

Supplementation of Vitamin C, L-ascorbyl-2-monophosphate-sodium-calcium for Sea Cage Reared Humpback Grouper (*Cromileptes altivelis*) Diets

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Introduction

Even though seed production of grouper culture in Indonesia has been successful (Sugama et al. 1998), there has been limited development of grow-out ventures. Several constraints of humpback grouper grow-out, especially in floating net cages, are still found; for example, slow growth and high mortality. Humpback grouper are very sensitive to improper handling and a sudden change of environmental conditions commonly leads to stress and mortality. Since very little information is known about the vitamin C requirement of humpback grouper, an experiment was carried out with fish reared in floating net cages to investigate the efficacy of stable vitamin C L-ascorbyl-2-monophosphate-Na-Ca (APNa).

Methods

A completely randomised design of five treatments with three replicates was applied in this experiment. The dietary treatments comprised three different levels of APNa at inclusion rates of 50, 100 or 150 mg/kg, a positive control (in which a standard commercial vitamin premix containing vitamin C as ascorbic acid was used) and a negative control (in which vitamin C was absent). All diets were formulated to be isonitrogenous and isoenergetic with specifications of 48.5% crude protein, 10.9% crude lipid and 3047 calories/g gross energy.



Maros researcher Ms Asda Laining inspecting farm-made pelleted fish feed at Iloilo, Philippines.

Hatchery-reared juvenile humpback grouper, *Cromileptes altivelis*, from the Research Institute

for Mariculture, Gondol, Bali, Indonesia and weighing 5–7 g, were distributed to each of 15 cages (1 × 1 × 2.5 m). During the two months of culture, fish were fed to satiation a pelleted diet, twice daily at 0700 and 1600 h. Feed ingredients and manufactured pellets were analysed for moisture crude protein, crude lipid, crude fibre, ash and energy. The vitamin C content of liver samples was determined using HPLC procedures. Fish were weighed and their length measured every two weeks and food intake reconciled over the same periods for determination of growth rates, feed efficiency and survival. Environmental characteristics of water temperature, salinity, transparency and dissolved oxygen were measured periodically during the 8-week experiment.

Results and Discussion

Fish growth rate was significantly improved with increasing APNa. The weight gain of fish on the vitamin C-free negative control diet was only half that of fish fed the highest APNa supplementation (150 mg/kg) diet (110% vs. 254% respectively) and not significantly different to the positive control diet (120%). Feed efficiency also improved with increasing dietary APNa

content, but feed intake did not differ significantly among dietary treatments (Table 1).

Fish survival rates improved with dietary APNa content and was best for the 150 mg/kg APNa supplemented diet (95%) and worst for the commercial premix control diet (72.5%) (Table 1). Mortalities first occurred in the third fortnight and continued during the final fortnight of the experiment. This corresponded with a decline of water quality around the sea cages during a period of heavy rainfall that washed silt and debris into the sea in the region of the fish cages (Table 2). A similar study undertaken by Subyakto (2000) and Giri et al. (1999), but under laboratory conditions, showed that including ascorbyl-2-monophosphate-Mg in the diet at a rate of 25–30 mg/kg was sufficient for rearing humpback grouper.

Vitamin C level in the liver increased with increasing dietary APNa supplementation (Table 3). The vitamin C content of the liver of fish fed the commercial premix diet was low (6.0 µg/g), similar to that of fish fed the vitamin C-free diet (4.2 µg/g) and only half that of similar fish sampled at the start of the experiment (12.3 µg/g).

Table 1. Weight gain, daily growth rate, feed intake, food conversion ratio (FCR) and survival rate of humpback grouper fed on diets containing different levels of APNa.

Variables	Commercial premix control	APNa level, mg/kg feed			
		0	50	100	150
Weight gain (%)	119.5	110	170	187	254
Daily growth rate (%/d)	1.40 ^a	1.32 ^a	1.78 ^b	1.88 ^b	2.26 ^c
Feed intake (g/g) ¹	19.4 ^a	19.7 ^a	18.7 ^a	19.4 ^a	21.0 ^a
FCR (g/g) ²	2.99 ^a	3.12 ^a	2.00 ^b	1.83 ^c	1.43 ^d
Survival rate	72.5	75.0 ^a	85.0 ^b	86.7 ^b	95.0 ^c

¹Apparent average daily feed intake of all fish in the tank.

²FCR determined as total weight gain (g) divided by total feed (g) dispensed.

Within response traits, values followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Water quality observed around the cages during the experiment.

Variables	Day			
	0–14	15–28	29–40	41–56
Temperature (°C)	29.5–30.4	29.1–30.4	25–27	27.9–28
Salinity (ppt)	34	32	25–26	27–31
Transparency (m)	4.0–5.8	3.9–4.5	1.0–1.4	3.1–3.9
Dissolved oxygen (ppm)	4.2–5.9	4.9–7.9	3.5–4.1	4.5–5.0

Table 3. Vitamin C contained in humpback grouper liver at the beginning and end of the experiment (mean \pm SD).

Treatments	Vitamin C in liver ($\mu\text{g/g}$)
Initial	12.3 \pm 4.03
Commercial premix control	6.1 \pm 0.25
Vitamin C-free control	4.2 \pm 1.25
50 mg APNa	26.1 \pm 2.20
100 mg APNa	47.6 \pm 2.14
150 mg APNa	94.2 \pm 0.35

Conclusions

- Supplementation of dietary APNa at 150 mg/kg diet resulted in the best biological performance of humpback grouper as indicated by growth rate, feed efficiency, survival rate and liver vitamin C content.
- Humpback grouper require a high dietary vitamin C level especially if fish are likely to be subjected to stressful conditions such as poor water quality.

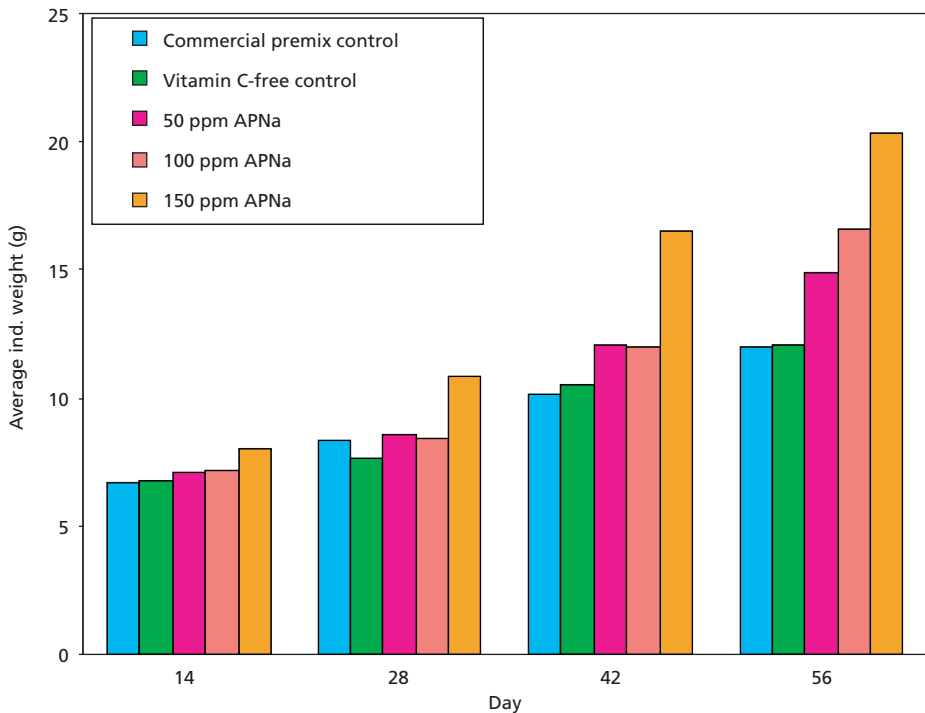


Figure 1. Average individual weight of humpback grouper fed with different levels of APNa.

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Utilisation of Different Dietary Carbohydrate Sources by Humpback Grouper (*Cromileptes altivelis*)

Usman, N. Palinggi and N.A. Giri

Introduction

Carbohydrates are an important macro-nutrient of feeds. They are a cheaper source of energy than either protein or lipid but only if they can be digested and the energy utilised by the animal. Carnivorous marine fish have little capacity to digest and utilise dietary carbohydrates as energy sources and much less than that of herbivorous or omnivorous freshwater fish (Wilson, 1994; Shiau, 1997). Utilisation of dietary carbohydrate is not only affected by species and the size of fish but also by the nature of the carbohydrate itself (Spannhof and Plantikow, 1983; Omondi and Stark, 1996; Peres and Oliva-Teles, 2002; Lee et al., 2003). As a broad generalization, freshwater fish utilise starch better than simple sugars (see Shiau 1997), whereas marine carnivorous fish appear better at utilising simple sugars than starch (Deng et al., 2001; Lee et al., 2003), but anomalies have been observed, e.g. *E. malabaricus* grouper in 23°C water utilised starch better than glucose (Shiau and Lin 2002).

It is not known if humpback grouper can utilise carbohydrate as an energy source or whether different types of carbohydrates differ in their usefulness as dietary constituents. To better understand the capacity of juvenile humpback groupers to utilise carbohydrates, diets providing different carbohydrate types were tested.

Methods

Hatchery-reared humpback grouper juveniles of initial weight 7.8 ± 0.4 g were held in 12 black polycarbonate tanks. Each tank was filled with 80 L of filtered seawater and stocked with

15 fish. Water flow was at 45 L/h and aeration was supplied to each tank. A randomised block design (three replicates) was used to examine four pelleted dry diets that differed only in the source of carbohydrate — glucose, sucrose, dextrin or starch — each being included at 20% of the diet. The dietary crude protein, crude fat and digestible energy specifications were 54%, 11% and 3.3 kcal/g, respectively. Fish were fed twice daily to satiation for six weeks. Weight and length measurement was carried out fortnightly.

Apparent digestibility of the diet was measured using chromic oxide as the digestibility marker (Takeuchi, 1988). At the conclusion of the growth assay, blood samples were taken at 0, 3, 6, 9, 12, 18 and 24 h after feeding and plasma glucose levels determined by the procedure of Wedemeyer and Yasutake (1977).

Results and Discussion

The type of carbohydrate in the diet had a significant effect on the productivity responses of the fish (Table 1). Feeding the glucose diet resulted in the best growth rate and feed efficiency being significantly better than in all other diets, while the starch diet resulted in the worst fish performance. Fish fed either the sucrose or dextrin diets produced an intermediate performance significantly better than the starch diet but inferior to the glucose diet. Protein retention rates were highest for glucose and dextrin diets and significantly better than starch and, in turn, better than the sucrose diet. More lipid was retained by fish fed the glucose diet (68%) but differences between other carbohydrate types were not significant (range 46% to 54%). There

Table 1. Protein retention (PR), lipid retention (LR), absolute growth rate (GR), feed consumption (FC), feed efficiency (FE), and survival rate (SR) of humpback grouper fed diets containing different types of carbohydrate.

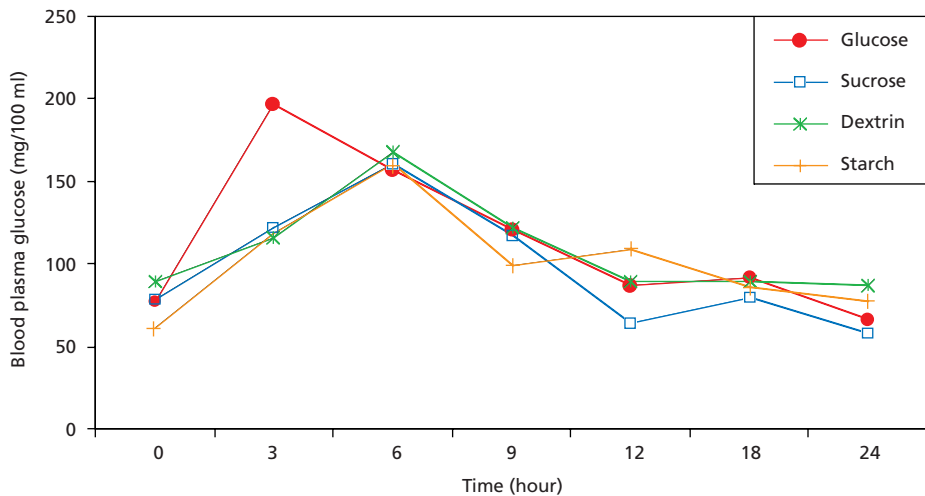
Nutrient	Carbohydrate			
	Glucose	Sucrose	Dextrin	Starch
PR (%)	35 ± 1.4 ^c	26 ± 1.8 ^a	33 ± 1.8 ^c	30 ± 0.9 ^b
LR (%)	68 ± 5.4 ^b	46 ± 2.5 ^a	54 ± 2.8 ^a	50 ± 6.9 ^a
GR (g/d)	0.40 ± 0.03 ^c	0.27 ± 0.00 ^a	0.34 ± 0.02 ^b	0.34 ± 0.03 ^b
FC (%)	247 ± 14.5 ^b	218 ± 6.1 ^a	235 ± 6.4 ^{ab}	234 ± 11.6 ^{ab}
FE (%)	101 ± 2.3 ^c	79 ± 2.7 ^a	91 ± 4.2 ^b	90 ± 3.3 ^b
SR (%)	100 ^a	100 ^a	100 ^a	100 ^a

Means within rows with a common letter are not significantly different ($P > 0.05$).

Table 2. The apparent digestibility of nitrogen free extract (ADNFE), crude protein (ADCP) and lipid (ADL) of diets containing different types of carbohydrate when fed to juvenile humpback grouper.

Nutrient	Kind of carbohydrate			
	Glucose	Sucrose	Dextrin	Starch
ADNFE (%)	96.6 ± 1.42 ^c	87.7 ± 2.86 ^b	82.8 ± 2.58 ^b	96.3 ± 2.94 ^a
ADCP ADL (%)	94.4 ± 0.28 ^b	93.4 ± 0.87 ^a	94.6 ± 0.23 ^b	94.9 ± 0.45 ^b
(%)	97.2 ± 1.11 ^a	96.2 ± 0.83 ^a	95.6 ± 0.18 ^a	95.3 ± 1.46 ^a

Means within rows with a common letter are not significantly different ($P > 0.05$).

**Figure 1.** Change of rate and pattern of blood plasma glucose in humpback grouper (*C. altivelis*) fed different types of dietary carbohydrates.

were no fish losses on any of the treatments during the experiment. These results accord with the findings of Shiau and Lin (2002) for *E. malabaricus* grouper held in cool (23°C) water, but contrast with their earlier observations (Shiau and Lin, 2001) where starch and glucose were equally well utilised by *E. malabaricus* that were held in warm (29°C) water.

The type of carbohydrate in the diet significantly affected the apparent digestibility of nitrogen free extract (NFE) and protein, but not the digestibility of lipid (Table 2). NFE and protein digestibility was highest for the glucose diet and significantly higher than all other diets in the case of NFE, but only for the sucrose diet in the case of protein. It is difficult to understand

why the apparent digestibility of protein should have been depressed by the inclusion of sucrose and yet not with starch. However, the absence of any effect of carbohydrate type on the apparent digestibility of lipid agrees with similar findings for striped bass and sunshine bass fed diets containing glucose, maltose or dextrin (Rawles and Gatlin III, 1998).

The ability of fish to absorb and metabolize dietary carbohydrate can be gauged from the rate and pattern of change in blood plasma glucose. Humpback grouper fed the glucose diet resulted in faster glucose absorption and attained a higher plasma glucose level than fish fed diets containing other carbohydrate types (Fig. 1). Hung et al. (1989) reported that white sturgeon fish are more able to utilise dietary glucose and maltose and had higher glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase enzyme activities, compared to fish fed diets containing fructose, sucrose, dextrin or starch. Subsequent studies (Deng et al., 2001) confirmed the ability of white sturgeon fish to utilise glucose and maltose more efficiently than starch or dextrin. In this regard, humpback grouper appear to mimic other marine carnivorous fish in being able to utilise simple carbohydrates such as glucose better than more complex sources such as starch and dextrin.

Conclusions

- The type of carbohydrate in the diet affects the apparent digestibility of both NFE and protein, but not lipid, and consequently growth rate, feed efficiency and nutrient retention responses of humpback grouper.
- Blood plasma glucose concentration increased most rapidly and attained the highest value at 3 h post-feeding when glucose was included in the diet. Including sucrose, dextrin or starch in the diet resulted in a similar pattern of plasma glucose with peak concentrations at 6 h post-feeding.
- The best dietary carbohydrate source for juvenile humpback grouper is glucose followed by dextrin, starch and sucrose.

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Utilisation of Dietary Dextrin by Juvenile Humpback Grouper (*Cromileptes altivelis*)

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Introduction

As a cheaper energy source than either protein or lipid, carbohydrate should be considered when formulating cost-effective and environmentally-friendly compounded grouper grow-out feeds. Generally, carnivorous marine fish are not as good as herbivorous or omnivorous freshwater fish in utilising carbohydrate as a source of energy. For example, growth of greasy grouper *Epinephelus coioides* and Atlantic salmon *Salmo salar* was optimised when diets contained 12% and 11% of carbohydrate, respectively (Shiau and Lan, 1996; Grisdale-Helland and Helland, 1997), whereas tilapia *Oreochromis niloticus* × *O. aureus* could effectively utilise and spare for protein when included at dietary concentrations of up to 41% (Shiau and Peng, 1993).

Since information regarding the utilisation of carbohydrates in grouper diet is still very limited (Usman, 2002), an experiment was conducted to determine the optimum level of carbohydrate in diets for juvenile humpback grouper.

Methods

The experiment was a completely randomised design and comprised five treatments and three tank replicates. The dietary treatments provided graded inclusions of dextrin as the carbohydrate source from 0% to 28% at 7% increments. All diets were formulated to be isonitrogenous and isocaloric and were prepared as a dry pellet.

Hatchery-reared juvenile humpback grouper of average initial body weight 8 ± 0.3 g were stocked at a rate of 11 fish per tank into 30 L polycarbonate tanks. Each tank was supplied

with a flow through water system and individual aeration to maintain good water quality during the rearing period. Fish were fed twice daily to satiation for 63 days. At the end of the experiment, two fish from each tank were taken randomly and the liver and muscle removed for glycogen analysis (Wedemeyer and Yasutaka, 1977). The hepatosomatic index (HSI) was also determined as: $HSI = 100 \times (\text{wet liver weight} / \text{total wet fish weight})$ with all weights in grams.

Results and Discussion

Percentage weight gain and feed conversion ratio (FCR) of fish improved as the amount of dextrin in the diet increased from 0 to 14% with no significant productivity change at higher dextrin levels (Table 1). Regression analysis showed that percentage weight gain and FCR of the fish



Experimental pellet diets for groupers, Pegametan, Bali, Indonesia.

improved curvilinearly with increasing dietary dextrin with asymptotic maximum responses at 18% and 21% dextrin, respectively.

Liver glycogen and lipid concentration increased curvilinearly with increasing dietary dextrin (Table 2), with asymptotic maximum responses occurring at dietary dextrin contents of 24% and 25%, respectively. Shimeno *et al.* (1979) reported that glycogen liver content of yellowtail (*Seriola quinqueradiata*) with a weight of 144 g, increased when fed carbohydrate levels up to 14%, and then decreased at higher levels. Muscle glycogen and lipid concentration similarly increased with increasing dietary dextrin (Table 2) but the asymptote was beyond the range of dietary dextrin examined in the experiment. Liver size was also affected by the dietary carbohydrate levels as indicated by the increasing hepatosomatic index (HSI) with increasing dietary dextrin (Table 2). Regression analysis showed that the HSI attained a maximum value with a dietary dextrin content of 18%. Based on these results, it appears that humpback grouper have a reasonably good capacity to utilise carbohydrate as an energy source.

Table 1. Percentage weight gain, feed efficiency, and feed conversion ratio (FCR) of juvenile humpback grouper fed experimental diets.

Dietary dextrin level (%)	Weight gain (%)	Feed efficiency	FCR ¹
0	222 ± 5.1 ^a	0.77 ± 0.04 ^a	1.37 ± 0.06 ^b
7	251 ± 8.8 ^b	0.86 ± 0.03 ^{ab}	1.16 ± 0.04 ^{ab}
14	268 ± 8.2 ^b	0.92 ± 0.09 ^b	1.02 ± 0.07 ^a
21	249 ± 9.9 ^b	0.93 ± 0.05 ^b	1.03 ± 0.05 ^a
28	259 ± 14.6 ^b	0.91 ± 0.01 ^b	1.07 ± 0.03 ^a

¹Weight of dry feed as fed (g)/fish weight gain (g).

Means in the same column with a common superscript letter are not significantly different ($P > 0.05$).

Table 2. Glycogen and lipid concentration of liver and muscle and the hepatosomatic index (HSI)¹ of juvenile humpback grouper fed experimental diets containing graded amounts of dextrin.

Parameter	Dietary dextrin level (%)				
	0	7	14	21	28
Liver					
Glycogen (%)	2.54 ± 0.97 ^a	5.28 ± 0.44 ^b	7.84 ± 0.56 ^c	7.96 ± 0.31 ^c	8.40 ± 0.2 ^c
Lipid (%)	17.17 ± 1.14 ^a	18.49 ± 0.94 ^{ab}	19.84 ± 0.55 ^{ab}	21.25 ± 2.16 ^b	20.52 ± 3.18 ^b
Muscle					
Glycogen (%)	0.01 ± 0.01 ^a	0.04 ± 0.02 ^{ab}	0.05 ± 0.02 ^b	0.07 ± 0.02 ^b	0.07 ± 0.02 ^b
Lipid (%)	16.37 ± 1.37 ^a	17.85 ± 1.65 ^a	18.92 ± 1.98 ^a	18.40 ± 0.74 ^a	19.76 ± 3.90 ^a
HSI (%)	2.07 ± 0.28 ^a	3.51 ± 0.07 ^b	3.63 ± 0.27 ^b	3.41 ± 0.44 ^b	3.46 ± 0.64 ^b

¹HSI = 100 × (wet weight of liver (g)/total wet weight of fish (g)).

Means in the same column with a common superscript letter are not significantly different ($P > 0.05$).

Conclusion

- Juvenile humpback grouper were able to efficiently utilise dextrin as a dietary energy source at inclusion rates of up to at least 14%.
- Further work is warranted to see if carbohydrates such as dextrin can be used by humpback grouper to spare dietary protein and so reduce the amount of nitrogen excreted by the fish.

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Replacement of Fish Meal by Animal By-product Meals in a Practical Diet for Grow-out Culture of Grouper (*Epinephelus coioides*)

O.M. Millamena

Introduction

Grouper culture has been dependent mainly on trash fish as feed (Boonyaratpalin 1993). Artificial diets have been developed for grouper (Chen and Chen 1986; Chen et al. 1987) but these diets have a high content of fish meal, the most common protein source in aquafeeds. With an increasing population and increased fishing pressure, the global production of fish meal has been in a state of decline whilst the demand for aquaculture has been steadily increasing (Tacon 1996). There is an urgent need to find suitable alternatives to fish meal. The objective of this study was to develop compounded feeds for juvenile grouper that have a low content of fish meal, and as an alternative to trash fish feeding.

Methods

Experimental diets

The basal diet contained 44.4% dietary protein supplied mainly by Chilean fish meal (40%), shrimp meal *Acetes* sp. (10%), soybean meal (6%) and squid meal (1%). The abattoir by-products, consisting of 4:1 combination of processed meat meal and blood meal, were incorporated to replace fish meal protein at increasing percentage replacements of 0% to 100% on an iso-nitrogenous basis in diets 1–8 (Tables 1 and 2). The 100% fish meal diet (diet 1) and trash fish as sole feed (diet 9) served as control treatments.

Culture

E. coioides juveniles, initial mean body wt (BW) = 6.0 ± 0.2 g, were stocked in 36-units of 250-L

fiberglass tanks at 25 fishes per tank. Tanks were supplied with sand-filtered seawater in a flow-through system provided with a standpipe at the center and cut PVC pipes to serve as shelter for the fish. Fish were initially fed on trash fish then gradually acclimatised to the diets for five days prior to start of the experimental run. Eight dietary treatments representing increasing (0%, 10%, 20%, 30%, 40%, 60%, 80%, 100%) percent replacements of fish meal protein with 4:1 combination of meat meal and blood meal were tested in quadruplicate groups of fish arranged in a completely randomised design. Fish were fed the diets twice per day at a daily feeding rate of 5–6% of BW and trash fish at 10–12% of BW for 60 days. Parameters used to determine diet efficiency were growth expressed as percent weight gain and specific growth rate (SGR), survival, food conversion rate (FCR), and body composition of grouper juveniles.

Results and Discussion

Table 3 shows the mean values of percent weight gain, SGR, survival, and FCR of *E. coioides* juveniles fed the diets. At the end of culture, grouper juveniles attained a weight gain of 448.0–570.4%. Specific growth rate ranged from 2.83% to 3.13%. Percentage weight gain and SGR tended to increase up to 20% replacement (diet 3) of fish meal with processed animal by-products, followed by a decreasing trend up to the highest level (100%, diet 8) of fish meal substitution. There were no significant differences ($P > 0.05$) in growth among fish fed diets

Table 1. Composition of the experimental diets on a dry basis in g per 100g dry diet.

Ingredients	Diets (% Replacement)							
	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)	6 (60%)	7 (80%)	8 (100%)
Chilean fish meal	40	36	32	28	24	16	8	0
Meat meal ^a	0	4	8	12	16	24	32	40
Blood meal	0	1	2	3	4	6	8	10
Shrimp meal	10	10	10	10	10	10	10	10
Soybean meal	6	6	6	6	6	6	6	6
Squid meal	1	1	1	1	1	1	1	1
Wheat flour	15	15	15	15	15	15	15	15
Vitamin mix	4	4	4	4	4	4	4	4
Mineral mix	3	3	3	3	3	3	3	3
Cod liver oil	6	6	6	6	6	6	6	6
Rice bran	15	14	13	12	11	9	7	5

^a Processed meat meal and blood meal produced by Consolidated Meat Group, Australia.

Table 2. Proximate composition (%) of the experimental diets on wet weight basis.

Diet	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	NFE* (%)	Ash (%)
1	2.4 ± 0.02	44.4 ± 0.62	12.2 ± 0.01	3.7 ± 0.03	23.0 ± 0.18	14.3 ± 0.00
2	2.8 ± 0.04	43.3 ± 0.22	11.9 ± 0.16	3.6 ± 0.11	24.2 ± 0.16	14.2 ± 0.05
3	2.8 ± 0.02	44.8 ± 0.25	11.7 ± 0.06	3.7 ± 0.08	22.2 ± 0.30	14.6 ± 0.09
4	2.8 ± 0.03	43.8 ± 0.13	12.1 ± 0.11	2.2 ± 0.14	22.9 ± 0.13	15.9 ± 0.19
5	2.7 ± 0.02	43.7 ± 0.25	11.7 ± 0.04	2.1 ± 0.13	23.8 ± 0.16	16.0 ± 0.21
6	4.0 ± 0.06	43.9 ± 0.04	11.5 ± 0.05	1.7 ± 0.16	22.6 ± 0.19	16.3 ± 0.06
7	4.0 ± 0.02	43.6 ± 0.13	11.3 ± 0.11	1.7 ± 0.63	22.8 ± 0.09	16.6 ± 0.17
8	3.8 ± 0.02	44.0 ± 0.71	11.5 ± 0.01	1.8 ± 0.12	22.2 ± 0.45	16.9 ± 0.17
9	4.2 ± 0.02	68.2 ± 0.05	5.5 ± 0.02	0.07 ± 0.01	2.0 ± 0.10	20.1 ± 0.04

*NFE; nitrogen free extract.

1–7 (0–80% fish meal replacement) including the trash fish control (diet 9). However, fish fed diet 3 had significantly higher ($P < 0.05$) growth than those fed diet 8 (100% fish meal replacement). Survival among fish fed the experimental diets did not significantly differ (96–100%) but was significantly higher ($P < 0.05$) than survival (90%) of fish fed trash fish. Likewise, feed conversion ratios were low and ranged from 0.93 to 1.05.

This study has demonstrated that replacement of up to 80% fish meal protein with processed slaughterhouse by-products allowed growth rates similar to or better than those exhibited by the control groups (fish meal based diet and trash fish feeding). Possible reasons for the reduced growth of grouper at total replacement may be due to deficiencies in essential nutrients. Fish meal, in general, has a good amino acid and fatty acid profile for fish. On the other hand, the animal by-product meals that were used to replace fish meal were lower in essential amino acids (methionine, lysine and

isoleucine) compared with those in grouper juveniles (Table 4).

Deficiencies in essential amino acids may explain the decline in growth performance of juvenile grouper particularly at full replacement levels of fish meal. Furthermore, animal meat meals are high in saturated fat and like other terrestrial proteins are characterised by high levels of n-6 polyunsaturated fatty acids but low levels of n-3 highly unsaturated fatty acids that are required by marine fish.

Another possible explanation for the reduced performance at increasing levels of fish meal substitution may be the resulting effect on diet digestibility. High ash content in meat meals may lower the digestibility of the diets and this may have caused the reduction in growth rates. In this study, the increase in ash content from 14.2% to 16.9% with increasing levels of animal by-product meals was reflected in the proximate analysis of the diets.

Table 3. Weight gain, specific growth rate (SGR), survival and food conversion ratio (FCR) of grouper fed the experimental diets for 60 days¹. Data are presented as mean \pm SE, n = 23–25 fish.

Diet/% meat replacement	% Weight gain	SGR ²	Survival (%)	FCR ³
1 (0)	502 \pm 38.3 ^{ab}	2.95 \pm 0.1 ^a	95 \pm 0.8 ^a	1.00 \pm 0.03
2 (10)	539 \pm 43.7 ^{ab}	3.06 \pm 0.1 ^a	100 \pm 0.8 ^a	0.99 \pm 0.02
3 (20)	570 \pm 36.6 ^a	3.13 \pm 0.2 ^a	99 \pm 1.8 ^a	0.95 \pm 0.03
4 (30)	530 \pm 78.6 ^{ab}	3.04 \pm 0.3 ^a	96 \pm 1.8 ^a	0.98 \pm 0.05
5 (40)	494 \pm 82.8 ^{ab}	2.93 \pm 0.3 ^a	99 \pm 1.8 ^a	1.02 \pm 0.06
6 (60)	501 \pm 75.6 ^{ab}	2.95 \pm 0.3 ^a	100 \pm 0.0 ^a	1.05 \pm 0.04
7 (80)	492 \pm 85.4 ^{ab}	2.92 \pm 0.3 ^{ab}	99 \pm 1.8 ^a	1.04 \pm 0.07
8 (100)	448 \pm 87.3 ^b	2.82 \pm 0.2 ^b	96 \pm 3.4 ^a	0.99 \pm 0.16
9 (TF)	525 \pm 62.0 ^{ab}	3.02 \pm 0.3 ^a	90 \pm 6.4 ^b	0.93 \pm 0.06

¹Treatment means with different superscripts within column are significantly different (P < 0.05).

Table 4. Comparison of the amino acid content in Chilean fish meal, meat and bone meal and blood meal (4:1) mixture in experimental diets (1–8) with the EAA pattern of grouper juveniles in g per 100 g TCA precipitable protein.

Amino Acid	Grouper juvenile	Diet (% replacement)							
		1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)	6 (60%)	7 (80%)	8 (100%)
Arg	2.50	3.00	3.09	3.19	3.28	3.38	3.56	3.76	3.94
His	1.20	1.73	1.79	1.84	1.90	2.01	2.07	2.18	2.29
Ile	1.66	2.48	2.33	2.19	2.04	1.88	1.60	1.30	1.01
Leu	5.04	6.31	6.46	6.60	6.75	7.00	7.19	7.48	7.77
Lys	4.60	5.22	5.11	5.00	4.88	4.82	4.55	4.32	4.10
Met	1.82	2.05	1.95	1.84	1.74	1.64	1.43	1.23	1.02
Phe	2.47	2.34	2.45	2.57	2.68	2.85	3.02	3.25	3.47
Thr	2.76	2.83	2.84	2.84	2.85	2.87	2.87	2.88	2.90
Tryp	—	—	0.04	0.09	0.13	0.18	0.27	0.36	0.45
Val	1.89	2.86	3.00	3.14	3.29	3.49	3.71	4.00	4.28

n = 4 replicate injections in the HPLC.

Conclusions

- Up to 80% of fish meal protein can be replaced by processed meat meal and blood meal coming from terrestrial animals with no adverse effects on growth, survival and feed conversion efficiency of *E. coioides* juveniles.
- Use of animal by-product meals as a protein source substantially lowers the level of fish meal required in juvenile grouper diet. Furthermore, the diet can be effectively used as a substitute for trash fish feeding, thereby reducing the requirements for fishery resource.
- From an economic standpoint, replacement of fish meal with cheaper animal by-product meals in a practical diet for grouper can alleviate the problem of low fish meal availability and high cost.

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The Use of Shrimp Head Meal as a Substitute to Fish Meal in Diets for Humpback Grouper (*Cromileptes altivelis*)

Rachmansyah, A. Laining and T. Ahmad

Introduction

Fishmeal is the main source of protein in fish feed manufactured in Indonesia and most of this (about 147,000 tonnes and valued at US\$123 million) is imported (Anonymous, 1998). Shrimp head meal originates from shrimp processing plant waste and contains about 50% protein. It is therefore a good potential candidate for the replacement of fishmeal. Moreover, the apparent digestibility of the protein of shrimp head meal is quite high (78.0%) and not much lower than for local fishmeal (82%) (Laining et al., 2003). The total replacement of fishmeal with a combination of meat and soybean meals in diets for juvenile barramundi, *Lates calcarifer*, resulted in equivalent fish growth (Williams et al., 2003). Grouper may also have a similar capacity to utilise protein sources other than fishmeal. The extent to which locally produced shrimp head meal can substitute for fishmeal in diets for juvenile humpback grouper was examined in this study.

Methods

The experiment was a completely randomised block design and comprised five dietary treatments and three replicates. The dietary treatments comprised graded inclusions of shrimp head meal from 0% to 40% in 10% increments, which replaced an isonitrogenous amount of fishmeal in a basal diet. Hence, all diets had a similar crude protein (CP) content of 45% and were formulated to the same gross energy specification of 4 kcal/g. The hatchery-reared fish

were stocked into 15 1 × 1 × 1.2 m floating net cages set in a raft in the sea. Stocking rate was 20 fish/m³ and the average initial individual weight of the fish was 15.9 g. Fish were fed twice daily to satiation. The experiment was carried out for 60 days and the fish were weighed and their length measured every fortnight. Apparent digestibility of the diets was determined at the conclusion of the growth assay with chromic oxide being used as a digestibility marker.

Results and Discussion

Replacing fishmeal with shrimp head meal adversely affected ($P < 0.05$) growth rate, feed conversion ratio and protein efficiency ratio responses of the fish and protein digestibility of the diet was reduced at shrimp head inclusion rates above 10% (Table 1). However, survival



Research Institute for Coastal Aquaculture experimental grow-out cages, Barru, southern Sulawesi, Indonesia.

Table 1. Biological response of humpback grouper fed different level of shrimp head meal.

Variables	Shrimp head meal (%) in the diet				
	0	10	20	30	40
Weight gain (%)	101.5 ^a	102.6 ^a	76.8 ^b	67.9 ^b	27.8 ^c
Survival rate (%)	100 ^a	96.7 ^a	95.0 ^a	98.3 ^a	96.7 ^a
Daily growth rate (%/day)	1.17 ^a	1.14 ^a	0.85 ^b	0.84 ^b	0.51 ^c
Feed conversion ratio	1.52 ^a	1.55 ^a	1.79 ^b	1.78 ^b	2.64 ^c
Feed intake ¹	23.2 ^a	23.0 ^a	19.6 ^b	19.0 ^b	13.8 ^c
Feed efficiency ²	66 ^a	67 ^a	62 ^a	59 ^b	41 ^c
Protein efficiency ratio	1.35	1.36	1.34	1.19	0.89
App. digestibility coefficient. (%)	85.2 ^a	86.9 ^a	81.3 ^b	79.6 ^b	81.9 ^b

¹Feed intake: Total daily feed intake (dry)/(total fish at start + total fish at the end) × 0.5.

²Feed efficiency: 100 × (Weight gain (g)/feed intake (g)).

rate was unaffected. The highest daily growth rate was observed in the fish fed the basal (zero shrimp head meal) diet, but this was not significantly better than the diet with 10% shrimp head meal. The apparent protein digestibilities of the basal and 10% shrimp head meal diets were similar (85% cf 87%, respectively) and significantly better than for diets with higher inclusions of shrimp head meal. This indicates that humpback grouper have some, though limited, capacity to digest the chitin-protein complex of shrimp head meal. Chitin is a long chain polysaccharide which is not well digested by marine carnivorous teleosts (Saleh et al., 1998; Angka and Suhartono, 2000). In parallel with changes in the protein digestibility of the diet, feed efficiency and protein efficiency ratio deteriorated as shrimp head meal was used at fishmeal substitution rates above 10%. Surprisingly, the reduced digestibility of high shrimp head meal diets did not stimulate a compensatory increase in feed intake. Instead, average feed intake decreased with increasing shrimp head meal inclusion, thus compounding a depression of fish growth rate.

Conclusion

- Shrimp head meal is not well digested by humpback grouper and its use as a replacement for fishmeal should be limited to no more than 10% of the diet.

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Development of Formulated Feeds for Grow-out Culture of Grouper (*Epinephelus coioides*) — Tank and Field Studies

O.M. Millamena and J.D. Toledo



Southeast Asian Fisheries Development Centre Aquaculture Department staff checking growth of groupers fed experimental diets in replicate cage/pond trials.

Introduction

The availability of a practical diet for grouper is a major constraint to grow-out production. Nutritional studies on grouper include dietary protein to energy ratio (Serrano and Apines 1996; Shiao and Lan 1996), optimum dietary lipid level (New 1987), essential fatty acid requirement (Millamena and Golez, unpublished

data) and vitamin requirements (Boonyaratpalin 2002). This information was used as a basis in developing a formulated diet for juvenile grouper. The objective of this study was to compare the performance of a Southeast Asian Fisheries Development Centre formulated diet with a commercial feed for grow-out culture of grouper and to transfer technology on grouper diet developed at SEAFDEC to industry.

Methods

Experimental diets

The percentage composition of a SEAFDEC formulated diet and proximate analyses of diets are shown in Tables 1a and 1b. In the tank trial, the SEAFDEC diet was prepared at the SEAFDEC Feed Laboratory, while a commercial feed miller prepared the commercial feed. In field trials, both the SEAFDEC diet and commercial feed were compounded into feeds by a commercial feed mill.

Tank Study

Grouper *E. coioides* juveniles were reared in 12 units of 150 L circular fibreglass tanks at 15 fish per tank with four replicates per treatment. Tanks were supplied with sand-filtered seawater in a flow-through system with adequate aeration and cut PVC pipes as shelter for the fish. Fish were fed the diets at a feeding rate of 5–6% of body weight (BW) and trash fish at 10–12% BW per day for 60 days. The tanks were cleaned of excess food and faeces before feeding each morning. Every 20 days, fish were bulk weighed to determine weight gain, which was used as the basis for adjustment of the feed ration. At the end of culture, the parameters used to determine diet efficiency were growth, expressed as percentage weight gain, specific growth rate (SGR), survival and food conversion ratio (FCR). The essential amino acid composition of the diets and commercial feed were compared with essential amino acid profiles of grouper juveniles.

Field Study

In the SEAFDEC Dumangas Brackishwater Station feeding trial, fish stocked were variable in size and grouped into two size groups. Treatments were arranged in a randomised complete block design with size groups as block. Each size group was stocked in two replicate, or a total of four replicate, cages per dietary treatment. Grouper juveniles, with initial BW of around 50 and 100 g, were reared 12-units of 2 m × 2 m × 1 m deep net cages installed in brackishwater ponds at six fishes per net cage. Thirty six fishes were stocked per size group or a total of 72 fishes for the 12 cages. Formulated feeds were given twice a day. Daily feeding rates were 5–6% of BW for the feeds and 10% of BW for trash fish. Fish were sampled every 20 days to determine weight gains for adjustment of the feed ration. The field trial was terminated after 120 days.

Results and Discussion

Tank Study

After 60 days of feeding, grouper juveniles attained weight gains of 215% (SEAFDEC diet), 118% (commercial feed) and 222% (trash fish), respectively (Table 2). Survival was 73%, 68% and 63%, respectively. Correspondingly, the FCRs were 1.5, 1.83 and 1.62. The commercial feed gave significantly lower growth, survival and FCR compared with SEAFDEC diet and trash fish control. The commercial feed had low protein content (Table 1a) that is below the established protein requirement of juvenile grouper

Table 1a. Proximate analysis (%) of experimental diets on dry matter (Tank study).

Diets	Moisture (%)	Crude Protein	Crude Fat	Crude Fibre	NFE ¹	Ash
SEAFDEC	4.64	44.06	7.22	3.22	33.35	12.15
Commercial	3.98	38.98	11.51	4.50	33.37	11.70

Table 1b. Proximate analysis (%) of experimental diets on dry matter (Field study).

Diets	Moisture (%)	Crude Protein	Crude Fat	Crude Fibre	NFE ¹	Ash
SEAFDEC	4.64	44.06	7.22	3.22	33.35	12.15
Commercial	3.98	44.74	7.54	3.48	32.02	12.02

¹NFE, nitrogen free extract.

Table 2. Weight gain, survival, specific growth rate and food conversion ratio (FCR) of juvenile grouper fed the experimental diets for 60 days (Tank study).

Diet	Weight gain (%)	SGR	Survival (%)	FCR
SEAFDEC	215 ± 31 ^a	1.88 ± 0.2 ^a	73 ± 7 ^{ab}	1.50 ± 0.05 ^{bc}
Commercial	118 ± 14 ^b	1.29 ± 0.1 ^b	68 ± 6 ^{ab}	1.83 ± 0.04 ^a
Trash fish	222 ± 70 ^a	1.85 ± 0.3 ^a	63 ± 5 ^a	1.62 ± 0.10 ^{ab}

Figures are presented as mean ± SE. For each column, values with different superscripts are significantly different ($P < 0.05$).

at 44% protein. The feed was also grossly deficient in four essential amino acids: methionine, isoleucine, lysine, and threonine (Table 3). Levels of these essential amino acids were relatively low compared with the amounts that were present in grouper juveniles. The commercial feed formulator was then informed of the results of chemical (proximate and amino acid) analyses and advised to improve the feed formulation to achieve the desired protein levels and amino acid composition.

Table 3. The essential amino acid content of the SEAFDEC diet, commercial feed and grouper juveniles (g/100g sample).

Amino Acid	Grouper juvenile	SEAFDEC	Commercial
Arginine	1.02	1.70	1.68
Histidine	0.43	0.66	0.05
Isoleucine	0.75	1.30	0.93
Leucine	1.75	2.32	1.74
Lysine	1.59	1.79	1.28
Methionine	0.62	0.57	0.32
Phenylalanine	0.87	1.46	1.05
Threonine	0.98	1.21	0.85
Tryptophan	0.03	—	—
Valine	0.65	1.21	1.11

Field Study

After 123 days of culture, the mean values of percent weight gains and SGR in two size groups are: SEAFDEC diet (504% and 2.58), commercial feed (445% and 2.49), and trash fish (522% and

2.78) (Table 4). Correspondingly, the feed conversion ratios were 3.52, 3.84, and 3.50. Survival rates were high in all treatments at 100%, 96% and 96%, respectively. Results of field trials at grow-out ponds did not show significant differences in growth performance, survival and FCR of grouper juveniles fed with the diets. The proximate composition of diets used in field studies was found to be similar in levels of crude protein, fat, fiber, ash and nitrogen free extract (NFE) (Table 1b). Both the SEAFDEC diet and commercial feed conformed to the established protein requirement of juvenile grouper. It should be noted that the present commercial feed had a higher protein content compared with the formulation that was pre-tested in tanks. This could explain the marked improvement in growth performance of grouper fed with the commercial feed.

Conclusions

- In tank trials, the poor performance of commercial feed was attributed to the low protein content and deficiencies in essential amino acids as confirmed by analysis of the amino acid composition.
- Improvement in growth performance of the commercial feed was achieved in field trials by increasing the dietary protein level and improving the amino acid composition to match that of grouper juveniles.

Table 4. Weight gain, survival, specific growth rate and food conversion ratios (FCR) of juvenile grouper fed the experimental diets for 123 days (Field study).

Diet	Weight gain (%)	SGR	Survival (%)	FCR
SEAFDEC	504 ± 146 ^a	2.58 ± 0.3 ^a	100 ± 0 ^a	3.52 ± 0.71 ^b
Commercial	445 ± 81 ^a	2.44 ± 0.1 ^a	96 ± 4 ^a	3.84 ± 0.59 ^a
Trash fish	522 ± 127 ^a	2.78 ± 0.3 ^a	96 ± 4 ^a	3.50 ± 0.42 ^a

Figures are mean ± SE. For each column, values with different superscript are significantly different ($P < 0.05$).

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SECTION 4

THE ASIA-PACIFIC GROUPEL NETWORK

M.A. Rimmer, M.J. Phillips and S.Y. Sim

The Asia-Pacific Marine Finfish Aquaculture Network, which was established (as the Asia-Pacific Grouper Network) in 1998, has grown rapidly. Network activities have contributed to improving the overall progress of developing sustainable grouper aquaculture in the Asia-Pacific region by supporting improved communication and providing opportunities for enhanced cooperation between participating agencies. Technology transfer has been a major focus for the network, with innovative use of modern electronic communication strategies and direct technology transfer through technical training. The outcome of these activities has been improved information access for researchers and industry and the development of mechanisms to spread project impacts widely throughout the Asia-Pacific region, beyond the agencies directly involved in projects.

Introduction

One of the constraints to the development of sustainable grouper aquaculture in the Asia-Pacific region has been the uncoordinated nature of the substantial regional research effort that has taken place over the last two decades. Researchers and practitioners felt they were working in isolation and were unaware of the many similar lines of research being undertaken by other laboratories.

In response to the identified need to improve communication and coordination of research effort, the Asia-Pacific Grouper Network was

established in 1998 at a grouper aquaculture workshop held in Bangkok, Thailand. The network is coordinated by the Network of Aquaculture Centres in Asia-Pacific (NACA) and has received support from the Australian Centre for International Agricultural Research (ACIAR) and the Asia-Pacific Economic Cooperation (APEC), through its Fisheries Working Group.

Recognising the importance of marine fish farming in the Asia-Pacific region, senior government representatives at the NACA 13th Governing Council Meeting in 2002 absorbed the grouper network into NACA's core program, to ensure its long-term sustainability. The coverage of the network was also expanded to include other species such as sea bass, snapper,



Students in the Gondol grouper hatchery training course being shown broodstock management techniques.

cobia, tuna and marine ornamentals and the name was changed to the Asia-Pacific Marine Finfish Aquaculture Network (APMFAN).



Demonstration of tank management and feeding techniques.

The overall objective of the network is to promote cooperation to support responsible development of marine finfish aquaculture within the Asia-Pacific region. Network activities are particularly directed at development of marine finfish aquaculture that:

- provides an alternative source of income and employment for coastal people, especially those currently engaging in destructive fishing practices;
- provides a quality alternative source of fish to wild-caught species, including fish fingerlings, that may be captured using destructive fishing techniques;
- contributes to protection of endangered reefs and reef fish from the pressures of illegal fishing practices through responsible aquaculture development;
- promotes environmentally sustainable marine fish culture practices by addressing

environmental constraints to marine fish culture associated with present practices, such as feed and fingerling supply; and

- promotes diversification of marine fish culture species appropriate to local economies and markets.

With such diverse and complex problems there is a need to share knowledge and experience to assist in finding solutions. The network provides the platform for cooperation in the Asia-Pacific region where aquaculture specialists can work with government agencies, non-government organisations, the private sector, communities and markets to ensure that aquaculture is integrated into broader objectives of conservation and poverty alleviation in coastal areas.

Communication

Facilitating communication between researchers, managers and industry is a central platform for the APMFAN.

Electronic communication

The communication strategies adopted by the network reflect the rise of internet-based communication methods, particularly e-mail and the World Wide Web. The use of electronic communication strategies allows rapid and widespread dissemination of information at relatively low cost.

The network produces two e-newsletters:

- A fortnightly e-news service with brief items on recent developments in marine finfish aquaculture; and
- A quarterly e-magazine that covers research and development issues in more depth, including invited contributions from network participants.

The APMFAN web site (www.enaca.org/grouper/) provide an information resource on marine finfish aquaculture, including archived articles from technical experts throughout the Asia-Pacific region, workshop proceedings and presentations, and contact details for those wishing to obtain more information about the subject.



Course participants observe the preparation of live feeds culture.



Students obtain 'hands-on' experience in the grading and sorting of juveniles.

Workshops

Workshops have proven to be an ideal forum for facilitating an exchange of ideas and experiences between grouper aquaculture researchers, aquaculture managers and industry. The high level of regional interest in marine finfish aquaculture has supported workshops at various centres throughout the region, including Thailand, Australia, Indonesia, the Philippines and Vietnam. This ability to utilise network resources to hold workshops in different locations has allowed many local representatives to participate, who would otherwise find it difficult to attend.

A major feature of the workshops has been the development of individual projects to support the network's research, development and extension program (see below). For example, the network workshop held in Hat Yai, Thailand, in April 1999 identified a number of needs for enhancing the sustainability of grouper aquaculture in the region with particular emphasis on grouper viral diseases. Based on these recommendations, network participants developed several projects that were subsequently funded by APEC, including:

- the publication of a husbandry and health manual for grouper, coordinated by the Southeast Fisheries Development Centre's Aquaculture Department; and
- the development of a regional research program on grouper virus transmission and vaccine development, assisted by the fish health section of the Asian Fisheries Society and the Aquatic Animal Health Research Institute, Thailand.



On completion of the course participants were presented with an official certificate of accomplishment.

Publications

Publications developed by the network are listed in Appendix 2. An excellent example of the strength of the networking approach to developing extension information is the Husbandry and Health Manual for Grouper. Access to network participants provided the coordinating agency, SEAFDEC AQD, with information and

experience from grouper aquaculture researchers and practitioners throughout the Asia-Pacific region. Following publication of the original English version, network participants provided translation into local languages: Filipino, Indonesian, Mandarin, Thai and Vietnamese. The result was a high-quality publication of direct application to farmers in the major grouper farming countries of Southeast Asia.

Staff exchanges

To encourage cooperation and information exchange amongst APMFAN partners, the network has supported staff exchanges between participating institutions (funded by both ACIAR and APEC). These exchanges have supported the development of human resources, provided a basis for capacity building, and ensured the transfer of new technology on various aspects of grouper culture to participating economies.

Research, development and extension coordination

A major focus of APMFAN has been to provide a structure to help coordinate the overall research effort within the region. This approach has been used to minimise overlap and prevent duplication of research effort on marine finfish aquaculture.

To achieve this, APMFAN has developed a program/project structure, where individual projects contribute to a program of activities. The structure of the APMFAN program is:

- 1 Production technology
 - 1.1 Broodstock
 - 1.2 Larviculture
 - 1.3 Nursery
 - 1.4 Grow-out
 - 1.5 Post-harvest
- 2 Environment
- 3 Marketing and Trade
- 4 Food safety and certification
- 5 Socio-economics and coastal livelihoods
- 6 Fish health
- 7 Training and extension

The network works with institutions and projects operating throughout the region undertaking research, development and extension activities on these different components in

a complementary and structured way, sharing experiences through the network, and, where possible, integrating activities between network partners.

The program structure facilitates gap analyses to identify research needs. For example, while there was a relatively high level of effort focussed on developing production technology for groupers and other high-value marine finfish, there had been relatively little work done on the socio-economic aspects of marine finfish aquaculture. Identification of this gap in the program allowed the development of a socio-economic study of Indonesian marine finfish hatcheries carried out by staff of SEAFDEC AQD, QDPI and NACA and funded by APEC and ACIAR (Siar et al. 2002). This socio-economic assessment indicated that these hatcheries are an important source of employment and economic benefits in northern Bali, and that the continued development of the marine finfish hatchery sector can provide valuable livelihoods for coastal communities.

Technology uptake

APMFAN has a strong focus on 'hands-on' training to facilitate technology uptake by farmers. An example of this is the Regional Grouper Hatchery Production Course, run at the Gondol Research Institute for Mariculture, Bali, Indonesia, for the last two years. The Gondol course provides hands-on training for a limited number (~15) of participants at a centre renowned for its excellence in developing production technology for marine finfish, particularly groupers.

The success of the course is evident from the results that have been achieved by course participants. In Thailand, Indonesia, Vietnam, Malaysia and Australia course graduates have been able to apply the techniques learnt from the training and have successfully produced grouper fingerlings, including *Epinephelus coioides*, *E. fuscoguttatus* and *Cromileptes altivelis*. Further courses are planned based on these successes.

Other network partners have also incorporated recent research results into their training courses. For example, SEAFDEC AQD has incorporated recent technological improvements

in grouper hatchery production into their regular Marine Finfish Hatchery course, and the Department of Primary Industries and Fisheries, Queensland, has run a series of workshops for farmers interested in grouper aquaculture in Australia. The Gondol Research Institute for Mariculture has run several courses in Indonesia for local farmers and fisheries officers.

Through these training courses, APMFAN has spread the impact of the network's research outcomes, including those of the ACIAR project, beyond the agencies that are formally involved in the project, and has provided direct technology transfer to farmers.

Conclusion

The coordinated and structured approach adopted by the network has proved to be effective in supporting research in marine finfish aquaculture, and in translating some of the research outcomes to development activities. APMFAN will continue to share knowledge and experience across the region. It is presently building its scope of activities to cover a wide range of marine fish and other species. Further work is also being undertaken on formalising

the participation of institutes within the network. The model is also being considered for other mariculture species and commodities, thus providing a wide range of mariculture options for coastal development in the region.

The building of further partnerships with government, the private sector and NGOs will be essential to continue the success of the network, as part of a concerted Asia-Pacific regional collaborative effort to address unsustainable fishing practices and poverty in coral reef and other coastal areas through responsible marine fish aquaculture development.

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APPENDIX 1

Development of Sustainable Marine Finfish Aquaculture in the Asia-Pacific Region

Needs Evaluation

To support the widespread dissemination of project results, the ACIAR project organised a *Regional Workshop on Sustainable Marine Finfish Aquaculture for the Asia-Pacific*, held at HaLong City, Vietnam, from 30 September to 4 October 2002. The workshop was funded by ACIAR, the Australian Academies of Technological Sciences and Engineering (through the Department of Education Science and Training, Frontiers of Science and Technology Missions and Workshops element of the Innovation Access Program) and by the Government of Vietnam.

The workshop attracted over 80 participants from Australia, Brunei Darussalam, China, Hong Kong SAR, India, Indonesia, Malaysia, Myanmar, Philippines, Solomon Islands, Thailand, Vietnam, and Europe, including representatives of the Asia-Pacific Economic Cooperation Fisheries Working Group, Food and Agriculture Organisation of the United Nations, International Marinelife Alliance, Marine Aquarium Council, Network of Aquaculture Centres in Asia-Pacific, The Nature Conservancy, and The WorldFish Centre.

The overall objectives of the workshop were to:

1. Provide detailed technical results of ACIAR project *FIS/97/73 Improved hatchery and*

grow-out technology for grouper aquaculture in the Asia-Pacific region.

2. Provide a forum for young researchers involved in the development of sustainable marine finfish aquaculture in the Asia-Pacific region to present their results and interact with other researchers.
3. Review the R&D needs for sustainable marine finfish aquaculture development in the Asia-Pacific region.
4. Identify potential collaborative projects to assist the development of sustainable marine finfish aquaculture development in the Asia-Pacific region.

To achieve the latter two objectives, the workshop participants formed discussion groups to:

- Identify constraints to the development of sustainable marine finfish aquaculture in the Asia-Pacific region;
- Identify activities required to address these constraints/issues (these may include: research and development, policy development, training, extension, etc.);
- Prioritise these activities (assigned high (H), medium (M) or low (L) priority).

Discussion Group	Chair	Rapporteur
<i>Hatchery</i>		
Broodstock	Joebert Toledo	Elizabeth Cox
Larval rearing	Ketut Sugama	Mike Rimmer
Larval feeds	Kevin Williams	Richard Knuckey
<i>Grow-out</i>		
Nursery	Clarissa Marte	Elizabeth Cox
Grow-out	Le Thanh Luu	Mike Rimmer
Environment	Yvonne Sadovy	Mike Phillips
<i>Other issues</i>	Pedro Bueno	Mike Rimmer

During the subsequent plenary sessions, the results of each discussion group were presented

to the workshop for further discussion and clarification. As well, workshop participants identified the institutions which are currently undertaking or are prepared to undertake research in each topic area.

The outcomes of this review are listed here to provide an overview of regional research needs to support the continued development of sustainable marine finfish aquaculture, and to indicate where research, development and extension efforts are already taking place in regard to these topics.

Hatchery

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Broodstock				
Broodstock supply	History of wild caught broodstock unknown (age, reproduction longevity, does spawning and/or egg quality decrease in older fish?)	<ul style="list-style-type: none"> • Document stock mortalities, age and record reproductive history of individuals across centres • Database, sharing of information between centres; long-term goals due to nature of collecting data • Centralisation of otolith reading to standardise results 	H	
	Identification of species selection criteria; use existing selection criteria matrices	<ul style="list-style-type: none"> • Individual countries to develop criteria most suitable to local markets/conditions; exchange of these criteria between countries? (The mechanism for exchange needs to be determined). Assistance from for example NACA • Importance of economic surveys in species selection 	H	
	Impact of fishing pressure on spawning aggregations in the wild How will this affect the supply of broodstock for captive breeding?	<ul style="list-style-type: none"> • Policy — protection for some spawning aggregations (Note: this is a broader fisheries sustainability issue — the discussion here focused on aquaculture aspects) • Possible source to access spermiating males (collect milt and return to wild) 	H	
	Difficulty of accessing males of some species	<ul style="list-style-type: none"> • Techniques already developed for some species • Further work may be required with specific species 	L	
Broodstock management	Development and assessment of captive breeding populations Important for future sustainability to reduce our reliance on wild caught fish as broodstock	<ul style="list-style-type: none"> • Husbandry techniques — feed and feeding, holding systems, disease prevention and control • Water quality management 	Very high	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
	Implications of maintaining genetic diversity in captive breeding programs Option to exchange captive bred broodstock between centres to maintain genetic diversity (Note issues regarding disease transfer and limited knowledge regarding genetic populations across regions) Benefit to reduce the need for individual institutions to hold large numbers of broodstock	<ul style="list-style-type: none"> Population genetics across geographic areas to identify different strains within species 	M	
Spawning	Optimising hormone induction techniques — dosages, frequency, sexes induced	<ul style="list-style-type: none"> Collation/dissemination of reproduction techniques used 	H	
	Control of seasonal reproduction techniques Cryo-preservation of sperm	<ul style="list-style-type: none"> Environmental control of reproduction 	H	
Egg supply/ quality	Effect of broodstock nutrition on egg quality	<ul style="list-style-type: none"> Expansion into other areas (to be identified) of nutrition required 	H	
	Development of egg quality criteria	<ul style="list-style-type: none"> Certification of standards (for egg sales) 	M	<ul style="list-style-type: none"> Note: Existing work (fatty acids) on snapper, grouper at SEAFDEC.
Disease	Develop techniques/protocols to acquire and maintain pathogen-free broodstock	<ul style="list-style-type: none"> Research required to increase our understanding of susceptibility to and transmission of pathogens Training and dissemination regarding collection and handling Study on vertical transmission of passive immunity from broodstock to larvae 	H	
	Need for coordination of viral disease testing protocols	<ul style="list-style-type: none"> Coordinated facility(ies) for nodavirus testing; one central facility to provide expertise, primers, etc. 		Current APEC involvement in disease issues: Refer to APEC Regional Research Program on Grouper Virus Transmission and Vaccine Development — needs to be taken forward.
Larval rearing				
Pre-feeding stages	Egg quality	<ul style="list-style-type: none"> Broodstock nutrition improved 	H	
	Yolk absorption rate — should have yolk left when mouth opens Handling of eggs and larvae	<ul style="list-style-type: none"> Information on egg handling needs to be transferred to private sector — training, extension 	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Early feeding stage (rotifer)	Suitable natural feed for initial feed	• Technology transfer for SS-strain rotifer culture — training, extension	L	
	Some countries — difficulty in producing SS-strain rotifers Difficulty in producing suitable numbers of copepods New species (Napoleon wrasse, coral trout, blue tang) — lack of suitable first feed organism	• Research on copepod culture technology	H	
Late feeding stage (<i>Artemia</i>)	<i>Artemia</i> expensive	• Research: optimal enrichment method	H	
	Nutrition — requires enrichment Nutritional requirements may change through development stages Rearing tank management	• Training in rearing procedures		
Metamorphosis	Cannibalism	• Research to reduce cannibalism, for example grading, feeding frequency, exercising, fish density — behavioural studies • Training in grading, management techniques	H	
Disease	VNN	• Optimal management will reduce incidence of VNN • Research on nodavirus	H	
	Bacterial diseases	• Improve immune response — research • Long-term research: vaccine • Probiotics	L	
	Parasites in extensive pond larval rearing	• Research on egg washing (ozone, iodine, UV) — effects on eggs and embryos • Research on prevention/control of parasites in ponds	H	
Larval biology, nutrition		• Research in larval biology • Research in larval nutrition	L	
Chemical use	Deformities in larvae/juveniles	• Training and extension on use of chemicals	H	
	Chemicals (and antibiotics) used prophylactically	• Policy development/education on responsible use of chemicals and antibiotics — good practice guidelines/standards • Research on chemical application	H	M
Larval Feeds				
Rotifers	Need for improved management	• Contact Europeans (and others) who have developed intensive rotifer culture	H-	immediate need
	Identify local small strains (SSS-rotifer)	• Develop culture method that continually harvests the smallest rotifers		
	Overcome problems in countries where rotifers are sourced from open ponds	• Transfer of existing technology, extension • Disease transfer, disinfection methods for all live-prey species		

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Copepods	Difficulty in maintaining culture Difficulty in getting numbers of n1–n2 nauplii Species selection	<ul style="list-style-type: none"> Identify why copepod is better feed for larvae Try to compensate deficiency in rotifer Develop mass culture technology 	M-H (long-term)	
<i>Artemia</i>	Not issue at the moment but still reliant on Great Salt Lake supply	<ul style="list-style-type: none"> Training for local farmers on best practice use, decapsulation, nutritional enhancement etc. Put in place an effective extension operation which can work with local bodies to design extension specific to the country, companies can be involved (INVE) 	L	
Nutritional enhancement	Most enhancement geared around enrichment for temperate species; need more results for tropical species Best-practice for use of commercial products Use of bacteria, probiotics	<ul style="list-style-type: none"> More nutritional information on requirements of target species Extension Protocol where you design specific enrichment composition 	H	
Artificial feeds	Under-utilised Have to change total management of farm, water management, tank design Reluctance to use, high cost	<ul style="list-style-type: none"> Need information on how to wean onto artificial diets Weaning methods and water quality management 	M-H	
Microalgae	Quality control of microalgae, maintenance of cultures	<ul style="list-style-type: none"> Use of algal concentrates — survey and assess available concentrates Develop a protocol for their use 	L	

Grow-out

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Nursery				
Holding systems Stage: post-metamorphosis to 2–3 cm plus 'tinies'. Duration = approx. 2 months	<p>Holding systems — high mortality of wild caught fry, no standardised management protocols</p> <p>Mortalities during and after transportation (of hatchery bred fry and wild caught juveniles).</p> <p>No current management protocols, particularly for wild caught juveniles; no standardised nursery systems</p> <p>Information about management of nursery systems is available</p>	<ul style="list-style-type: none"> Collation of existing practices and development of standardised procedures for handling and transport Information is available for some species Desired outcome — preparation of a manual that addresses optimal and standardised procedures for transportation and holding system maintenance Submit to APEC for possible funding 	M (easily done)	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Feeds	Difficulties weaning wild caught juveniles onto artificial diets	• Need to develop better weaning protocols	H	
	Farmers do not readily adopt artificial diets; there is a preference to continue feeding trash fish.	• Training/demonstrations to farmers on best practice weaning techniques	H	
	Feed availability/cost is an issue	• Need to develop an on-farm feed preparation method using local ingredients	M	
	Need to develop <i>Artemia</i> replacement diet for nursery phase	• Necessary to provide an information guide and training on feed composition, compile from existing information	H	
	Lack of information on some of the important micronutrients	• Further research is required to determine micronutrient requirements		
	Is wide size variation during nursery phase due to poor feeding management?	• Need further work on feed distribution and stocking density, shelters; work on feeding frequency has been done	H	
Cannibalism	Need to reduce the frequency of grading, stressful to fish	• Development of grading systems suited to species behaviour is needed; suggest assessment of available graders and associated mortalities	H	
	No knowledge about why cannibalism occurs	• Study behaviour of juveniles to assist in the development of effective solutions	H	
Disease	Transfer of diseases between centres/regions	• Develop quarantine procedures to address quality of seed for sale (local/import/export) • A future need to address certification of some hatchery operators in regard to developed quarantine protocols	H	
	Significant mortalities from disease outbreaks including viral and bacterial still occur			This has been addressed by the health and husbandry
	Grouper deformities — cause unknown	• Research focus may need to start in the hatchery phase, for example, with nutrition, physical handling	M	manual developed for grouper disease management.
	No prevention for viral diseases	• Development of vaccines and vaccination procedures and immuno-stimulants	H	
Grow-out				
Grow-out systems	Lack of information on pond grow-out (Indonesia, Vietnam and China have major grow-out systems) Remediation of effluent from pond culture	• Research: stocking density, pond management, water quality management; pond rotation, polyculture options	M	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Post-harvest				
Transport systems — live product	Sea transport for live fish — high mortality, high cost	• Research: improved transport	M	
	Chemical (anaesthetic) use in transport	• Extension, education	L	
Chilled and frozen product	Product quality at point of sale	• Research need	L	
	Product quality	• HACCP implementation • Value-adding opportunities	L (no immediate need)	
Product	Impact of feed substitution on product quality	• Research: product development and evaluation	L	
	Fish colour – market demand	• Research: optimise environmental conditions, feeds	L	
Environment				
Planning	Need for equity among resource users, access to common property	• Govt policy to deal with equitable planning: including mapping of suitable areas (including bio-physical, social, economic, environmental, and legal/ institutional aspects)	H	
	Need to identify proper places, potential areas, zones, and resource allocation			
	Need for awareness of environmentally sound planning and operational practices at both govt policy and producer level	• Planning stages to incorporate carrying capacity, and environmental assessment, take account of other sectors (land and water based activities that may affect aquaculture) and promote integrated planning	H	
	Lack of cooperation/coherence between policy makers, private sector and researchers	• Consider community based management options	H	
	Lack of capacity to develop and implement planning and management for coastal mariculture	• Information on good practices for planning, extension of such practices	H	
	'Clustering' behaviour and impacts	• Prepare a set of recommendations on good planning practice guidelines for adoption by APEC economies	H	
Carrying capacity (<i>capacity of area to sustain cages, and limits of coastal environment to sustain mariculture</i>)	Lack of assessment methodologies for practical application in tropical fish culture	• Develop principles and practical guidelines (rules of thumb) for applying carrying capacity in tropical environments	H	
	Use of trash fish: (1) price, industry sustainability; (2) impacts on wild fisheries; (3) water pollution	• Extend feed research results to feed companies	L/M	
	Lack of understanding/ consideration of biological limits to marine fish culture (feed, wild juvenile supply)	• Promote awareness of unsustainability of wild juvenile supply	L	
	Deterioration of coastal environments	• Monitoring of aquaculture development	M	
		• Licensing based on suitability and carrying capacity	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Impact assessment	EA is tedious and costly to apply, particularly for small-scale developments	• Promote continuous monitoring	M/H	
	Lack of information on nutrient loadings — characterisation of waste	• Assessment of nutrient loadings and budgets from marine fish culture	H	
		• Clarify responsibilities and scope/ requirement for EA (ideally within the planning process)	H	
Disease	Disease outbreaks, particularly viral diseases (VNN, iridovirus)	• Development and use of hatchery reared SPF stock	H (long-term)	
	Trans-boundary spread of pathogens	• Research on vaccines	H	
	Lack of knowledge	• Implement Asia regional guidelines on health management and responsible movement		
		• Further extension efforts on health management based on 'good husbandry practices' (health management manual)	H	

Socio-economics, marketing

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
Socio-economics, livelihoods	Need to identify beneficiaries of aquaculture development Willingness of people in coastal communities to accept alternative livelihoods	• Socio-economic evaluation of aquaculture • Need to ensure that there is a technology transfer phase • Encourage network participants to share experiences in this area as part of the STREAM APEC study on mariculture to provide alternative livelihoods		
Aquaculture/capture fisheries interactions	Seed supply — competition between fisheries and aquaculture sectors	• Better documentation on seed transportation		
	High levels of mortality in seed capture and transportation Destructive/wasteful fishing practices	• Extension material to improve seed handling techniques • Policy development: local seed used locally (increases appreciation of value of resource)		
	Use of 'trash' fish	• Policy development: promotion of compounded feed use, for example licence condition.		
Market	Certification, eco-labelling	• Voluntary codes of practice	M	
	Better meeting market requirements	• Market demand information • Forecasting	H	

Topic	Constraint/issues	Activities required	Priority (H/M/L)	Institutional commitment
	Need to improve market chain	<ul style="list-style-type: none"> • Increase communication and interaction between producers and market end • Develop farmer cooperatives to improve bargaining power • Promote aquacultured fish as higher quality, ciguatera free product 	H	
	<p>Lack of understanding/uncertainty on long-term market demand for marine finfish</p> <p>Consumer perception regarding quality of aquaculture/wild product, especially fat quality</p> <p>Focus has been on high-value species</p>	<ul style="list-style-type: none"> • Include market assessment for non-live fish markets • Feeds development research to incorporate assessment of end-product quality (see grow-out) • Need to focus on other species that are maricultured • Market study for full range of marine fish in A-P region 		
GMOs	<p>Attitude to GMO technology</p> <p>Market access versus improved productivity</p>		L	
General recommendations on networking	Information	<ul style="list-style-type: none"> • Prioritise activities of network — seek funding from donor agencies for specific activities/projects (APEC, ACIAR) • Hold additional marine finfish aquaculture workshop in Vietnam (invitation of Vietnamese Government) • Ensure that information is further disseminated to national/regional extension services 		
	Training, technical exchanges	<ul style="list-style-type: none"> • (many covered in recommendations) 		
	<p>Institutional commitment</p> <p>Private sector involvement</p> <p>Many fish farmers are small-scale operations, cannot attend large regional workshops</p>	<ul style="list-style-type: none"> • Need to ensure that research results are extended to private sector • Need to ensure that small-scale fish farmers are kept informed of technological improvements 		

APPENDIX 2

Project and Asia-Pacific Marine Finfish Aquaculture Network Publications

Asia-Pacific Grouper Network Publications

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