DEVELOPMENT OF PELLETED FEED FOR HUMPBACK GROUPER, *CROMILEPTES ALTIVELIS*

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ABSTRACT

DEVELOPMENT OF PELLETED FEED FOR HUMPBACK GROUPER, *CROMILEPTES ALTIVELIS*

Four growth trials were conducted in the effort to develop cost-effective diets for the humpback grouper, *Cromileptes altivelis*, a notoriously slow growing carnivorous marine fish species. The first three trials were related to the most important macro-nutrients in fish diets, namely protein, lipid, and carbohydrate, respectively. The findings from these trials were used as the basis for formulation of cost-effectives diets for humpback grouper in the final trial. In the first trial, six experimental diets were formulated to replace fish meal protein with a local poultry by-product meal (PBM) at 50, 75 or 100 % level, or an imported PBM at 75 or 100 % replacement level, respectively. The results from this study indicated that good quality terrestrial PBM can successfully replace more than half the protein from marine fish meal in the diets for humpback grouper. However, total replacement of fish meal with PBM might be constrained by lowered nutrient digestibility and limiting essential amino acids. In the second trial, five fish meal-based practical diets were formulated to contain iso-ingredients but with different sources of lipids [crude palm oil (CPO), refined, bleached, deodorized, palm olein (RBDPO), soybean oil (SBO), or canola oil (CNO)] and their performance compared with the control diet that contained cod liver oil (CLO) as the added lipid source. There were no significant differences ($P>0.05$) in terms of growth, feed utilization, body indices, and whole-body proximate composition among dietary treatments. Replacement of dietary CLO with the tested vegetable oils produced fish with lower n-3 highly unsaturated fatty acids and increased levels of 18:2n-6 in the muscle and liver. The present study demonstrated that various vegetable oils can be used in fish meal-based dietary formulation for humpback grouper without compromising growth or feed utilization efficiency. In the third trial, the effect of dietary carbohydrate sources and levels on growth and feed utilization of humpback grouper was examined. Six experimental diets were formulated to contain corn starch, tapioca starch or dextrin at 10% and 20% inclusion levels. The growth, feed utilization efficiency, and body indices of humpback grouper were independent of carbohydrate levels and sources. In view of the lower price of tapioca starch than the price of corn starch and dextrin, inclusion of tapioca starch in the practical diets for humpback grouper will be able to reduce feed cost. In the final trial, two diets (U-MS/SM1 and U-MS/SM2) were formulated with a blend of ingredients based on the result of the previous three trials. The performance of the experimental diets was compared with a locally sourced marine fish feed, an imported marine fish feed, and trash fish. Growth and FCR of humpback grouper fed experimental diet U-MS/SM2 were superior to other fish groups. FCRs of the pelleted feed ranged from 1.3 to 2.4 and significantly better ($P<0.05$) than the trash fish (5.0). The survival rate of fish fed trash fish was also significantly lower ($P<0.05$) than that of other fish groups. The cost analysis indicated that, feeding humpback grouper with either diets U-MS/SM1 or U-MS/SM2 were able to reduce the feed cost per kg of humpback grouper produced. The diets formulated in the present study can be used by grouper farmers as alternatives to the current practice of feeding trash fish. In conclusion, the success in using reduced amount of fish meal and fish oil in humpback grouper diets will be able to provide economical and ecological benefits to the local and global aquaculture industry.

ABSTRAK

Empat percubaan tumbesaran telah dijalankan dalam usaha untuk menghasilkan diet berkos-efektif untuk kerapu tikus, Cromileptes altivelis, spesies ikan marin karnivor yang mempunyai tumbesaran yang perlahan. Tiga percubaan yang pertama masing-masing adalah berkaitan dengan makro-nutrien yang paling penting dalam makanan ikan iaitu protein, lipid, dan karbohidrat. Hasil kajian dari ketiga-tiga percubaan ini dijadikan asas dalam formulasi diet berkos-efektif pada percubaan yang terakhir. Dalam percubaan pertama, enam diet diformulasi untuk mengandungi tepung hasil sampingan ternakan ayam (PBM) pada kadar menaik. Tepung ikan digantikan dengan PBM tempatan masing-masing pada kadar 50, 75, atau 100%, ataupun dengan PBM impot masing-masing pada kadar 75 atau 100%. Hasil kajian ini menunjukkan bahawa PBM yang berkualiti tinggi boleh menggantikan lebih daripada separuh protein yang berasal daripada tepung ikan. Walaubagaimanapun, penurunan penghadaman nutrient dan asid amino penghad mungkin menjadi penghalang kepada penggantian tepung ikan secara keseluruhan. Dalam percubaan yang kedua, lima diet praktikal yang berasaskan tepung ikan dihasilkan dengan mengandungi bahan-bahan yang sama tetapi sumber lipid yang berbeza [minyak mentah kelapa sawit (CPO), minyak masak kelapa sawit (RBDPO), minyak kacang soya (SBO), dan minyak canola (CNO)] dan dibandingkan prestasinya dengan diet yang berasaskan minyak hati ikan kod (CLO). Tidak terdapat perbezaan bererti ($P>0.05$) dari segi pertumbuhan, penggunaan makanan, index badan, dan komposisi proksimat badan ikan yang diberi makan diet berbeza. Penggantian CLO dengan minyak sayuran telah menyebabkan penurunan kandungan asid lemak n-3 tidak tepu dan meningkatkan kandungan 18:2n-6 di dalam otot dan hati ikan. Hasil kajian ini menunjukkan bahawa pelbagai jenis minyak sayuran boleh digunakan di dalam diet ikan yang berasaskan tepung ikan tanpa menjejaskan pertumbuhan dan kecekapan penggunaan makanan. Dalam percubaan yang ketiga, kesan sumber dan kandungan karbohidrat berbeza terhadap pertumbuhan dan kecekapan penggunaan makanan kerapu tikus telah dikaji. Enam diet diformulasi untuk mengandungi kanji jagung, kanji ubi kayu, atau dextrin pada kadar 10 dan 20%. Tumbesaran, kecekapan penggunaan makanan, dan index badan tidak dipengaruhi oleh jenis dan kadar karbohidrat dalam diet. Memandangkan kanji ubi kayu lebih murah daripada kanji jagung dan dextrin, penggunaanya dalam diet praktikal kerapu tikus akan dapat mengurangkan kos makanan. Dalam percubaan yang terakhir, dua diet telah diformulasi (U-MS/SM1 and U-MS/SM2) menggunakan campuran bahan-bahan berasaskan keputusan tiga kajian yang terdahulu. Prestasi diet ini dibandingkan dengan makanan ikan marin tempatan, makanan ikan marin impot, dan ikan baja. Tumbesaran dan kadar penukaran makanan (FCR) ikan yang diberi makan U-MS/SM2 lebih baik daripada kumpulan ikan lain. FCR bagi makanan buatan adalah dalam julat 1.3 - 2.4 dan lebih rendah daripada ikan baja (5.0). Kadar kemandirian bagi ikan yang diberi makan ikan baja juga lebih rendah ($P<0.05$) daripada kumpulan ikan lain. Analisa kos menunjukkan penggunaan diet U-MS/SM1 atau U-MS/SM2 boleh mengurangkan kos makanan dalam penghasilan kerapu tikus. Formulasi diet ini boleh digunakan oleh penternak kerapu sebagai alternatif kepada penggunaan ikan baja yang sedang diamalkan. Sebagai kesimpulan, kejayaan mengurangkan kandungan tepung ikan dan minyak ikan dalam diet akan memberi faedah ekonomi dan persekitaran kepada industri akuakultur tempatan dan global.

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%	percentage
°C	degree celcius
ppt	part per thousand
kg	kilogram
g	gram
mg	milligram
ug	microgram
L	liter
mL	milliliter
min	minute
IU	international unit
α	alfa
h	hour
d	day
♀	female
♂	male
n-3	omega-3
n-6	omega-6

LIST OF ABBREVIATION

ADC	Apparent digestibility coefficient
ANOVA	Analysis of variance
BBM	Broad bean meal
BM	Blood meal
CGM	Corn gluten meal
CNO	Canola oil
CNP	Canola protein concentrate
CO	Corn oil
ComDiet1	Commercial diet 1
ComDiet2	Commercial diet 2
CP	Coffee pulp
DHA	Docosahaexanoic acid
DM	Dry matter
EAA	Essential amino acid
EPA	Eicosapaentanoic acid
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
FM	Fish meal
FO	Fish oil
FPBM	Feed grade poultry by-product
GM	Gambusia meal
HE	Hexane:ethyl acetate
HSI	Hepatosomatic index
HUFA	Highly unsaturated fatty acids
IPF	Intraperitoneal fat
LCFA	Long-chain fatty acids
LM	Lupin kernel meal
LO	Linseed oil
MBM	Meat and bone meal
MCFA	Medium-chain fatty acids
ME	Metabolizable energy

MM	Meat meal
NPU	Net protein utilization
PBM	Poultry by-product meal
PER	Protein efficiency ratio
PPBM	Pet food grade poultry by-product
PSM	Pea seed meal
PUFA	Polyunsaturated fatty acids
RBDPO	Refined, bleached, deodorized palm olein
SBM	Soy bean meal
SBO	Soy bean oil
SFO	Sun flower oil
SGR	Specific growth rate
SPC	Soy protein concentrate
TM	Turkey meal
U-MS/SM1	Universiti-Malaysia Sabah/Sains Malaysia Diet 1
U-MS/SM2	Universiti-Malaysia Sabah/Sains Malaysia Diet 2
VSI	Viserosomatic index
WGM	Wheat gluten meal

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Aquaculture of high-value marine finfish continues to develop rapidly in the Asia-Pacific region as a result of growing market demand for live reef food fish (Rimmer, 2008). Grouper is one of the most popular species traded in the live reef food fish markets, especially in Hong Kong and Southern China (Laining *et al.*, 2003). Global grouper production increased dramatically in recent years, recorded as 79,816 in 1990 and increased to 189,789 metric tones in 2005 (FAO, 2005). Grouper production through aquaculture alone was reported to increase from 59,146 to 65,362 tonnes from 2004 to 2005, an increase of 11% mainly in the Asia-Pacific region (Rimmer, 2008). Recent years have witnessed an increasing interest in the commercial culture of various grouper species especially in Taiwan, Indonesia, Thailand, and Malaysia. They are preferred in aquaculture because of their desirable taste, hardiness in captivity, efficient feed conversion and rapid growth (Boonyaratpalin, 1997; Millamena, 2002), apart from high market demand.

Humpback grouper, *Cromileptes altivelis* is one of the most sought after grouper species mainly due to its high value and stable price in the live fish trade. It is listed in the World Conservation Union (IUCN) red list of threatened species. The culture started using the traditional method, which is characterized by the use of wild seed and trash fish feeding. In recent years, improved breeding and culture techniques of humpback grouper have led to increasing interest in the commercial culture of this species (Toledo *et al.*, 1993; Sugama *et al.*, 2000; Senoo *et al.*, 2004). Unfortunately, this particular grouper species is a notoriously slow growing fish which attains market size in more than one year. This long grow-out period implies higher feed input and cost of maintenance. Increasing price, shortage of supply, variable quality and poor feed conversion ratios when trash fish is used for feeding cultured grouper indicates that trash fish is not an economical diet. The cost of marine fish

production was estimated to be reduced by 20% and profit may also be doubled by switching from trash fish to pelleted feed (Orachunwong *et al.*, 2005). Therefore, the development of cost-effective diets is critical towards the success of any commercial culture of the humpback grouper.

Protein, lipid, and carbohydrate are the three major components of pelleted fish feeds. Protein and lipid are indispensable in fish diets due to the requirement of fish for essential amino acids and fatty acids. Addition of carbohydrate in fish diets is known to improve utilization of protein and lipid. Humpback grouper requires high protein of about 50% and moderate lipid level of about 9-12% for optimal growth and nutrient retention (Williams *et al.*, 2004). No consistent results about the utilization of different carbohydrates with varying complexities among fish species of different food habits have been achieved, including grouper species (Shiau & Lin, 2001; Shiau & Lin, 2002).

Considerable attempts have been carried out to evaluate alternative dietary ingredients in fish diets, especially on the replacement of expensive fish meal and fish oil with more cost-effective alternative protein and lipid sources. These efforts have been focused on identifying and utilizing feed ingredient sources whose global production can keep pace with the growth of the aquaculture sector. Of the different sources of animal proteins and dietary fats available for use within compound aquafeed, the largest in terms of volume available are terrestrial animal by-product meals and plant oils (Tacon *et al.*, 2006). The potential of these feed ingredients in grouper feeds requires thorough evaluation. In the present study, efforts to reduce the use of fishery resources as feed inputs in grouper feeds were carried out through a series of trials using humpback grouper fingerlings at the hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah (Figure 1.1).

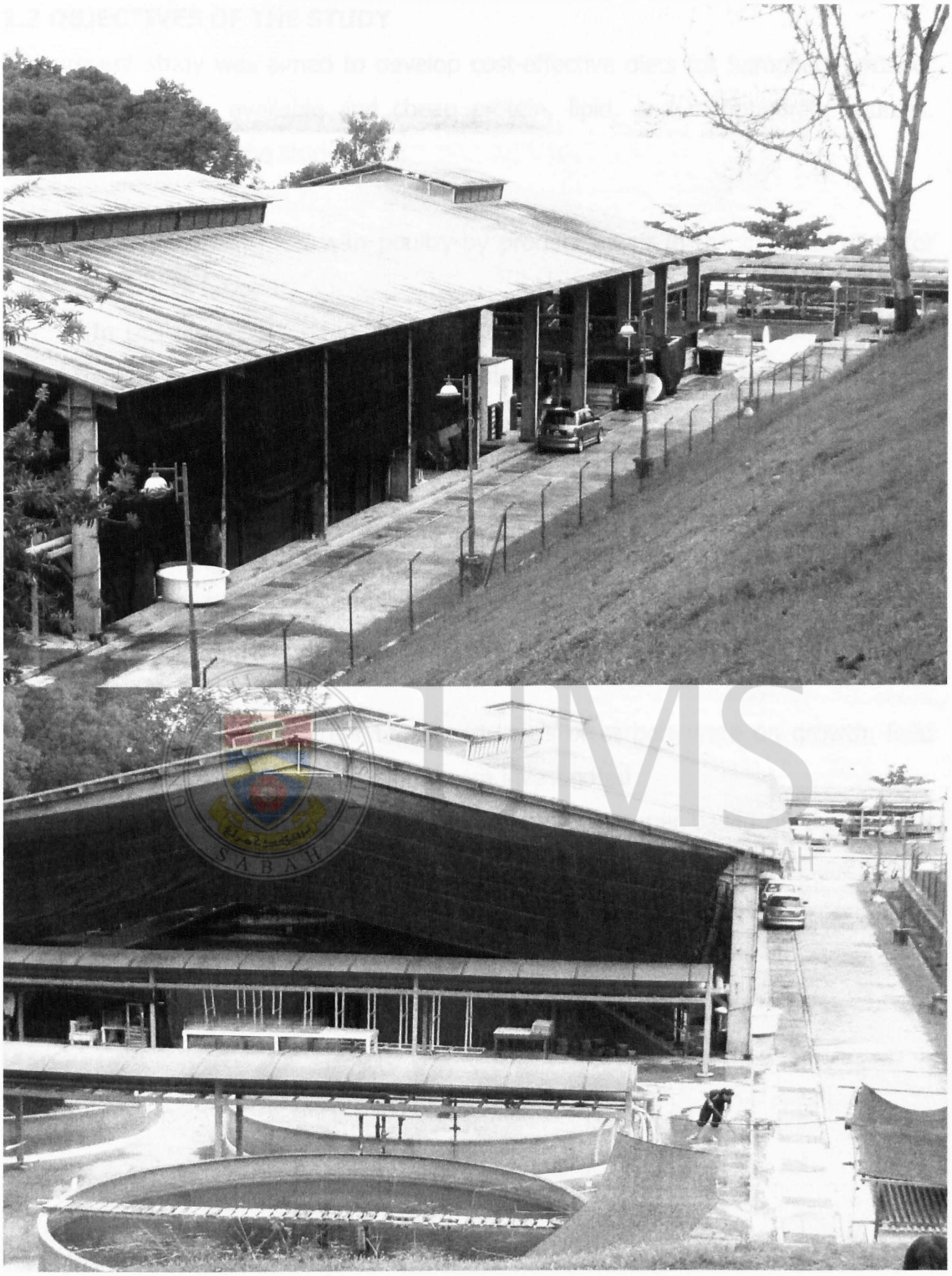

Figure 1.1: Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah

1.2 OBJECTIVES OF THE STUDY

The present study was aimed to develop cost-effective diets for humpback grouper using commercially available and cheap protein, lipid, and carbohydrate sources. Specific objectives of the study were:

a. To replace fish meal with poultry-by product meals in the practical diets for humpback grouper.

   -In this study, the effect of partial and total replacement of fish meal with poultry by-product on growth, feed utilization, nutrient digestibility, and body composition of fish were studied.

b. To replace fish oil with vegetable oils in the practical diets for humpback grouper.

   -The effects of fish oil replacement with vegetable oils on growth, feed utilization, and muscle and liver fatty acid composition were investigated.

c. To investigate the utilization of different types and levels of carbohydrate by humpback grouper.

   - The effects of different types and levels of carbohydrate on growth, feed utilization and body composition were investigated

d. To develop cost-effective diets for humpback grouper based on the findings of the first three trials.

   -Growth, feed utilization, body composition, and cost analysis of experimental diets were compared with commercial diets and trash fish.

CHAPTER 2

LITERATURE REVIEW

2.1 TAXONOMY, BIOLOGY AND DISTRIBUTION OF GROUPERS

Groupers are classified under the serranid Subfamily Epinephelinae, which belongs to the order Perciformes. The family contains about 159 species of marine fishes in 15 genera. Groupers are sedentary fish species, which tend to spend considerable time in rocky cervices, seagrass beds, and sandy or silty areas, in tropical to sub-tropical waters along the coasts (Heemstra & Randall, 1993). Most grouper species are sequential hermaphrodites, starting out as male and changing the sex to female later in life (Shapiro, 1987). They are also highly fecund fish, with a predictable spawning cycle (Toledo *et al.*, 1993). Groupers can withstand salinity ranging from 15 to 45 ppt, with optimum temperature ranges from 22-28°C (Boonyaratpalin, 1997). Most groupers are predators, which feed on a variety of prey fishes, larger crustaceans, and cephalopods (Heemstra & Randall, 1993).

The genus *Cromileptes* is quite distinct from other grouper genera. The shape of the head is unique among serranid fishes, whereby they have ten dorsal-fin spines, which cannot be found in other grouper species (Figure 2.1). The body is compressed and covered with black-spots (Heemstra & Randall, 1993). *Cromileptes altivelis* is distributed widely in the Western Pacific from the southern Japan to Palau, Guam, New Caledonia, and southern Queensland (Australia); and in the eastern Indian Ocean from the Nicobars to Broome, Western Australia. *C. altivelis* was identified ad protogynous hermaphrodite. Males were present in the population as young as 4 years of age. A number of sexually transitional individuals between 6 to 10 years of age were also recorded. Sexual maturity was estimated to occur at 2 years of age and 330 mm fork length (Davies et al., 2006).

It is commonly known as humpback grouper, mouse grouper, high finned grouper or polka dot grouper. It is known as 'barramundi cod' in Australia, 'kerapu

tikus' or 'kerapu bebek' in Indonesia, 'señorita' in the Phillipines (Rimmer, 2004), and 'sarasa-hata' in Japan (Heemstra & Randall, 1993). Its popular local name is 'kerapu tikus' in Malaysia. In Sabah, this species is commonly called as 'kubing'.

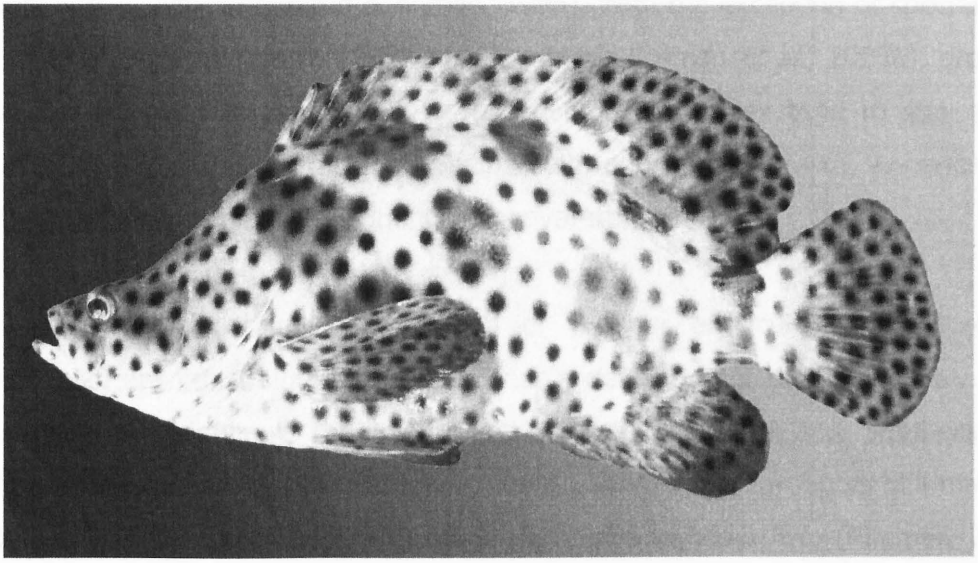

Figure 2.1: Humpback grouper, *Cromileptes altivelis* (photo credit: Prof. Shigeharu Senoo)

2.2 STATUS OF GROUPER AQUACULTURE

Grouper aquaculture has been practiced since long in many parts of the world, especially in the Asia-Pacific region. It was started using cage culture system along the coastal area. Worldwide, Taiwan and Indonesia are the major producers of cultured grouper, followed by Thailand and Malaysia (Rimmer, 2004). Most grouper species are high value marine fish in the live fish trade in Southeast Asia, commanding a wholesale price of up to around US$90-100/kg (Lau & Perry-Jones, 1999; Laining *et al.*, 2003). Humpback grouper normally fetch higher price than any other grouper species in this trade. Locally, the market price of humpback grouper in the seafood restaurant can reach RM135-180 per kg, and could increase up to RM160-350 per kg during festive seasons, especially the Chinese New Year (Senoo, 2002; Figure 2.2). The regional total production of groupers through aquaculture in 2001 may have been more than 23,000 metric tons and valued at around US$160 million (Rimmer, 2004). In Malaysia, grouper aquaculture started in the 1970s using brackish water ponds and floating net cages (Teng *et al.*, 1978). Production of

grouper through aquaculture in Malaysia was about 2,280 metric tons, with estimated value of around RM54,600 (Department of Fisheries (DOF), 2004-2005). Major cultured species of groupers are *Epinephelus coioides*, *E. malabaricus*, *E. tauvina*, *E. fascoguttatus*, *E. lanceolatus*, *Plectropomus leopardus*, *P. maculatus*, E. *areolatus*, *E. bleekeri*, and *C. altivelis* (Hussin & Ahmad, 1996). With the exception of species such as *E. akaara* (Boonyaratpalin, 1997) and *C. altivelis* (Usman *et al.*, 2005a), groupers exhibit rapid growth in captivity. As a sedentary fish, they tend to stay at the bottom of cages. This characteristic helps save energy for growth, enhance feed efficiency and reduce feeding frequency (Boonyaratpalin, 1997).

Despite the continuing expansion of grouper aquaculture, there remain several important constraints to the sustainable development of this industry. The two major constraints are the insufficient supply of fish fry and problems with nutrition (Boonyaratpalin, 1997; Rimmer, 2004). Wild-caught groupers still make up the bulk of the seedstock supply in many parts of Southeast Asia (Rimmer, 2004). Low survival rate at larval stages was reported as the main problem in hatchery production of groupers (Husain & Higuchi, 1980; Rimmer, 1997). Nevertheless, seed production techniques of several grouper species such as *Epinephelus sp.* and *C. ativelis* have been developed (Toledo *et al.*, 1993; Sugama *et al.*, 2000; Senoo *et al.*, 2004).

Trash fish has been traditionally used as the main feed source for cultured groupers. This method of feeding is preferred by most farmers due to the availability of trash fish, the perception of lower cost when using trash fish, compared to high price and limited supply of pelleted feed (Williams & Rimmer, 2005). The low usage of pelleted feed is also due to inadequate information on the use of the feeds and limited supply of weaned stock (Che Musa & Nuruddin, 2005). In 1998, The Asia-Pacific Marine Finfish Aquaculture Network was established to contribute to the improvement of 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. 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) (Rimmer *et al.*, 2004).