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Feed and feeding practices at farm level for marine finfish aquaculture in Asia-Pacific

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There are many marine finfish species cultured in the Asia-Pacific region and many of these still rely on trash fish as the sole food source for nursery and grow-out stages. Although there are some commercially produced formulated feeds, Asia farmers, particularly small scale operators, prefer the traditional methods of feeding trash fish to carnivorous species such as snapper, Asian seabass (barramundi) and groupers because of its availability and the farmer's view that pelleted feeds are more expensive. In contrast, Australian fish farmers exclusively use pelleted dry feeds for almost all farmed fish, including barramundi, groupers, snappers and Atlantic salmon. Southern blue fin tuna is the only marine finfish species in Australia where fresh fish (usually pilchards) is fed but even this industry is slowly changing to feeding pelleted dry feeds as better tuna formulations are developed.

Farm worker feeding pelleted feed at a Penang seabass farm.

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Feeding pelleted feed to threadfin at a marine fish farm in Johor.

Malaysia

Marine finfish farmers in Malaysia use a limited commercial formulated feed. Most carnivorous species are cultured on trash fish although several carnivorous and omnivorous species (eg. *Lates calcarifer* Asian seabass, *Eleuthonema tetradectylum* fourfingers threadfin and *Trachinotus blochii* pompano) use generic Asian seabass feed as the main feed source, supplemented with trash fish. Snapper and grouper species are mostly fed with trash fish. This is often of poor quality and consists of a mixture of unwanted fish species, usually very small in size.

However, other marine finfish species such as milkfish are fed with industrial bakery rejects or low-grade wheat flours. The growth rate of these fish is very slow, requiring a total of 18 months for 1 cm fry to reach a marketable size of around 300-500 grams.

Indonesia

The farming practice in Indonesia is a mixture of feeding pelleted dry feed produced by commercial feed companies, or trash fish. Although commercial feed for grouper is available in Indonesia, the price is still high, about US$1/kg. By comparison, the cost of trash fish is about US$ 0.35-0.59 per kg, depend on species and season. However, since trash fish contains a lot of water, around 70% or more, whereas pelleted dry feed contains only about 10% water, the pelleted feed is less expensive than trash fish when the comparison is made on the basis of the amount of nutrients each provides. Cost is only one factor to consider as some fish do not readily take to pelleted feed unless they have been weaned onto it from an early age.

Most humpback groupers (*Cromileptes altivelis*) are fed on pelleted

Farm worker feeding formulated feed to pompano fingerlings at a floating cage farm in Johor.

Farm worker cleaning fresh sardine, headed, gutted and de-boned for feeding to groupers.

Trash fish cut into different sizes to suit various sizes of groupers in different cages.

dry feeds while other grouper species (*Epinephelus* spp and *Plectropomus* spp.) are mostly fed on trash fish.

Normally, commercial feed is broadcast into the cages and the fish fed to apparent satiety based on the judgment of the workers. Trash fish is usually chopped into pieces according to the size of groupers. Some farmers will remove the head and gut before feeding to the grouper, while others who are running on a larger scale simply cut the whole fish into chunk-size pieces, gut included.

The species of trash fish used varies from place to place and the time of the year. Most of the trash fish used in Indonesia is of good quality.

Thailand

Formulated pelleted dry feed is used for most seabass farming in Thailand. However, for other carnivorous species such as grouper, snapper and trevally most farmers still prefer to use trash fish. The quality of trash fish is generally quite good, with a price of around US$ 0.20-0.28 per kg depending on season and species. Currently, there is no commercial feed available locally for snapper or groupers.

Hong Kong and China

In Hong Kong most cultured marine finfish species are carnivorous with species including seabreams, snappers, amberjack, cobia and pompano. Most farms are still feeding trash fish but some farms use dry pellets, with a few using moist pellets for grow-out practices.

Several species of marine finfish farmed in the southern part of China are fed mostly on trash fish, particularly for aggressive feeders such as amberjack and cobia. Fresh trash fish cost around US$ 0.20-0.25 per kg. Other species such as tiger grouper (*Epinephelus fuscoguttatus*), tongue soles (*Cynoglossus* spp.) and flounders (*Pseudorhombus* spp.) are mostly fed with farm made moist feed, with local trash fish as the main ingredient and mixed with fishmeal. When the supply of trash fish is scarce, particularly during closed fishery season, formulated dry feed is used as a supplement. Dry feed costs around US$0.85/kg.

Farmer feeding trash fish to groupers in Southern Thailand.

Trash fish and fishmeal mixed to form a "block" of moist feed for nursery of five centimeter grouper fingerlings.

Milkfish feeding on industrial bakery rejects.

Typical polar cages used for Atlantic salmon culture in Tasmania.

Vietnam

Vietnam farms a wide range of marine carnivorous finfish species including snappers, seabreams, groupers and cobia. Most of the feeding is based on trash fish particularly in farming areas at Cat Ba Island and Halong Bay. The trash fish cost around US$ 0.19-0.45/kg. Most of the trash fish are freshly caught by small-scale fishers and delivered to the farms by boat daily.

Australia

The main marine species cultured in Australia are southern bluefin tuna (*Thunnus maccoyii*), Atlantic salmon (*Salmo salar*), Asian seabass/barramundi (*Lates calcarifer*) and yellowtail king fish (*Seriola lalandi*). There is also some production of snapper (*Pagrus auratus*), groupers (*E. coioides*), mulloway (*Argyrosomus hololepidotus*) and black bream (*Acanthopagrus butcheri*). All marine finfish farming in Australia uses extruded pelleted dry feed except for southern bluefin tuna where a transition is occurring from feeding fresh pilchards to extruded pellets. Depending on the species, these feeds are produced as slow-sinking pellets (for most seacage farms) or as a floating pellet. Floating pellets are used for aggressive feeders such as yellowtail king fish and especially for barramundi cultured in freshwater impoundments where the turbidity of the water encourages the fish to feed at the surface. Atlantic salmon, southern bluefin tuna and yellowtail king fish are cultured in large polar cages of 80 m diameter or bigger while barramundi are cultured in rectangular or polar cages of varying sizes. Smaller and typically rectangular cages (lengths of 4 to 6 m) are used for fish cultured in freshwater impoundments while larger rectangular or polar cages (lengths or diameters of 20+ m) are used for fish in seawater. Atlantic salmon and barramundi feeds are formulated specifically for these species while these or slight variants of them are used for the other species. Barramundi feeds typically contain 45-50% crude protein and 13 to 18% lipid and cost from US$1.10 to 1.20 per kg. Atlantic salmon feeds are 40-42% crude protein and 28-30% lipid, costing from US$1.30 to 1.45/kg.

Feeding commercial slow sinking feed to groupers in Lampung.

Farm workers are feeding trash fish to cobia in Cat Ba Island.

Sea cages used at Marine Harvest's Tiwi Island (near Darwin) barramundi farm, which produces about 1,500 tonnes of fish per year. Problems with crocodile and shark predation has been overcome using stainless steel mesh.

ACIAR grouper grow-out feeds program and related CSIRO research

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Groupers are highly prized fish in the live reef fish markets of Hong Kong and other Asian cities where prices (US$/kg) range from 8-11/kg for gold spot grouper (*Epinephelus coioides*), $15-20 for tiger grouper (*E. fuscoguttatus*), $30-40 for coral trout (*Plectropomus* spp) and red grouper (*E. akaara*) and $80-95 for mouse grouper (*Cromileptes altivelis*). The recent development of technology for large-scale hatchery production of grouper fry in Indonesia and the Philippines, largely as a result of research carried out in the collaborative ACIAR grouper project, is producing a plentiful supply of grouper fingerlings for aquaculture on-growing. At the time that the ACIAR grouper project began in 1999, information on the nutritional requirements of groupers for grow-out from fry to market was almost non-existent. To address this need, the ACIAR grouper project collaborators in Australia, Indonesia, the Philippines and Vietnam embarked on grow-out feeds research with the primary aim of developing cost-effective pelleted grouper grow-out feeds. Our approach was to:

i) define the requirements of groupers for the key nutrients that determine the rate at which fish grow;
(i) determine the nutritive value of locally available marine and terrestrial feed ingredients; and

iii) examine the extent to which high cost marine protein feed ingredients could be replaced using cheaper and more renewable terrestrial protein feed ingredients.

The ultimate goal is to develop pelleted grouper feeds as a more sustainable, lower-polluting and cost-effective alternative to the feeding of fresh fishery bycatch. If achieved, this would provide a more sustainable and profitable way of culturing groupers and at the same time reduce competition between man and aquaculture for a dwindling supply of fishery catch and lessen aquaculture's impact on the surrounding environment.

This article details the project research carried out by CSIRO and summarizes other grow-out feeds research in the ACIAR grouper project. Other articles in this series describe in more detail the project work that was carried out in Indonesia and Vietnam.

Flow-through constant temperature seawater experimental array consisting of 48 x 100 L insulated tanks at CSIRO used for grouper nutrition research.

ACIAR Grouper Project grow-out feeds team

The research was a collaborative effort between four laboratories that were formal participants in the ACIAR Grouper project and a fifth institution, which became affiliated with the project through a complementary Australian AusAID CARD project. The collaborating institutions and team leaders were: Dr Kevin Williams, CSIRO Marine Research, Cleveland, Australia; Dr N. Adiasmara Giri, Research Institute for Mariculture, Gondol, Bali, Indonesia; Ms Asda Laining, Research Institute for Coastal Aquaculture, Maros, South Sulawesi, Indonesia; Dr Oseni Millamena, South East Asian Fisheries Development Centre, Tigbuaan, Ilioli, the Philippines; and Dr Nguyen Dinh Mao, University of Fisheries, Nha Trang, Vietnam.

CSIRO studies on protein and lipid utilization in mouse groupers *C. altivelis*

A series of growth assay and digestibility studies were carried out under controlled laboratory conditions (filtered flow-through seawater at 29ºC and 12:12 photoperiod) to determine the optimum dietary protein and lipid specification for mouse grouper fingerlings. Additional metabolic studies were undertaken to see if feeding lipids comprised mostly of medium chain fatty acids (12-14 carbon chain length) would stimulate the fish to better use lipid as an energy source and so spare or reduce the amount of protein needed in the diet. Key findings of the research are outlined below.

Optimal dietary protein and lipid content

An 8-week comparative slaughter growth assay and digestibility study involving 10 pelleted dry feeds and 4 replicate tanks of fish per treatment (10 g start weight) was carried out to determine the optimal dietary crude protein (CP) and lipid specifications for juvenile mouse grouper. Fish growth rate, food conversion ratio, nutrient retention and protein digestibility of the diet improved linearly as the amount of CP in the diet was increased from 38 to 59%. However, increasing the amount of lipid in the diet from 14 to 22% reduced energy digestibility but growth rate of the fish was unaffected. Fish fed the higher lipid diets were much fatter (Table 1).

As the digestible protein (as N) content of the diet increased, the amount of digestible N required per kg fish weight gain increased: linearly for the 14% lipid diets, and curvilinearly for the 22% lipid diets (Figure 1). This shows feeding the higher lipid diet did bring about some sparing of protein for energy.

A second comparative slaughter growth assay and digestibility experiment employing the same culture conditions as for the first experiment was carried out to see if supplying lipid at moderate (15% added oil) or high (30% added oil) concentrations in the diet and in the form of either long-chain fatty acids (LCFA, as olive oil) or medium-chain fatty acids (MCFA, as coconut oil) affected the way the fish used the lipid as an energy source. Five diets, a low-lipid (6%), high-protein (76% CP) control diet and four 'lipid' diets that made up a 2 x 2 factorial of the two types and two concentrations of lipid, were fed to six replicate tanks of fish (300 fish in total; 14 g average initial weight) for 8 weeks. The formulation of the 'lipid' diets was identical to the control except that the required amount of lipid was included at the expense of defatted fishmeal with a concomitant lowering of the dietary CP from 76 (control diet) to 64 and 53% for the 15 and 30% lipid treatments, respectively.

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

Metabolism of fatty acids

The 'lipid' diets fed in the aforementioned Experiment 2 were used in this study except that radioactively-labeled fatty acids were incorporated to allow the metabolism of these fatty acids to be traced. As a marker for MCFA, ^{14}C-octanoic acid (an 8 carbon chain fatty acid) was used while ^{14}C-oleic acid (an 18 carbon chain fatty acid) and ^{14}C-palmitic acid (a 16 carbon chain fatty acid) were used as markers of LCFA. Diets with these labeled fatty acids were fed to fish in metabolic chambers (7-9 replicates per treatment) and the amount and rate of lipid oxidation quantitatively determined by measurement of the ^{14}C label present in the fish, in the expired CO_2 and in the dissolved and particulate organic matter in the water of the metabolic chamber.

The lipid in the MCFA diets, irrespective of whether present at 15 or 30% of the diet, was oxidized far more rapidly than the LCFA lipid, with far more of the ^{14}C label appearing in the respired CO_2 and less remaining in the fish (Table 2). The amount of lipid in the diet appeared to have no effect on the amount of lipid oxidized. Moreover, respiration rates increased dramatically when fish were fed the MCFA compared to the LCFA diets, indicating a physiological response of the fish to remove CO_2 produced as a result of lipid oxidation (Figure 2).

Conclusions

- Diets for fingerling mouse grouper should contain not less than 41% digestible protein (about 55% CP) on an as fed (~92-93% DM content) basis.

Laboratory Technician Leigh Whitlock Laboratory carrying out lipid analyses at CSIRO's Cleveland laboratory.

- Increasing the lipid content of the diet above about 15% did not promote greater fish growth but rather led to increased body fat deposition and a reduction in food intake. There was some evidence of protein sparing by dietary lipid.
- Replacement of LCFA lipids (such as fish or long-chain vegetable oils) with MCFA lipids (such as coconut oil) did increase the rate of fat oxidation but had a detrimental effect on food intake, and consequently also on growth rate.

Other grow-out feeds research in the ACIAR Grouper Project

Digestibility of feed ingredients

The apparent digestibility of a comprehensive range of ingredients available in the Philippines and Indonesia was determined for gold spot grouper (*E. coioides*) and mouse grouper (*C. altivelis*), respectively. CP in both marine and terrestrial animal meals was well digested (above 76%) by both grouper species with the exception of oven-dried blood meal, which was poorly digested (55%). The protein digestibility of plant products was more variable (from 43 to 100%) with high fibre meals such as rice bran and lucaena (ipil-ipil) meal being poorly digested. The DM digestibility of the meals was adversely affected by the amounts of ash and fibre they contained. A collation of the DM and CP apparent digestibility values of the tested ingredients is presented in Table 3.

Nutrient requirements of juvenile groupers

Growth rate and survival of sea-caged mouse groupers were improved when diets were supplemented with up to 150 mg/kg of vitamin C as the heat-stable form of L-ascorbyl-2-monophosphate-Na-Ca. This benefit of vitamin C supplementation was most apparent following heavy flood rains, which caused a marked deterioration in the turbidity and dissolved oxygen content of the water around the cages. The dietary requirement for the essential omega-3 highly unsaturated fatty acids (n-3 HUFA) was examined for mouse and tiger grouper. Increasing the supplementation rate up to 1-1.5% of the diet resulted in improved fish growth rates and better survival. In studies examining the capacity of mouse grouper to utilize different types of carbohydrate as energy sources, best results were achieved using glucose while starch and sucrose were the least effective.

These nutrient requirement studies indicate that juvenile groupers require diets that are high in digestible CP (around 45-50%), moderately low in lipid (around 10%) and contain not less than 1.0% and preferably 1.5% of n-3 HUFA. Addition of at least 100 mg of a heat stable form of vitamin C per kg of diet is recommended and this should be increased to 150 mg/kg if stressful culture conditions are likely to occur.

Fishmeal replacement studies

In studies examining the ability of terrestrial protein meals to substitute for fishmeal in formulated feeds for juvenile gold spot grouper, a 4:1 combination of meat meal and ring-dried blood meal, respectively was able to replace up to 80% of fishmeal protein in the diet without adverse effects on growth, feed conversion or survival of the fish. Other terrestrial protein meals such as cowpea, corn gluten, lucaena (ipil-ipil) meal and soybean meal were less successful as fishmeal replacements. With mouse grouper, growth rate and feed conversion deteriorated markedly when shrimp head meal was used at inclusion rates above 10% as a replacement for fishmeal protein.

In laboratory and field cage studies, a practical low-cost pelleted dry diet was formulated on a digestible nutrient basis to meet the requirements of juve-

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

Response	CP (%)					Lipid (%)	
	38	43	49	54	59	14	22
ADCP (%)	46.8^{C}	55.3^{BC}	58.5^{A}	69.7^{A}	74.0^{A}	59.8	61.9
ADE (%)	59.9^{A}	58.4^{B}	51.3^{C}	61.3^{B}	68.1^{A}	62.2^{X}	57.5^{Y}
SGR (%/d)	1.12^{C}	1.11^{C}	1.26^{B}	1.42^{A}	1.52^{A}	1.31	1.26
FCR (g:g)	1.58^{C}	1.49^{C}	1.24^{B}	1.08^{A}	1.00^{A}	1.28	1.27
BF (%)	23.5	23.2	23.7	23.1	23.5	21.7^{X}	25.1^{Y}
RDN (%)	58.6^{A}	48.8^{B}	50.3^{B}	42.3^{C}	38.8^{C}	48.9	46.7
RDE (%)	35.0^{C}	38.6^{C}	52.3^{A}	47.5^{B}	44.2^{B}	40.7^{Y}	46.3^{X}

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

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

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

A,B,C Means in the same column with different letters differ (P <0.05).
DOM = Dissolved organic matter in metabolic chamber water.
POM = Particulate organic matter (faeces and in some cases, regurgitated feed) in metabolic chamber water.

Figure. 1: Relationship between the digestible N content of the diet and the amount of digestible N required per kg weight gain of fish fed diets containing either 14 (▲) or 22 (■) % lipid.

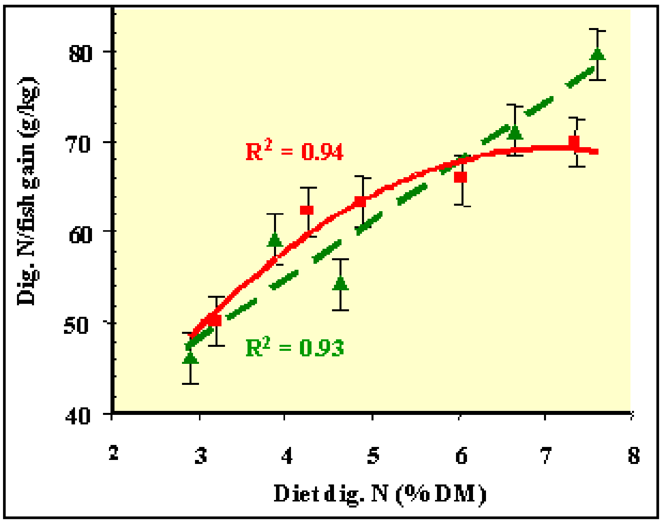

Figure. 2: The amount of consumed dietary lipid retained as body fat or oxidized by fish fed either a low lipid (6%) control (Con) diet or diets with 15 or 30% added olive oil (LCFA) and 15 or 30% added coconut oil (MCFA).

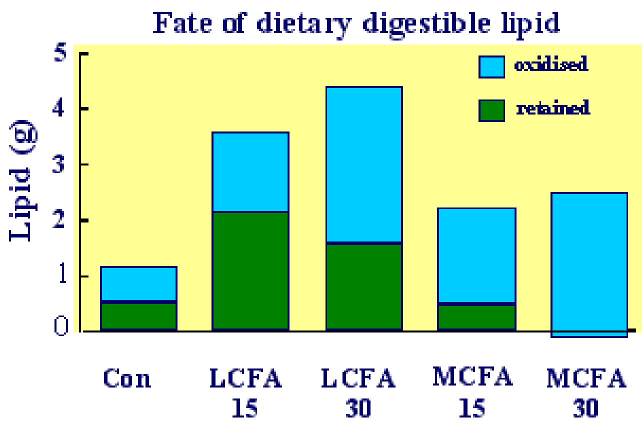

Figure 3: Respiration rate of fish following ingestion of diets containing coconut oil (MCFA) or olive oil (LCFA) added at 15 or 30% of the diet.

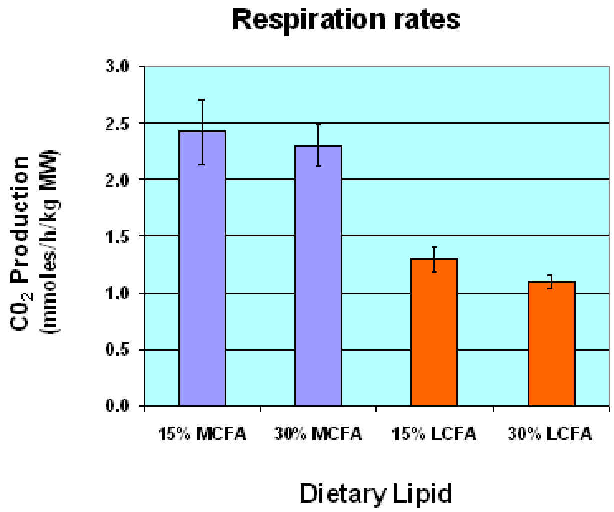

Table 3. The dry matter (DM) and crude protein (CP) apparent digestibility (AD) of selected feed ingredients determined for gold spot grouper in the Philippines and for mouse grouper in Indonesia

Feed ingredient	Gold spot grouper		Mouse grouper	
	DMAD[1]	CPAD[1]	DMAD[1]	CPAD[1]
Marine product				
Fishmeal (Chilean 65% CP)	84 ±3.1	98 ± 0.7		
Fishmeal (mixed 45% CP)	59 ±1.2	82 ± 2.0	59 ± 1.2	82 ± 2.0
Fishmeal (sardine 65% CP)			87 ± 2.5	93 ± 1.4
Fishmeal (tuna 50% CP)	75 ± 3.6	76 ± 1.9		
Fishmeal (white 69% CP)	89 ± 1.7	99 ± 0.3		
Shrimp meal (Acetes 72% CP)	76 ± 4.0	95 ± 0.7		
Shrimp head meal (50% CP)			59 ± 3.3	78 ± 1.3
Squid meal (71% CP)	99 ± 1.0	94 ± 0.2		
Terrestrial animal product				
Blood meal (Australian ring 84% CP)				
Blood meal (oven dried 84% CP)			48 ± 0.9	55 ± 1.4
Blood meal (formic 87% CP)			68 ± 1.6	88 ± 0.6
Blood meal (propionic 84% CP)			62 ± 2.6	84 ± 0.7
Meat meal (Australian 44% CP)	61 ± 0.8	99 ± 1.3		
Meat meal (Philippine 45% CP)	78 ± 0.1	84 ± 1.7		
Meat solubles (73% CP)	99 ± 0.5	98 ± 0.1		
Poultry feather meal (67% CP)	74 ± 3.1	82 ± 2.6		
Plant product				
Corn germ meal (8% CP)	85 ± 2.8	83 ± 4.7		
Corn gluten meal (56% CP)	94 ± 2.0	99 ± 0.7		
Cowpea meal (white 24% CP)	74 ± 3.1	94 ± 1.2		
Lucaena (ipil-ipil) meal (19% CP)	56 ± 0.1	79 ± 2.6		
Lupin albus meal (26% CP)	54 ± 1.2	98 ± 3.7		
Palm oil cake meal (11% CP)			45 ± 2.4	81 ± 1.3
Rice bran meal (11-14% CP)	69 ± 7.0	43 ± 5.4	22 ± 1.5	60 ± 1.4
Soybean concentrate (54% CP)	76 ± 4.9	86 ± 0.4		
Soybean meal (full-fat 41% CP)			55 ± 2.7	67 ± 1.3
Soybean meal (solvent 51% CP)	76 ± 1.7	96 ± 0.1		
Wheat flour (9% CP)	73 ± 0.9	83 ± 1.3		
[1] Mean ± SD.				

nile gold spot grouper and compared with feeding either a commercial pellet diet or fresh fishery bycatch. In both studies, fish fed the project formulation diet survived and grew as well as those fed the fresh bycatch. In the laboratory study, fish fed the commercial pellet diet grew significantly slower and converted feed less efficiently than those fed either the project diet or fresh bycatch. The analysis of the commercial pellet diet showed a sub-optimal specification. When the commercial mill adjusted the formulation to meet these specifications, fish fed that diet in the field study performed as well as those fed either the project diet or fresh bycatch.

Conclusions

The research carried out in the project has conclusively shown that juvenile groupers will readily accept pelleted dry diets. Diets formulated to meet the fish's requirements for digestible nutrients, and not containing excessive amounts of plant protein meals, will enable juvenile groupers to grow as well, if not better, than those fed fresh fishery bycatch. Further research is needed in the areas of essential fatty acid requirements of tiger groupers (*E. fuscoguttatus*) and to examine whether the nutritional requirements of groupers above 200-300 g are different to juveniles of 10-100 g size. Another area of potential research is to develop nursery feed formulations and to develop management practices for successful weaning of fry from live/fresh feeds to a pelleted dry feed.

Feed development and application for juvenile grouper

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Groupers are potentially important aquaculture species since they have a high economic value. Groupers, predominantly *Epinephelus spp*, have been cultured throughout Asia for many years with commercial production based on captured wild seed and the fish reared on trash fish. Recently, hatchery technology has been developed for seed production of some grouper species and this has stimulated interest in grow-out farming. However, the continued use of trash fish as a feed source for groupers should be discouraged because of the risk of disease transfer and the environmental problems associated with its use.

Feed is often the single largest cost item in fish culture. Although trash fish is presently the first choice of farmers for on-growing groupers, its availability can be limited and varies with season. Information on the nutrient requirements of groupers is still very limited and it is imperative that this is addressed if cost-effective and high performing artificial feeds are to be developed to replace the feeding of trash fish. Based on the limited available information on the nutrient requirements of groupers, some feed companies have produced feeds for nursery and grow-out. However, these have not been well accepted by grouper farmers because they are thought to be expensive and the fish do not accept it as well as trash fish. In order to develop a better artificial feed for juvenile groupers, a series of experiments have been conducted at Gondol to increase our knowledge about the dietary requirements of several grouper species.

Nutrient requirements of juvenile groupers

Dietary protein and lipid requirements

Tiger grouper (*Epinephelus fuscoguttatus*) and humpback or mouse grouper (*Cromileptes altivelis*) are carnivorous fish and thus naturally have a high requirement for dietary protein. Grow-out studies have examined the optimum dietary protein and lipid specifications for juvenile tiger and humpback groupers. A significant interaction between dietary protein and lipid has been observed for growth rate. Although fish productivity generally increases with increasing dietary protein, feeding with 9% lipid feed was found to be better than feeding either the 6 or 12% lipid feeds. Food conversion ratio (FCR) improved and more dietary lipid was retained as dietary protein increased; retention of dietary lipid also increased with increasing lipid content of the diet. There was a slight difference between tiger and humpback groupers in the optimal dietary protein specification: 47% for juvenile tiger grouper and 54% for humpback grouper. In another series of experiments, five levels (0.0, 3.0, 6.0, 9.0, and 12%) of dietary lipid were fed to juvenile tiger and humpback grouper. This confirmed the earlier results with the optimal dietary lipid found to be 12 and 9%, respectively for juvenile tiger and humpback grouper. Thus for juvenile tiger and humpback groupers, pelleted diets of about 90% dry matter (DM) should contain around 50% protein, 9-12% lipid, about 4.5 kcal gross energy/kg (18.8 MJ/kg) and a protein to energy ratio of 120 mg/kcal (27 g/MJ).

Essential fatty acid requirements

Omega 3 or n-3 highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential dietary fatty acids for marine fish. Requirement for n-3 HUFA varies with the species and size of fish. Experiments have examined the n-3 HUFA requirement of juvenile humpback and tiger groupers. Juveniles of average starting body weight of 5 ± 0.7 g were reared for 9 weeks in 100 L tanks and supplied with flow through seawater. Fish were fed one of six feeds that provided n-3 HUFA levels of 0, 0.5, 1.0, 1.5, 2.0 or 2.5%. Based on growth rate and FCR, the optimal dietary n-3 HUFA specification was found to be 1.5 - 2.0% for humpback grouper and 1.75 –2.8% for tiger grouper.

Vitamin requirements

Similar to all other carnivorous marine fish, groupers require a dietary source of vitamin C (ascorbic acid). A lack of vitamin C in very young fish will result in skeletal deformities such as lordosis and scoliosis (curvature of the skeleton) and deformity or absence of the gill opercula; in older fish a deficiency is exhibited as slow growth, weak sickly fish, anemia and death in severe cases. Vitamin C is very important for maintaining a strong immune system and any deficiency will increase the fish's susceptibility to stress and disease. Pure ascorbic acid is very easily destroyed by mild heating and exposure to light. To overcome the low stability of ascorbic acid, other more stable types of vitamin C have been developed. Some products are simply ascorbic acid coated by glycerine or some other film which helps to reduce exposure of the ascorbic acid to light and so slow its destruction. However, these coated ascorbic acid products are still not very stable and are mostly destroyed during any type of hot pelleting process. Ascorbyl phosphate is a much more stable form of vitamin C, which has been shown in other fish species to be effective in meeting the fishes nutritional needs. To evaluate its effectiveness for groupers, juvenile humpback groupers of about 14 g starting weight were fed feeds containing 0, 15, 30, 60, 120 or 250 mg/kg of ascorbyl phosphate magnesium (APM) for 126 days. Based on the results of this experiment, it is recommended that pelleted grouper feeds should contain not less than 30 mg APM/kg for maximum fish growth and to ensure that the fish's immune system is fully functional.

Another experiment has been conducted to determine the requirement of vitamin B6 (pyridoxine). This is another important and essential vitamin for fish and in severe deficiency neurological disorders such as erratic and spiral swimming, shock reaction to stress or handling and death are seen. In a less severe deficiency, the signs are fairly non-specific such as poor appetite and slow growth rate of the fish. Fortunately, pyridoxine hydrochloride, which is the commonly available form of pyridoxine, is very stable to heat and light. To determine the pyridoxine requirement of groupers, six levels of pyridoxine (0, 20, 40, 60, 80 or 160 mg/kg) were fed to juvenile humpback groupers for 98 days. Using the increase in blood haemoglobin as an index of pyridoxine adequacy of the feed, the optimal specification for maximum blood haemoglobin was 60 mg/kg. This is much higher than the 15-20 mg/kg dietary specification recommended for many other marine fish. Since pyridoxine HCl is quite soluble and groupers are fairly slow feeders, this higher specification may be due to leaching of pyridoxine from the feed. In any event, it is recommended that grouper feeds have a dietary pyridoxine HCl specification of 40-60 mg/kg to maintain the healthy condition of fish.

Table 1. Formulation and macro nutrient composition of the Gondol practical feed formulation for juvenile groupers

Ingredient	Formulation (%)	Nutrient	Composition (% of diet)
Fishmeal (65% CP)	55	Crude protein	46-50
Soybean meal	10	Total lipid	9-13
Squid liver meal	14.5	Fiber	6-7
Mysid shrimp meal	6	Ash	18-25
Squid oil	6		
Trace mineral mix	2.5		
Vitamin mix	2.0		
CMC binder	4.0		
Total	100		

Table 2. Result of a 4-month feed comparison trial with humpback grouper in net cages

Productivity traits	Test diets		
	Gondol feed	Commercial feed	Trash Fish
Initial weight (g)	36.0	36.0	36.0
Final weight (g)	147.6	132.8	133.4
Survival rate (%)	98.7	98.0	95.1
FCR	1.39	1.54	5.82
Haematocrit (%)	37.3	38.2	24.2

Practical feed development for juvenile groupers

Fishmeal replacement

Fish meal is the main source of protein in conventional pelleted feeds for most fish species. Because capture fisheries production has not increased since the early 1990's and production of fishmeal has similarly not increased but aquaculture's demand has skyrocketed, it is not surprising that the price of fishmeal continues to increase at rates above inflation. In an attempt to offset the spiraling cost of fishmeal as an ingredient in pelleted fish feeds, there has been a lot of research worldwide to find cheaper protein alternatives. At Gondol, we have examined the extent to which soybean meal can be used to replace

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Present status in the development of culture technology for the silver pomfret *Pampus argenteus*

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Silver pomfret about 35 days old after hatching.

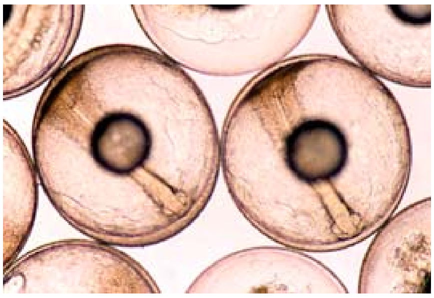
The silver pomfret *Pampus argenteus,* locally known in Kuwait as 'zobaidy', is a high priced food fish having worldwide market demand. It is also extensively distributed in different regions of the world from the East China Sea to Southeast Asia, Indian Ocean and in the Arabian Gulf. In recent years the silver pomfret capture fishery from the wild has been declining in Kuwait and in other regions due to over fishing. In spite of the decline in wild stock and increasing market price, there has been little research on developing technology for the hatchery and grow-out culture of this species until recent years. During June 1997, in a preliminary attempt to develop hatchery and larval rearing techniques, the Aquaculture Fisheries and Oceanography Department (AFOD) of the Kuwait Institute for Scientific Research (KISR) succeeded for the first time in the world in larval rearing and fry production of *Pampus argenteus* under hatchery culture conditions. This was achieved using eggs stripped from wild spawners caught by drifting gillnets, with 45 larvae successfully grown to juvenile stage. Pursuant to the success of 1997 larval rearing, a five-year research project was initiated in April 1998 to assess the technical feasibility of silver pomfret culture in Kuwait. Since then the project has successfully produced several thousand silver pomfret fingerlings for nursery and grow-out production and developed larval rearing techniques to grow-out fish to marketable size. Continued efforts are underway to develop domesticated broodstock for spawning under controlled culture conditions.

Hatchery performance

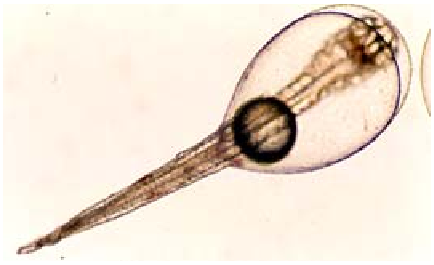
The present source of eggs for larval rearing is based on stripping wild caught spawners. Fully mature males and gravid females are found during June to September in the coastal waters of Kuwait at about a depth of 5-20 m. They are captured with drifting gillnets in the daytime during spring tides at new and full moon. During the spawning period, the surface seawater temperature ranges from 28-33 ºC and the water salinity ranges from 35-39 ‰. The peak spawning period is June-July when the water temperature ranges from 28-29ºC.

Fully ripe gravid females, with hydrated and free-flowing eggs, occur between 15:00-18:00 hours during ebb tides. In the gillnet catch, fully mature males outnumber the females at an average ratio of 5:1. Males also mature earlier with a much lower gonado-somatic index (GSI) than females. Fecundity and histological studies indicate that the broodstock is indeterminate showing that a female may spawn at least six batches of eggs from June to August with an average relative batch fecundity of 170.6 eggs/g of somatic weight. Total fecundity of a 500g female is about 350,000 eggs. An average of about 40,000-60,000 viable eggs can be collected from each ripe female, depending on the size and condition of the ovary. The percentage of viable eggs depends on the fishing season/month and accordingly the highest percentage of viable eggs are collected during June. The fertilized eggs are spherical, transparent and pelagic and about 1.1mm diameter in size. The egg hatching time is 15 hours at 29-30 ºC. The hatching rate depends on the

Stripping for egg collection from wild spawners.



Above: Fertilized eggs and embryo development of silver pomfret. Below: Newly hatched larvae.



egg collection time and conditions and normally it ranges from 28-50%. The newly hatched larvae have a large ellipsoid yolk and measure about 2.4 mm in total length.

Larval rearing

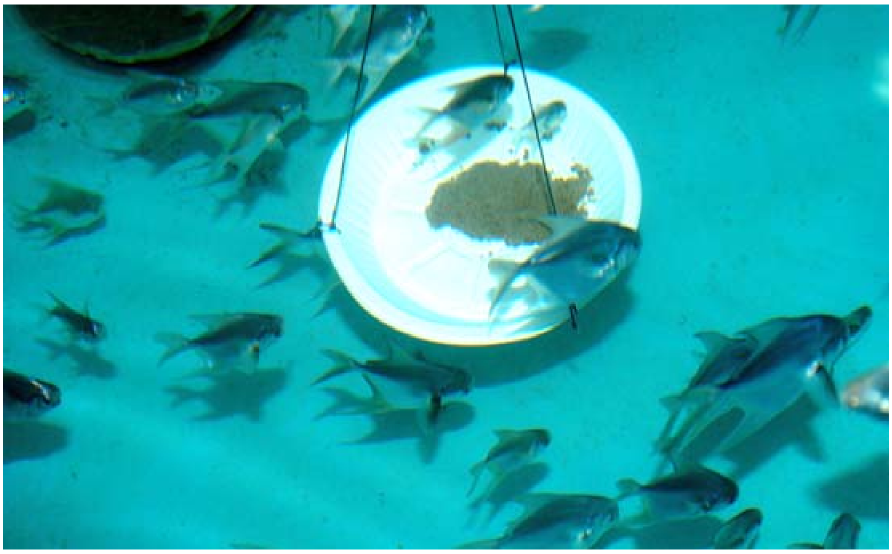
The optimal temperature for rearing larvae is 27-29 ºC and salinity 39-40‰. The newly hatched larvae are generally stocked at a density of 30-120 larvae per litre. However, stocking rates of 20-40 larvae per litre are conventionally used without any significant difference in larval survival at these stocking densities. Significantly higher survival rates at 12 days after hatching were observed for larvae fed on a combination of mixed species of micro-algae consisting of *Chlorella, Isochrysis* and *Nannochloropsis* at a cell density of 1 million cells per ml in the larval rearing waters along with nutritionally-enriched rotifers, compared to those reared using a single algal species. First larval feeding is with rotifers at a density of 5 rotifers per ml until 6 days after hatching, and then increased to 10-15 rotifers per ml since prey consumption by the larvae increases significantly at 8 days until they are weaned to newly hatched *Artemia* nauplii. Larval feeding on rotifers is significantly higher in cultures with mixed species of algae compared to those using a single algal species in the culture system. Furthermore, the essential ω3 HUFA were also significantly higher in rotifers treated with mixed species of algae along with commercial enrichment media ‘Super Selco’ and ‘DHA Protein Selco’. Within a week of feeding on *Artemia* nauplii, the larvae could be weaned to a formulated paste feed. The larvae are also easily trained to accept the paste feed kept in trays suspended in the water column. Under experimental culture conditions the survival was up to 4.2% to juvenile stage. Mass larval rearing and fry production using 1m^3 and 4m^3 capacity round fiberglass tanks showed that better larval survival is achieved in larger tanks. One of the reasons for larval mortality in the hatchery is due to a behavior of the larvae in swallowing tiny air bubbles at the water surface. This is due to the innate feeding habit of silver pomfret, which consume small jellyfish and medusae. As tiny air bubbles may mimic jellyfish in appearance, the larvae consume them and tend to float, losing their balance in the water column. This phenomenon increases during the later part of the larval rearing period from day 25. Larval mortality due to air-bubble ingestion has been substantially reduced by introducing indirect aeration techniques instead of using air-diffusers directly in the larval tanks. Larval mortality due to cannibalism is low in the hatchery phase compared to other marine fish species. The lack of cannibalism and ready acceptance of forumulated feeds make the hatchery techniques developed thus far promising for commercial ventures. Further research studies to refine larval rearing are in progress, focussing on feed and tank management techniques to enhance fry production.

Grow-out culture

To date the grow-out culture of silver pomfret has been restricted to land-based culture systems using different capacity round circular tanks of up to 65 m^3 with flow-through seawater. Screening for a commercial feed suitable for use in the grow-out phase is still in progress. In the meantime, based on the results obtained from various experiments, a semi-moist feed is used. The feed is kept in a plastic bowl, which is suspended in the water column. In trials between 1998-2003, the average weight gain for fish of less than 50g was up to 0.34g per fish per day and 0.23 g per day in fish above 50g. Due to feed improvements made through feed additives in 2004, the growth rate for fish less than 50g in size has been increased to 0.62g per fish per day and in the above 50g size group it has been increased to around 1.32g per fish per day. During this period the specific growth rate (SGR) was up to 5.98% per day for fish in the below 50g size group and about 2.13% per day for fish above 50g. The growth rate declines during the winter period

About 45 days old after hatching.

in Kuwait from November to March when the water temperature drops to 13 ºC. Growth performance of this fish stocked at different densities of 60, 80, 100 and 120 fish/m^3 in a re-circulating tank culture system showed that better survival could be obtained at a stocking density of 60 fish/m^3. Growth performance of silver pomfret using earthen ponds and sea-cages is yet to be investigated.



Grow-out culture of silver pomfret.

Domesticated broodstock development

During the grow-out culture, fast growing selected fish were maintained in separate tanks for the development of domesticated broodstock. Histological studies have shown that silver pomfret are a gonochoristic species, in which the male and female sex exists in separate individuals. The studies also show that the processes of sexual differentiation for the male and female are completed at the ages of 115 and 135 days after hatching from the egg, respectively. Under tank culture conditions, fully grown broodstock size ranged from 124 to 823g. The fish selected for broodstock development were found to undergo gonad maturation in captivity as evidenced by the occurrence of fully ripe male producing milt at a size of 134g in body weight and 17cm in fork length (FL). The first occurrence of female maturation in the broodstock holding facility was with stage-2 gonad at the size of 222.5g in body weight and 19.5cm in FL. During July 2004 a 408g female with a stage-IV ovary was observed. However, the broodstock holding tank was infested with *Cryptocarion* and mass mortality of the fish occurred hampering further progress in spawning of the broodstock during 2004. Further investigations are in progress towards developing domesticated broodstock from hatchery produced individuals. Towards achieving spawning in the broodstock holding facility, efforts made to inject the fish with chorionic gonadotrophin (HCG) failed to yield any results. The potential spawners were also not tolerant to handling and injection stresses, particularly in the case of wild brooders. Techniques for the spontaneous natural spawning of the captive broodstock using improved tank management and feeds are yet to be developed.

Hatchery produced potential broodstock.

Future prospects

In general, developing commercial culture technologies for marine fish species takes many years of research and development. Compared to some of the economically important farmed tropical marine fish species, the research on silver pomfret has only been initiated relatively recently and should be considered at the initial stages of development, although substantial progress has been made towards commercial applications. Based on the results achieved so far, the silver pomfret *Pampus argenteus* seems to be an excellent candidate for aquaculture

A 408g cultured female with stage-IV ovary

Best practices for using trash fish in the culture of juvenile Malabar grouper, *Epinephelus malabaricus*

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University of Fisheries' nutritional wet laboratory comprising 48 experimental tanks.

Malabar grouper *Epinephelus malabaricus* is a highly valued species in the live fish markets of Vietnam and throughout Asia. The recent development of hatchery production technology for Malabar grouper fry and its successful exploitation by a commercial hatchery in Vietnam has resulted in a promising supply of fry for aquaculture. Commercial culture of grouper in Vietnam relies almost entirely on the feeding of low-value fish and fishery by-product ('trash fish'). In Viet Nam, the species of trash fish most commonly fed are lizard fish (*Saurida* spp.), pony fish (*Leiognathus* spp.), cardinal fish (*Apogon* spp.), anchovy (*Stolephorus* spp.), pomfret (Psenes spp.) and spinefoot (*Siganus* spp.). Some farmers have a preference for using lizard fish and cardinal fish. Although grouper aquaculture production in Vietnam is increasing each year and was 3,000 metric tonnes in 2003, the size of the industry is still not large enough to get aquafeed companies involved. 'Trash fish' will continue to be used in Vietnam for some years as a major feed for groupers. It is a comparatively inexpensive source of feed that is available locally, although its availability and price does fluctuate during the year and it can be scarce and expensive during the winter typhoon season. However, poor husbandry and feeding practices can result in a lot of the trash fish being wasted and as a consequence, can cause serious downstream pollution problems. For these reasons, determining best practices for using trash fish is a high priority for the Vietnamese grouper industry. This paper describes an experiment comparing different types of trash fish and feeding rates for culturing juvenile Malabar grouper.

How the research was done

A 3 x 4 factorial design was used in an 8-week experiment to investigate the effects of feeding juvenile Malabar grouper of initial size 10 ±0.3 g either of three different types of trash fish – cardinal fish, lizard fish or a combination of equal amounts of cardinal fish and lizard fish – and four daily feeding rates – 5, 7, 9 or 11% of fish biomass. A total of 480 fish were stocked equally into 48 x 100 L polyethylene tanks (four tank replicates per treatment) under controlled laboratory conditions. Each tank was supplied with flow-through bio-filtered seawater (33 to 35 g L^{-1}) at an exchange rate of 500% day^{-1} and with supplementary aeration.

The feed processing laboratory.

By-catch was bought fresh from the Cu Lao Trung Fishing Port, Nha Trang city and immediately weighed into plastic bags and stored at -20ºC until required to be fed. The trash fish was thawed and chopped into pieces immediately prior to feeding with the daily allocation being fed in two equal amounts twice daily at 0730 and 1530 h. Uneaten food was collected, oven dried and its weight used to calculate actual food intake after correcting for leaching loss.

What was found

There were some very minor interactions between the type of trash fish fed and feeding rate but these were so inconsequential in comparison to the main treatments that they will be ignored in this article. However, productivity of the groupers were markedly affected by both trash fish type and feeding rate.

Effect of type of trash fish

Growth rate, expressed as average weekly gain (AWG) or as specific growth rate (SGR), as-fed and dry matter (DM) food conversion ratio (FCR) and fish survival were generally best when cardinal fish and lizard fish were fed as the combination while feeding cardinal fish by itself resulted in the worst productivity (Table 1).

Lizard fish has a higher lipid and lower ash (and thus, higher energy) content than cardinal fish (Table 2) and this most likely explains why the Malabar grouper grew better when fed the lizard fish. However, why a combination of the two trash fish types should result in better grouper productivity than either alone is not so easily explained. Clearly, the combination of trash fish was more preferred by groupers than either type alone since food intake was significantly higher for the combination. This perhaps explains the better growth of the groupers on the combination but it does not explain the significantly better DM FCR that was also observed for the combination. The improved FCR suggests that the combination provided an overall better nutritional profile for the groupers but in what respects is not known.

Effect of feeding rate

In line with the applied treatment, daily food intake increased incrementally with increasing feeding rate up to the 9% level with a non-significant increase thereafter for the 11% rate. As a consequence, fish weight gain showed a parallel response with significantly better grouper growth rates as the feeding rate was increased from 5 to 9% (Table 3). FCR was almost identical for the three lower feeding rates but worsened slightly when the feeding rate was increased to 11%. While this may be a genuine reduction in the efficiency with which the grouper used the food, it equally could have been due to incomplete collection of uneaten food since some feed refusal was common at this feeding rate.

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Table 1. Effect of trash fish type on the productivity responses of juvenile Malabar grouper.

Response trait	Type of trash fish fed			
	Cardinal	Lizard	Combined	± SEM
Weekly gain (g)	4.36^{C}	4.92^{B}	5.13^{A}	0.017
SGR (%/d)	2.61^{C}	2.78^{B}	2.85^{A}	0.013
Daily intake (g as-fed/d)	2.94^{C}	3.03^{B}	3.15^{A}	0.007
FCR (as-fed)	4.70^{B}	4.29^{A}	4.29^{A}	0.017
FCR (DM)	1.19^{C}	0.99^{A}	1.04^{B}	0.004
Survival (%)	93.1^{B}	95.6^{AB}	97.5^{A}	0.98

^{A,B,C} – Within each response trait, means without a common superscript letter are different ($P < 0.05$).

Table 2. Whole body chemical composition (± SD) of common trash fish species caught in the central coast region of Vietnam.

Analysis	Trash fish species			
	Cardinal	Lizard	Anchovy[1]	Spinefoot
	Fresh basis			
Moisture	74.7±0.99	74.8±0.44	75.7±0.42	74.6±0.42
Ash	5.3±0.14	3.7±0.08	3.1±0.04	6.5±0.08
Protein	16.2±0.07	17.3±0.65	14.9±0.30	13.1±0.74
Lipid	1.7±0.22	2.6±0.37	1.6±0.19	2.0±0.23
	Dry matter basis			
Ash	21.0±0.55	14.7±0.33	12.9±0.15	25.4±0.30
Protein	64.0±0.09	68.7±0.88	61.3±0.60	51.6±0.98
Lipid	6.7±0.65	10.3±0.75	6.6±0.38	7.9±0.46

[1] Long-jaw anchovy.

Table 3. Effect of feeding rate on the productivity responses of juvenile Malabar grouper.

Response trait	Feeding rate (% of biomass per day)				
	5	7	9	11	± SEM
Weekly gain (g)	3.06^{C}	4.44^{B}	5.87^{A}	5.84^{A}	0.020
SGR (%/d)	2.15^{C}	2.70^{B}	3.05^{A}	3.08^{A}	0.015
Daily intake (g as-fed/d)	1.90^{D}	2.76^{C}	3.65^{B}	3.85^{A}	0.008
FCR (as-fed)	4.36^{A}	4.36^{A}	4.36^{A}	4.63^{B}	0.020
FCR (DM)	1.06^{A}	1.06^{A}	1.05^{A}	1.12^{B}	0.005
Survival (%)	90.8^{C}	95.0^{B}	99.2^{A}	96.7^{AB}	1.13

^{A,B,C} – Within each response trait, means without a common superscript letter are different ($P < 0.05$).

Grouper grow-out feeds research at Maros Research Institute for Coastal Aquaculture, South Sulawesi, Indonesia

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In Sulawesi, the development of grouper aquaculture is hindered by dependence on the fluctuating and uncertain supply of perishable trash fish. While some commercially produced pelleted grouper feed is imported from Java and Thailand, it is expensive and fish that have previously been fed on trash fish do not readily adapt to dry pellets, resulting in slower fish growth. To address this problem, grouper feed research at Maros has focused on examining the potential of locally available meals as ingredients in farm-made feeds. This has entailed determining the apparent digestibility of locally available feed ingredients and examining their potential as partial replacements for imported fishmeal, and investigating the nutrient requirements of juvenile mouse (*Cromileptes altivelis*) and tiger (*Epinephelus fuscoguttatus*) groupers. While much of this past research has involved feeding acclimatized fish on laboratory-made dry pellets, our current research emphasis is to adapt these findings for the production of moist pelleted feeds that can be made on-farm using simple equipment. Moist pelleted feeds are well accepted by juvenile groupers and can be used as the only food source or alternatively as a way of training the fish to accept pelleted feeds without losing condition that otherwise frequently occurs when only dry feed pellets are fed.

Results of past research are outlined in this article and some information is given about producing farm-made moist pellets for feeding juvenile mouse and tiger groupers.

Apparent digestibility of Sulawesi feed ingredients

To date, the apparent digestibility of nine locally available feed ingredients have been determined for mouse

Dry ingredients and fish oil being weighed prior to being hand mixed together with the minced fish.

grouper. These feed ingredients are available throughout the year and in sufficient quantities to justify this research. Each of the feed ingredients was incorporated in a reference diet at an inclusion rate of typically 30% and its apparent dry matter, crude protein and energy digestibility determined by reference to the digestibility marker, chromic oxide. Although these studies were carried out using mouse groupers, it is expected that other grouper species would show similar results. The outcomes of the experiments are summarized in Table 1.

The data shows that protein contained in shrimp head, formic and propionic blood, sardine, mixed-fish and palm oil cake meal is well digested by mouse grouper. However, the low dry matter and energy apparent digestibility of palm oil cake meal and rice bran meal indicates that groupers do not use these meals very well. The comparatively poor digestibility of soybean meal, especially that of protein, was surprising and indicate that it may not be very suitable as a replacement for fishmeal in compounded feeds for groupers. Not unexpectedly, the oven-dried blood meal was not well digested but this could be markedly improved by organic acid fermentation.

Replacement of fishmeal

A 9-week growth experiment was carried out with mouse grouper to see if shrimp head meal could be used as a partial replacement for fishmeal. Fish were held in 1 x 1 m seacages and fed twice daily. Five diets were examined in which shrimp head meal at 10% inclusion increments from 0 to 40% were used to substitute for fishmeal on an isonitrogenous basis. Table 2 shows the results of this experiment.

Although survival rate was unaffected by the amount of shrimp head meal in the feed, most other response characteristics became worse with increasing inclusion of shrimp head meal. The reduced intake associated with increasing amounts of shrimp head meal is most likely a dual effect of the fish not liking this feed and the smaller size of the fish resulting from the lower biological value of feeds containing the shrimp head meal. The results suggest that shrimp head meal should not be used at inclusion rates above 10% as a substitute for fishmeal in grouper feeds. The high content of chitin in the shrimp head meal may explain the poor performance of the fish fed these feeds since the chitin would likely be poorly digested by the fish.

Preparation of moist feed pellets (noodles) using simple on-farm equipment. A kitchen meat mincer is used to mince the trash fish. The mixed dough is extruded through the meat mincer and the resultant noodles subsequently broken into suitable lengths for feeding to groupers.

What carbohydrate is best for groupers?

A 6-week growth and nutrient retention experiment was carried out to compare four different types of carbohydrate – dextrin, starch, sucrose and glucose – when each was included at a similar inclusion of 16%. The object of this study was to see if including carbohydrate in the feed formulation would provide the fish with an alternative source of energy instead of breaking down absorbed protein for this purpose. If carbohydrate can be digested and effectively metabolized it would enable the amount of protein in the feed to be reduced, lowering both the cost of the feed and reducing the excretion of nitrogen to the water. The results of the work are shown in Table 3.

As shown by the data in Table 3, glucose was the most effective type of carbohydrate, resulting in higher growth rates and higher retentions of protein and energy. Starch and dextrin were the next best while sucrose was the least effective type of carbohydrate.

Table 1. Dry matter, crude protein and gross energy apparent digestibility coefficients (%) of locally available feed ingredients in Sulawesi.

Ingredient	Dry matter	Crude protein	Gross energy
Shrimp head meal	59	78	64
Soybean meal (full-fat)	55	67	51
Palm oil cake meal	45	81	40
Dried blood meal[1]	48	55	ND
Formic blood meal[2]	68	88	ND
Propionic blood meal[3]	62	84	ND
Local sardine meal	87	93	85
Local mixed-fish meal	59	82	77
Rice bran meal	22	60	44

ND: Not determined as insufficient faecal sample for energy analysis.
[1] Whole cattle blood recovered from slaughterhouse and oven dried.
[2] Oven dried cattle blood following fermentation using formic acid.
[3] Oven dried cattle blood following fermentation using propionic acid.

Since glucose is not a standard feed ingredient and starch and sucrose were not very effective as alternative energy sources, it seems that carbohydrates will not have a useful role in grouper feeds for protein sparing.

Vitamin C requirements

Carnivorous fish such as groupers do not have the capacity to synthesize vitamin C and neither do they carry sufficient microorganisms in their digestive tract to provide a non-dietary supply of this vitamin. Vitamin C has many physiological functions, including maintenance of epithelial tissue functionality and maintaining the fish's immunity system. In larval fish, a deficiency of vitamin C leads to skeletal and other deformities while in older fish, a deficiency will cause slow growth, increased mortality and reduced resistance to disease. Ascorbic acid, which is the active chemical of vitamin C, is very easily destroyed by ultra violet light and heating. To overcome this problem, and especially where feed is pelleted by hot extrusion or steam press, more stable forms of Vitamin C have been developed. One such product is L-Ascorbyl-2-phosphate-sodium-calcium (APNaCa). An 8-week experiment was carried out with groupers held in seacages to test the efficacy of this product as a source of vitamin C. In the experiment, four incremental levels of APNaCa from 0 to 150 mg/kg were examined and compared with feed of the same formulation but in which a proprietary vitamin premix was used. The vitamin C in the proprietary premix was in an unstable form. The results of this study are shown in Table 4.

In the experiment, fish performance improved with increasing supplementation of APNaCa with the best growth seen at the highest amount of 150 mg/kg. Interestingly, most of the difference in growth of the fish occurred in the last few weeks of the experiment when severe flooding caused a marked deterioration in water quality. Compared to fish fed the low vitamin C feeds, those fed the higher APNaCa feeds were better able to handle the water quality stress with high survival rates and continued good growth. The fish fed the commercial vitamin premix containing non-stabilized ascorbic acid grew poorly, hardly better than those fed the zero APNaCa feed, and survival was low. Adding APNaCa at rates of 150 mg/kg feed is advised and especially if culture conditions may be stressful.

Moist feeds

Although the above studies were carried out using pelleted dry feeds, current research is examining moist pellets as a cost-effective way of providing feeds for groupers. Moist pelleted feeds have many advantages for the small-scale grouper farmer:

Table 2. Biological response of mouse grouper to feeds containing increasing amounts 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]
DGC (%/d)	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 (g/week)	23.2[a]	23.0[a]	19.6[b]	19.0[b]	13.8[c]
Protein efficiency ratio (g:g)	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]
Survival rate (%)	100[a]	96.7[a]	95.0[a]	98.3[a]	96.7[a]

[a,b,c] Values in the same row with a similar superscript letter are not significantly different (P>0.05).
DGC – Daily growth coefficient (100 times the difference between start and end weights raised to the one third power and divided by the number of days on experiment).

Table 3. Biological response of mouse grouper fed diets containing different types of carbohydrate.

Variables	Source of carbohydrate			
	Glucose	Sucrose	Dextrin	Starch
Absolute growth rate (g/d)	0.40[c]	0.27[a]	0.34[b]	0.34[b]
Feed efficiency (%)	100.8[c]	78.6[a]	91.2[b]	90.1[b]
Protein retention (%)	35.0[c]	26.2[a]	33.1[c]	29.7[b]
Lipid retention (%)	67.8[b]	45.7[a]	53.9[a]	49.8[a]
Survival rate (%)	100[a]	100[a]	100[a]	100[a]
App. digestibility of NFE	96.6[a]	87.7[b]	82.8[b]	69.3[a]
App. digestibility of protein	94.4[b]	93.4[a]	94.6[b]	94.8[b]
App. digestibility of lipid	97.2[a]	96.2[a]	95.6[a]	95.3[a]

[a,b,c] Values in the same row followed by a similar superscript letter are not significantly different (P>0.05).

Table 4. Weight gain, daily growth rate, feed efficiency and survival rate of mouse grouper fed different levels of APNaCa.

Variable	Comm. premix (control)	APNACa level (mg/kg)			
		0	50	100	150
Weight gain (%)	120[a]	110[a]	170[b]	187[b]	254[c]
DGC (%/d)	1.40[a]	1.32[a]	1.78[b]	1.88[b]	2.26[c]
Feed efficiency (%)	33.5[a]	32.0[a]	49.8[b]	54.7[c]	69.9[d]
Survival rate (%)	72.5[a]	75[a]	85[b]	86.7[b]	95[c]

[a,b,c,d] Values in the same row followed by a similar superscript letter are not significantly different (P>0.05).
DGC – As for Table 3.

- They can be easily made on-farm with simple equipment (some form of meat grinder to mince and cold-extrude the mixed dough to form the noodle pellet).
- Reduced dependence on trash fish since only a quarter or less is required compared to using trash fish as the sole source of food.
- Enables locally-available ingredients to be used in the formulation.
- Enables effective vitamin enrichment (often deficient when trash fish only is fed).
- Reduces food wastage (and consequential pollution of the water) as moist pellets are eaten by the fish with minimal loss.
- Moist pellets can be used to train fish to accept dry pellets.

There are a couple of disadvantages. Moist feeds have to be prepared daily (unless refrigeration is available) and preparation can be time consuming and labor intensive. Additionally, some knowledge about what feed ingredients can be used is essential otherwise a nutritionally unsatisfactory feed might be produced. However for a small-scale farmer, the advantages far out weigh the disadvantages.

Examples of suitable moist pellet formulations for feeding juvenile groupers are shown in Table 5 and the ease with which they can be made on-farm using simple equipment is illustrated in.

Table 5. Examples of formulations and gross nutrient composition for producing moist pelleted feed for juvenile groupers

Ingredient	1	2	3	4
	Kg (as used)			
Trash fish	50	50	60	60
Fishmeal (65% CP)	13	12	10	11
Mussel/snail meat		7	0	0
Mysid meal	5	0	0	0
Soybean meal (solvent)		0	15	0
Groundnut meal[1]		0	0	15
Dry blood meal		10	0	0
Rice bran	16	15	9	0
Tapioca/cassava starch		0	0	9
Wheat gluten (80% CP)	10	0	0	0
Fish oil[2]	4	4	4	3
Additives[3]	2	2	2	2
Total	100	100	100	100
	Nutrient composition (% as fed)			
Moisture	42	47	50	50
Crude protein	29	26.5	24.5	24
Lipid	7.5	7.5	7.5	7
Ash	7.5	6.5	6.5	5.5

[1] Do not use unless known to be free of fungal mycotoxins such as aflatoxin.
[2] Can be deleted but inclusion will produce better fish growth.
[3] Use quality vitamin and trace mineral premixes.

Future research

Past work has focused on feed development for mouse grouper and particularly for juvenile fish of 20 to 150 g size. Our focus will now change to examine fish in the size range of 200 g to market size and with an increasing emphasis on tiger grouper since its faster growth lends itself better as an aquaculture species. Research will also continue to determine apparent digestibility of locally available feed ingredients and the further development of practical moist feed formulations using locally available ingredients.

Grouper grow-out feeds... continued from page 17...

Conclusions

This research has demonstrated that trash fish can be an excellent food source for culturing Malabar groupers with DM FCR's of less than 1.0 able to be achieved under experimental conditions. It has also demonstrated that the type (species) of trash fish used can significantly affect the productivity of the grouper. In this study, lizard fish was a better species to feed than cardinal fish although a combination of both gave the best overall result. These productivity response differences of the grouper are thought to be due to differences in the macro- and/or micro-nutrient composition of the trash fish being fed. The best rate to feed the trash fish was found to be 9% of the fish biomass with the total amount fed as two equal meals a day. Not only did this rate result in the best growth and FCR of the fish but survival was almost 10% higher than feeding at a 5% rate.

Future research

The above study was carried out under closely controlled laboratory conditions. It will be important to see if these findings can be validated under normal seacage farming conditions. Under such conditions, nutrient losses from the trash fish are likely to be greater (leading to poorer FCRs and growth rates) and the freshness of the trash fish at feeding is often far from satisfactory. The addition of a vitamin supplement and some simple form of binding the trash fish into a more stable food may overcome both of these problems.

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Feed development and application for juvenile grouper... ***continued from page 11...***

fishmeal in grouper feeds. Unfortunately, using soybean meal in amounts exceeding 10% of the diet as a protein replacement for fishmeal caused growth rate and food conversion of the fish to worsen.

Practical feed formulation for juvenile groupers

A suitable practical feed formulation for juvenile groupers has been developed at Gondol (Table 1).

In a 4-month trial with humpback grouper held in a floating net cage, the effectiveness of the Gondol feed formulation has been compared against a commercial pelleted grouper feed and trash fish.Table 2 shows the results of this comparison. Growth rate and FCR were best for grouper fed the Gondol feed. In terms of growth rate and FCR, the commercial feed was as good as the vitamin-supplemented trash fish. Interestingly, the haematocrit value (proportion of red cells in the blood – an indirect measurement of haemoglobin content) of the grouper fed trash fish was quite low, suggesting either a vitamin deficiency (even though a vitamin supplement was mixed with the trash fish) or some other toxic or infectious agent present in the grouper.

Future research

Gondol will continue research to increase our knowledge about the nutrient requirements of groupers with tiger grouper being the main species to be used for these investigations. A priority area is to develop successful nursery feeds in order to quickly and easily wean fry from live feed (or trash fish) to pelleted dry feeds. This work will be coordinated with other grouper feeds research that will be carried out in the ACIAR Marine Finfish Technology Improvement project at Maros (Indonesia).

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