grouper, growth rate and feed conversion deteriorated markedly when shrimp head meal

Table 1. The dry matter (DM) and crude protein (CP) apparent digestibility (AD) of selected feed ingredients determined for gold spot grouper in the Philippines and for humpback grouper in Indonesia.

Feed ingredient	Gold spot grouper		Humpback grouper	
	DMAD[1]	CPAD[1]	DMAD[1]	CPAD[1]
Marine product				
Fishmeal (Chilean 65% CP)	83.6 ± 3.09	98.0 ± 0.72		
Fishmeal (mixed 45% CP)	59.1 ± 1.23	82.4 ± 1.99	59.1 ± 1.23	82.4 ± 1.99
Fishmeal (sardine 65% CP)			87.2 ± 2.53	92.5 ± 1.40
Fishmeal (tuna 50% CP)	75.4 ± 3.61	76.2 ± 1.92		
Fishmeal (white 69% CP)	89.2 ± 1.69	98.6 ± 0.31		
Shrimp meal (Acetes 72% CP)	76.0 ± 4.00	95.0 ± 0.72		
Shrimp head meal (50% CP)			58.8 ± 3.33	78.0 ± 1.32
Squid meal (71% CP)	99.4 ± 0.95	94.2 ± 0.21		
Terrestrial animal product				
Blood meal (Australian ring 84% CP)				
Blood meal (oven dried 84% CP)			48.1 ± 0.85	55.2 ± 1.35
Blood meal (formic 87% CP)			67.9 ± 1.63	87.5 ± 0.55
Blood meal (propionic 84% CP)			61.7 ± 2.60	84.2 ± 0.69
Meat meal (Australian 44% CP)	60.8 ± 0.80	98.9 ± 1.32		
Meat meal (Philippine 45% CP)	77.7 ± 0.09	83.8 ± 1.66		
Meat solubles (73% CP)	99.3 ± 0.45	97.6 ± 0.08		
Poultry feather meal (67% CP)	74.3 ± 3.06	81.8 ± 2.58		
Plant product				
Corn germ meal (8% CP)	85.2 ± 2.81	82.9 ± 4.71		
Corn gluten meal (56% CP)	94.0 ± 2.03	99.5 ± 0.65		
Cowpea meal (white 24% CP)	74.2 ± 3.14	93.5 ± 1.22		
Lucaena (ipil-ipil) meal (19% CP)	56.0 ± 0.04	78.8 ± 2.64		
Lupin albus meal (26% CP)	54.1 ± 1.24	97.5 ± 3.65		
Palm oil cake meal (11% CP)			45.3 ± 2.37	80.5 ± 1.30
Rice bran meal (11–14% CP)	68.5 ± 7.02	42.7 ± 5.38	22.2 ± 1.52	59.5 ± 1.41
Soybean concentrate (54% CP)	76.3 ± 4.88	85.5 ± 0.40		
Soybean meal (full-fat 41% CP)			54.8 ± 2.72	67.2 ± 1.29
Soybean meal (solvent 51% CP)	75.7 ± 1.69	96.0 ± 0.13		
Wheat flour (9% CP)	72.8 ± 0.85	82.9 ± 1.26		

[1] Mean ± SD.

was used at inclusion rates above 10% as a replacement for fishmeal protein.

In laboratory and field cage studies, a practical low-cost dry pelleted diet was formulated on a digestible nutrient basis to meet the requirements of juvenile gold spot grouper and compared with feeding either a commercial pellet diet or fresh fishery bycatch (Millamena 2002). In both studies, fish fed the project formulation diet survived and grew as well as those fed the fresh bycatch. In the laboratory study, fish fed the commercial pellet diet grew significantly slower and converted feed less efficiently than those fed either the project diet or fresh by-catch. The analysis of the commercial pellet diet showed a sub-optimal specification. When the commercial mill adjusted the formulation to meet these specifications, fish fed that diet in the field study performed as well as those fed either the project diet or fresh by-catch.

The research carried out in the project has conclusively shown that juvenile groupers will readily accept pelleted dry diets. Diets formulated to meet the fish's requirements of digestible nutrients and not containing excessive amounts of plant protein meals will enable juvenile groupers to grow as well as those fed fresh fishery by-catch.

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Apparent Digestibility of Selected Feed Ingredients in Diets for Grouper (*Epinephelus coioides*) Juveniles

P.S. Eusebio, R.M. Coloso and R.E.P. Mamauag

Introduction

Cultured grouper are commonly fed trash fish. Because of the insufficient supply, high cost and variable quality of trash fish, there is a need to develop cost-effective and environment-friendly formulated diets (Tokwinas 1989). Inexpensive feed formulation can be achieved with incorporation of a variety of feed ingredients (Boonyaratpalin et al. 1998). Effective incorporation of an ingredient however, requires information on its digestibility for the target species. This study was conducted to determine the quality of selected feed ingredients as protein sources in grouper diets based on their nutrient composition and apparent digestibility coefficients for dry matter (ADMD) and crude protein (APD).

Methods

Grouper juveniles were used in a series of feeding experiments. A total of 56 juveniles (mean body weight ± standard deviation (s.d.) = 85.4 ± 5 g) were used for the first batch of test ingredients (Chilean fish meal, white fish meal, shrimp meal, defatted soybean meal, white cowpea meal and ipil-ipil leaf meal). 54 juveniles (mean body weight ± s.d. = 125.8 ± 5 g) were used for the second batch (squid meal, local meat and bone meal, meat solubles, soy protein concentrates, and rice bran), 72 juveniles (mean body weight ± s.d. = 198.2 ± 8 g) for the third batch (tuna fish meal, imported meat and bone meal, blood meal, corn gluten meal and wheat flour) and 48 juveniles (mean body weight ± s.d. = 211.4 ± 6 g) for the fourth batch (poultry feather meal, lupin seed meal and corn germ meal). White cowpea (*Vigna unguiculata*) and lupin (*Lupinus albus*) seeds were used in their raw dried state. Ipil-ipil (*Leucaena leucocephala*) leaves were soaked in tap water for 24 h, drained, rinsed with water and air-dried. All feed ingredients were oven-dried for 12 h at 60°C, ground and sieved using a No. 60 mesh size. Samples were taken for proximate analysis using standard methods (AOAC 1990).

Apparent digestibility coefficients were measured *in vivo* by using a flow through modified Guelph faecal collection system with filtered aerated sea water (flow rate = 800–1000 ml/min for 45–65 days. The method by Cho et al. (1982) was adapted using a ratio of 70:30 (reference diet to test ingredient) in each test diet (Table 1). A reference diet was formulated to meet the known nutrient requirement of grouper (45% protein, 10% fat and 375 kcal/kg metabolizable energy). All experimental diets contained 1% Cr_2O_3 as an external indicator and 1% carboxymethylcellulose (CMC) as binder. The fish were acclimated with reference diet (without Cr_2O_3) for 5 days before feeding them experimental diets twice daily (08:30 h and 14:30 h) at a rate of 5–8% of body weight. The seawater temperature and salinity ranged from 27°C to 28°C and 30 ppt. to 31 ppt., respectively. Each tank was provided with a faecal decantation column. Water from the 60L tank (first two batches) or 250 L tank (3rd and 4th batch) flowed through the decantation column into an attached clear plastic bottle where the faecal material (voided from 17:30 h to 07:30 h) settled and remained until collected.

The tanks and the faecal collection apparatus were cleaned twice daily; 2 h after feeding in the

Advances in Grouper Acquaculture
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ACIAR Monograph 110 (printed version published in 2004)

afternoon and before feeding in the morning. Faecal collection was started when the colour of the faeces became greenish, and were collected every morning (08:00 h) from the plastic bottle, washed three times with distilled water, freeze-dried and prepared for crude protein and Cr_2O_3 analyses. Apparent protein digestibility (APD) of the feed ingredients was computed using the formula described by Spyridakis et al. (1989) and Forster (1999). Apparent dry matter digestibility (ADMD) of feed ingredients was computed following the formula of Spyridakis et al. (1989) and Cho et al. (1982).

All data were analyzed using ANOVA for a completely randomized design. Treatment means were compared by Duncan's New Multiple Range Test. Differences were considered significant at $P<0.05$.

Results and Discussion

The proximate composition (g/100g dry weight) of various feed ingredients is shown in Table 2. Protein levels in fish meals and other feedstuffs of animal origin were generally high (47–87%). The protein content of feed ingredients from plant ranged only from 11% to 61%. Likewise, the levels of ash in fish and shrimp meals were higher (15–16%) compared with those in feed ingredients of plant origin (7–12 %). The levels of fiber in rice bran and ipil-ipil leaf meal (10–16%) were higher compared with those of other feed ingredients (<7%). Rice bran had the highest fat content (11%).

Table 3 shows the apparent digestibility coefficients for dry matter (ADMD) and crude protein. No significant difference was observed among the ADMD or APD of the reference diets in all batches of experiments, which indicated that variations in time, body weight of experimental fish and size of tanks have not affected the apparent digestibility measurements. ADMD and APD values for a reference diet in each batch were the constants used in the computation of ADMD and APD of the respective feed ingredients.

Table 1. Proximate composition of various feed ingredients for *in vivo* digestibility experiment (g/100 g dry weight)[1].

Test ingredients	%H_2O	Protein	Fat	Fibre	NFE[2]	Ash
Animal by-product						
Blood meal[3]	3.27	87.33	4.36	0.04	6.02	2.05
Fish meal, Chilean	10.03	73.57	7.99	0.08	2.09	16.27
Fish meal, tuna[4]	9.56	56.76	10.94	0.88	11.66	19.76
Fish meal, white[5]	7.64	74.63	7.59	0.00	1.72	16.06
Meat and bone meal[3]	10.40	49.05	9.02	1.18	9.41	31.34
Meat and bone meal[4]	4.95	46.96	10.49	0.78	4.75	37.02
Meat solubles (Protamino Aqua)	4.20	76.52	1.16	0.20	10.34	11.78
Poultry feather meal[4]	4.82	70.88	17.68	0.62	8.32	2.50
Shrimp meal, *Acetes* sp.	7.40	72.39	2.89	2.80	6.82	15.10
Squid meal	6.70	76.50	4.00	0.60	11.00	7.90
Plant by-product						
Corn germ meal[4]	4.51	8.54	47.35	6.38	36.91	0.82
Cowpea meal, white[6]	7.06	25.62	0.54	6.23	63.19	4.42
Corn Gluten meal[4]	7.96	60.62	6.96	3.36	27.84	1.22
Soy protein concentrates (HP 300)	5.73	56.87	1.03	5.09	28.71	8.30
Ipil-ipil leaf meal[7]	9.40	21.37	7.34	15.50	46.59	9.20
Lupin (*Lupinus albus*) seed meal[4]	7.62	28.54	5.52	14.24	48.72	2.98
Rice bran	8.78	12.26	10.46	10.32	55.24	11.72
Soybean meal, defatted	7.82	54.82	0.92	5.62	31.76	6.88
Wheat Flour	13.18	10.93	1.09	0.58	86.90	0.50

[1] Analysis done in Centralized Analytical Laboratory at SEAFDEC/AQD.
[2] Nitrogen free extract = 100 – [% crude protein + % ash + % crude fiber + %crude fat].
[3] Australia.
[4] Philippines.
[5] Alaskan white fish meal, USA.
[6] *Vigna unguiculata*, whole seeds in their raw state.
[7] Native ipil-ipil (*Leucaena leucocephala*) leaves soaked in water for 24 hours.

Table 2. The composition of reference and test diets for *in vivo* digestibility experiment of various feed ingredients (g/100 g feed).

Feed Ingredient	Reference Diet	Test Diet
Fish meal, Chilean	37.00	25.90
Squid meal	5.00	3.50
Shrimp meal, *Acetes* sp.	10.00	7.00
Soybean meal, defatted	13.00	9.10
Wheat flour	7.80	5.46
Rice bran	13.94	9.14
Cod liver oil	2.50	1.75
Soybean oil	2.50	1.75
Vitamin mix[1]	4.20	2.94
Mineral mix[1]	2.00	1.40
Ethoxyquin	0.05	0.05
Phosphitan C[2]	0.01	0.01
Chromic oxide (Cr_2O_3)	1.00	1.00
Carboxymethylcellulose,CMC	1.00	1.00
Test ingredient	—	30.00

[1] Biomin, commercially available vitamin and mineral mixture for shrimps, Overseas Feeds Corporation, Cebu City, Philippines.
[2] Ascorbic acid monophosphate, feed grade, Showa Denko K.K. Japan.

The apparent digestibility coefficients for ADMD ranged from 37% to 99% where squid meal and meat solubles had the highest and blood meal the lowest coefficients. Dry matter digestibility of reference diets were comparable with those of Chilean fish meal, white fish meal, tuna fish meal, poultry feather meal, *Acetes sp.,* local meat and bone meal, soy protein concentrates, defatted soybean meal, white cowpea meal, corn gluten meal and corn germ meal (74–94%). Low ADMD values seem to indicate that the overall efficiency of grouper to utilise the nutrients decreased as dietary fiber in feedstuff increased (de Silva et al., 1990). Reduced ADMD can also be associated with an increase in the nitrogen free extract of the respective feed ingredients, which also suggests that grouper had limited ability to digest starch and other carbohydrate components of the feed ingredients.

The apparent protein digestibility (APD) of all feed ingredients tested were relatively high (79–99%), except rice bran (43%) and blood meal (15%). APD values for the reference diets were comparable with those of white fish meal, Chilean fish meal, *Acetes* sp., squid meal, meat

Table 3. Apparent digestibility coefficients for dry matter and crude protein of various feed ingredients (%).

Batch No.	Diet/Feed Ingredient	ADMD[1]	APD[2]
1	Reference diet[3]	85.37 ± 0.29^{bc}	97.16 ± 0.10^{a}
	Fish meal, Chilean	83.56 ± 3.09^{bc}	98.03 ± 0.07^{a}
	Shrimp meal[4]	75.98 ± 4.00^{cd}	95.01 ± 0.72^{a}
	Soybean meal, defatted	75.68 ± 1.98^{cd}	96.03 ± 0.13^{a}
	Fish meal, white	89.22 ± 1.69^{b}	98.57 ± 0.31^{a}
	Cowpea meal, white	74.17 ± 3.14^{cd}	93.53 ± 1.22^{a}
	Ipil-ipil leaf meal	55.97 ± 0.04^{e}	78.83 ± 2.64^{b}
	Squid meal	99.37 ± 0.95^{a}	94.21 ± 0.21^{a}
	Meat and bone meal[5]	77.73 ± 0.09^{c}	83.79 ± 1.66^{b}
	Soy protein	76.32 ± 4.88^{cd}	85.50 ± 0.40^{b}
	Meat solubles	99.33 ± 0.45^{a}	97.59 ± 0.08^{a}
	Rice bran	68.50 ± 7.02^{d}	42.70 ± 5.38^{c}
3	Reference diet[3]	84.14 ± 1.14^{bc}	93.16 ± 0.41^{a}
	Meat and bone meal[6]	60.80 ± 0.80^{de}	98.91 ± 1.32^{a}
	Blood meal[6]	36.85 ± 0.98^{f}	15.45 ± 2.01^{d}
	Corn Gluten meal	93.98 ± 2.03^{ab}	99.52 ± 0.65^{a}
	Fish meal, tuna	75.39 ± 3.61^{cd}	76.24 ± 1.92^{b}
	Wheat flour	72.75 ± 0.85^{d}	82.86 ± 1.26^{b}
4	Reference diet[3]	84.35 ± 1.06^{bc}	94.58 ± 0.30^{a}
	Corn germ meal	85.15 ± 2.81^{bc}	82.86 ± 4.71^{b}
	Lupin seed meal	54.11 ± 1.24^{e}	97.48 ± 3.65^{a}
	Poultry feather meal	74.32 ± 3.06^{cd}	81.83 ± 2.58^{b}

[1] ADMD = Apparent Dry Matter Digestibility, Spyridakis et al., 1989; Cho et al., 1982.
[2] APD = Apparent Protein Digestibility, Spyridakis et al., 1989; Forster, 1999.
[3] ADMD and APD = Spyridakis et al., 1989.
[4] *Acetes* sp.
[5] Philippines.
[6] Australia.

solubles, imported meat and bone meal, defatted soybean meal, white cowpea meal, corn gluten meal, and lupin seed meal (93–99%). These were significantly higher than those of tuna fish meal, local meat and bone meal, poultry feather meal, soy protein concentrates, wheat flour, ipil-ipil leaf meal and corn germ meal (76–85%). Low APD values for rice bran and ipil-ipil leaf meal may be due to their low protein or high fiber contents (McGoogan and Reigh 1996). High APD values were obtained for some feed ingredients with high protein content. Increases in protease enzyme activity were observed when dietary protein increased suggesting that enzyme activity was related to the amount of protein in the gut (Eusebio and Coloso 2002). However, blood meal with high protein content had low APD value, which can be attributed to the processing method used in its preparation. This method can damage the amino acids, thus contributing to low nitrogen digestibility.

Conclusions

- ADMD values vary with the levels of fiber and other carbohydrate substances in feed ingredients.
- Grouper can utilise dietary protein efficiently regardless of whether it is of animal or plant origin.
- High APD values are generally obtained in feed ingredients with high protein content.
- Low digestibility coefficients for feed ingredients can also be attributed to the processing methods used in their preparation.

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Evaluation of some Terrestrial Proteins in Complete Diets for Grouper (*Epinephelus coioides*) Juveniles

P.S. Eusebio, R.M. Coloso and R.E.P. Mamauag

Introduction

Various protein sources have been evaluated with the objective of partially or completely replacing fish meal in fish diets. Some plant protein sources have promoted reasonable growth even at high (≥25%) dietary inclusion levels (Jackson et al. 1982; Elangovan and Shim 2000; Gouveia and Davie, 2000). Also, other plant and animal protein sources were used in the diets for carnivorous fish without negative effects on growth (Gomez et al. 1995; Carter and Hauler 2000; Eusebio and Coloso 2000). This study was undertaken to determine the nutritive value of some of the more widely available protein sources in the diets for grouper juveniles based on apparent digestibility coefficients for dry matter (ADMD) and crude protein (APD), food conversion ratio (FCR), specific growth rate (SGR) and survival.

Methods

A series of feeding experiments were conducted to determine the growth performance of grouper juveniles (initial body weights = 2–5 g). The test diets were formulated for growth (4 replications/treatment) and digestibility experiments (3 replications/treatment). Each diet contained a test ingredient: experiment 1 (white fish meal, white cowpea meal and ipil-ipil leaf meal), experiment 2 (local meat and bone meal, soy protein concentrates and meat solubles) and experiment 3 (imported meat and bone meal, blood meal and corn gluten meal). The APD values for feed ingredients obtained from the previous experiments[1] were considered in the formulation. The composition and chemical analyses (AOAC 1990) of the formulated diets is shown in Tables 1–3.

A feeding trial for each experiment was conducted for 85 days in a flow-through system with filtered and aerated seawater. Ten and twenty juveniles were stocked in each of 60 L and 250 L oval fiberglass tanks, respectively. The fish were acclimated for five days prior to feeding them test diets. Feed was given twice daily (8:00 h and 16:00 h) at 15% of body weight at the start and computed thereafter to 8% towards the end of the feeding period. Water temperature and salinity ranged from 27–28°C and 29–30 ppt., respectively. Other water quality parameters were within the ranges appropriate for growth. The fish were weighed as a group every 20 days for 80 days. The growth performance of grouper juveniles was evaluated based on SGR (% day^{-1}), FCR and survival. Apparent digestibility coefficients (ADMD and APD) of formulated diets were also measured using the modified Guelph Faecal Collection System with Cr_2O_3 as an external indicator (Eusebio and Coloso 2000). The feeds and faeces were analyzed for moisture and protein using standard methods (AOAC 1990), and Cr_2O_3 (Carter et al. 1960). ADMD and APD were

[1] Eusebio, P.S., Coloso, R.M. and Mamauag, R.E.P. Apparent digestibility of selected feed ingredients in diets for grouper (*Epinephelus coioides*) juveniles. This volume, pp. 75–78.

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Table 1. Composition of the control and test diets (Experiment 1)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3
Fish meal, Chilean	39.00	—	38.00	35.00
Fish meal, white	—	30.00	—	—
Cowpea meal, white	—	—	20.50	—
Ipil-ipil leaf meal	—	—	—	15.10
Squid meal	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00
Soybean meal, defatted	17.00	24.40	12.96	13.21
Wheat flour	9.80	6.20	—	7.00
Rice bran	7.74	12.84	1.28	2.63
Cod liver oil	2.10	2.15	2.50	2.40
Soybean oil	2.10	2.15	2.50	2.40
Vitamin mix[2]	4.20	4.20	4.20	4.20
Mineral mix[2]	2.00	2.00	2.00	2.00
Ethoxyquin	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00
Proximate Composition[3]				
Crude protein (N × 6.25)	48.6	48.3	47.5	46.5
Crude fat	9.3	9.2	9.2	9.8
Nitrogen-free extract[4]	23.4	24.7	23.4	25.6
Crude fiber	2.9	3.5	3.5	2.9
Metabolisable energy (kcal/100g)[5]	370	372	365	372
Calculated digestible protein	42.6	42.6	43.0	42.0

[1] As fed basis (g/100g feed).
[2] Biomin, commercially available vitamin and mineral mixtures, Overseas Feed Corporation, Cebu City, Philippines.
[3] Dry weight basis (g/100g feed).
[4] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[5] Metabolizable energy was calculated based on the standard physiological values of 4.5kcal/g protein, 3.3kcal/g carbohydrate and 8.0kcal/g fat (Brett and Groves, 1979).

Table 2. Composition of the control and test diets (Experiment 2)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3
Fish meal, Chilean	39.00	30.00	14.00	—
Meat and bone meal[2]	—	16.00	—	—
Soy protein concentrates	—	—	35.00	—
Meat solubles	—	—	—	30.00
Squid meal	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00
Soybean meal, defatted	17.00	14.50	8.30	21.50
Wheat flour	9.80	10.40	—	4.70
Rice bran	7.74	3.34	14.74	14.04
Cod liver oil	2.10	2.00	2.60	3.25
Soybean oil	2.10	2.00	2.60	3.25
Vitamin mix[3]	4.20	4.20	4.20	4.20
Mineral mix[3]	2.00	1.50	2.50	3.00
Ethoxyquin	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00
Proximate Composition[4]				
Crude protein (N × 6.25)	48.6	47.7	43.9	46.9
Crude fat	9.3	9.3	9.2	9.9
Nitrogen-free extract[5]	23.4	24.8	31.1	27.9
Crude fiber	2.9	2.8	3.9	3.1
Metabolisable energy (kcal/100g)[6]	370	371	374	381
Calculated digestible protein	42.6	42.2	40.1	42.4

[1] As fed basis (g/100g feed).
[2] Locally available.
[3] Biomin, commercially available vitamin and mineral mixtures, Overseas feed Corporation, Cebu City, Philippines.
[4] Dry weight basis (g/100g feed).
[5] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[6] Metabolizable energy was calculated based on the standard physiological values of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8.0 kcal/g fat (Brett and Groves, 1979).

Table 3. Composition of the control and test diets (Experiment 3)[1].

Feed Ingredient	Control	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal, Chilean	36.00	25.00	—	12.00	18.00
Meat and bone meal[2]	—	19.00	—	—	—
Blood meal[2]	—	—	27.00	—	—
Corn gluten meal	—	—	—	31.00	—
Fish meal, tuna	—	—	—	—	22.00
Squid meal	5.00	5.00	5.00	5.00	5.00
Shrimp meal, *Acetes* sp.	10.00	10.00	10.00	10.00	10.00
Soybean meal, defatted	16.50	14.00	20.00	13.00	18.50
Wheat flour	9.80	12.00	7.60	3.60	8.80
Rice bran	10.96	3.74	14.24	12.54	6.14
Cod liver oil	2.24	2.25	3.20	2.30	2.15
Soybean oil	2.24	2.25	3.20	2.30	2.15
Vitamin mix[3]	4.20	4.20	4.20	4.20	4.20
Mineral mix[3]	2.00	1.50	4.50	3.00	2.00
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Phosphitan C	0.01	0.01	0.01	0.01	0.01
Carboxymethylcellulose	1.00	1.00	1.00	1.00	1.00
Proximate Composition[4]					
Crude protein (N × 6.25)	47.5	47.2	48.1	47.6	47.7
Crude fat	11.4	11.4	10.4	13.8	12.0
Nitrogen-free extract[5]	23.7	23.00	28.4	25.5	24.5
Crude fiber	3.4	2.2	2.2	2.6	1.4
Metabolisable energy (kcal/100g)[6]	384	379	394	409	391
Calculated digestible protein	43.0	43.5	28.3	43.3	40.6

[1] As fed basis (g/100g feed).
[2] Australia.
[3] Biomin, commercially available vitamin and mineral mixtures for shrimps, Overseas Feed Corporation, Cebu City, Philippines.
[4] Dry weight basis (g/100g feed).
[5] NFE = 100 – (% crude protein + % crude fat + % crude fiber + % crude ash).
[6] Metabolizable energy was calculated based on the standard physiological values of 4.5 kcal/g protein, 3.3 kcal/g carbohydrate and 8.0 kcal/g fat (Brett and Groves, 1979).

computed using the formula of Spyridakis et al. (1989).

The data were analyzed using ANOVA for a completely randomized design. Treatment means were compared by the use of Duncan's Multiple Range Test (SAS Institute Inc. 1988). Differences were considered significant at $P < 0.05$.

Results and Discussion

Experiment 1

Table 4 shows the growth performance of grouper and digestibility of the diets used in the growth experiment. Based on SGR, the growth performance of grouper juveniles (initial body weight ± standard error (s.e.) = 3.7 ± 0.6g) fed control diet, white fish meal and white cowpea meal-based diets was comparable (3.2–3.3% day^{-1}). Fish fed ipil-ipil leaf meal-based diet had the poorest growth performance (2.7% day^{-1}). No significant difference was observed between the FCR of white fish meal and white cowpea meal-based diets, and the control diet (1.3–1.4). Ipil-ipil leaf meal-based diet with FCR value of 1.6 was less efficient than the other three diets. Survival was 100% in all treatments. ADMD values for the control diet, white cowpea meal and ipil-ipil leaf meal-based diets were not significantly different (65–72%) but lower than that of the white fish meal-based diet (81%). The APD value for white fish meal-based diet was the highest (95%), followed by the control diet (91%) and white cowpea meal-based diet (88%). Ipil-ipil leaf meal-based diet had the lowest APD value of 79%. The poor growth performance of grouper fed ipil-ipil leaf meal-based diet can be associated with the low APD value of the respective diet. Jackson et al. (1982) found that ipil-ipil leaf meal (25% replacement of fish meal) in a diet for tilapia resulted in poor growth, which can be attributed to the toxic effect of mimosine present in ipil-ipil leaves.

Experiment 2

The growth performance of grouper juveniles (mean initial weight ± s.e. = 2.5 ± 0.1g) and digestibility of the diets used in the growth experiment are shown in Table 5. The SGR of fish fed the control diet was comparable with that of fish fed local meat and bone meal-based diet (3.6% day^{-1}). The growth of fish (SGR = 3.4% day^{-1}) fed soy protein concentrates-based diet was not as excellent as that of the control fish, which can be associated with the lower values for ADMD (58%) and APD (93%) of their diet compared with the control diet (ADMD = 73%; APD = 95%). Fish fed meat solubles-based diet (FCR = 2.7) had the poorest growth performance (SGR = 2.4% day^{-1}) because the diet that was given to them was not as efficient as the other diets (FCR = 1.4–1.5). However, no significant difference was observed on the survival of fish fed the four diets (80–93%). Also, the poor growth performance of fish given soy protein concentrates and meat solubles-based diets can be due to the processing methods used in the preparation of the respective feed ingredients. Heat can damage the amino acid components of the feed ingredients thus making them unavailable to the fish (Opstevedt et al. 1984).

Experiment 3

The SGR of fish (mean initial weight ± s.e. = 3.9 ± 0.4g) fed the control diet and imported meat and bone meal, corn gluten meal and tuna fish meal-based diets were comparable (3% day–1), but significantly higher than that of fish given blood meal-based diet (2% day–1) (Table 6). FCR values for the control diet, meat and bone meal

Table 4. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 1)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	ADMD[4]	APD[5]
Control diet	3.71 ± 0.24^{a}	3.22 ± 0.03^{a}	1.43 ± 0.02^{a}	71.90 ± 2.21^{b}	90.65 ± 0.43^{b}
Fish meal, white	3.7 ± 0.35^{a}	3.34 ± 0.05^{a}	1.28 ± 0.07^{a}	80.75 ± 1.79^{a}	94.99 ± 0.33^{a}
Cowpea meal, white	3.67 ± 0.37^{a}	3.24 ± 0.05^{a}	1.38 ± 0.10^{a}	64.72 ± 1.80^{b}	87.84 ± 0.41^{b}
Ipil-ipil leaf meal	3.68 ± 0.38^{a}	2.67 ± 0.10^{b}	1.64 ± 0.05^{b}	72.45 ± 0.50^{b}	78.74 ± 0.14^{c}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively); survival was 100% in all treatments.
[2] Specific growth rate = 100 × (ln W_{final} – ln $W_{initial}$/time (days).
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % DM_{faeces}/% DM_{diet} × 100)].
[5] APD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % $protein_{faeces}$/% $protein_{diet}$ × 100)].

Table 5. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 2)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	Survival rate (%)	ADMD[4]	APD[5]
Control	2.54 ± 0.06^{a}	3.63 ± 0.02^{a}	1.41 ± 0.08^{a}	80.00 ± 5.47^{a}	73.26 ± 1.08^{a}	94.59 ± 0.71^{a}
Meat and bone meal (Philippines)	2.49 ± 0.09^{a}	3.60 ± 0.05^{a}	1.38 ± 0.04^{a}	91.67 ± 4.01^{a}	64.14 ± 2.67^{b}	91.87 ± 0.59^{c}
Soy protein concentrates (HP 300)	2.49 ± 0.08^{a}	3.38 ± 0.05^{b}	1.53 ± 0.10^{a}	81.67 ± 6.01^{a}	58.45 ± 1.99^{c}	92.55 ± 0.37^{bc}
Meat solubles (Protamino Aqua)	2.48 ± 0.08^{a}	2.43 ± 0.04^{c}	2.72 ± 0.09^{b}	93.33 ± 3.33^{a}	70.97 ± 0.39^{a}	93.74 ± 0.18^{ab}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively).
[2] Specific growth rate = 100 × (ln W_{final} – ln $W_{initial}$/time (days).
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % DM_{faeces}/% DM_{diet} × 100)].
[5] APD = 100 – [(% Cr_2O_{3diet}/% $Cr_2O_{3faeces}$ × % $protein_{faeces}$/% $protein_{diet}$ × 100)].

Table 6. Growth performance of grouper, *Epinephelus coioides* juveniles and apparent digestibility coefficients (%) for dry matter and protein of the diets (Experiment 3)[1].

Dietary treatment	Initial weight (g)	Specific growth rate (% day^{-1})[2]	FCR[3]	Survival rate (%)	ADMD[4]	APD[5]
Control	3.92 ± 0.55^{a}	3.10 ± 0.10^{a}	1.08 ± 0.05^{a}	97.5 ± 0.01^{a}	79.7 ± 1.3^{a}	94.6 ± 0.3^{a}
Meat and bone meal (Australia)	3.93 ± 0.48^{a}	3.00 ± 0.01^{a}	1.09 ± 0.09^{a}	97.5 ± 0.01^{a}	60.4 ± 3.6^{b}	87.6 ± 1.1^{b}
Blood meal (Australia)	3.92 ± 0.43^{a}	2.44 ± 0.13^{b}	1.44 ± 0.02^{c}	95.0 ± 0.02^{a}	82.9 ± 0.9^{a}	89.4 ± 0.5^{b}
Corn gluten meal	3.93 ± 0.36^{a}	2.82 ± 0.13^{a}	1.24 ± 0.02^{b}	90.0 ± 0.05^{a}	73.8 ± 1.5^{a}	93.3 ± 0.6^{a}
Fish meal, tuna	3.91 ± 0.31^{a}	3.05 ± 0.08^{a}	1.10 ± 0.04^{a}	97.5 ± 0.01^{a}	78.6 ± 2.0^{a}	93.1 ± 0.6^{a}

[1] Treatment means in columns followed by different superscripts are significantly different ($P < 0.05$; mean value ± standard error of the mean; n = 4 and 3 for growth and digestibility experiments, respectively).
[2] Specific growth rate = $100 \times (\ln W_{final} - \ln W_{initial}/\text{time (days)}$.
[3] Feed conversion ratio = dry weight feed (g)/wet weight gain (g).
[4] ADMD = $100 - [(\% Cr_2O_{3diet}/\% Cr_2O_{3faeces} \times \% DM_{faeces}/\% DM_{diet} \times 100)]$.
[5] APD = $100 - [(\% Cr_2O_{3diet}/\% Cr_2O_{3faeces} \times \% \text{protein}_{faeces}/\% \text{protein}_{diet} \times 100)]$.

and tuna fish meal-based diets were comparable (1.1) and better than that of corn gluten meal-based diet (1.2). Blood meal-based diet was the least efficient with FCR value of 1.4. The poor growth performance of fish fed blood meal-based diet can be associated with the poor efficiency and low APD of the diet. Also, Allan et al. (2000) observed that the poor availability of isoleucine in blood meal for rainbow trout was associated with its low isoleucine and high leucine contents. ADMD values for the blood meal, tuna fish meal and corn gluten meal-based diets, and the control diet were comparable (74–83%). The imported meat and bone meal-based diet had the lowest ADMD value (60%). On the other hand, APD coefficients for the control diet, and corn gluten meal and tuna fish meal-based diets were comparable (93–95%), but were higher than those of the imported meat and bone meal-based diets (88%) and blood meal-based diets (89%). Furthermore, no significant difference was observed on the survival of fish fed the five diets (90–98%).

Conclusions

- White cowpea meal (20.5% incorporation), local (16% incorporation) and imported (19% incorporation) meat and bone meals can partially replace fish meal in the diets for grouper juveniles without affecting their growth.
- Low ADMD and APD values for the processed feed ingredients (meat and bone meal, soy protein concentrates and blood meal-based diets) can be associated with the processing methods used in the preparation of the respective feed ingredients, which can damage the amino acids and contribute to low nitrogen digestibility.
- Apparent digestibility coefficients (ADMD and APD) and growth can be used as indicators to determine the nutritional value of feed ingredients. However, the availability and optimal balance of amino acids must also be considered.

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Apparent Digestibility of Selected Local Feed Ingredients for Humpback Grouper (*Cromileptes altivelis*)

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Introduction

The main objective of this study was to determine the apparent digestibility of several feed ingredients locally available in South Sulawesi for juvenile humpback grouper. Feedstuff digestibility assessment in fish is essential for determining nutrient requirements, screening the potential nutritive value of alternative feed ingredients and in the development of nutritionally adequate diets at least cost (Hajen et al. 1993). At present, digestibility coefficients of common feed ingredients have been reported for only a few species of warmwater marine carnivorous fishes (NRC 1983) and none for humpback grouper.

Methods

A reference diet and nine test diets, namely shrimp head meal, three types of blood meal (dried blood meal, formic acid-preserved blood meal, propionic acid-preserved blood meal), two types of fish meal (sardine meal, local-mixed fish meal), soybean meal, palm oil cake meal and rice bran were tested (Table 1). Chromic oxide was added to the diets at an inclusion rate of 1% as the digestibility marker.

Three experiments were carried out sequentially. Each experiment constituted a 4×4 latin square design in which four diets (one reference diet and three test ingredient diets) were examined over four collection periods. In each experiment, 20 fish of 20g initial weight from each cage were transferred to a 200L cylindro-conical faecal collection tank that was fitted with a faeces collection chamber (Allan et al. 1999). Following a five-day acclimatisation period, during which the fish were fed their prescribed diet twice daily to satiety, faeces were collected. The collected faeces were oven-dried (40°C) and stored in a sealed bottle at –40°C until analysed. Faecal collection continued for five to seven days when it was judged that a sufficient sample had been collected for chemical analysis. After each faecal collection period, diets were reallocated to the collection tanks in accordance with the latin square design and faecal collection recommenced after a further five-day acclimatisation period. This process was repeated for each of the four collection periods and sequentially for each of the three experiments.

The nutrient content of feed and faecal samples was analysed by AOAC (1990) procedures. After acid digestion, chromium concentration was determined by a spectrophotometric method (Furukawa and Tsukahara 1966). The apparent digestibility coefficient (ADC) of a nutrient in an ingredient was calculated according to procedures described by Foster (1999).

Results and Discussion

Humpback grouper generally showed a high capacity to digest protein. The apparent digestibility of plant protein (67.2% to 80.5%) was almost as good as that of animal protein (78.0% to 92.5%) except for the poorly digested oven-dried blood meal and rice bran meal. However, the apparent digestibility of dry matter (DM) was generally poor and especially for plant feed ingredients where values ranged from 22.2% for rice bran meal to 54.8% for soybean meal. By comparison, the DM apparent digestibility of

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Table 1. Formulation (g/kg, air dry) of experimental diets.

Ingredient and international feed number (IFN)		Diet		
Ingredient	**IFN**	**Reference**	**Test diet 1**	**Test diet 2**
Fish meal	5-01-985	570	342	399
Soybean meal; roasted; full-fat	5-14-005	80	48	56
Wheat gluten	—	100	60	70
Wheat flour	4-05-199	60	36	42
Rice bran	4-03-928	80	48	56
Fish oil	7-08-049	40	24	28
Squid oil	—	30	18	21
Vitamin mix[1]	—	30	18	21
Mineral mix [2]	—	10	6	7
Test ingredient 1 (animal origin)[3]	—	0	400	0
Test ingredient 2 (plant origin)[4]	—	0	0	300
Chromic oxide	—	10	10	10

[1] At 30 g/kg inclusion level, provided in 1 kg of final diet: retinol, 540 mg; cholecalciferol, 9.125 mg; α-tocopherol, 212.4 mg; menadione, 375 mg; thiamin, 300 mg; riboflavin, 750 mg; pyridoxine, 300 mg; cyanocobalamin, 3.5 mg; ascorbic acid, 4500 mg; folic acid, 150 mg; nicotinic acid, 1800 mg; d-pantothenic acid, 1500 mg; biotin, 3.75 mg; and d/l methionine, 1500 mg.

[2] At 10 g/kg inclusion level, provided in 1 kg of final diet: Ca, 3.25 g; P, 1.0 g; Fe, 60 mg; Mn, 40 mg; I, 0.75 mg; Cu, 3 mg; and Zn, 37.5 mg.

[3] Animal origin:

Shrimp head meal (5-04-226) and Local mixed fish meal (5-01-974): Manufactured by TAS Coy, Makassar, South Sulawesi.

Sardine meal(5-02-015): Dried sardine from fish landing site and extruded at RICF feed mill.

Blood meal (5-00-380): Oven-dried (60°C) and ground fresh bovine blood from local abattoir, Makassar, South Sulawesi.

[4] Plant origin:

Soybean meal (5-14-005): Whole soybean seed supplied by PT Inti Tani and heat-extruded at RICF feed mill.

Palm oil cake (5-04-487): Manufactured by PT. Pertani, Luwu, South Sulawesi.

Rice bran (4-03-928): Supplied by PT. Pertani, Sidrap, South Sulawesi.

Table 2. The dry matter (DM), crude protein (CP) and gross energy (GE) apparent digestibility coefficients (%) of diets and of substituted test feed ingredients examined in three experiments. Each coefficient is the mean of four replicates.

Diet and ingredient designation	Dry matter	Crude protein	Gross energy
Shrimp head meal	58.5 ± 3.33^{a}	78.0 ± 1.32^{b}	63.6 ± 0.89^{a}
Soybean meal (full-fat)	54.8 ± 2.72^{b}	67.2 ± 1.29^{c}	51.1 ± 0.89^{b}
Palm oil cake meal	45.3 ± 2.37^{c}	80.5 ± 1.30^{a}	40.4 ± 3.74^{c}
Dried blood meal	48.1 ± 0.85^{c}	55.2 ± 1.35^{c}	nd
Formic blood meal	67.9 ± 1.63^{a}	87.5 ± 0.55^{a}	nd
Propionic blood meal	61.7 ± 2.60^{b}	84.2 ± 0.69^{b}	nd
Local sardine meal	87.2 ± 2.53^{a}	92.5 ± 1.40^{a}	85.2 ± 0.90^{a}
Local mixed-fish meal	59.1 ± 1.23^{b}	82.4 ± 1.99^{b}	77.2 ± 1.91^{b}
Rice bran meal	22.2 ± 1.52^{c}	59.5 ± 1.41^{c}	44.3 ± 0.97^{c}

[a,b,c,d] Treatment means within each column with the same superscript letters are not significantly different ($P > 0.05$).
nd Not determined as insufficient faecal sample for energy analysis.

animal feed ingredients was higher, especially for the local sardine meal (87.2%). The local mixed-fish meal and the shrimp head meal were far less digestible with a DM apparent digestibility of only 59.1% and 58.5%, respectively, and lower than either of the two fermented blood meals (61.7% and 67.9%). The apparent digestibility of gross energy (GE) was comparatively high for the two fish meals, with the local sardine meal being significantly more digestible than the local mixed-fish meal (85.2% compared with 77.7%, respectively). Although, the apparent energy digestibility of the shrimp head meal was low (63.6%), it appeared to be higher than for each

of the three plant feed ingredients; full-fat soybean meal (51.1%), palm oil cake meal (40.4%) and rice bran (44.3%).

The low energy digestibility of the plant feed ingredients can be attributed to their high carbohydrate content and poor digestibility by carnivorous fish (Lupatsch et al. 1997). Other warmwater carnivorous marine fish, such as Asian sea bass and red drum, appear to have a higher capacity to digest plant ingredients than humpback grouper (Gaylord and Gatlin 1996).

Generally, freshwater and warmwater fish appear to digest carbohydrates more effectively than marine fish and coldwater fish (Wilson 1994). This study has shown that humpback grouper are able to efficiently digest the protein of both plant and animal feed ingredients. However, they have a very limited capacity to digest carbohydrate-rich products such as many plant feedstuffs and shrimp head meal that additionally has a high ash content (25.1%). Blood meal was digested better than plant meals and thus has greater potential to be used as a dietary replacement of fish meal in humpback grouper diets, particularly if the nutritive value of blood meal is enhanced through preservation. The two types of local sources of fish meal were digested well by humpback grouper and thus have good potential to substitute for imported fish meal. However, use of these products as aquaculture ingredients would directly compete with their traditional use for human consumption.

Conclusions

- Humpback grouper are able to digest the protein of both plant and animal feed ingredients.
- Humpback grouper have a very limited capacity to digest carbohydrate-rich products found in many plant feedstuffs, and shrimp head meal which additionally has a high ash content.
- Several feed ingredients could be used as protein sources as fish meal replacement.

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The Optimal Dietary Protein and Lipid Specification for Rearing Humpback Grouper (*Cromileptes altivelis*) Fingerlings

K.C. Williams, D.M. Smith, I.H. Williams, S. Irvin, M. Barclay and M. Jones

Introduction

A major objective of this project was to develop compounded grouper grow-out feeds that are more cost-effective and less environmentally damaging than the alternative of feeding fresh fishery by-catch. Information on the nutritional requirements of epinepheline groupers is limited (Boonyaratpalin 1997; Chen 2001) and non-existent for humpback grouper. Moreover, it is not known whether or not groupers can effectively utilise dietary lipid as an energy source in order to spare protein and so reduce nitrogen (N) discharge to the environment. As a first step in the development of formulated grow-out feeds for humpback grouper fingerlings, a series of growth, digestibility and metabolic assays were carried out to determine the optimum dietary protein and lipid specification for maximising productivity of humpback fingerlings.

CSIRO researcher Dr Kevin Williams assisting RICA staff to design digestibility experiments.

Methods

The optimum dietary protein to lipid ratio for humpback grouper was determined by feeding fingerlings one of 10 pelleted diets in which the dry matter (DM) crude protein (CP) concentration varied from 41% to 63% at 5.5% increments and in combination with either 15% or 24% DM lipid (a 3:1 mixture of fish oil and soybean oil). These diets were fed to four replicate tanks of fingerlings in an 8-week comparative slaughter growth and nutrient digestibility assay.

A second comparative slaughter growth and digestibility assay, employing the same culture conditions as before, was carried out to see if supplying dietary lipid at moderate (15% added oil) or high (30% added oil) concentrations and in the form of either long-chain fatty acids (LCFA, C18+, as olive oil) or medium-chain fatty acids (MCFA, C12–C16, as coconut oil) affected the way the fish used the lipid as an energy source. Five diets, a low-lipid (7% DM), high-protein (82% CP DM) control diet and four 'lipid' diets that together comprised a 2×2 factorial of the two types and two concentrations of lipid, were fed to six replicate tanks of fingerlings for eight weeks. The formulation of the 'lipid' diets was identical to the control except that the required amount of lipid was included at the expense of defatted fish meal with a concomitant lowering of the dietary CP from 82% to 69% and 57% DM for the 15% and 30% lipid treatments, respectively. These same 'lipid' diets were radioactively labelled with ^{14}C-octanoic

acid (as a marker of MCFA) and ^{14}C-oleic acid and ^{14}C-palmitic acid (as markers of LCFA) were fed to fish and the fate of the labelled ^{14}C was determined using metabolism chambers. For these studies, seven to nine replicates of each treatment were used and the presence of the ^{14}C in the fish, in the chamber water and in the respired CO_2, was quantitatively determined for the ensuring 22 hr post-feeding.

Results and Discussion

Fish productivity and CP digestibility improved linearly with increasing dietary CP; energy digestibility was lower for the high lipid diets and fish on these diets were fatter, but did not grow faster, than those fed low lipid diets (Table 1). Asian seabass, *Lates calcarifer*, showed a similar improvement in growth rate with

Table 1. Apparent digestibility (AD) of crude protein (CP) and energy (E) of diets and specific growth rate (SGR), dry matter (DM) food conversion ratio (FCR), DM body fat (BF) and retention of digestible N (RDN) and digestible E (RDE) of fish.

Response	CP (%)					Fat (%)	
	41.0	46.5	52.0	57.5	63.0	15	24
ADCP (%)	46.8^{C}	55.3^{BC}	58.5^{A}	69.7^{A}	74.0^{A}	59.8	61.9
ADE (%)	59.9^{A}	58.4^{B}	51.3^{C}	61.3^{B}	68.1^{A}	62.2^{X}	57.5^{Y}
SGR (%/d)	1.12^{C}	1.11^{C}	1.26^{B}	1.42^{A}	1.52^{A}	1.31	1.26
FCR (g:g)	1.58^{C}	1.49^{C}	1.24^{B}	1.08^{A}	1.00^{A}	1.28	1.27
BF (%)	23.5	23.2	23.7	23.1	23.5	21.7^{X}	25.1^{Y}
RDN (%)	58.6^{A}	48.8^{B}	50.3^{B}	42.3^{C}	38.8^{C}	48.9	46.7
RDE (%)	35.0^{C}	38.6^{C}	52.3^{A}	47.5^{B}	44.2^{B}	40.7^{Y}	46.3^{X}

$^{A,B,C; X,Y}$ Within comparisons, means without a common letter differ ($P < 0.05$).

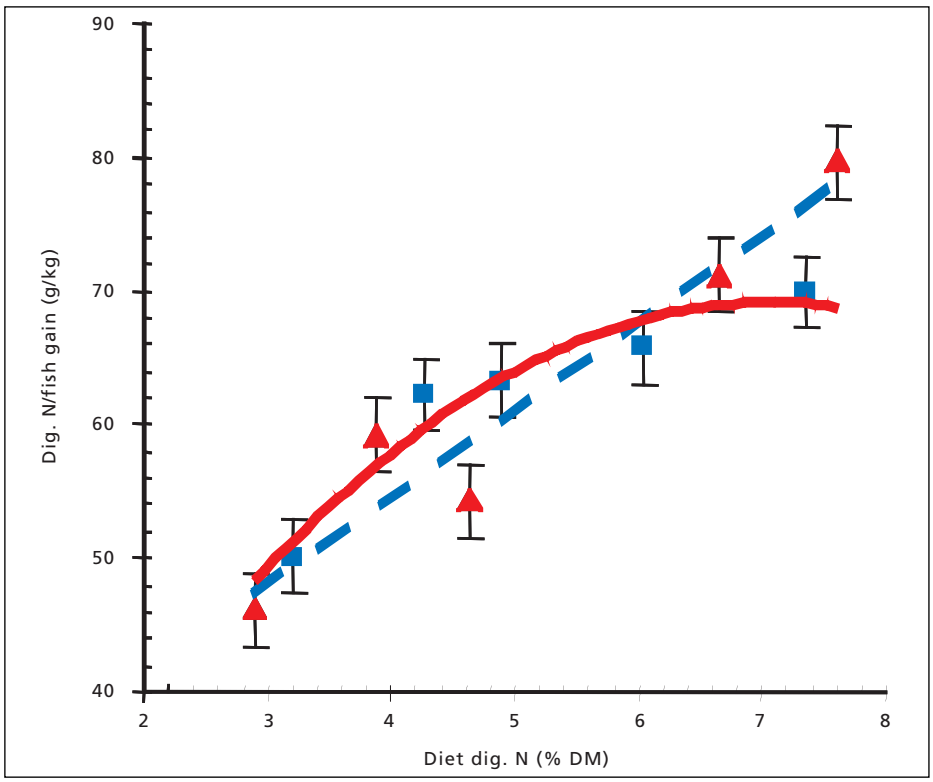

Figure 1. Relationship between the digestible N content of the diet and the amount of digestible N required per kg weight gain of fish-fed diets containing either 15 (▲; $R^2 = 0.93$) or 24 (■; $R^2 = 0.94$)) % fat.

increasing dietary CP content, but differed in that growth rate and FCR improved incrementally with increasing dietary lipid content (Williams et al. 2003). Humpback grouper required significantly more digestible N per unit weight gain with increasing dietary CP content and this relationship was unaffected by the amount of lipid in the diet (Fig. 1).

Increasing the amount of lipid in the control diet by adding 15% of olive oil (LCFA) at the expense of fish meal resulted in a 14% to 20% improvement in growth rate and food conversion, a doubling of the body fat content of the fish (from 15% to 29% DM) and the retention of dietary protein was increased by 28% (from 25% to 32%). A higher addition of olive oil (30%) reduced voluntary food intake by 40%, and consequently depressed growth rate by 32% while protein retention and body fat content were unchanged. Adding coconut oil (MCFA) instead of olive oil depressed food intake by 59%, with a similar reduction in growth rate and no increase in protein retention. The amount of dietary lipid retained as body fat in the fish relative to that oxidised for energy decreased with increasing dietary lipid and was less for MCFA than for LCFA lipids (Fig. 2).

The percentage distribution of radioactivity following ingestion of ^{14}C-labelled diets containing either olive oil or coconut oil at inclusion rates of 15% or 30% showed by humpback grouper oxidised MCFA far more rapidly than LCFA (Table 2). The respiration rate of fish fed diets containing MFA was significantly higher than those fed LCFA (Fig. 3).

Table 2. Percentage distribution of radioactivity following ingestion of ^{14}C-labelled diets containing varying inclusion rates of either coconut oil (MCFA) or olive oil (LCFA).

Diet lipid	Distribution of radioactivity (%)			
	Fish	Respired CO_2	DOM	POM
15% LCFA	70^{B}	15^{B}	11^{B}	3.9^{B}
30% LCFA	67^{B}	11^{B}	11^{B}	11.5^{C}
15% MCFA	23^{A}	51^{A}	26^{A}	0.6^{A}
30% MCFA	17^{A}	49^{A}	34^{A}	0.6^{A}

DOM = Dissolved organic matter in metabolic chamber water.

POM = Particulate organic matter in metabolic chamber water.

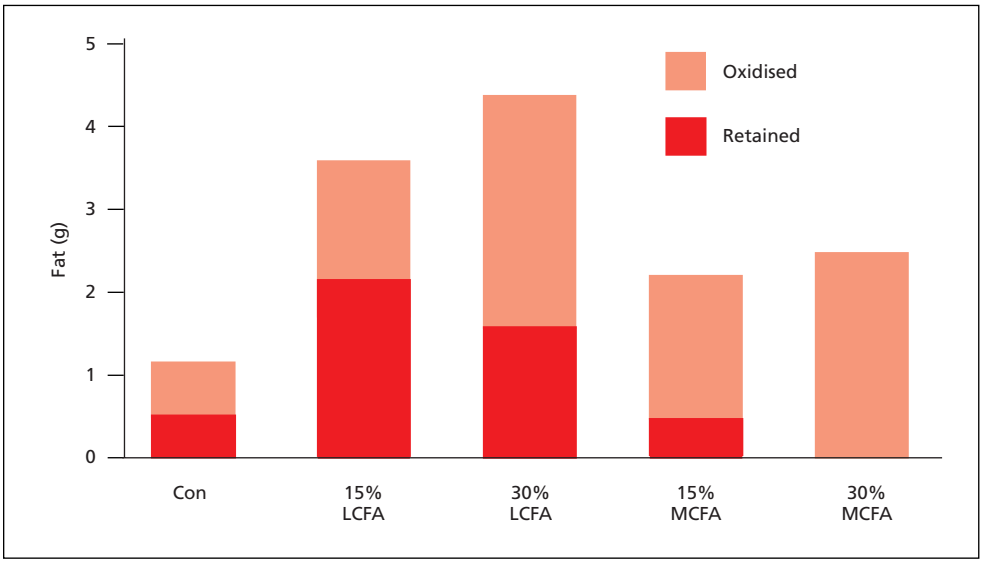

Figure 2. The amount of consumed dietary lipid retained as body fat or oxidised by fish fed either a low lipid (7% DM) control (Con) diet or diets with either 15% or 30% added olive oil (LCFA) or coconut oil (MCFA).

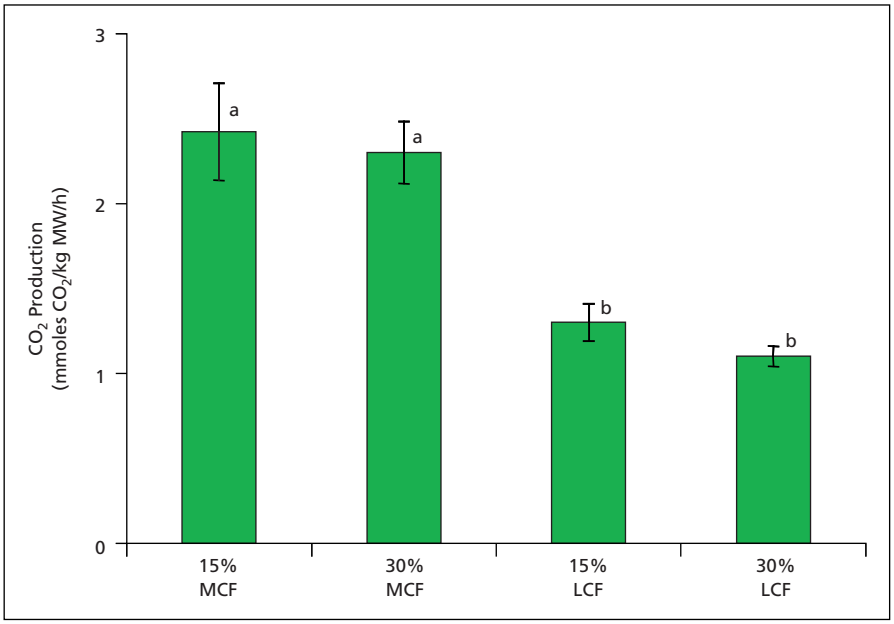

Figure 3. Respiration rate as measured by CO_2 production of fish following ingestion of diets containing 15% or 30% of either coconut oil (MCFA) or olive oil (LCFA).

Conclusions

- Diets for fingerling humpback grouper should contain not less than 44% DM digestible protein (about 60% CP).
- Increasing the lipid content of the diet above about 15% did not promote greater oxidation of the fat but rather led to increased body fat deposition, a reduction in food intake and a slowing of growth rate.
- Replacement of LCFA lipids (such as fish or long-chain vegetable oils) with MCFA lipids (such as coconut oil) did increase the rate of fat oxidation but had a detrimental effect on food intake, and consequently also on growth rate.

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Optimum Level of Dietary Protein and Lipid for Rearing Juvenile Tiger Grouper (*Epinephelus fuscoguttatus*)

N.A. Giri, K. Suwirya and M. Marzuqi

Introduction

Live tiger grouper, *Epinephelus fuscoguttatus*, is a commercially high-value fish. Recently, success in propagation of this species has been reported (Sudjiarno et al. 2001) following success in the development of hatchery technology for humpback grouper, *Cromileptes altivelis* (Sugama et al. 2001). Generally, cultured groupers are primarily fed trash fish. Feed represents a large part of production costs during intensive aquaculture. As protein represents the most expensive component in fish diet, it is important to determine the optimal level of dietary protein for growth of fish. Some researchers have reported that the dietary protein requirement of groupers ranges from 47.8–60%, depending on species, but there is no information on dietary protein requirement for tiger grouper. Dietary lipid is a source of energy and essential fatty acids for fish. Some researchers have reported that the optimum dietary lipid requirement for groupers varies between 9–13.5% depending on species (Boonyaratpalin 1997, Lin and Shiau 2003, Williams et al. 2004). There is no information on lipid requirement for *E. fuscoguttatus*. The balance of dietary protein and lipid is an important aspect in diet formulation to minimise utilisation of protein for energy source and also to produce a cost-effective diet.

Methods

Two series of experiments were conducted to determine crude dietary protein (CP) and lipid requirement for growth of juvenile tiger grouper. For the first experiment, six dry diets were prepared to contain graded levels of CP from 32% to 57% DM at 5% increments. Diets were formulated using fish meal, casein, mysid shrimp meal and squid liver meal as the protein source. The energy content of the experimental diets was adjusted by inclusion of dextrin. Hatchery produced juveniles of tiger grouper of 11.9 ± 0.02 g average body weight were stocked in 30 litre polycarbonate tanks with the density of 10 fish per tank. Each tank was equipped with flow-through seawater and aeration to maintain good water quality in the rearing tank. Fish were fed twice every day at satiation level for 42 days. The experiment was a completely randomised design with six treatments and three replicates for each treatment.

For the second experiment, six experimental dry diets were prepared to contain graded levels of lipid from 0 to 50% DM at 3% increments. Diets were formulated using casein and chloroform-methanol extracted fish meal, squid liver meal and mysid meal as the main protein source. The gross energy content of the experimental diets was adjusted by adding dextrin and α-starch so that all diets contained 4.0–4.2 kcal/g diet. Hatchery produced juveniles of tiger grouper of 4.7 ± 0.40 g average body weight were stocked in 30 L polycarbonate tanks with a density of 12 fish per tank. Each tank was equipped with flow-through seawater and aeration to maintain good water quality in the rearing tank. Fish were fed twice daily at satiation level for 56 days. The experiment was a completely randomised design with six treatments and three replicates for each treatment.

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Results and Discussion

Dietary CP significantly affected final weight, percent weight gain and feed efficiency responses of the fish (Table 1). Fish fed 47% dietary CP showed the highest weight gain (45.9 g) and percent weight gain (287%), but not significantly better than fish fed 52% or 57% CP diets. As for growth rate, feed efficiency improved with increasing dietary CP up to 47% but worsened at higher levels of dietary CP. Fish fed the 47% CP diet showed the best protein efficiency ratio and protein retention. These results indicate that juvenile tiger grouper require a diet of 47% CP for best growth. This finding is similar to that of Chen and Tsai (1994) who reported a dietary CP requirement of 48% for the marbled grouper *E. malabaricus*. Vergara *et al*. (1996) reported that dietary protein levels above the optimum level caused growth rate and feed efficiency of juvenile gilthead sea bream, *S. aurata*, to worsen. They attributed this result to insufficient energy being consumed by the fish with a net loss of energy due to inefficient deamination of absorbed excess amino acids and excretion of nitrogenous waste products. In the present juvenile tiger grouper study, increasing dietary protein above 47%, while not adversely affecting fish growth rate, did cause a significant impairment of feed efficiency.

Fish performance was significantly influenced by the lipid content of the diet (Table 2). Fish fed the diet without lipid supplementation had the lowest survival and the worst final weight, percent weight gain and feed efficiency. These response traits improved significantly as the dietary lipid content increased up to 9% and plateaued thereafter. These results are similar to those reported by Chu *et al*. (1996), who found that the grouper, *E. areolatus*, required a dietary lipid level of 9% for good growth. Increasing the dietary lipid content to 12% or 15% did not improve growth of tiger grouper. Whole body lipid content and protein retention of fish also increased as the dietary lipid level increased up to 9% (Fig. 1). This result indicates that juvenile tiger grouper have a limited ability to metabolize dietary lipid as an energy source.

Table 1. Final weight, weight gain, feed efficiency and protein efficiency ratio of juvenile tiger grouper fed experimental diet[1].

Protein level	Final Weight (g)	Weight gain (%)	Feed Efficiency[2]	Protein efficiency ratio[3]
32	37.6 ± 0.5^{a}	217.2 ± 6.1^{a}	69 ± 0.01^{a}	2.05 ± 0.02^{a}
37	38.6 ± 0.8^{a}	225.4 ± 6.6^{a}	72 ± 0.01^{a}	1.98 ± 0.03^{a}
42	41.3 ± 0.8^{b}	248.0 ± 6.7^{b}	81 ± 0.02^{b}	1.99 ± 0.03^{a}
47	45.9 ± 0.8^{c}	286.7 ± 6.9^{c}	99 ± 0.03^{c}	2.15 ± 0.02^{b}
52	44.9 ± 0.7^{c}	279.1 ± 5.7^{c}	88 ± 0.02^{d}	1.69 ± 0.04^{c}
57	44.4 ± 0.5^{c}	274.5 ± 4.5^{c}	87 ± 0.03^{d}	1.55 ± 0.04^{d}

[1] Initial weight = 11.9 ± 0.02 g. Values within the column with a common letter are not significantly different ($P > 0.05$).
[2] Feed efficiency: 100 × Weight gain (g)/feed intake (g).
[3] Protein efficiency ratio = $\frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$.

Table 2. Final weight, weight gain, survival, and feed efficiency of juvenile tiger grouper fed experimental diet[1].

Lipid level	Final Weight (g)	Weight gain (%)	Survival (%)	Feed Efficiency[2]
0	16.57 ± 0.72^{a}	251.4 ± 14.5^{a}	75.1 ± 0.0^{a}	73 ± 0.08^{a}
3	19.30 ± 0.95^{abc}	307.3 ± 21.1^{abc}	97.2 ± 4.8^{b}	94 ± 0.09^{ab}
6	18.73 ± 2.41^{ab}	294.3 ± 50.1^{ab}	83.3 ± 0.0^{c}	93 ± 0.17^{ab}
9	21.93 ± 0.47^{c}	360.4 ± 10.7^{c}	100.0 ± 0.0^{b}	112 ± 0.04^{b}
12	18.80 ± 0.30^{ab}	296.1 ± 6.5^{ab}	91.7 ± 8.4^{b}	97 ± 0.10^{ab}
15	19.30 ± 2.98^{bc}	314.5 ± 58.7^{bc}	100.0 ± 0.0^{b}	99 ± 0.17^{ab}

[1] Initial weight = 4.7 ± 0.4 g. Values within the column with a common letter are not significantly different ($P > 0.05$).
[2] Feed efficiency: 100 × Weight gain (g)/feed intake (g).

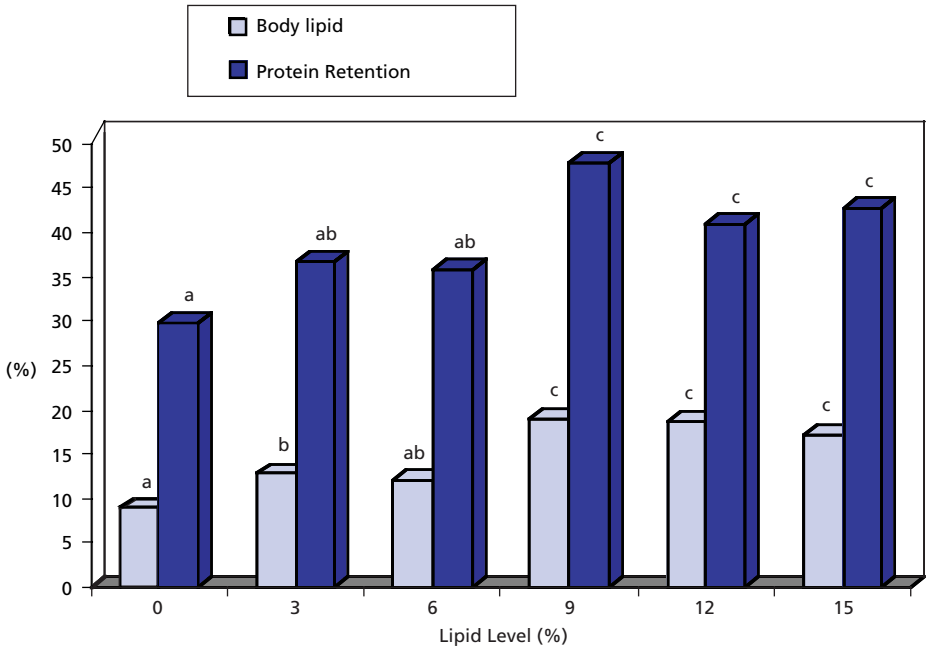

Figure 1. Whole body lipid content and protein retention of juvenile tiger grouper fed experimental diets with different lipid levels.

Conclusions

- The optimal dietary CP and lipid specifications for juvenile tiger grouper are 47% and 9% respectively.

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Dietary Optimum Protein for Tiger Grouper (*Epinephelus fuscoguttatus*) Diet Reared in Floating Net Cages

A. Laining, N. Kabangnga and Usman

Introduction

Generally, aquatic animals — particularly marine fishes — require high protein for their maximum growth. Several investigations on protein requirement of groupers have been reported such as juvenile *Ephinephelus striatus* requires more than 55% protein, (Ellis et al. 1996); *E malabaricus* 47.8% (Chen and Tsai 1994), while humpback grouper (*Cromileptes altivelis*) requires 52% (Giri et al. 1999). Moreover, it has also been reported that humpback grouper require lipid at a level of 9–11% and vitamin C in the form of L-ascorbyl-2-monophosphate-sodium-calcium at a rate of 150 ppm (Laining et al. 2002). Humpback grouper have the capability to utilise glucose as a carbohydrate source at 16% (Usman 2002). This experiment was conducted to provide preliminary information regarding the optimum level of dietary protein for a tiger grouper diet.

Methods

A 17-week experiment was carried out to determine the appropriate level of dietary protein and its effects on biological responses and apparent crude protein (CP) and dry matter (DM) digestibility of tiger grouper. The experiment was a randomised block design of five treatments and three replicates. Diets containing graded levels of protein: from 35% to 50% at 5% increments were fed to tiger grouper raised in floating sea cages. All diets were formulated to be isocaloric (4.7 kcal/g).

Tiger grouper were transferred from the Research Institute for Mariculture, Gondol, Bali and sorted into three different weight groups namely, small (53–65 g), medium (75–85 g) and large (97–105 g). Fish were stocked into fifteen $1 \times 1 \times 2.5$m cages with 12 fish per cage. The fish were fed twice daily to satiety.

The parameters measured were growth rate, feed efficiency, survival rate and the protein digestibility coefficient. Determination of the apparent digestibility coefficient was done after growth assay using chromium oxide as an inert marker.

Research Institute for Coastal Aquaculture staff feeding juvenile *Cromileptes altivelis* in experimental cages, Barru, Indonesia.

The response traits measured were growth rate, feed efficiency, survival rate and DM and CP apparent digestibility. Chromium oxide was used as the marker for determining apparent digestibility with faecal collection being carried out for this purpose upon the completion of the growth assay.

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Results and Discussion

Growth rate significantly improved as dietary protein increased, with the diet containing 50% protein resulting in the highest percent weight gain (266%), while the smallest weight gain was achieved by fish fed the 35% CP diet (77%) (Table 1). The change in average individual weight of fish over the course of the 17-week experiment is shown in Figure 1. Feed efficiency and survival rate showed a similar improvement, with increasing dietary CP. Increasing the dietary protein content from 35% to 50% resulted in almost a doubling of both the survival rate and a similar magnitude of improvement in feed efficiency. Based on broken-line analysis of

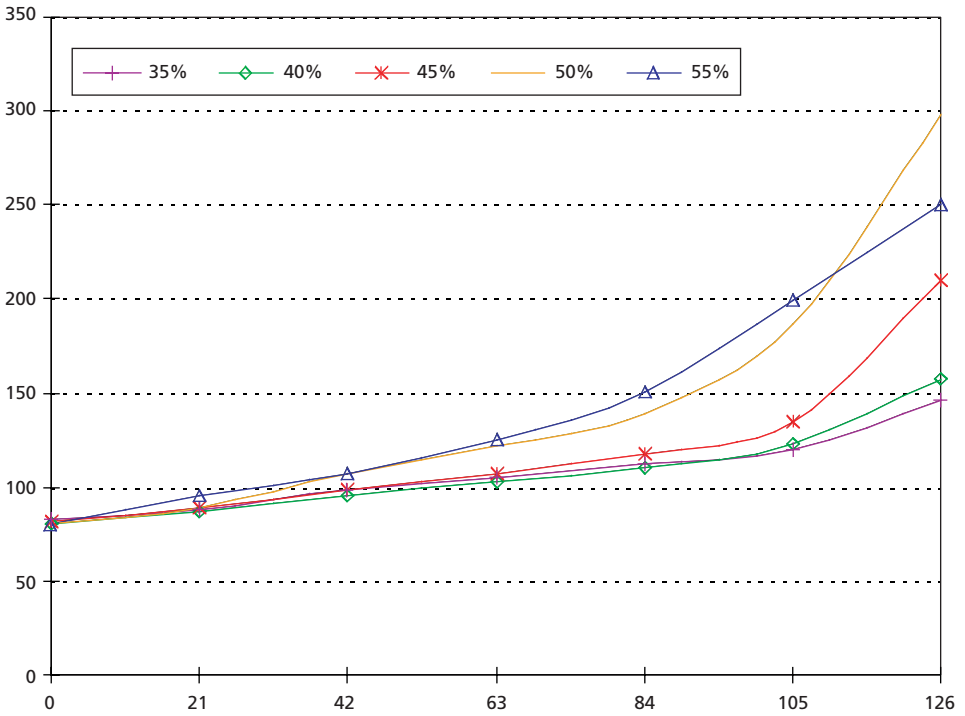

Figure 1. Average individual weight gain of tiger grouper after 126 days of culture.

Table 1. Biological performances of tiger grouper fed diets with different levels of protein.

Biological Parameter	Kadar protein/*Protein levels* (%)				
	35	40	45	50	55
Initial weight (g)	82.6	80.6	81.8	80.7	80.4
Final weight (g)	145.9	156.7	210.3	298.3	250
Weight gain (%)	77.2[a3]	94.9[a]	158.6[b]	266.2[d]	210.9[c]
Absolute growth (g/d)	0.5[a]	0.6[a]	1.0[b]	1.7[d]	1.3[c]
Survival rate (%)	41.7[a]	44.4[a]	50[ab]	72.2[c]	61.1[bc]
Feed intake (g/)[1]	168.6[a]	176.9[a]	200[ab]	274.2[c]	255.2[bc]
Feed efficiency (%)[2]	37.4[a]	48.8[a]	64.3[b]	78.6[c]	71.3[bc]
Dry matter digest.coefficient (%)	47.33	50.49	48.50	53.90	50.28
Protein digest.coefficient (%)	72.15	71.66	76.59	80.96	79.86

[1] Feed intake: Total daily feed intake/0.5 × (total fish at start + total fish at the end).
[2] Feed efficiency: Weight gain (g)/feed intake (g) × 100%.
[3] Value in rows followed by the same superscript are not significantly different (P > 0.01).

weight gain (Jobling, 1994), a dietary CP specification of 51% was determined to be optimal for tiger group over the weight range of 80 g to 300 g.

The DM apparent digestibility was not significantly affected by dietary CP (Table 1). However, CP apparent digestibility significantly increased as dietary CP increased from 35% to 50%, but with no further improvement with the 55% CP diet.

Conclusions

- Productivity responses of juvenile tiger grouper improved as the dietary CP content increased up to 50%.
- The apparent digestibility of CP, but not DM, also improved with increasing dietary CP up to 50% and protein digestibility of tiger grouper also improved.
- For tiger grouper reared from 80 g to 300 g, the optimum dietary CP specification was determined from broken-line regression analysis to be 51%.

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Effect of Dietary n-3 HUFA on Growth of Humpback Grouper (*Cromileptes altivelis*) and Tiger Grouper (*Epinephelus fuscogutatus*) Juveniles

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Introduction

Humpback and tiger grouper are two candidate species for aquaculture in Indonesia, as well as in other countries. Research is ongoing to improve the propagation techniques for these species and to increase the production of juveniles. However, little information is available on the nutritional requirements of these species and especially their requirement for the omega-3 highly unsaturated fatty acids (n-3 HUFA). Marine fish are unable to synthesise n-3 HUFA *de novo* and thus they need to be supplied in the diet for optimal health and growth (Watanabe 1993; Wanakowat et al. 1993; Lochmann and Gatlin 1993). The objective of this study was to determine the n-3 HUFA requirement of juvenile humpback and tiger grouper.

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Methods

Six pelleted diets were formulated in which the total dietary n-3 HUFA content was varied from 0% to 2.5% dry matter at 0.5% increments. In a separate 8-week growth assay experiment for each fish species, diets were fed to three replicate tanks of juveniles and measurements made of percentage weight gain, feed intake and feed efficiency.

Results and Discussion

Productivity responses of juvenile humpback grouper fed diets varying in n-3 HUFA content are presented in Table 1. Neither total feed intake nor survival rate was significantly ($P > 0.05$) affected by the amount of n-3 HUFA in the diet. However, weight gain increased curvilinearly with increasing dietary n-3 HUFA, with a maximum response at the 1.0% supplementation rate. This result demonstrates that the minimum dietary n-3 HUFA requirement for humpback grouper juveniles is 1.0% dry matter.

Responses of tiger grouper to the feeding of diets varying in n-3 HUFA content are presented in Table 2. Feed intake was unaffected by the amount of n-3 HUFA in the diet. Percentage weight gain and feed efficiency improved linearly and curvilinearly, respectively, as the amount of n-3 HUFA in the diet increased. This result implies a dietary requirement for n-3 HUFA of at least, and possibly more than, the maximum supplementation rate of 2.5% examined in this experiment.

Table 1. Percentage weight gain, total feed intake and survival rate of humpback grouper juveniles, fed diets varying in n-3 HUFA content.

Dietary level of n-3 HUFA (%)	Weight gain (%)[1]	Feed intake (g/ind)[2]	Survival rate (%)
0	115[a]	17.9[a]	100
0.5	136[ab]	18.1[a]	100
1.0	182[c]	19.4[a]	100
1.5	169[c]	19.1[a]	100
2.0	183[c]	19.4[a]	100
2.5	189[c]	19.7[a]	100

[1] Weight gain (%) = 100 × (average final weight – average initial weight)/average initial weight.
[2] Feed intake = 0.5 × sum of daily DM feed allocation/(total fish at start + total fish at end).
Values in columns followed by the same superscript letter are not significantly different ($P > 0.05$).

Table 2. Percentage weight gain, feed efficiency and total feed intake of juvenile tiger grouper fed diets varying in n-3 HUFA content.

Dietary n-3 HUFA (%)	Weight gain (%)[1]	Feed efficiency (%)[2]	Feed intake (g/ind)[1]
0.0	509 ± 10.3[a]	0.71[a]	15.2 ± 1.06[a]
0.5	528 ± 29.4[ab]	0.74[ab]	16.1 ± 0.32[a]
1.0	560 ± 11.9[ab]	0.79[ab]	15.7 ± 0.69[a]
1.5	605 ± 50.6[bc]	0.80[b]	15.2 ± 0.27[a]
2.0	621 ± 27.0[c]	0.86[b]	14.8 ± 2.62[a]
2.5	650 ± 13.7[c]	0.85[b]	15.0 ± 2.78[a]

[1] Weight gain (%) = (average final weight – average initial weight)/average initial weight × 100.
[2] 100 × (Total weight gain (g)/total feed intake (g))
means within a column with the same superscript are not statistically different ($P > 0.05$).

A lack of n-3 HUFA in the diet of marine fish has been reported to increase mortality, decrease growth rate and result in the development of an abnormal swim bladder (Sorgeloos, et al., 1988; Webster and Lovel, 1990; Koven, et al., 1990). The dietary requirement of n-3 HUFA varies with species and the size of fish. For example, the dietary n-3 HUFA requirement of gilthead sea bream larvae is about 2.2% (Salhi, et al., 1994), whereas only 0.5–1% is required for juvenile red drum (Lochmann and Galtin, 1993) and about 0.9% for juvenile Korean rockfish of 6 g size (Lee, et al., 1993). A similar dietary n-3 HUFA specification of 1% appears adequate for juvenile Asian sea bass (Wanakowat et al., 1993). Our findings suggest that the dietary n-3 HUFA requirement of humpback grouper fingerlings is only about 1%, whereas tiger grouper require much higher levels of at least 2.5%. Further studies are needed to confirm that these two grouper species do have such differing requirements for dietary n-3 HUFA.

Conclusions

- The dietary n-3 HUFA specification for optimal growth of juvenile humpback and tiger groupers should be not less than 1.5% and 2.5%, respectively.
- Further research is required to confirm the differences between these grouper species.

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