THE NUTRITION AND FEEDING OF A NATIVE THAI SPECIES, THE MARBLE GOBY (*Oxyeleotris marmoratus*), INVOLVING ON-FARM AND EXPERIMENTAL STUDIES

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

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Declaration

I hereby declare that this thesis has been composed entirely by myself and has not been submitted for any other degree. Information from the works of others (Published and unpublished) and their contributions have been acknowledged.

Jatuporn Bundit

This thesis is dedicated in loving memory to my father and mother;

Patthana and Sri Bundit

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Abstract

In Thailand, culture and production of a high value freshwater fish, the marble goby, is dependant upon farm-made feeds using marine and freshwater trash fish as primary ingredients. However, there is lack of nutritional research regarding the use of such farm-made feeds and their impacts on the nutritional status, growth and health of marble goby. The aims of the present study were to evaluate the effects of farm- made feeds on slaughter indices, fish lipid classes and fatty acid profiles, nutrient composition and digestibility. In addition it was intended to improve on-farm feed quality for both current practical feeds as used by farmers and alternative feeds using rice bran and tilapia with reference to biochemical composition, growth performance and haematology of marble goby.

Nutritional evaluation of farmed fish compared to their wild counterparts indicated that fatty acid composition of farmed marble goby was markedly influenced by diet. Marble goby appeared to utilize MUFA preferentially as an energy source compared to SFA. Fish muscle was characterised by higher n-6 PUFA; arachidonic acid (20:4n-6, AA) and docosapentaenoic acid (22:5n-6, DPA). Eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22: 6n-3, DHA) comprised the majority of n-3 PUFA found in fish muscle and can potentially be enriched into marble goby muscle through the diet. The significantly higher ratios of neutral liver lipid to polar lipid (NL/PL) indicated the imbalance of dietary lipid and energy of on-farm feeds. Moreover, findings on slaughter indices and lipid peroxidation of farmed fish; higher HSI, VSI, liver TBARS

and the pale lipid-rich liver of farmed fish indicated problems with the nutritional quality of lipid in farm-made feeds.

Experiments aimed to improve farm-made feeds using supplemental vitamin E in the form of α-tocopherol. These demonstrated that supplementation of α-tocopherol to oxidised diets, both mackerel and tilapia based, did not result in a significant beneficial effect in reducing mortality, and improving growth and haematology in marble goby. However, dietary α-tocopherol supplementation helped in reducing fish muscle peroxidation but was not related to muscle α-tocopherol levels. In mackerel based diets containing lipid peroxidation up to 250-300 µmols MDA g^{-1}, α-tocopherol supplementation appeared not to help in reducing liver peroxidation.

The alternative use of tilapia as a feed for marble goby resulted in growth and survival rates similar to those of fish fed mackerel based diets. Tilapia contained intrinsic α-tocopherol levels that appeared to be sufficient to reduce marble goby tissue peroxidation. The synergic effects on antioxidant activities between α- tocopherol supplement and natural E vitamer contained in rice bran helped to reduce tissue TBARS and improve haematology in fish fed combination diets of oxidised tilapia and rice bran.

The inclusion of rice bran in farm based diets resulted in decreased tissue peroxidation, an adverse affect on dry matter and protein digestibility; and lower fish feed intake, growth and survival rate when 25% of rice bran was added into practical mackerel based diet. Overall, formulated feeds showed promising growth and survival rate in marble goby but more research on dietary nutrients and energy balances are required.

Abbreviations and Acronyms

AA	Arachidonic Acid
ACIAR	Australian Centre for International Agricultural Research
ADC	Apparent Digestibility Coefficient
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
DOF	Department of Fisheries (Thailand)
FAO	Food and Agriculture Organisation
NFE	Nitrogen Free Extract
TBARS	Thiobarbituric Acid Reactive Substances
MDA	Malondialdehyde
FAME	Fatty Acid Methyl Esters
HSI	Hepatosomatic Index
DWG	Daily Weight Gain
VSI	Viscerosomatic Index
EFA	Essential Fatty Acid
DPA	Docodapentaenoic Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
PUFA	Poly-Unsaturated Fatty Acid
HUFA	Highly Unsaturated Fatty Acid
FCR	Feed Conversion Ratio
PER	Protein Efficiency Ratio
APHA	American Public Health Association
MUFA	Mono-Unsaturated Fatty Acid
SFA	Saturated Fatty Acid
TAG	Triacylglycerol
NL	Neutral Lipids
PL	Polar Lipids

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Chapter1 General Introduction

The marble goby *Oxyeleotris marmoratus* is considered to be one of the most promising finfish for aquaculture in Thailand (Suwanjarat et al. 2005) and is also a good candidate species for research (Leatherland et al. 1990; Jow et al. 1999; Sayer, 2005; Masaya et al. 2006). Marble goby has several biological advantages for culture such as they can live in a wide range of salinities (Sunit and Jenjit, 2000). As it remains virtually motionless it requires only to be kept a little moist during live transportation, thus only the minimal use of water and oxygen is required (Rakbankerd, 2005). In addition, marble goby appear to be able to detoxify endogenous ammonia to glutamine in the muscle reducing endogenous ammonia production and excretion (Jow et al. 1999). Marble goby are nutritionally highly prized by Asian consumers, both at home and overseas. This because they are lean, boneless and their firm, white flesh has no off-flavour taste (Sompong, 1980). Amongst ethnic Chinese marble goby is widely believed to be an aphrodisiac, mostly by men, and is served in expensive restaurants (Suraniranat, 1998).

In Asia, marble goby are considered a species of high economic value and they fetch the highest price of any edible freshwater fish with a farm gate price of around £7 or £12 per kilogram have been reported in Thailand and Taiwan respectively (Ausyfish, 2006). In Thailand marble goby are considered to have great international export potential. The market demand and price profile of marble goby have meant relative stability in both domestic and export markets. It is particularly important in terms of export to countries where there are consumers of Asian origin, especially ethnic Chinese (Rakbankerd, 2005). The main markets are China, Hong Kong, Taiwan, Singapore,

Malaysia and Japan (Suraniranat, 1998; Rakbankerd, 2005).The market demand from such consumers always appears to outstrip supply (Rakbankerd, 2005; Phoomthai, 2007).

Marble goby have long been cultured in Thailand and other South-East Asian countries particularly Malaysia, Singapore, Taiwan and Vietnam. In 2000, 207 tonnes of marble goby were produced by aquaculture in Malaysia, Singapore, and Thailand. This represents about 74% of the total global aquaculture of gobioid fishes (282 tonnes) for 2000 (GALE, 2005).

Until recently in Thailand marble goby production has been reported from three main sources. These are cage culture, semi-intensive polyculture systems in earthen ponds where marble goby is raised with other species such as giant freshwater prawn *Macrobrachium rosenbergii* (Lin et al. 2002) tilapia *Oreochromis niloticus* (Sompong, 1980) and sea bass *Lates calcarifer* (Seenoo et al. 1994a) and capture-based fisheries. The cage culture and semi-intensive polyculture have been largely responsible for grow-out production (Edwards et al. 2004) and cage culture has long been a majority contributor (Varangkana, 1986; Lin and Kaewpaitoon, 2000; Rakbankerd, 2005). The capture-based fisheries where production is considerably lower and yield is unpredictable (Amornsakun et al. 2002). The total export of 13.2 and 38.7 tonnes in 2005 and 2006 of value of 1,995,956 (£28,514) and 12,356,666 Thai Baht (£176,524) respectively have been reported by DOF (2005), unfortunately, there is no data available for exact source and production.

The supply of marble goby seed is still principally from capture fisheries. However, it has been successfully propagated through induced spawning using hormonal injections (Tay and Seow, 1971), and several technical improvements to the basic procedure have been reported (Tan and Lam, 1973; Manop, 1984; Seenoo et al.

1994a; Seenoo et al. 1994a; Harmin et al. 1997). Nursing hatchery-based fry at early stages using live feed has been accomplished (Tawee et al. 1986; Tavarutmaneegul and Lin, 1988). However, this approach has received only limited research attention although there is increasing interest in this area. More recently a number of researchers have reported attempts to develop culture techniques and further understanding of the life-history of marble goby fry at in early developmental stages (Panu et al. 1989; Seenoo et al. 1994a; Pham, 2001; Amornsakun et al. 2002; Udompo, 2002; Abol-Munafi et al. 2005; Van et al. 2005). In addition genetic improvement of marble goby to improve growth, disease resistance and adaptability to farming systems have been investigated by many scientists (FAO, 1995).

Due to its economic value, marble goby is highly attractive to fish farmers who have always had a keen interest in farming this species. Unfortunately, attempts to sustain marble goby using complete artificial diets have not so far been successful since fish at various stages of development are reluctant to accept pellets and no commercial feeds exist for this species (Nanthiya, 1989; Basa, 1989; Lin and Kaewpaitoon, 2000; Pham, 2001; Edwards et al. 2004). This has been a common problem in the early nutritional development of feeds for capture-based carnivorous finfish species such as snakehead (*Chana striata*) (Jantrarotai and Jantrarothai, 1993) and grouper *Epinephelus* spp. (Kevin and Michael, 2005). Feeds and feeding of marble goby has largely been derived from the knowledge and experience of farmers gathered over several decades of fish culture. There is little or no scientific information published on the nutrition of marble goby. There is also a lack of current research on using farm-made aquafeeds for marble goby even though grow- out production has long been significantly supported by farm-made aquafeeds (Edwards and Allan, 2004; Suchart et al. 2005).

The benefits of using farm-made aquafeeds for small-scale aquaculture producers in Asia and the Pacific have long been recognized (Tacon, 1997; Tacon and Silva, 1997). Asia is by far the leading region for global aquaculture production and Asia accounted for 85%, 89% and 92% of the world aquaculture production in 1991, 2002 and 2004 respectively (FAO, 2004; FAO, 2006). About 33% of Asian finfish and crustacean production has been achieved at least partially using farm-made aquafeeds (Kee-Chai, 1993; Francesca et al. 2004). FAO (1993) forecasted that in 2004 approximately 2.0-2.3 million metric tonnes (mmt) of global production of carnivorous finfish and approximately 25 mmt of crustacean were produced without the use of commercial feed. Unfortunately, no statistical data has been reported to prove the forecasting since the last report by FAO in 2003. In fact more than 90% of world aquaculture production in 2004 was from developing Asian countries with about 74% originating from China (FAO, 2006) where on-farm feeds have historically been regarded as a majority feed input in their culture systems (Felicitas, 1996; Lamgsen and Beiping, 2002; Edwards and Allan, 2004). This perhaps could indicate the remaining importance of the farm-made feed to aquaculture of the region.

Farm-made aquafeeds allow farmers to adapt feed inputs to their own financial resources and requirements. They also facilitate the use of locally available agricultural by-products which would otherwise have limited use within the community (ACIAR, 2004). Farm-made aquafeeds are also potentially cheaper for farmers than commercial aquafeeds.

Research that develops and improves existing on-farm aquafeeds together with identification and utilization of alternative potential feed ingredients is required to sustain and expand marble goby production. It should be noted that a key issue is that so far seed used for marble goby culture is still obtained from the wild. Developing a

successful artificial diet could be a complicated task because such feeds are not well accepted by this species and their nutritional requirements are not fully known.

In addition, nutritional research on the effects of existing farm-made feeds on marble goby carcass composition would allow some understanding of their nutritional status. It might also permit some extrapolation to consideration of their nutrient requirements that could then be used to develop advanced diets to support marble goby culture in the future.

The aims of the present research are specifically to;

1) Evaluate on-farm feeds and feeding strategies on fish nutrient composition of marble goby

2) Assess the use of farm based diets on fish growth, feed digestibility, feed intake, nutrient utilization and chemical composition of marble goby.

3) Improve existing practical farm-made feeds to ensure nutritional quality in terms of fish carcass analysis and haematological responses.

4) Investigate the use of alternative feeds on fish growth performance, fish nutritional quality and fish health.

Chapter 2 Literature Review

2.1 Biological characteristics

The sand goby, marble goby or marbled sleeper goby are common names for the carnivorous fish *Oxyeleotris marmoratus* which is a member of the family Eleotridae. This species is commonly found in freshwater and brackishwater bodies throughout Indo-China (Sompong, 1980). It is one of the largest goby-like fishes in the world and grows up to 50 cm in total length and reaches maturity at approximately 7 cm (Tan and Lam, 1973). The young are free-swimming initially and assume the bottom-dwelling habits of the adults later on (Seenoo et al. 1994a).

Marble goby differs from true-gobies in that the pelvic fins are separated whereas in true gobies these are combined to make a cup-shaped sucker. In most other respects they are similar to the true gobies (Kelvin and Peter, 2002). Marble goby are comparatively dark brown on the dorsal side and pale brown on the ventral side. The body has a series of long dark blotches. The fins are without spines and have black or dusty bands. Colouration is variable and depends on the environment of the habitat bottom and lighting. The body is elongated and without a lateral line. The flattened-upper-side of the head has ocelli. The mouth has a deep cleft directed obliquely upward (as shown in Figure 1) (Kottlelat et al. 1993). This species is sluggish and prefers to spend most of its time half-buried in the habitat substrate with eyes protruding to ambush its prey (Kelvin and Peter, 2002).

2.2 Fish breeding strategies

Tavarutmaneegul and Lin (1988) claimed that marble goby are capable of multiple spawning and can spawn throughout the year under suitable conditions. This species can acclimatise to, and reproduce in, both freshwater and brackishwater (Masaya et al. 2006). In nature marble goby breeds intensively during the rainy season, May to August, and less during other months, particularly November and December when temperatures are lower (Sompong, 1980).

Figure 1 Adult male marble goby (*Oxyeleotris marmoratus*) of 1.22 kg in body weight and 32.5 cm. in total length.
Source: Kasimoff (2005)

Natural and artificial spawning have resulted in successful breeding of marble goby (Tay and Seow, 1971). In nature, spawning usually occurs at dawn when females lay eggs in nests such as wood, stone and among fine-leaved plants. During spawning the male sheds its milt and engages in brood care by fanning the eggs using its pectoral fins. The amber-coloured fertilized eggs have a bundle of adhesive filaments (Tan and

Lam, 1973). Eggs hatch in about 28h at optimal temperatures of 25-27 °C (Panu et al. 1989).

Larvae exhibit a typical S-posture and horizontal swimming around two days after hatching. The feeding of fry on zooplankton is recommended on the 3rd day after hatching when the eyes, the optic vesicle with ciliated epithelium, the free neuromasts on the head and the trunk and the ciliate olfactory epithelium are functional (Seenoo et al. 1994a). The first taste buds appear in the oral cavity 6 days after fertilization (Seenoo et al. 1994b). Marble goby change their habit from pelagic to benthic at 35 days after hatching and show positive phototaxis during the early stages (Seenoo et al. 1994a).

Marble goby fecundity is considered exceedingly high. It has been estimated that mature female marble goby has a mean relative fecundity ranging between 130-300 eggs per gram body weight with younger fish producing more eggs per gram body weight (Pisarn and Wichaen, 1977; CFRDB, 2006). While, Kok (1982) and Ali (1999) reported that snakeheads: *Ophiocephalus punctatus* and *Channa striata* had average fecundity ranging between 2-17 and 11-36 eggs per gram body weight, respectively.

Fish breeding using Human Chorionic Gonadotrophin (HCG) to induce maturation and artificial fertilization in marble goby has been reported to be successful in Thailand and Malaysia (Kamthorn, 1972; Tan and Lam, 1973). Although a high percentage (90%) of fertilization and hatching was achieved, newly hatched larvae did not survive for more than a few days due to starvation.

Semi-natural breeding using artificial substrates to collect fertilized eggs before transfer to hatchery aquaria for incubation is one of the practical techniques used in Thailand (Sunit and Jenjit, 2000). The hatchlings are kept in a hatching tank for 3-5 days until the yolk sacs are absorbed. The newly developed fry are then transferred to outdoor concrete tanks containing ‘green water’. The fry are initially trained to eat the

exogenous live feeds that are summarized in Table 2. Fry at the age of 30-60 days are nursed in organically fertilized ponds and fed supplementary feeds (Tavarutmaneegul and Lin, 1988; Sunit and Jenjit, 2000).

Despite the relatively large fecundity and high hatching rate, fingerling production is reduced by severe mortality during larval development and during the young post-larval stage. The vulnerable stage occurred when young post-larvae shifted from endogenous to exogenous food sources. There are two causes of vulnerability postulated. Firstly, high mortality because relatively inactive feeding behaviour makes them poor feeders (Seenoo et al. 1994b) and secondly, starvation due to a small mouth size (~0.1mm.) that limits the availability of food organisms, since most common zooplankton species are larger than the mouth opening (Seenoo et al. 1994b). With improved feeding of first feeding larvae, higher production can be anticipated (Pham, 2001; Van et al. 2005).

2.3 Nutritional qualities of marble goby

Rakbankerd (2005) revealed that marble goby contain a high proportion of edible flesh (58%) that is boneless, firm and has a mild flavour. It has relatively high protein and low fat content; higher protein-to-fat ratio compared to other tropical economically important carnivorous species (Table 1).This could be influenced by type of feed consumed (Jauncey, 1982; Ohta and Watanabe, 1996), feeding strategy (Jobling et al. 1998) and its genetics (De-Santis and Dean, 2007). However, until recently there has been little research on nutritional requirements and the nutritional profile of marble goby.

2.4 Feeding habits

In nature, marble goby is considered a motionless, carnivorous fish. It is a bottom-feeding fish, preying on small fish, crustaceans, insects and molluscs (Sompong, 1980). Evaluation of feeding habits using stomach content analysis Pisarn and Wichaen (1977) found that fingerling and adult of wild marble goby feed mainly on small fish, small freshwater prawns and benthos. The proportion of small fish found in stomach increased as fish size increased as well as with seasonal availability of prey (Vu et al. 2005).

Table 1 Chemical composition (% wet weight of whole fish) of mature tropical carnivorous freshwater species

	Snake head (*Channa striata*)	Hybrid catfish (*Clarias batrachus*)	Basa catfish (*Pangasius bocourti*)	Striped catfish (*Pangasius hypophthalmus*)	Marble goby *O. marmoratus*
Constituents (%)					
Crude protein	17.1	15.35	11.3	13.1	15.0
Crude Lipids	0.6	6.09	11.6	3.6	0.2
Ash	1.1	2.02	3.8	4.4	0.6
Moisture	79	74	75	74	79

Sources: Kok, (1982); Erfanullah and Jafri, (1998);Hung et al (2004); Rakbankerd (2005).

Note: The constituents are given as wet weight.

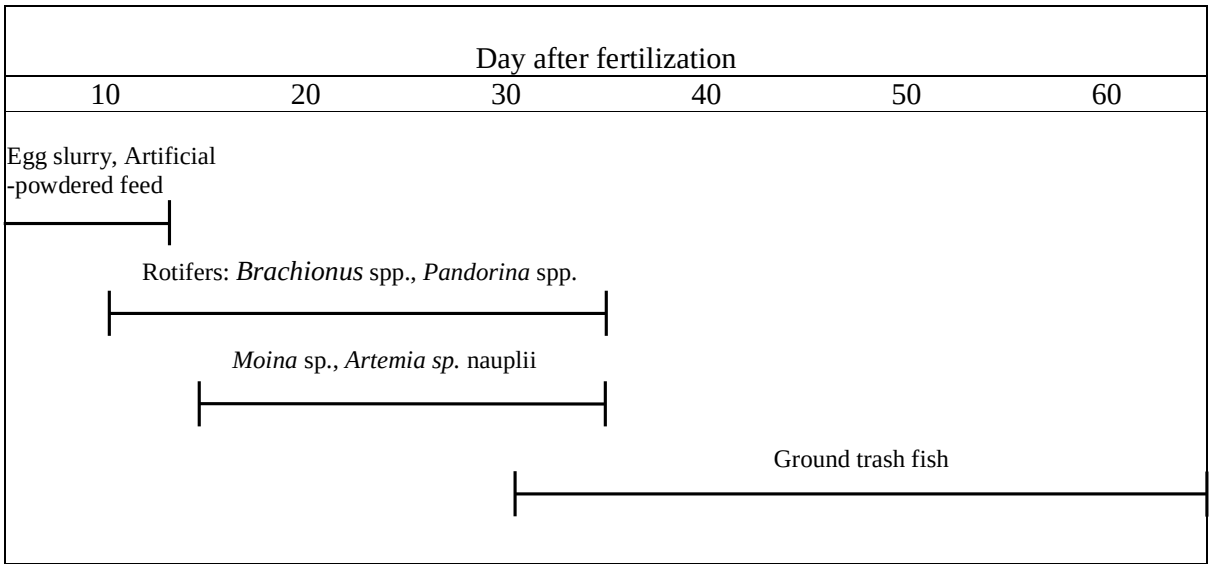
2.5 Fry nursing, growout feeds and feeding.

A histological study on digestive tract development of marble goby larvae by Pham (2001) found that the larvae started to develop intestines on day 1 after hatching, while the stomach developed on days 10-15 after hatching. Micro-algae play an important role in digestion during the early larval stages of marble goby (Seenoo et al. 1994b). During the post larval stage, marble goby first consume phytoplankton then, commence feeding on micro-zooplankton such as ciliates and rotifers (Seenoo et al.

1994b).The presence of algae in rearing water can influence the activity of digestive enzymes in developing marble goby larvae. This is supported by the work of Van et al (2005) who reported that the trypsin and chymotrypsin levels in 2-6 days after hatching rate of marble goby larvae fed with rotifers (*Brachionus* sp.) and nauplii of copepods and moina were higher in fry raised in green water than in those reared in clear water.

First feeding was found at the average body length of 1mm with mouth size openings of 0.008-0.2mm (Tavarutmaneegul and Lin, 1988). The recommended feeding schedules for various feeds used in rearing marble goby fry are summarized in Table 2. Seenoo et al (1994b) reported that the selection of live feeds used is directly related to the mouth sizes and mobility of fry rather than development of sense organs.

Table 2 Schedules for the variety of feed used in rearing *O. marmoratus* larvae (an average individual size of 4 to 25 millimetre in total length)



Sources: Tavarutmaneekul and Lin (1988); Seenoo et al (1994a).

Panu et al (1989) reported that size differences in the larvae of marble goby were found to be the most important factor affecting cannibalism while inadequate and unsuitable food supply can further increase cannibalism. Cannibalistic behaviour is

recognized as occurring in larvae and continuing until the juvenile stage (Tawee and Amnaj, 1980; DOF, 2000). It has been reported by Anusorn and Sujitra (1989) that mortalities due to cannibalism can be effectively reduced when larvae are raised in plankton-rich water and an increased water current. However, thus far no one has reliably reduced cannibalism-induced mortality to acceptable levels. Grading is necessary in intensive operations to remove the fastest growing cannibals (DOF, 2000).

Table 3 Feed and feeding of marble goby fry and fingerlings in different culture systems

Initial stocking size	**Rearing system**	**Stocking density**	**Culture period**	**Feed types/feeding strategy**	**Growth /Survival rate**	**References**
Fry at 5 days after hatching	Hapas in cement tank + aeration + green water flow thought system	No report	24 days	Fed on egg slurry and for first 10 days. After that fish were fed by using rotifers.	1) Fry reached total length of 1.5 – 2 cm 2) Survival rate was 80%.	Sompong (1980)
0.35 –0.37 g	Cement tank (2×3 m.)	600 fish L^{-1}	3 months	Feeding: Trash fish and fresh water shrimp paste which were mixed of 1% of concentrate feed in each.	1) Final weight of larvae fed trash fish and Freshwater shrimp pasted were 0.6 and 0.8 g 2) Survival rate were 10.7 % and 5.6%	Tawee and Amnaj (1980)
0.04-0.39 g	Earthen pond of the size of 800 m^2	13 fish m^{-2}	30 days	Combination of natural foods through organic fertilization using dried chicken manure and supplementary feed; trash fish+ rice bran + mineral & vitamin premix. The hiding substances were also provided. • Feeding rate of 10% of body weight.	1) *Final weight* was 2.4 g 2) Survival rate was 40%	Tawee et al (1986)
0.32 cm	Fibre glass of volume of 250 L	24 fish L^{-1}	60 days	To compared the growth and survival rate of larvae raised in different salinities; 0, 10, 20 ppt Feeding: using *Chlorella* sp. to maintain population of Rotifers before feeding to fish. *Moina* sp. was applied at the 20 days of culture period until harvesting.	1) Survival rate of larvae raised in 0, 10 and 20 ppt.were 0%, 97% and 98%, respectively 2) Final length were 1.94 and 1.82 cm. in 10 and 20 ppt, respectively	Sunit and Jenjit (2000)
8.25 g	Floating cages in Earthen pond	285 fish m^{-2}	24 days	Fed on supplementary feed; Trash fish (94%) + rice bran (5%)+ min.&vit. premix (1%)	1) *Final weight* was 8.60 g 2) Survival rate was 35%	Tawee and Panida (1978)

Table 4.1 Feed and feeding of growout marble goby in different culture systems

Initial stocking size.	**Rearing system**	**stocking density**	**Culture period**	**Feed types/feeding strategy**	**Growth /Survival rate**	**References**
132-176g of individual weight (collected from wild)	Cages (1.5×2×1.5m.) in pond	60 fish m^{-2}	5 months	1. Supplementary feed: Formula 1. (64% trash fish + 18% rice bran + 15% binder, 2% mineral & vitamin premix + 1% soybean oil) Formula 2. (64% trash fish + 9% rice bran + 19% binder, 2% mineral & vitamin premix + 6% soybean oil) Formula 3 (32% trash fish + 32% rice bran + 17% binder, 2% mineral& vitamin remix + 3.5% soybean oil) Feeding rate was 4% body weight, once a day	1) Final weight were 326, 291 and 326g 2) Daily weight gain were 1.12, 0.87 and 1.12, respectively 3.) Survival rate were 73%, 71% and 63 % in fish fed formula 1, 2 and 3 feed, respectively (P>0.005)	Anusorn et al. (1988)
60-200g (collected from wild)	Earthen pond	1 fish m^{-2}	10 months	Feeding by using supplementary feed; The ratio of refused meat: rice bran = 1:1 by weight • Feeding rate of 10% body weight	1) Final weight of 300-800g could be obtained	Field survey at Samuth Prakarn Province by Kamthorn (1972)
(Polyculture) tilapia = 85g marble goby = 70g of individual weight	Earthen pond of the size of 200m^2	No report	6 months	To compared growth of fish raised in different stocking densities: T1 = Tilapia 0.5 fish/m^2 + marble goby 0.1 fish m^{-2} T2 = Tilapia 1 fish/m^2 + marble goby 0.1 fish m^{-2} • Feeding by using rice bran and pellet • Feeding rate was 3% body weight	1) Mean final weight of marble goby was about 220 g in both treatments	Sompong (1980)
80g individual weight	Earthen Pond of the size of 200 m^2	1 fish m^{-2}	8 months	To compare growth of fish fed with different feeds: T1 = Pellet T2 = Supplementary feed (The ratios of rice bran: trash fish: cooked rice was 3:5:2 by weight • Feeding of 5% body weight, once a day	1) Total production of 140 kg and 157kg 200 m^{-2} in T1 and T2, respectively	Somprasong (1972)

Table 4.2 Feeds and feeding of grow-out marble goby in different culture systems (cont.)

Initial stocking size.	Rearing system	stocking density	Culture period	Feed types/feeding strategy	Growth /Survival rate	References
81g individual weight	A net fenced-cove in reservoir (5.24 ha in area), in South Vietnam	Marble goby were stocked at 960 fish ha^{-1}. Fingerling of common carp (31g), silver carp (14g) and bighead carp (17g) were stocked at 470 fish ha^{-1} each, while grass carp (20g) was stocked at 170 fish ha^{-1}. Giving an overall stocking density of 2540 fish ha^{-1}	7 months	● Fish relied on natural foods in water body (freshwater fish prawn, small fish, benthos and etc.) ● Neither fertilizer nor feed was added to the cove	● Gross yield of marble goby was 251.1 kg ha^{-1} $crop^{-1}$, while gross yields of silver carp, bighead carp, common carp and grass carp were 90.5, 114.3, 84.6 and 35.0 kg $ha^{-1}crop^{-1}$, respectively. ● Survival rate was 73.7% for marble goby and 55.1–62.8% for carps	Vu et al (2005)

A summary of feed and feeding practices at in early-life history and grow-out stages of the marble goby in Thailand with different culture systems described in the text is presented in Table 2, Table 3, Table 4.1 and Table 4.2

Marble goby fry raised in earthen ponds had shown a low survival rate (25 -50 %) (Panu et al. 1984; Tawee et al. 1986; Anusorn and Sujittra, 1989) in particular, predators, stress and improper collection were main causes of fry mortality. The marble goby's habit of hiding in muddy water makes fry collection difficult as ponds need to be drained before harvesting (Rakbankerd, 2005). It is preferable that survival rate of fry can be improved by providing suitable natural foods at the right time and with appropriate husbandry. In addition, juveniles seem to grow faster when supplementary feeds consisting of high protein animal sources and natural foods are presented in sufficient quantity (Anusorn et al. 1988; DOF, 2000). However, there is a lack of knowledge on the culture requirements of marble goby as no information is available on the successful nursing of fry up to sub-adult size of 50-100g. The sub-adult size is considered an important stage in supporting production of the marble goby since it is the size that is required for growout cage culture.

Culture of fish in pond sizes of $800m^2$-$1600m^2$ employs stocking rates of 10,000-24,000 fingerlings (Anusorn and Sujittra, 1989; DOF , 2000). A stocking rate of 100-150 $fish.m^{-2}$ of 50-100 g in individual weight is preferable for cage culture (Sompong, 1980). Harvesting is performed periodically by marketable size selection. The marketable size is about 400-1,200g and it usually takes 6-10 months for fish to reach the marketable size (Rakbankerd, 2005).

Until recently, marble goby culture systems do not rely on pelleted feeds. Feeding of this species is still predominantly traditional. Their foods are usually marine

trash fish, rice bran, poultry slaughter house by-products or a combination of these (Edwards and Allan, 2004; Suchart et al. 2005).

2.6 Environmental effects

Marble goby could be regarded as an amphibious species. It can survive in terrestrial conditions for up to 72 hrs without having an accessory breathing organ (David, 2000) and relies on its ability to reduce endogenous ammonia production and detoxify ammonia to glutamine that accumulates in the muscle tissue (Jow et al. 1999). A study by Ip et al (2004a) found that marble goby activates hepatic glutamate dehydrogenase and glutamine synthetase at levels of threefold and 30-fold, respectively, when out of water in order to detoxify ammonia to glutamine.

Water quality at 3-4 m in Boraphet reservoir, Thailand where marble goby seed were found in abundance was recorded as: dissolved oxygen, total alkalinity, pH and temperature were 6.0-7.0 ppm, 70-80 ppm, 6.5 –7.2 and 24-30°C, respectively (Manop, 1984).

As yet there is no published information on the tolerance limits and physiological responses of marble goby to accumulated waste metabolites.

2.7 Diseases and parasites

One of the most critical problems in marble goby culture is the high mortality of fish caused by disease (Somkiat, 1997; Lin and Kaewpaitoon, 2000; Pham, 2001; Prasankok et al. 2002). It appears from the literature that Epizootic Ulcerative Syndrome (EUS) is the most serious of disease in marble goby. It is a seasonal disease that usually occurs just after heavy rain and during cooler weather (December-February). This disease causes ulcerative lesions on the body and/or head of the affected

fish. The primary causative agent is still unknown and it has been suggested that a complex of agents is involved in the epizootic (Somkiat, 1997; Prasankok et al. 2002). The major pathogens are viruses: binavirus, iridovirus; *Oxyeleotris marmoratus* ranavirus (OMRV), bacteria; *Aeromonas hydrophila*, and fungus; *Aphanomyces invadans*, have so far been found to be associated with EUS (Hedrick et al. 1986; Saitanu et al. 1986; Somkiat, 1997; Prasankok et al. 2002; Saitanu et al. 1986; Somkiat, 1997). However, the role of viruses in such a wide spread epizootic is still unclear. Similarly, the studies of bacterial disease of the marble goby intensively cultured in wooden cages in the Nan River, Thailand, found that the majority of bacterial infections were caused by *Aeromonas hydrophila*. The combination of low dissolved oxygen, an abrupt change of water temperature and pesticide residue (Dieldrin and Endrin) in water were considered as predisposing factors to stress and infection (Supamataya, 1984). The marble goby is reported to be one of the most sensitive fish to stress caused by handling, transportation and transference between habitats. Supamataya (1984) and Koolvara (1985) claimed that intraperitoneal injection with oxytetracycline-HCl or tetracycline-HCl into fish infected with *Aeromonas hydrophilla* at a dose of 30 mg kg^{-1} of fish body weight resulted in a 100 percent survival rate. Moreover, the parasites *Henneguya* sp., *Dactylogyrus* sp., *Lernaea* sp. and *Ergasilus* sp. were commonly found in specimens of marble goby (Supranee, 1999; Janejira et al. 2006; Shariff, 1982). However, the presence of these parasites is not normally a serious problem affecting the culture of marble goby in Thailand.

2.8 Roles of farm-made feeds in aquaculture

The FAO (FAO, 1993) suggested that farm-made feeds be defined as feeds in pellet or other forms, consisting of one or more artificial and/or natural feedstuffs,

produced for the exclusive use of a particular farming activity and not for commercial sale or profit. The benefit of using farm made feeds is to promote fish growth, cost efficiency and reducing dependency on off-farm feed ingredients.

It has been estimated that over 1 million tonnes of farm-made feeds have been produced annually in Asia and that about one-third of Asian finfish and crustacean production has been achieved partially through their use (Kee-Chai, 1993; Francesca et al. 2004). Besides, it was estimated by FAO (1993) that by the year 2000, around 2.0-2.3 million ton (1.9 million tons in 1990) of global production of carnivorous finfish, crustacean, common carp, tilapias and milkfish would be produced without the use of commercial feed. Farm-made aquafeeds are thus clearly important for aquaculture production and development.

A wide range of farm-made aquafeeds in eleven leading Asian aquaculture countries are from single feeds available on-farm such as grass or rice bran to farm-made formulated feeds. These include aquatic and terrestrial plants (duckweeds, azolla, water hyacinth etc.), aquatic animals (snails, clams etc.) and terrestrial-based live feeds (silkworm larvae, maggots etc.), plant processing (de-oiled cakes and meals, beans, grains and brans). Fresh trash fish, fish meal and animal-processing by-products (blood and feather meal, bone meal etc.) are the commonest animal proteins used in farm-made aquafeeds (FAO, 1993).

The traditional feeding method for carnivorous fish of using low value/“trash fish” remains the method of choice for many farmers in Asia and is likely to remain so for some time to come. This is particularly true for those farming presently low-volume species such as snapper (*Lutjanidae* spp.), grouper (*Epinephelus* spp.) and many other marine fish where aquafeed manufacturers find it difficult to develop economically competitive pelleted feeds as an alternative to trash fish. Even though feeding for high

volume species where compounded, soft-dry, dry or extruded feeds are available as alternatives to trash fish is increasingly becoming the preferred source of feed, the availability of a compounded feed for a particular species will not necessarily mean that farmers will use it. As long as trash fish remains locally available, at a price which makes profit achievable, it will continue to be used. For example, even though more than 120,000 tonnes of compounded feed are currently being fed to cultured yellowtail in Japan, farmers still prefer raw fish for yellowtail (FAO , 2005a). A similar trend has been found in sea bass (*Lates calcarifer*) and grouper (Serranidae) cage culture, which dominate marine fish culture in Thailand. Total sea bass and grouper production had increased significantly between 1985-2004 due to high demand and high farm gate prices. In 2004, the price of sea bass was about 150 baht kg^{-1} and grouper was sold at about 200-250 baht kg^{-1} (Pakjuta et al. 2005). It should be emphasised here that in Thailand, until recently, feeding of these two species had largely relied on trash fish (Boonyaratpalin, 1997; Pakjuta et al. 2005; Supis, 2005). Besides, throughout the Asia-Pacific region, feeding carnivorous fish using trash fish is likely to remain so for some time yet (Kevin and Michael, 2005).

In Thailand, since 1990, fish production from aquaculture has increased significantly. Aquaculture continues to play an important role in food security of the country; providing employment and generating foreign income (Somsueb, 1993; Pakjuta et al. 2005). Thailand was 7^{th} in the top ten of global aquaculture producers in 2004 and produced 1,172,866 tonnes or 2.2 % of world aquaculture production of 53,212,177 tonnes (FA0, 2006). In particular, freshwater fish production has increased throughout the country and this is currently concentrated in the central and North-Eastern region. Freshwater fish production in the country has increased four times from 22,200 tonnes (15% of total finfish production) to 96,560 tonnes (20% of total finfish

production) in 1990 and 2004, respectively. (Piumsomboon, 2001; Pakjuta et al. 2005). The pattern of culture, either monoculture or polyculture, varies according to species raised. Monoculture in both land and water-based systems is commonly practiced for carnivorous species. In contrast, polyculture is generally practised for raising herbivorous species in which pond polyculture systems are the dominant production systems in the country (WorldFish Center, 2002). Economically important cultured freshwater finfish in Thailand are tilapia sp., silver barb (*Puntius gonionotus*), catfish (*Pangasius sutchi*), snake skin gourami (*Trichogaster pectoralis*), walking catfish (*Clarias* spp.), striped snakehead (*Channa striata*) and marble goby (*Oxyeleotris marmoratus*). The striped snakehead (*Channa striata*) and marble goby (*Oxyeleotris marmorata*) are the main carnivorous species cultured in North-East Thailand. The annual aquaculture production in 2002 of these fish was 540 and 0.7 metric tonnes, respectively (DOF, 2004b).

A large number of farmers in Thailand use traditional methods for culturing fish and no additional feed. Many farmers use feed occasionally, some utilize by-products from raising poultry. Every now and then, fertilizer is used to generate natural fish feed. Generally, the common farm-made feeds used range from single ingredient (mainly trash fish), and simple mixtures (usually of rice mill products and fresh trash fish) to quite complex formulated feeds. Premixed vitamins are already available in Thailand (Tacon and Silva, 1997; Edwards and Allan, 2004).

2.9 Using freshwater trash fish for aquaculture

Within Asia, the working definition of “trash fish” is fish that have a low commercial value by virtue of their low quality, small size or low consumer preference.

They are either used for human consumption or used to feed livestock/fish, either directly or through reduction to fish meal/oil (FAO, 2005c; Pakjuta et al. 2005).

Most trash fish in Thailand comes from marine fisheries. Based on official statistics, the total marine fish catch from 1981 to 2002 was 1.35-2.40 million tonnes per year. Of this total marine fish catch, trash fish accounted for 0.69-1.10 million tonnes or 31-59 % (DOF, 2004a). A significant part of this low value/trash fish is made up of juveniles of commercially important species that could produce a more valuable catch if the juveniles were given time to grow to adulthood (Supis, 2005).

The use of low value trash fish as feeds for high-value species is increasing to support the expansion of aquaculture, especially in central Thailand where the culture of economically viable freshwater species is dependant upon marine trash fish. This is placing pressure on the long-term sustainability of the fisheries in which trash fish are caught. However, when human food fish species are caught and ‘‘processing wastes’’ of fish are utilized in feeding for aquaculture their use is considered economically sustainable (FAO, 2005b). For example, the processing wastes of the Indo-Pacific mackerel caught in the Gulf of Thailand and transported to processing plants in the northeast are used in feeding freshwater striped snakehead (*Channa striata*) which is a unique type of striped snakehead pond culture in Nakhon Rachasima province (Suchart et al. 2005). Similarly, in Nakhon Phanom, northeast Thailand, by-products of *Cirrhinus mrigala* and *Labeo rohita* imported from Central Thailand to make ‘‘Som Doh’’ (a type of popular fermented fish recipe) is used as feed for caged *Pangasius bocourti* culture (Supis, 2005; Suchart et al. 2005; Blake and Pitakthepsombut, 2006). Additionally, fish offal from both freshwater and marine fish processing plants is used to produce fish hydrolysate (fish silage) widely used for feeding pigs, poultry, ruminants and fish (Bykowski and Dutkiewicz, 1996). Offal has been used in

commercial salmon farming in Denmark and Norway and in experiments in developing countries involving warm-water species, namely the snake-head in Thailand, and the carp in Indonesia (Disney and James, 1980; SEPA, 2005).

Because of economic factors and problems with diet formulation, feed storage and distribution, trash fish is still the mainstay of a number of cage fish farms (Edwards and Allan, 2004). While trash fish may seem ideal, it suffers from a number of disadvantages. Many readily available species of trash fish, especially members of the sardine and mackerel family, have fat contents that are too high for some cultured species, while others contain high levels of thiaminase which can lead to thiamine deficiency if not heat treated (Wilson, 1991; Sim et al. 2005). Trash fish also has high moisture content and is expensive to transport. It is therefore fresgwater trash fish that have been used as an alternative for growing some species, particularly in the lower Mekong basin in Thailand and Lao PDR where farms are located near freshwater trash fish sources. A survey by Suchart et al (2005) described small-scale marble goby (*Oxyeleotris marmoratus*) cage culture on the Songkhram river in Nakhon Phanom province, Northeast Thailand, using two small 3x6x3 m cages. Each cage was stocked with 300 wild juveniles, 100-200g, collected by the farmer. Caged fish were fed fresh fish collected from the river by the cage owner once a day. Pelleted feed and golden apple snail (*Pomacea canaliculat*) flesh were used as a supplement. The quantity of feed increased from 2 kg day^{-1} in the first month to approximately 7 kg day^{-1} in the month preceding harvesting. The culture period lasted 12 months and resulted in a total production of 300 kg with a 50-60% survival rate, 1.0-1.2 kg in individual weight. This can be explained by the farm's small size which ultimately meant that the daily amount of trash fish required for feed was small enough to be available in the environment. This small scale operator sells live marble goby at prices as high as 300 Baht kg^{-1}. In Nam

Ngum reservoir, Lao PDR, 2.5 % (48 metric tonnes) of 1,920 metric tonnes of Pa Keo (*Clupeichthys aesarnensis*) of total caught was used for aquaculture feeds in cages (Mattson et al. 2001; Suchart et al. 2005). In Thailand, there is little qualitative and quantitative information and research on the use of freshwater trash fish as a feeding strategy.

An FAO survey (Suchart et al. 2005) in early 2005 found that there were only farmers in North-East Thailand using freshwater trash fish. The field survey in 2004 conducted in this study found there were many marble goby cage farms that had been feeding their fish using freshwater trash fish in Kanchanaburi province, Central Thailand and Ubon Rachathani province, Northeast Thailand.

The aim of the current research was to evaluate the use of culture-based freshwater trash fish that already available in the growing area, for example tilapia as an alternative feed for growing marble goby. Tilapia are considered good candidates as they reproduce rapidly and tend to be overpopulate in mixed-sex pond culture systems (Graham et al. 1995). Tilapia can produce fingerlings all year round and can potentially provide sufficient supplies to use as feed for marble goby instead of selling at a low market price. Ecologically an additional benefit would be a reduction in the use of marine trash fish in aquaculture.

Using live mixed tilapia as forage fish has been satisfactory as a method of controlling the population of tilapia and at the same time enhancing predator production through polyculture with predatory species such as grouper (*Epinephelus malabaricus*), sea bass (*Lates calcarifer*), largemouth bass (*Micropterus salmoides*) and snakehead (*Channa striata*) (John and Tucker, 1999; William et al. 2001; Yi et al. 2002).

Even though the use of tilapia as traditional trash fish feed has been reported in silver barramundi (*Lates calcarifer*) in India and grouper (*Epinephelus malabaricus*) in

Vietnam (ACIAR, 2004), there is still a lack of information and research regarding the effects of its use in both single and combined feed, with other ingredients, on growth performance, survival rate, nutrient utilization and carcass quality of cultured species.

Chapter 3 General Material and Methods

The methodology outlined in this chapter describes the laboratory techniques used in chemical analysis of experimental fish, feed ingredients and feeds. The description for the specific techniques used is given in material and methods of each chapter. The laboratory analysis was done at Institute of Aquaculture, the University of Stirling.

3.1 Chemical analysis

3.1.1 Proximate analysis of feedstuffs and fish samples

A proximate analysis is the determination by prescribed methods of moisture, ash, crude protein, crude lipid and nitrogen free extract. Proximate analysis was performed by standard methods (AOAC, 1990). Analysis included determination of dry matter, ash, crude protein, crude lipid and nitrogen free extract (as carbohydrate). Details are summarised below.

Moisture

Moisture content was determined by oven drying at 110ºC overnight.

Ash content

Ash content was determined by loss on ignition in a muffle furnace at 550ºC overnight.

Crude protein determination

Crude protein was determined by kjeldahl using a Tecator Kjeldahl Auto 1030 using standard procedures as described by AOAC (1990).

Crude lipid

Crude lipid was determined by the Soxhlet method using petroleum ether as a solvent (Tecator Soxtec System 1043, manufacturer's details).

Crude fibre

Crude fibre was determined using a Tecator Fibertec 1020 (manufacturer's details) and standard protocols.

Nitrogen free extract (NFE)

Nitrogen Free extract (carbohydrate equivalent) was calculated by subtracting the moisture, crude protein, crude lipid, ash and crude fibre from 100%.

NFE (%) = 100 – (Moisture + Crude protein +Crude lipid +Ash +Crude fibre)

3.1.2. Gross energy content of feeds

Gross energy in feed was determined using an adiabatic bomb calorimeter (Gallenkamp) using standard protocols.

3.1.3 Glycogen analysis

Measurement of glycogen in fish muscle and liver tissues was performed using a modified method described by Teresa et al (Teresa et al. 1998). The method consists of a 2-step process; 1) digestion and extraction of glycogen from tissue and 2) quantification.

3.1.4 Measurement of lipid peroxidation

The level of tissue and feed lipid peroxidation was determined by assessment of malondialdehyde (MDA) as total thiobarbituric acid (TBARS)-reactive products using a method adapted from that of Burk et al (Burk et al. 1980).

3.1.5 Lipid analysis

The lipid analysis of fish, the raw material and feed samples involved lipid extraction, lipid class and fatty acid analysis. Prior to analysis, fish tissue samples were separated from any blood residuals, skin and bones by dissection. Precaution was taken at each stage to minimize the risk of auto-oxidation of polyunsaturated fatty acids or hydrolysis of lipids. Samples of dried feeds and raw materials were ground using a food grinder to provide fine particles and hence homogenous samples.

Lipid extraction

Lipid extraction was performed using the Folch and Sloane (1957) method.

The lipid extract was then dissolved in Chloroform:Methanol (2:1 v/v) containing 0.01% BHT to give a standard concentration of 10 mg of lipid mL^{-1} solution. Finally, the solution was transferred to a labelled glass vial that was immediately filled with nitrogen and stored at -20 °C prior to further analysis.

Lipid class analysis

Separation and quantification of lipid classes were performed by high-performance thin-layer chromatography (HPTLC), followed by calibrated scanning densitometry (Bell et al. 1993).

Fatty acid analysis

Fatty acid methyl esters (FAME) from total lipids were prepared by acid-catalysed transmethylation for 16 h at 50ºC, using heptadecanoic acid (17:0) as internal standard (Christie, 2003). Fatty acid methyl esters were extracted and purified as described by Tocher and Harvie (Tocher and Harvie, 1988) and were separated in a Fisons GC8000 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica wall coated capillary column (30 m×0.32 mm i.d., Chrompack UK Ltd.

London), on-column injection system and flame ionization detection. Hydrogen was used as the carrier gas with a oven thermal gradient from an initial 50 ºC to 150 ºC at 40 ºC min^{-1} and then to a final temperature of 230 ºC at 2 ºC min^{-1}. Individual FAME was identified by comparison with known standards, a well-characterized fish oil and by reference to published data as described by Tocher and Harvie (Tocher and Harvie, 1988) and quantified using a PC with chromcard software (Thermoquest Italia SPA, Milan, Italy).

3.1.6 Measurement of vitamin E content

Vitamin E (α-tocopherol) was determined by High Performance Lipid Chromatography (HPLC) with fluorescence detection as described in Huo et al (Huo et al. 1996). The method also permits the measurement of different forms of vitamin E; thus, total vitamin E was measured.

3.2 Apparent dry matter digestibility and apparent nutrient digestibility (AND)

Diets and faecal samples were analysed for dry matter and crude protein contents following AOAC (1990); dry matter by oven-drying at 105°C overnight; crude protein by micro-Kjeldahl.

Feeds and faecal samples were digested in nitric acid, followed by oxidation with perchloric acid and the quantity of chromic oxide (Cr_2O_3) present was determined using atomic absorption spectrophotometry (Forster, 1999).

Apparent Dry matter digestibility and apparent nutrient digestibility (AND) were calculated according to the equation below;

$$\text{AND (\%)} = 100 - \left[\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}} \right]$$

3.3 Growth performance and slaughter indices

Growth performance and slaughter indices of fish were evaluated according to the recommendations by Martino et al (Martino et al. 2005) and Du et al (Du et al. 2005). The following parameters were considered:

1) Weight gain = (final weight –initial weight)

2) Daily weight gain: DWG = fish weight gain (g) /culture period (days)

3) Feed conversion ratio: FCR = dry feed weight intake (g) /fish wet weight gain (g)

4) Protein efficiency ratio: PER = (final body weight × final body protein) – (initial body weight × initial body protein)/total protein intake (g)

5) %Viscerosomatic index: VSI = (viscera weight (g) /fish body weight (g)) ×100

6) %Hepatosomatic index: HSI = (liver weight (g) /fish body weight (g)) ×100

3.4 Data evaluation

Data for fish lipid class and fatty acid compositions were from individual fish and treated as independent samples. The mean values obtained from each treatment for proximate analysis, vitamin E and haematological analysis were from pooled samples. Statistical analysis of data was performed using SPSS version 13 for Windows statistical software package.

3.4.1. Statistical data analysis in samples of on-farm survey

A parametric statistical test was used where the samples were normally distribution and homogeneity of variance was accepted (the test statistic's significance was greater than 0.05), one-way analysis of variance (ANOVA) with unequal sample sizes was then tested. The differences between means were determined using the Gabriel post hoc range test and pair-wise multiple comparison. Where the sample was

normally distributed and heterogeneity of variance was found, the Games-Howell method was used to determine mean differences (Jerrold, 1999).

A non-parametric statistical test was also used when conditions for parametric tests were not met; samples were not normally distributed and could not be corrected using data transformation.

The Kruskal-Wallis one way analysis of variance with tied ranks is a non-parametric method. The test assumes that the sampled populations have the same dispersion and shapes, but is still robust if that is not the case (Jerrold, 1999; Calvin, 1999). After ranking all data from all groups together then the Kruksal -Wallis test was calculated manually as

$$\text{Kruskal-Wallis (K)} = 12 /N(N+1) \sum_{i=1}^{k} R2i /ni -3(N+1)$$

Where k = number of groups

ni = number of observations in group i

N = total number of observations in all k groups (Σ ki=1 ni)

Ri = sum of the ranks of the ni observations in group i

If the observed value of K was equal to or higher than a reference critical value obtained from statistical table of Chi square ($\chi 2$) with k-1 degree of freedom (for k more than 5, in our present study k = 8 groups of fish), then the hypothesis H0 (H0 = HA) is rejected at the level of significance, (p=0.05) (Jerrold, 1999). The significance of the K test was done using SPSS statistical package version 13.0.

Non-parametric multiple comparisons with unequal sample sizes was applied when the null hypothesis was rejected and tied mean ranks were present. The significance of individual pairs of data was obtained according to conditions below;

$$Q \geq Q(\alpha, k)$$

$$Q = (RA-RB) / SE$$

Where, Q = a test statistic based on calculation

Q = Critical values of Q for non-parametric multiple comparison testing obtained from statistical tables

α = level of significance (0.05)

k = number of groups

RA and RB = a mean rank of group A and group B, respectively.

$$SE = \text{square root of } (N(N+1)/12)-(\Sigma t/12(N-1))x\ (1/nA +1/nB)$$

Where N = number of cases

Σt = number of groups of tied ranks

n = number of observations in a group (unequal sample size)

Non-parametric Turkey –type multiple comparisons was also used when equal sample sizes were applied (in fish proximate composition and slaughter indices data) (Jerrold, 1999).

3.4.2 Statistical data analysis in on-station experiments

Data from experiments were analysed statistically using a parametric statistical test when samples were normally distributed and homogeneity of variance was accepted, the one-way analysis of variance (ANOVA) with equal sample sizes was then tested. Any mean differences were confirmed using Tukey's test. Meanwhile, Dunnett's T_3 was applied when samples were normally distributed and heterogeneity of variance was not found (Jerrold, 1999).

The Kruskal-Wallis one way analysis of variance by ranks (K) was used when parametric test was not possible.

When the K test gave significant differences, Non-parametric Tukey-type multiple comparisons with equal sample sizes using the Nemenyi test was done (Jerrold, 1999). The significance of individual pairs of data was obtained using equations below;

$$q \geq q(\alpha, v, k)$$

$$q = (RA - RB) / SE$$

Where, q = a test statistic based on calculation

q = ''the studentized range'' which is dependent upon the significance level (α).

(For K test the used ''studentized range'' was $q(\alpha, \infty, k)$ in statistical table.

α = level of significance (0.05)

v = the error degree of freedom for the analysis of variance

k = number of groups

RA and RB = a mean rank of group A and group B, respectively.

SE = square root of $(n(nk)(nk+1))/12$

Where k = number of groups

n = number of observations in a group (equal sample size)

Chapter 4 A Review of Farms Survey on Current Practice, Feed Sources and Feeding Strategies in Culturing Marble Goby in Thailand

4.1 Introduction

The aim of this survey was to evaluate the effects of on-farm feed types and feeding strategies on fish nutritional status, total lipid content, fatty acid and lipid class composition, fish growth and production

Quantitative and qualitative information was obtained from cage and pond farmers fish seed traders and fish exporters using semi-structured interviews. In addition, an expert Dr Tawee Wiputthanumat, at the Department of Thai Fisheries, Bankok and biologists at Nongsua Department of Fisheries (DOF) research station, Pathum Thani were also interviewed regarding culture techniques and related information on marble goby. Secondary data were collected from a database on farmers at AIT Aquaculture Training & Consultancy Unit (TCU) and also annual reports from Nongsua DOF. Numbers of farms and their details were initially obtained from the TCU database, although, unfortunately, the database did not provide sufficient information to categorise differences in terms of feed type and feeding management.

Over the last decade there has been a lack of published information on use of farm-made aquafeeds for culturing marble goby in Thailand. Therefore in order to obtain up to date information and to find more farms employing a range of different feed types and/or feeding management strategies, the potentially informative social network among farmers was used and as many marble goby farmers were interviewed as possible.

The field visits and interviews were carried out in farms located in provinces of Central (Suphanburi, Ayuttaya, Samuth prakarn, Kanchanaburi), Northern (Nakhon sawan), Eastern (Chanthaburi) and Northeastern (Khonkaen, Ubon Rachathani) Thailand (Figure 2)

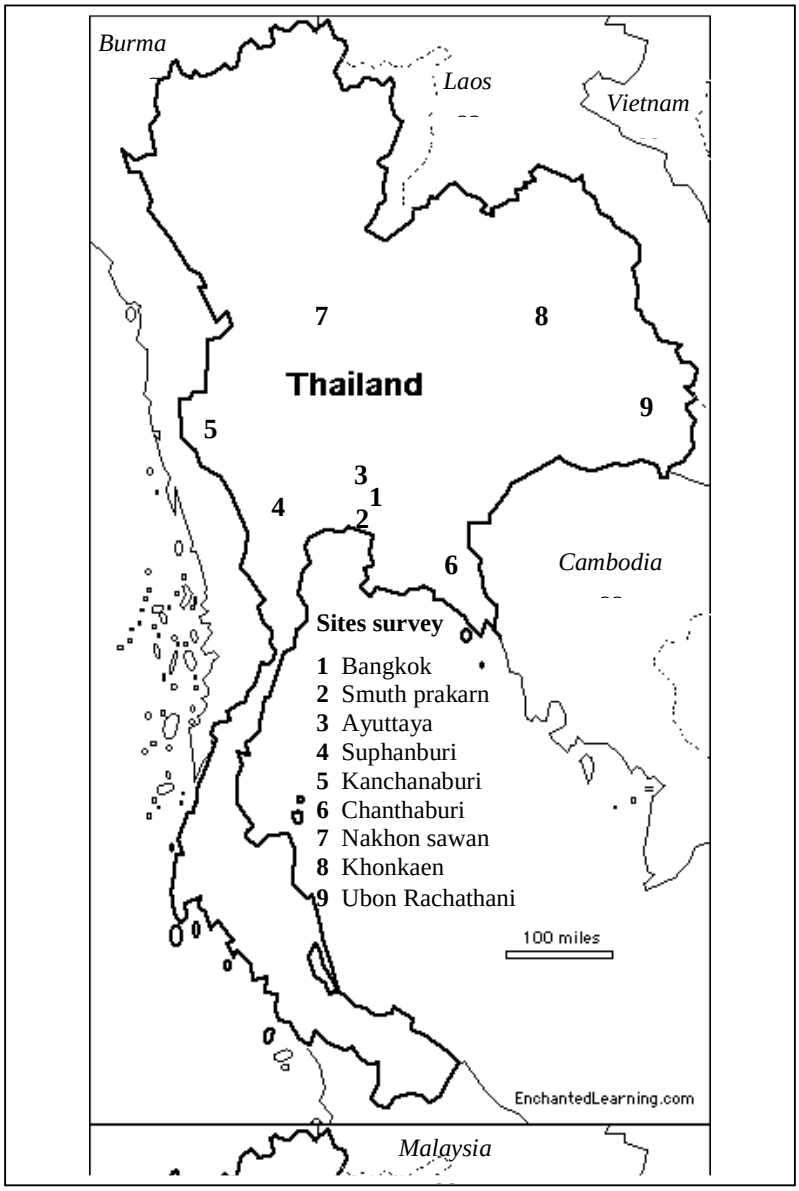


Figure 2 Map of surveyed sites in Thailand.

Source: applied from www.enhantedlearning.com

4.2 Results of the survey

4.2.1 Culture System

4.2.1.1 Water-based system

Cage culture was the most commonly practiced method in the study area, especially where farmers could have free-access to culture areas such as rivers and reservoirs, both in deep static and free flowing water. In rivers, there was a minimum water depth of 1 m of clear water below the bottom of the cage to keep it clear of the mud and sediment. In reservoirs deeper water (> 10 m. depth) areas were used for the setting of cages.

There were two common types of cages. Firstly there was a wooden cage made of soft wood as whole parts of cage and a blue nylon netting cage bag of mesh size of 2-2.5 mm. with bamboo poles serving as its frame and float. These two materials, wood and bamboo, have long been recognised as a popular type of cage and have been used since over the last decade for marble goby culture (DOF , 2000; Lin and Kaewpaitoon, 2000). The common cage size was 3 × 1.5 ×1.5 m. The costs of construction were 2,000-3,000 (£30-43) and 4,500-5,000 Thai Baht (£59-75) for the bamboo and wooden cages respectively. Also cages had an average life range of between 1-2 and 3-5 years respectively. The payback period could be achieved in the first year of culturing. The second type was a polyethylene netting bag of mesh size of 2.0-2.5 cm hanging on a frame made of galvanised steel and plastic materials. An oil drum served as a float. The cage size of 3.0 × 3.0 × 2.0 m costs 9,500-11,000 Thai Baht (£135 -157). The cage frame can have a life span of 10 years with proper care and the cage bag may last for 3-4 years which an occasional maintenance is required.

4.2.1.2 Land-based system

Ponds were prepared by applying lime ($Ca(OH)_2$) to the drained pond at the rate of 25 kg per 0.04 ha (0.25 rai /400m^2) and the pond was then left to dry under sunlight at least 2-3 days till rain comes. The pond was then allowed to fill with rainwater to a depth of 1.5-2.0m. Subsequently the pond was fertilized using chicken manure purchased from nearby chicken farms at the rate of 50 kg per 0.04 ha (0.25 rai /400m^2). The fertilizer was applied to increase the production of phytoplankton such as chlorella sp. and to enhance natural foods growth; benthos such as *Bellamya filosa* (Gastropoda), small freshwater crab *Esanthelphusa* spp. (Decapoda) and small freshwater prawns such as freshwater prawn *Macrobrachium lanchesteri*, so more food would be available for marble goby. The pond size was dependant on available area, labour and available capital. The pond sizes generally ranged from 0.04 to 0.16 ha. In one small scale farm in Chantaburi province, the house-wife was the key person looking after fish. She preferred to have 2 small grow-out ponds (20 × 20 m, 400m^2) and kept a larger pond for water storage. This made it easier to manage feed preparation, (chopping trash fish), feeding fish and also disease treatment. Larger pond sizes of 1,200 and 1,800 m^2 were found in Khonkaen province, where the farm was operated on a large piece of land and hired outside labour.

4.2.1.3 Seed supply

The supply of marble goby seed for both cage and pond culture mainly depends on the seasonal availability of wild seed. Most seed were collected from natural water bodies using scoop-nets (‘Ai-ngo’ in Thai) during the rainy season. However, some fish collectors claimed that the seed was also available out of season, but demand was poor since most farmers avoided risking the high mortality experienced during lower

temperatures in November - February. Farmers preferred individual seed weights of 80-100g. The quantity of seed collected was unpredictable; the collectors normally take a while to complete the order. It took 1-2 month to complete 10,000 seed weight of 50-100g collected by a catching team (5 collectors) in Burirum province for instance.

Generally after fish seed was collected, fish were conditioned in cement tanks and given live small freshwater prawn; *Macrobrachium lanchesteri* as feed before grading by size and transporting to farmers. Many farmers in the Central region ordered seed from collectors in the Northeast, while farmers in the Eastern region purchased from catching teams within the region. Seed was sold by the kilogram. Individual seed sizes of 50-100g cost 100-250 Baht (£1.4-3.6) kg^{-1}. Cage farmers claimed the bigger fish at stocking to maximize fish survival rate. In this case, quality of seed varied depending on the skill of collectors in collecting, conditioning and transporting the seed. The collecting process required the proper type of fishing gear otherwise fish could be injured by bruising, and loss of scales. Conditioning of feeding fry is important to ensure fish are healthier and ready for transportation. Unhealthy fish detected during the conditioning process can obviously be eliminated. Conditioning required at least 1-2 weeks before transportation. However, a longer period of conditioning is preferred by grow-out farmers as mortality is then absorbed by the collector. In contrast, the collectors preferred to keep fish for only up to two weeks due to the high cost of live feed, especially the freshwater prawn *Macrobrachium lanchesteri* and fish mortality (up to 30%). The collectors claimed that fish mortality was due to stress as a result of shifting the culture environment from a natural habitat to a cement tank.

There were two means of transportation. Some collectors kept fish in plastic bags containing aerated water. Transportation was carried out during the night through public transport and fish were transferred to another contracted mobile trader in the

early morning of the following day. Fish were then delivered to farmers using pick-ups in the late morning before stocking into the pond. This type of seed transportation was commonly used when a small number of fish was ordered. Meanwhile some collectors carried fish in containers containing aerated water that were kept aerated throughout transportation. In the latter case, the quality of fish seed would be higher and hence the price of seed would normally be higher. On one particular farm in Ayuthaya province, the seed was supplied by different local fishermen at different times. In this case fish farmers normally paid a lower price for the seed, however, the quality of fish was uncertain, supply was unpredictable and size likely to be heterogeneous. Moreover, cage farmers in Wachiralongkorn reservoir, Kanchanaburi province, collected their own wild seed as they were also capture fishermen too.

4.2.1.4 Fish stocking density

Stocking density in cage monoculture was on average 80-100 fish m^{-2} with initial individual weight of 100-200g. The culture period was about 5-8 months.

In cage polyculture systems marble goby were stocked with yellow mystus (*Mystus nemurous*), and four other species (x); striped catfish (*Pangasius sutchi*), giant snakehead (*Channa micropeltes*), striped snakehead (*Channa striata*), Siamese giant carp (*Catlocarpio siamensis*). The stocking ratio was 1.5:2:1 of marble goby:yellow mystus:x. The stocking density was 30 fish m^{-2} in which the stocking size of all species was on average 100-300 g. The culture period was 8-12 months.

In pond monoculture systems, fish at an average size of 50-100g were stocked at the rate of 1-4 fish m^{-2}. In the semi-intensive pond polyculture system, marble goby was cultured as supplementary production with other species such as sex-reversed tilapia thus stocking density was lower compared to other systems; 50-100 fish per 1200 m^2.

4.2.2 Fish Feed, Feeding and Production

4.2.2.1 Marine trash fish

Previously, a marine yellow stripe trevally (*Selaroides leptolepis*) had widely been used for feeding marble goby. However, due to a decrease in supply, farmers have therefore been using Indian mackerel and jack mackerel instead, especially in Ayuthaya and Suphanburi Provinces in the Central region. Also in the Chantaburi province in the Eastern region where marine trash fish were collected from the Gulf of Thailand, Indian and jack mackerel were locally available from ports. In Ayuthaya, marine trash fish were delivered to cage farmers by local middlemen. Trash fish were purchased from the cold storage room before delivery to farmers. At farms, trash fish were kept in a container topped-up with ice. The price of trash fish was 7-10 Baht (£0.1-0.14) kg^{-1}. However, farmers were unable to purchase trash fish in bulk due to the lack of cold storage facilities on farms. In the Central region, trash fish were already found to be spoiled and quality had deteriorated on their arrival at farms. This could potentially lead to nutritional deficiency in farmed fish (Quinyuan et al. 2001; Chinabut, 2002). Similar survey results on trash fish utilization in Thailand was reported by Supis (2005) who reported the low quality of by-catch product, including trash fish due to improper handling, insufficient ice and a lack of or a poorly maintained cold room. Whereas, in the Eastern region where marine trash fish were locally available and fish could be transported daily from the port to farms, their freshness and hence nutritional quality was more certain.

Trash fish were freshly prepared by either chopping into pieces or ground using a hand operated-mincer. Feeds were given to fish just after preparation by placing them

into feeding trays. An estimated feeding rate of 5-7% of fish body weight and a feeding frequency of once a day in the morning were common practices by farmers.

4.2.2.2 Freshwater trash fish

The use of freshwater trash fish for feeding marble goby was commonly found in cage farms situated in Wachiralongkorn and Srinakharin reservoirs in the Central region and in Sirinthorn reservoir in the North-Eastern region. Cage culture relied on small fish caught in reservoirs such as a clupeid, the Thai river sprat; *Clupeichthys aesarnensis* Wongratana ('Pla sew kaew' in Thai), *Rasbara* spp. and *Puntius* spp. The Thai river sprat was found to be the main species used for feeding caged fish.

Fishing for the Thai river sprat to feed marble goby was done during the night. Fish were attracted to the light from a kerosene lamp, which was hung over the water surface at the centre of a trapping lift net. Feeding was done once a day in the morning. Whole trash fish were thrown directly into fish cages. Some cage farmers ground the fish before feeding using a hand operated-grinder. An estimated feeding rate was about 5-7% of fish body wet weight.

The Thai river sprat has so far been sufficiently available to meet cage culture demands due to its ability to reproduce all year round and it being a planktonic feeder (Jutagate et al. 2003). However, fishing due to high demand for this species might lead to over exploitation in the near future.

4.2.2.3 Chicken slaughter-house by products

Chicken offal has long been regarded as one of the commonest fresh by-product feeds for growing carnivorous fish in Thailand (FAO, 1993; Chinabut, 2002; Edwards and Allan, 2004). However, this was rarely used by marble goby farms since getting a contract with a slaughter-house to receive regular and sufficient supplies needed a

prohibitively large amount of initial capital. In this survey, only one commercial farmer in Khonkaen Province, North-Eastern region was using chicken offal to feed marble goby. Chicken offal was ground before feeding to the fish at the rate of 10-15 kg per pond size of 300 m^2 stocked with 8000-9000 fish. Feeding was done every 2 days. The farm owner claimed there was sufficient feed since fish also fed on small freshwater prawns and benthic fauna in the pond. However, it was his first crop of marble goby and his strategy was to produce broodstocks to begin the semi-natural fish breeding program in his farm.

4.2.2.4 On-farm formulated feed

The simple formula of moist farm-made feed for marble goby in one cage farm in Ayuthaya, Central region was composed of Indian mackerel trash fish, rice bran, salt and vitamins premixed in the ratio of 10:1:1:0.1 respectively. Feeding rate was 4-5% body weight every 2 days. The farmer's strategy was to use rice bran to make feed economically since it could reduce amount of trash fish. Farmers claimed that vitamins and salt helped to build up healthy fish and it was believed to be essential in preventing disease.

In the pond polyculture system, marble goby was stocked with tilapia. Where tilapia was the main production species farmers were more concerned with feeding tilapia than marble goby. Fish in the pond were routinely fed on rice bran manufactured feeds were also used occasionally to supplement fish nutrition, especially while fattening before harvesting.

4.2.3 Fish Harvesting, Growth and Production

Two general harvesting methods were used: partial harvest when only a portion of fish was harvested at one time and complete harvest where all fish were harvested.

The partial harvesting was usually found in both cage systems. Fish of individual weights of >400g were graded to sell during the culture period to prevent production lost caused by cannibalism.

Complete harvesting was common in pond culture where water was totally drained from the ponds. Partial harvesting using seine nets was not practiced by farmers since the marble goby is sensitive to handling stress, possibly resulting in higher mortality of the remaining fish.

The contractors, which in many cases were exporters, would normally contract fish farmers who were ready to harvest in advance. Being marble goby collector/middle man/contractor requires specialized skills, especially in handling and collecting live fish. Fish must be handled properly to be able to negotiate with exporters for a better price. To ensure that fish would remain healthy and live after harvesting, contractors normally preferred to come to farms to manage fish harvesting themselves. Moreover, most fish collectors operated on a cash basis on buying fish.

The farm-gate price of live fish of 400-700g was 350-600 Baht kg^{-1} (£5-9) depending on fish size. The growth pattern of fish was reported to be similar in all culture systems. Fish at average stocking size of 80-200g could easily reach marketable size (400-500g) within 5-8 months. Survival rates varied between 40-70%.

4.2.4 Marketing

The marble goby was always sold live since the price of chilled dead fish fell to only 30-50% of live fish prices. The marble goby market channel has been found to operate both internationally and domestically. In the international market, live fish was

preferred by the main importing countries. In the domestic market, both live and chilled fish have been distributed to wealthy urban and tourist restaurants. Markets have shown attractively stable and steady growth. Price negotiation for live fish depends on the size of fish; the bigger size of 1000g live fish could be sold individually for 1,000 Baht (£15) as compared to 350-500 Baht (£5-9) for sizes of 400-500g. Dead fish of the same size depreciated to 100 Baht kg^{-1} (£1.5) no matter how fresh. Interestingly, cultured fish were preferred by exporters to wild fish as farmed fish were considerably healthier; had a higher tolerance to handling stress and a high survival rate after transportation.

The main current international markets for live marble goby have already been established in Taiwan (preferably a fish size of 400-800g) followed by Malaysia (600 - 1,000g), Hong Kong and Singapore (≥ 800g). However, the quantities of fish produced by farmers have been very unpredictable which has made marketing plans for collectors difficult.

4.2.5 Constraints

4.2.5.1 Disease

The number of cage farmers has obviously been decreasing due to fish mass mortality. Farmers lack specific knowledge of the types and causes of disease that affect their stock (Thompson and Crumlish, 2000). Mortality often arises as a result of stress and nutritional imbalance (Jantrarotai and Jantrarothai, 1993). Many fish died just before reaching 300-400g, especially in cage fish fed on trash fish that had a relatively large and pale liver (Figure 3). Presumably, they either received prolonged feeding on oily trash fish or rancid feeds as a result of improper storage of trash fish and rice bran.

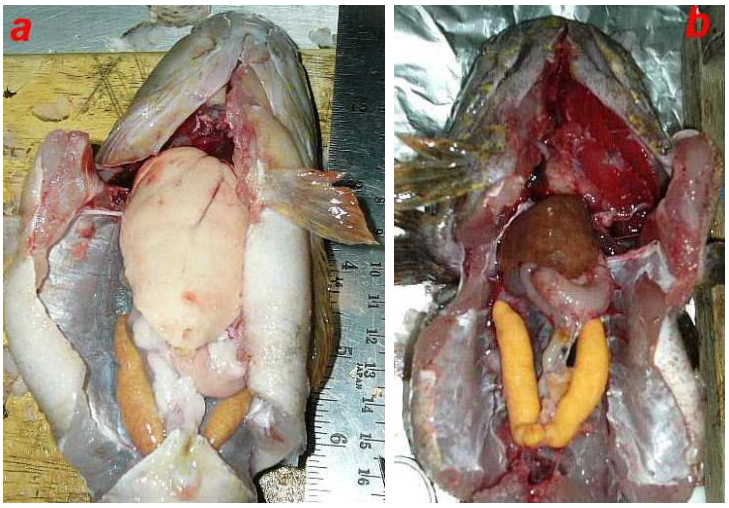


Figure 3 A fatty liver of fish fed trash fish (a) and a normal liver of wild fish (b)
Source: Bundit, J. (Farm Survey, 2004)

4.2.5.2 Seed supply

The quality and quantity of supplies of wild seed were unreliable and seasonal. The quantity was directed by the natural breeding cycle of the fish. Although successful artificial breeding and rearing of fry and fingerlings have been reported, technology is limited and confined to the Department of Fisheries (DOF). Other problems include the dwindling supply and increasing cost of wild seed. There is also growing concern over damage to the environment and resource from over-fishing and destructive collection of seed and adults from the wild.

4.2.5.3 Feed

As long as marble goby culture has relied on fish food from capture fisheries, the future supply of feed is questionable. Feeding fish with by-catch could potentially cause ecological and environmental impact and an unsustainable culturing technique.

4.2.6 Conclusion

Culture of marble goby is not new in Thailand but is still underdeveloped in terms of feed and feeding practices compared to other economic important species, catfish *Clarias* spp. for instance. In fact, marble goby presents great profit margins per unit production, a short payback period and also has great market potential both domestically and internationally.

The sourcing of seed from wild fisheries increases pressure on fisheries and is unsustainable in the long term. Consideration should be given to developing sustainable seed from hatcheries.

With respect to the feeds used, marble goby culture still relies mainly on marine and freshwater trash fish either as a single feed or combined with other ingredients. Although the cost of trash fish is rising, farmers will continue to use it as long as the selling price of marble goby is well above feeding costs. It is also the prevailing belief among farmers that trash fish is good for growing marble goby and that they are not well adapted to compound feed. In addition, small scale cage farmers normally do not have access to the financial resources necessary to invest in purchase of pellet diets or infrastructure such as refrigeration for feed storage. In fact, it is easier to collect trash fish/by-catch themselves, or in small amounts as and when financial or trash fish resources are available. Therefore, using freshwater trash fish /by- catch will continue to be a major feed source for marble goby culture for the foreseeable future.

Although, making moist farm-made feeds is practical and cost-effective for certain farms, it may not be so for others since the raw materials used are unconventional. Since marble goby rearing presently depends on wild freshwater capture fisheries, it will thus be difficult to increase the overall production, since farming is unlikely to be possible where capture fisheries are not available. Meanwhile

where farming exists, restriction rules and regulation in controlling over-fishing of by-catch will soon be applied. This will certainly cause limitations in food supplies for marble goby and fish production can not be expanded as result.

Farming practices and culture techniques have been transferred among farmers themselves. There is still lack of research and government involvement.

4.2.7 Further need

To sustain the culture and ensure consistent quality and quantity of seed, the government should pay attention to decentralisation of knowledge, techniques and practices of breeding and rearing of fry to local hatcheries.

Since fish disease has been affecting total production of grow-out farms, support in disease diagnosis will be needed by farmers. Efforts should be made to organize diagnostic support to identify and treat diseases and related conditions. Moreover, according to our farms survey, the nutritional pathological signs; the swollen pale fatty liver and an excessive visceral fat were observed in fish fed an oxidised dietary lipid particular the trash fish based diets. It should be noted that practical feeds prepared under tropical conditions is susceptible to lipid oxidation. Therefore, nutritional research on improvement using oxidised dietary lipid of practical diets is required.

Until recently, culture of marble goby still relied mainly on farm-made aquafeeds. Therefore, research to provide information of marble goby nutritional status fed existing farm-made aquafeeds would be a useful guide for future development of nutritional research.

Most farmers have widely used freshwater trash fish for feeding marble goby, further research is therefore required to evaluate the effects of its use on fish nutrient composition and growth performance. Moreover, to reduce the dependency on

overexploited freshwater capture fisheries and also to continue expanding the culturing of marble goby, development and improvement of alternative low-value freshwater farm-fish that are already available in the growing area as feeds for marble goby should potentially be investigated.

Chapter 5 Slaughter Indices and Fish Lipid Composition of Wild and Cultured Marble Goby (*O. marmoratus*) Fed Different Farm-made Feeds

5.1 Introduction

Dietary lipids are a major source of energy in all fish, especially for carnivorous species which have a limited ability to utilize dietary carbohydrate for energy (Pei et al. 2004; Gallagher, 1997). Lipids also assist in the absorption of fat-soluble vitamins (SRAC, 1998). Dietary lipid has been reported to have a protein sparing effect, replacing protein which may otherwise be used to provide energy (Watanabe, 1982; Tacon, 1997). Dietary lipids also serve as the source of essential fatty acids which are needed for normal growth and development, including reproductive development (Wilson, 1995; Sargent et al. 1999; Sargent et al. 2002).

Fatty acids that cannot be synthesised by an animal but are required are generally referred to as the essential fatty acids (EFA) and these maintain cell integrity and immune function (Sargent et al. 1999). In common with other vertebrates fish do not have the capability to synthesize either linoleic (18:2n-6) or linolenic (18:3n-3) fatty acid *de novo*. Hence one or both of these fatty acids must be supplied preformed in the diet, depending on the essential fatty acid requirements of any particular species (Wilson, 1995). It must be borne in mind, however, that the 'requirement' in this context refers to dietary fatty acids in relation to growth under the conditions of a particular experiment. It is likely that all fish have a requirement for both n-3 and n-6 fatty acids but that these may be for trace levels satisfied by body reserves and not detected in conventional feeding trials. Unlike marine fish almost all freshwater fish

have an innate ability to desaturate and elongate dietary 18:2n-6 to 20:4n-6 (arachidonic acid, AA) and 22:5n6, (docosapentaenoic acid, DPA), also 18:3n-3 polyunsaturated fatty acids to 20:5n-3 (eicosapentaenoic acid, EPA) and ultimately 22:6n-3 (docosahexaenoic acid, DHA) desatuarates (Steffens, 1997). In general freshwater fish require dietary linoleic and/or linolenic acid or both. Early studies of essential fatty acids requirements in common carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella* and Eel *Anguilla japonica* demonstrated that these fish require a combination of dietary 18:2n-6 and 18:3n-3 (Takeuchi and Watanabe, 1977a; Takeuchi et al. 1980; Takeuchi et al. 1991). Tilapia *Oreochromis zillii*, *Oreochromis nilotica* and hybrids *O. nilticus*× *O. aureus* have been shown to require n-6 and but not n-3 fatty acids (Takeuchi et al. 1983; Kanazawa, 1985; Huang et al. 1998). Channel catfish *Ictalurus punctatus*, in contrast, require n-3 but not n-6 (Satoh et al. 1989a).

It has been well documented that the fatty acid compositions of tissue lipids in fish usually reflect the fatty acid profile of dietary lipid inputs (Satoh et al. 1989a; Almansa et al. 1999; Cejas et al. 2003; Rodriguez et al. 2004). The examination of lipid contents and the fatty acid profiles of wild fish living in natural ecosystems and under natural feeding, which contributes to basic knowledge of lipid metabolism and provides useful information on fish fatty acids requirements, have been reported (Harrell and Woods, 1995; Bo-young et al. 2000; Morehead et al. 2001; Palacios et al. 2007). Comparisons of lipid compositions from tissues of wild fish with their cultivated counterparts has provided a good estimation of the suitability of the diet to their lipid nutrition (Castell et al. 1994; Melika et al. 1996; Rodriguez et al. 2004; Cejas et al. 2004). Besides, examining the concentrations of the different lipid classes allows us to understand how a fish allocates its energy resources (Sargent et al. 2002).

Thus, a comparison of lipid contents, slaughter indices and the fatty acid profiles and lipid classes of wild and captive fish can yield valuable information that may help to elucidate their essential fatty acid requirements. This is also practically important as a reference point for correct diet formulation in respect to the lipid used. No studies are available today which analyse the lipids of juvenile marble goby (*O. marmoratus*). Therefore the present study is a first attempt to provide information about the lipid requirements of juvenile marble goby through comparison of tissue lipid contents, fatty acid compositions and lipid classes in wild fish and cultured fish fed different farm-made diets.

The specific objectives of this study were to

1) Determine the effects of on-farm feeds on fish nutritional composition and slaughter indices of cultured marble goby
2) Evaluate the effects of different on-farm feeds on fatty acid profiles and lipid classes of cultured marble goby
3) Compare the fatty acid profiles and lipid classes between wild and cultured marble goby.

5.2 Material and methods

5.2.1 Fish sampling

The owners or managers of twelve farms were interviewed. Fish sampling was performed in only eight grow out farms due to the difficulty of sampling, especially in earthen pond farms. In addition the duration of feeding did not meet the requirements of the study; feeding period was less than 3 months for instance. The survey was carried out during September – October 2003. Information about culture systems and management practices for each farm is summarized in Table 5.1

In order to establish the effects of feeding practices on fish quality only farms where fish had been fed for a minimum of 3 months (based on farmer data) were sampled. Cultured fish were collected at an average size of 300-400 gram. Wild fish were collected by local fisherman using scoop nets (locally referred to as 'Ai-ngo'). Fish were transferred live using plastic containers with sufficient water and aeration was supplied. Fish were transported to the laboratory of the DOF provincial fisheries station nearest the collecting sites and transportation time was 30-60 minutes. Fish were then acclimatized to a larger cement tank system for 2-3 hrs. Fish were then anesthetized using ice. Biometric data were collected; an individual length and weight were measured before fish were sacrificed for dorsal muscle tissue and liver samples. The fish muscle and liver samples were then preserved in dry ice purchased from local ice-cream factories. Subsequently, samples were transported to the Asian Institute of Technology (AIT) and samples finally were kept in liquid nitrogen. Fish samples were then preserved in dry ice and transported to the University of Stirling for the proximate composition and fatty acid analysis.

Table 5.1 A summary of feeds and feeding practices from selected farms

Rearing system	Farmer name and location	Initial stocking size	Stocking density	Culture period	Feed types/feeding strategy	Growth / Survival rate/Production
Cage-Monoculture (FARM A) Floating wooden cage in river (Noi river, turbid water) ▪ Cage size of 3 × 1.5 × 1.5 m. ▪ There were 4 cages	Mr. Samraeng Pansuwan, 75 years old, Phakhai district, Ayuthaya province.	Fish at size of 100-300g, but preferred size of 200g	200 fish per cage (100 Baht(£1.4) kg^{-1}, bought from farmers in village)	6-8 months (Feb./March – Oct./Aug.)	● Feeding by using supplementary feed; marine trash fish (Indian mackerel) +rice bran + salt (NaCl) +mineral&Vit. premix; the ratio of 10:1:1:0.10 kg, using feeding tray ● Marine trash fish were purchased from local fish frozen manufacturing - Feeding once in every two days, 3-4% body weight	● 400-600g at harvesting size. ● Yield of 100-110 kg $cage^{-1}$ ● grading for sale every 2-3 months, remaining smaller fish were restocked again.
Cage-Monoculture (FARM B) ▪ Floating cage, Polyethylene cage bag in Srinakarin Reservoir, Srinakarin dam., Kanchana Buri, ▪Clear water ▪ Cage size of 2 ×1.5 × 1.5 m. ▪ 2 Cages	Mr Amnart Sarn-im, 27 years old, Ban Plai na suankao, Nasuan Sub-district, Srisawat district, Kanchana Buri province	100-200g fish were caught from reservoir	400 fish $cage^{-1}$	7- 8 months	● Feeding by using freshwater fish mainly was “Pla sew kaew”; Thai river sprat (*Clupeichthys aesarnensis*)which were collected from the reservoir ● 5kg $day^{-1}cage^{-1}$, once a day (morning)	● 400 – 800g ● 60% survival rate

Table 5.2 A summary of feeds and feeding practices in selected farms (cont.)

Rearing system	Farmer name and location	Initial stocking size	Stocking density	Culture period	Feed types/feeding strategy	Growth / Survival rate/Production
Cage-Monoculture (FARM C) ▪ Floating cage, Sirinthon Dam, Ubon Rachatani, NE Thailand. ▪ Cage size of 2 × 2× 2 m. ▪ 3 Cages	Mr Piboon Chaikaew, 38 years old, Pong Dindum village, Chongmek sub-district, Sirinthon district, Ubon Rachtani prov.	100-200g caught from reservoir using gill net.	200 fish cage^{-1}	7-10 months	● Feeding by using freshwater fish mainly was "Pla sew kaew"; Thai river sprat (*Clupeichthys aesarnensis*) and "Hang Daeng" (*Puntius sp*.) which were collected from the reservoir ● feed twice a day; morning and afternoon	No report due to it was the first crop growing during the interview
Cage-Polyculture (FARM D) ▪ Floating, polyethylene cage bag in reservoir, Wachilalongkorn Dam, Khanchana Buri province ▪ cage size of 3.5 × 4.5 × 1.5 m. ▪ There were 2 cages in total	Mr Prasert Majchukit, 49 years old Moo4. Ban Huay Kayeng, Thongphapoom district, Khanchana Buri prov.	● Mable goby 100 – 300g, fish seed were caught from reservoir ● Yellow Mystus (*Mystus nemurous*), Giant snake head (*Channa micropeltes*), Striped Snake head (*Channa stiata*), Sauvage (*Probarbus jullieni*)	● Mable goby 150 fish cage^{-1} ● Yellow mystus 200 fish cage^{-1} ● Others sp. 100 fish cage^{-1} - multiple stocking	8-12 months	● Feeding by using freshwater fish mainly was "pla sew kaew"; Thai river sprat (*Clupeichthys aesarnensis*) which were collected from the reservoir ● 3kg day^{-1}, once a day (morning)	

Table 5.3 A summary of feeds and feeding practices in selected farms (cont.)

Rearing system	Farmer name and location	Initial stocking size	Stocking density	Culture period	Feed types/feeding strategy	Growth / Survival rate/Production
Pond -Monoculture (FARM E) ● 2 Earthen ponds; 400 m^2 in area, 2 m. in depth. ▪ponds were covered by aquatic submerged weeds. ● small numbers of some unwanted fish were also found in the pond during harvesting; Three spot gourami, Tilapia, Anabas	Mrs Preeda Udomyat 45/1 Moo. 6, Ban Bore, Bore sub-district, Klung district, Chantaburi province	Fish sizes of 50-100g were bought from fish collector at Klung district.	400 fish $pond^{-1}$	8-9 months	● Feeding by using chopped yellow stripe trevally (*Selaroides leptolepis*), and indian mackerel purchased from a local fishing pier nearby; 5kg $pond^{-1}$ of feeding rate. ● Using feeding tray ● Fish were fed once a day in morning; ● Ponds were drained out once a year after harvesting and filled up again by rain ● Applied 10kg of commercial EM (Effective Microbial-organism) and 20 kg of salt (NaCl) into the pond whenever fish had shown disease signs (about 2-3 times a crop) ● Fish normally were infected by external parasite i.e., *Lernea* but never found secondary serious infection by other diseases.	● 400-800g of harvested size ● 70-80 % survival rate ● Gross income of 50,000 60,000 Baht $pond^{-1}crop^{-1}$

Table 5.4 A summary of feeds and feeding practices in selected farms (cont.)

Rearing system	Farmer name and location	Initial stocking size	Stocking density	Culture period	Feed types/feeding strategy	Growth / Survival rate/Production
Pond-Monoculture (FARM F) ▪ 2 ponds of size of 1,800 m^2 ▪ 1 pond of size of 1200 m^2	Mr Somboon Taweewiwathana (38 years old) 208 Moo 13, Ban Nong Ya Prak, Donhan sub-district, Muang district, Khonkaen province.	50 -100g from fish collectors in Sakon Nakhon province.	● 8,000-9,000 fish $pond^{-1}$ (1,800 m^2) ● 4,500 fish $pond^{-1}$ (1,200 m^2)	Fish have already been raising for 4 months and will continued until reaching brood stock size	● Feeding by using chicken by products (heads and internal organs) from chicken slaughter house which is located in the province. ● Feeding by using live common carp and silver barb fries was initially applied. Unfortunately many fish were appeared intestinal injury and died ultimately. Presumably (that what farmer assumed) because of dorsal spines and bones from eaten live fries. Afterwards, feed used has been completely shifted to be the chicken by products. ● He also attempted to produce the fry to support his grow-out system by application of natural breeding technique. Consequently, semi-natural breeding would possibly be the next trial for him.	● It was the first crop and Aquaculture is a totally new experience for him. ● The main purpose of culturing this crop was for being fish brood stocks. ●Major income has been from selling noodles produced by his own factory and from organic vegetable crops respectively.
Pond-Polyculture (FARM G)	Heatoo, Ladkrabang, SamutPrakarn province.	50-100g	50-100 fish /0.16 ha (1,200 m^2)	5-6 months	● Feeding by using rice bran and supplementary feed, Marble goby were cultured as additional production with Sex-reversal tilapia. The stocking density of the marble goby was 50-100 fish /0.16ha	● 2-3 fish kg^{-1} ● 25-40kg /0.16ha $crop^{-1}$

Table 5.5 A summary of feeds and feeding practices in selected farms (cont.)

Rearing system	Farmer name and location	Initial stocking size	Stocking density	Culture period	Feed types/feeding strategy	Growth / Survival rate/Production
Wild fish at Kiritarn reservoir, Kiritarn Dam, Chantaburi province	Ms Chalor, Kanchanaburi				Fish were collected by catching teams, fish caught were then sold to fish collector (middle man) and fish after recuperated for few days, graded small fish (5-10g) were distributed to aquarium fish traders in JJ market, Bangkok and also directly to aquarium fish exporters, and larger size (50g up) were usually sold to grow-out farmers nearby.	Marble goby of 2-10g in size were the major ordered from the aquarium fish market.

5.3 Results

5.3.1 Proximate analysis

Proximate analyses and TBARS for feeds used on the various farms are presented in Table 6.

The results of proximate analysis of whole body, muscle and liver tissues of fish are presented in Table 7. The moisture, ash and crude protein contents of muscle appeared to be relatively uninfluenced by feeds and culture regime. There were only minor differences between groups in total lipid contents of muscle which were higher in groups fed freshwater trash fish (Farm B, C, D and G). Percentage moisture content in whole body decreased as lipid content increased.

The muscle lipid level of wild fish was higher than of fish fed mackerel trash-fish diets (Farms A and E) and fish fed chicken offal (Farm F) ($p<0.05$). However, lipid levels were found to be similar among groups of fish given feeds based on freshwater trash fish ($p>0.05$). Whole body lipid content was highest in fish fed chicken offal (Farm F). Moreover, liver lipid contents were significantly lower in groups of wild fish, fish fed marine and rice-bran based diets (Farm-A); supplementary feed plus natural foods (Farm G) than remaining groups of fish.

5.3.2 Glycogen content, TBARS, Hepatosomatic index (HSI) and Vicerosomatic index (VSI)

Deposition of liver glycogen was influenced by inclusion of carbohydrate (rice bran) in feeds. Liver glycogen levels were higher in fish fed mackerel plus rice bran based feed (Farm-A) and supplementary plus natural foods (Farm-G) than in other

groups of fish ($p<0.05$) (Table 7). Liver glycogen contents did not vary significantly among groups fed only freshwater trash fish or marine trash fish.

Lipid oxidation (TBARS) in fish liver were relatively higher in farmed fish compared to wild fish, except for the groups of fish fed supplementary feed plus natural foods (Farm G) and mackerel trash fish based feed (Farm A). The greatest degree of lipid oxidation of fish muscle (0.029 ±0.004 µmols MDA mg^{-1}sample) and liver (1.162 ±0.042 µmols MDA mg^{-1}sample) occurred in fish fed single mackerel trash fish (Farm E) ($p<0.05$).

Viscerosomatic indices (VSI) and hepatosomatic indices (HSI) were higher in fish fed single freshwater trash fish (Farm C) which had VSI and HSI values of 10.55 and 5.38, respectively. HSI were higher in fish that had high liver lipid contents.

Table 6 Results of proximate analysis (% dry matter) and TBARS measurement (µmols MDA mg^{-1} of wet weight sample) of different feeds used on fish farms

Chemical composition	Feeds					
	Mackerel[1]	Yellow striped scad[2]	Thai river sprat[3]	Rice bran[4]	Chicken offal[5]	Herbivorous fish pellet[6]
Crude protein (%)	67.2 ±0.4	72.9 ±0.5	72.1 ±0.1	17.1 ±0.0	32.5 ±0.2	20.36 ±0.2
Crude lipid (%)	13.9 ±1.3	8.5 ±1.2	16.2 ±0.7	15.0 ±0.1	20.0 ±0.8	5.25 ±0.0
Ash (%)	14.7 ±3.8	16.5 ±0.1	11.0 ±0.1	13.4 ±0.1	7.4 ±0.2	9.3 ±0.1
Crude fibre (%)	na	na	na	4.6 ±0.5	na	5.5 ±0.4
TBARS (µmols MDA mg^{-1}wet weight sample)	0.32 ±0.01	0.16 ±0.00	0.12 ±0.01	0.04 ±0.00	0.05 ±0.00	0.02 ±0.00

Values are means of triple samples ±SD, na; not analysed.
[1]feed used in farm A and E
[2]feed used in farm E
[3]feed used in farm B, C, D
[4]feed used in farm A and G
[5]feed used in farm F
[6]feed used in farm G

Table7 Proximate composition, Hepatosomatic index, Vicerosomatic index, glycogen (% wet weight) and T-BARs values (µmols MDA mg^{-1}wet sample) for whole fish, fish muscle and liver samples collected from wild fish and various farms

Chemical composition	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F	Farm G	Wild fish
Moisture (%)[1]								
muscle	79.8 ±0.3^{bc}	81.2 ±0.2^{c}	79.6 ±1.0^{bc}	80.6 ±0.8^{bc}	78.8 ±0.9^{bc}	77.2 ±0.5^{a}	80.0 ±0.2^{ab}	79.1 ±0.9^{ab}
whole fish	78.1 ±1.0^{c}	75.1 ±0.6^{ab}	76.9 ±1.7^{bc}	76.8 ±0.2^{bc}	78.0 ±0.2^{c}	74.3 ±1.8^{a}	74.8 ±0.7^{ab}	78.2 ±0.2^{c}
Crude protein (%)[1]								
muscle	19.23 ± 0.15^{cd}	19.49 ±0.09^{d}	21.04 ±0.01^{f}	17.91 ±0.05^{a}	18.68 ±0.14^{b}	18.91 ±0.13^{bc}	20.30 ±0.09^{d}	20.12 ±0.05^{d}
whole fish	17.00±0.07^{ab}	17.07 ±0.12^{b}	16.73 ±0.10^{ab}	16.41 ±0.19^{a}	15.66 ±0.14^{a}	17.27 ±0.12^{b}	18.81 ±0.08^{b}	16.60 ±0.09^{a}
Ash (%)[1]								
muscle	1.25 ±0.00^{ab}	1.12 ±0.08^{a}	1.67 ±0.00^{b}	1.17 ±0.02^{a}	1.10 ±0.13^{a}	1.23 ±0.07^{ab}	1.22 ±0.04^{ab}	1.36 ±0.01^{b}
whole fish	4.62 ±0.08^{a}	5.40 ±0.00^{b}	5.34 ±0.20^{b}	5.84 ±0.30^{b}	4.53 ±0.14^{a}	4.15 ±0.03^{a}	5.59 ±0.43^{b}	4.13 ±0.16^{a}
Crude lipid (%)[1]								
muscle	0.58 ±0.05^{a}	0.90 ±0.07^{c}	0.76 ±0.05^{bc}	0.67 ±0.05^{ab}	0.57 ±0.07^{a}	0.57 ±0.02^{a}	0.81 ±0.10^{bc}	0.77 ±0.11^{bc}
whole fish	0.84 ±0.02^{a}	2.92 ±0.01^{b}	0.92 ±0.02^{a}	1.12 ±0.01^{ab}	1.83 ±0.09^{b}	4.35 ±0.67^{c}	1.31 ±0.02^{ab}	0.95 ±0.02^{a}
liver	10.79 ±0.18^{a}	38.29 ±0.95^{b}	34.91 ±1.03^{b}	33.33 ±0.20^{b}	34.71 ±3.62^{b}	36.42 ±1.37^{b}	10.22 ±1.77^{a}	12.37 ±1.82^{a}
Hepatosomatic index[2] (HSI) (%)	0.93 ±0.24^{a}	3.07 ±0.59^{c}	5.38 ±0.89^{d}	1.81 ±0.67^{abc}	2.55 ±0.86^{bc}	1.27 ±0.42^{ab}	1.42 ±0.18^{ab}	1.20 ±0.19^{ab}
Viscerosomatic index (VSI) (%)[2]	3.24 ±1.02^{a}	6.56 ±1.06^{c}	10.55 ±2.33^{d}	4.57 ±0.91^{abc}	6.26 ±1.66^{abc}	5.94 ±0.48^{bc}	4.81 ±0.70^{abc}	4.19 ±0.58^{a}
Glycogen (%)[3]								
muscle	0.76 ±0.48^{d}	0.56 ±0.04^{c}	0.88 ±0.12^{d}	1.22 ±0.05^{d}	0.93 ±0.03^{bc}	0.41 ±0.04^{b}	0.28 ±0.06^{a}	0.28 ±0.02^{a}
liver	11.99 ±0.60^{c}	8.67 ±0.27^{b}	7.02 ±0.71^{ab}	6.79 ±1.13^{ab}	7.93 ±0.48^{ab}	7.05 ±1.32^{ab}	11.95 ±1.06^{c}	6.03 ±1.15^{a}
T-BAR (µmols MDAmg^{-1}wet sample)[3]								
muscle	0.019 ±0.03^{b}	0.026 ±0.004^{c}	0.017±0.003^{b}	0.017 ±0.003^{ab}	0.029 ±0.004^{c}	0.015 ±0.00^{ab}	0.012 ±0.003^{a}	0.015 ±0.003^{a}
liver	0.169 ±0.005^{a}	0.626 ±0.021^{d}	0.646 ±0.009^{d}	0.315 ±0.014^{c}	1.162 ±0.042^{f}	0.847 ±0.019^{e}	0.225 ±0.013^{b}	0.195 ±0.007^{ab}

Mean ±SD of four and five replications for [1] and [3] in all farms and of seven and five replications for [2] in farm A, B, C, D, E, F and G, wild fish
In the same row, values that are not followed by the same superscript letter are significantly different at $p < 0.05$.

5.3.3 Fatty acid profile in fish muscle

Total lipid contents of feeds and fatty acids profile in fish muscle are presented in Table 9 and Table 8, respectively. Fatty acid compositions in all groups of fish were significantly different ($p<0.05$), except for 18:3n-3 and 18:3n-6 ($p>0.05$). However, there were no marked differences in fatty acid composition of muscle among the groups fed freshwater trash fish based feeds. Within the general patterns of fatty acids, the higher level of polyunsaturated fatty acids (PUFA) was primarily due to higher levels of 18:2n-6, 18:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6. The prominent saturated fatty acids (SFA) in fish muscles were 16:0, 18:0 and monounsaturated fatty acids (MUFA) were18:1n-9 and 18:1n-7. The proportion of PUFA was highest (47.6-55.9%) while that of MUFA was lowest (12.5 -15.5%). SFA showed an intermediate proportion (30.1-41.9%) between MUFA and PUFA. Moreover, there were no differences in proportions of SFA and MUFA among groups of fish, while PUFA showed significant differences ($p<0.05$)

The fatty acid composition of fish reflects, to some extent, that of the diet; higher levels of eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids, ratios of n-3/n-6, ∑PUFA/∑SFA, DHA/EPA were higher in fish fed feed which was rich in 22:6n-3; marine trash fish (Farm-E) than in remaining groups of fish ($p<0.05$). Feeding high level 16:0 ingredients resulted in high levels of 16:0 accumulated in fish muscle in all cultured fish groups. Although, the fatty acid profile of wild fish was similar to cultured fish, the concentrations of 20:4n-6 and total n-6 fatty acids were found to be relatively higher in wild fish than cultured fish groups.

Table 8 Fatty acid composition (% of total fatty acids on wet weight basis) of wild and cultured fish muscle collected from different farms.

Fatty acid	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F	Farm G	Wild fish
14:0	1.0 ±0.1^{bc}	0.9 ±0.2^{ab}	0.9 ±0.1^{abc}	0.9 ±0.1^{bc}	1.2 ±0.1^{c}	0.6 ±0.0^{a}	0.8 ±0.1^{ab}	0.9 ±0.1^{abc}
16:0	23.5 ±1.2^{b}	20.8 ±1.7^{ab}	19.2 ±0.9^{a}	18.7 ±0.3^{a}	19.7 ±1.3^{a}	21.0 ±1.0^{ab}	20.4 ±1.6^{ab}	19.6 ±0.4^{a}
16:1*	4.5 ±0.4^{ab}	4.0 ±0.6^{b}	3.6 ±0.9^{ab}	3.0 ±0.2^{ab}	7.6 ±1.6^{ab}	6.0 ±1.2^{ab}	3.5 ±0.2^{ab}	2.7 ±0.6^{a}
18:0	12.5 ±1.5^{ab}	11.4 ±0.1^{ab}	11.9 ±0.5^{ab}	11.7 ±0.2^{ab}	11.8 ±1.3^{b}	10.7 ±0.3^{a}	11.7 ±0.3^{ab}	11.8 ±0.5^{ab}
18:1*	10.0 ±0.7^{ab}	10.9 ±1.3^{c}	8.0 ±1.2^{bc}	10.1 ±0.9^{ab}	7.2 ±0.6^{a}	9.0 ±1.4^{bc}	10.7 ±0.4^{bc}	10.9 ±0.5^{bc}
18:2n-6	3.0 ±0.4^{ab}	6.4 ±1.8^{de}	3.4 ±0.9^{bc}	3.7 ±0.8^{bc}	1.7 ±0.3^{a}	7.8 ±1.4^{e}	4.9 ±0.3^{cd}	4.1 ±0.9^{bc}
18:3n-6	0.4 ±0.0	0.4 ±0.2	0.3 ±0.0	0.3 ±0.0	0.3 ±0.1	0.2 ±0.0	0.3 ±0.1	0.4 ±0.1
18:3n-3	0.7 ±0.1	1.7 ±0.1	1.7 ±0.1	1.4 ±0.2	0.7 ±0.2	0.6 ±0.1	2.3 ±0.3	1.4 ±0.5
20:1**	0.7 ±0.3^{ab}	0.5 ±0.0^{b}	-	0.1 ±0.0	-	0.2 ±0.1^{a}	0.3 ±0.0^{ab}	0.3 ±0.0b
20:3n-6	1.1 ±0.4^{b}	1.0 ±0.3^{ab}	0.8 ±0.3^{ab}	0.8 ±0.1^{ab}	0.4 ±0.1^{a}	0.9 ±0.1^{b}	1.0 ±0.1^{b}	0.9 ±0.3^{ab}
20:4n-6	5.3 ±1.6^{a}	15.6 ±0.4^{bcde}	15.2 ±0.1^{bde}	14.4 ±1.7^{bcde}	7.8 ±0.4^{abc}	9.8 ±0.3^{c}	12.8 ±1.0^{cde}	16.7 ±2.6^{e}
20:4n-3	0.2 ±0.1^{ab}	0.5 ±0.2^{ab}	0.4 ±0.0^{b}	0.3 ±0.1^{ab}	0.2 ±0.0^{a}	0.2 ±0.0^{a}	0.4 ±0.0^{b}	0.3 ±0.1^{ab}
20:5n-3	1.7 ±0.3^{a}	2.6 ±0.5^{ab}	2.9 ±0.1^{bc}	2.7 ±0.1^{ac}	3.7 ±0.7^{b}	0.8 ±0.4^{a}	3.3 ±0.6^{b}	2.8 ±0.5^{ab}
22:1**	0.3 ±0.4^{ab}	0.2 ±0.1^{ab}	0.2 ±0.1^{ab}	0.3 ±0.1^{ab}	0.1 ±0.0^{a}	0.2 ±0.1^{ab}	0.2 ±0.0^{b}	0.3 ±0.0^{b}
22:4n-6	5.1 ±1.5^{d}	4.5 ±0.1^{cd}	3.9 ±0.4^{cd}	3.8 ±0.4^{bcd}	1.9 ±0.0^{a}	2.3 ±0.0^{ab}	3.5 ±0.2^{bc}	4.0 ±0.5^{cd}
22:5n-6	5.8 ±1.1^{ab}	4.3 ±0.1^{a}	5.1 ±1.0^{ab}	5.4 ±0.7^{ab}	5.4 ±0.4^{ab}	5.4 ±0.3^{b}	4.2 ±0.6^{a}	4.5 ±0.7^{ab}
22:5n-3	2.7 ±0.6^{ab}	3.5 ±0.1^{b}	3.5 ±0.2^{b}	3.2 ±0.3^{ab}	3.5 ±0.3^{b}	2.3 ±0.3^{a}	3.8 ±0.3^{b}	3.0 ±0.2^{ab}
22:6n-3	12.8 ±0.1b	9.4 ±0.3^{a}	14.4 ±0.8^{bc}	13.6 ±0.5^{b}	26.0 ±3.3^{d}	18.7 ±2.5^{c}	11.0 ±0.3^{ab}	11.0 ±2.5^{ab}
∑SFA	41.9 ±3.7	34.7 ±1.2	33.5 ±1.3	33.0 ±0.6	30.1 ±3.8	33.3 ±0.6	34.3 ±1.6	33.2 ±1.5
∑MUFA	13.0 ±1.8	14.9 ±1.1	13.2 ±0.3	12.5 ±3.6	14.0 ±0.2	14.9 ±1.3	15.5 ±0.9	14.7 ±1.6
∑PUFA	47.6 ±1.2^{a}	50.9 ±2.3^{ab}	51.1 ±2.2^{ab}	54.6 ±3.0^{b}	55.9 ±6.0^{b}	51.8 ±0.7^{ab}	50.2 ±2.5^{ab}	53.3 ±1.3^{ab}
∑n-3	16.1 ±0.7^{a}	18.3 ±0.4^{a}	20.1 ±1.8^{ab}	21.2 ±1.1^{ab}	35.4 ±4.0^{b}	22.7 ±3.5^{ab}	21.6 ±0.6^{ab}	18.0 ±1.2^{a}
∑n-6	28.3 ±1.2^{ab}	29.2 ±2.8^{ab}	28.7 ±1.4^{ab}	28.9 ±2.0^{ab}	17.7 ±1.3^{a}	27.1 ±1.6^{ab}	26.2 ±2.2^{a}	33.0 ±2.5^{b}
(n-3)/(n-6)	0.6 ±0.1^{a}	0.6 ±0.1^{a}	0.8 ±0.1^{ab}	0.8 ±0.1^{ab}	2.0 ±0.1^{b}	0.8 ±0.2^{ab}	0.8 ±0.1^{ab}	0.5 ±0.1^{a}
∑PUFA/∑SFA	1.2 ±0.1^{a}	1.4 ±0.2^{ab}	1.6 ±0.2^{ab}	1.7 ±0.1^{ab}	1.9 ±0.6^{b}	1.6 ±0.0^{ab}	1.5 ±0.1^{ab}	1.6 ±0.2^{ab}
DHA/EPA[1]	3.7 ±0.3^{ab}	3.0 ±0.5^{a}	3.7 ±0.9^{ab}	4.3 ±0.5^{ab}	7.8 ±0.2^{b}	8.0 ±0.0^{b}	2.9 ±0.2^{a}	3.3 ±0.5^{ab}

Values are means ±SD of 3 and 4 replications for Farm A,B,C,D,E,F and n=4 for Farm G and wild fish) and fatty acids less than 0.1% are not reported. Different superscript letters within rows represent significant differences ($p<0.05$). *Contains n-9 and n-7 isomers.
** Contains n-11 and n-9 isomers [1]22:6n-3/20:5n-3

Table 9 Fatty acid compositions (% of total fatty acids on wet weight basis) of feeds.

Fatty acid	Rice bran	Indian mackerel	Yellow striped scad	Thai river prat	Chicken offal
14:0	0.3 ±0.1	4.7 ±0.1	1.8 ±0.2	0.4 ±0.1	0.4 ±0.1
16:0	19.1 ±0.2	21.5 ±0.3	2.4 ±0.4	25.8 ±1.0	23.2 ±2.2
16:1*	0.3 ±0.0	8.1 ±0.3	2.3 ±0.2	5.1±0.0	1.0 ±0.4
18:0	2.1 ±0.1	8.8 ±0.0	1.6 ±0.1	7.7 ±0.4	21.4 ±6.5
18:1*	44.0 ±0.6	8.1 ±0.1	2.6 ±0.3	22.7 ±1.0	14.8 ±2.1
18:2n-6	31.3 ±0.9	2.2 ±0.6	9.1 ±1.0	15.1 ±0.5	17.9 ±3.1
18:3n-6	-	0.4 ±0.0	0.8 ±0.3	0.9 ±0.28	-
18:3n-3	-	0.9 ±0.1	0.8 ±0.0	0.2 ±0.0	0.4 ±0.1
20:1**	0.6 ±0.0	0.3 ±0.0	0.3 ±0.1	0.6 ±0.1	0.1 ±0.1
20:3n-6	0.2 ±0.0	0.5 ±0.0	0.3 ±0.2	0.6 ±0.2	-
20:4n-6	0.1 ±0.0	4.3 ±0.0	0.3 ±0.0	2.6 ±0.1	9.9 ±2.4
20:4n-3	-	0.4 ±0.0	0.3 ±0.0	0.1 ±0.1	-
20:5n-3	-	9.8 ±0.4	4.0 ±0.4	0.3 ±0.0	0.6 ±0.2
22:1**	0.1 ±0.1	0.2 ±0.1	0.5 ±0.1	0.1 ±0.0	-
22:4n-6	-	0.5 ±0.0	0.5 ±0.1	0.52 ±0.1	0.1 ±0.0
22:5n-6	-	2.1 ±0.1	0.5 ±0.0	1.4 ±0.4	-
22:5n-3	0.7 ±0.2	2.4 ±0.1	1.9 ±0.3	1.4 ±0.2	0.6 ±0.2
22:6n-3	0.7 ±0.0	18.8 ±0.6	27.3 ±0.7	12.7 ±0.6	0.7 ±0.1
∑SFA	22.5 ±0.2	36.6 ±0.3	7.9 ±0.5	33.9 ±0.5	40.1 ±1.9
∑MUFA	44.4 ±2.0	17.7 ±1.0	5.7 ±0.9	25.8 ±0.7	16.1 ± 0.32
∑PUFA	33.1 ±1.2	45.8 ±1.0	85.6 ±1.3	34.2 ±1.4	35.61 ±5.4
∑n-3	2.0 ± 0.4	33.0 ±1.0	41.3 ±0.5	14.5 ±0.8	2.3 ±0.3
∑n-6	31.0 ±0.5	10.2 ±0.5	12.0 ±0.2	19.7 ±0.4	29.5 ±4.5
(n-3)/(n-6)	0.1 ±0.0	3.3 ±0.2	3.4 ±0.3	0.7 ±0.1	0.1 ±0.0

Results represent means in triple samples ±SD. ∑ include some minor components not shown. Fatty acids less than 0.1% are not presented.
*Contains n-9 and n-7 isomers.
** Contains n-11 and n-9 isomers

5.3.4 Lipid class composition

The total lipid (TL) contents and lipid class compositions of fish muscle and liver are presented in Table 10 and Table 11, respectively.

TL of fish muscle (% wet weight) ranged from 0.57% to 0.88% approximately. Cultured fish fed marine trash fish based feeds (Farm A and Farm E) displayed a smaller amount of total lipid (0.57% and 0.58%). The proportions of neutral lipid (NL) in fish muscle were significantly higher in all cultured fish (51.8 -66.4%) than in wild fish (32.5%) ($p<0.05$). The profiles of both polar lipids (PL) and NL in fish muscle for all fish groups showed a similar pattern. The prominent NL classes were cholesterol and triacyglycerols (TAG). The major lipid classes found in PL were phosphatidyl-choline (PC, 19.4-26.2%) and phosphatidyl-ethanolamine (PE, 8.9-22.6%). Proportions of phosphatidyl-inositol (PI, 3.0-4.8%), phosphatidyl-serine (PS, 2.2-4.0%) and springomyelin (SM, <0.01-5.7%) were considerably lower. The lowest value for TAG (3.9%) and highest value for PI (22.6%) was found in wild fish ($p<0.05$).

The ratio of NL/PL showed less marked differences among cultured groups which had an average NL/PL ratio of 1. The highest ratio of NL/PL (2.0) was found in fish that had highest value of TL (Farm B) meanwhile, the lowest value of 0.5 was observed in wild fish.

In the liver, the main lipid classes were the same as those found in muscles, but with relatively high values of TL (15-60 times). The ratios of NL/PL (4.7-51.6) in fish liver were higher and of greater variability than in muscles in which TAG was the prominent lipid (14.9-62.2%).

Table 10 Total lipid, and lipid class (% of total lipid expressed as wet weight) of wild and cultured fish muscle samples collected from various farms.

	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F	Farm G	Wild fish
Total lipid (TL) (g/100g wet sample)	0.58 ±0.05^{a}	0.90 ±0.07^{c}	0.76 ±0.05^{b}	0.67 ±0.05^{ab}	0.57 ±0.07^{a}	0.57 ±0.02^{a}	0.81 ±0.10^{bc}	0.77 ±0.11^{bc}
Lipid classes								
Total neutral lipids (% of TL)	**51.8 ±0.5^{b}**	**66.4 ±0.5^{c}**	**55.5 ±1.2^{b}**	**53.2 ±0.5^{b}**	**55.4 ±2.1^{b}**	**53.5 ±1.1^{b}**	**54.7 ±3.1^{b}**	**32.5 ±3.1^{a}**
Cholesterol	25.3 ±0.4^{d}	17.6 ±0.3^{b}	18.8 ±1.5^{bc}	19.0 ±0.1^{bc}	16.9 ±0.1^{c}	20.8 ±0.8^{c}	20.3 ±0.8^{c}	12.2 ±0.5^{a}
Free fatty acids	7.9 ±3.2^{a}	9.6 ±0.1^{a}	10.5 ±1.7^{ab}	10.3 ±1.5^{ab}	6.6 ±0.8^{a}	6.8 ±0.3^{a}	7.8 ±2.0^{a}	16.4 ±3.7^{b}
Triacylglycerols	8.7 ±3.7^{a}	22.3 ±0.0^{b}	21.7 ±3.2^{b}	23.1±2.0^{b}	24.1 ±0.6^{b}	23.5 ±1.3^{b}	16.1 ±0.8^{a}	3.9 ±1.0^{a}
Sterol ester	9.9 ±0.9^{e}	16.9 ±0.0^{f}	4.5 ±0.7^{d}	0.8 ±0.1^{b}	7.9 ±0.6^{c}	2.5 ±0.2^{c}	10.5 ±1.2^{e}	nd
Total polar lipids (% of TL)	**48.2 ±0.5^{b}**	**33.6 ±0.5^{a}**	**44.5 ±1.2^{b}**	**46.8 ±0.5^{b}**	**44.6 ±2.1^{b}**	**46.5 ±1.1^{b}**	**45.3 ±3.1^{b}**	**67.5 ±3.1^{c}**
Springomyelin	5.7 ±1.0^{b}	nd	2.0 ±2.6^{ab}	4.0 ±0.7a^{b}	4.0 ±0.3^{ab}	1.0 ±0.0^{a}	0.5 ±0.7^{a}	4.3 ±0.6^{b}
Phosphatidyl-choline	24.8 ±0.1^{b}	19.4 ±0.2^{a}	23.1 ±2.1^{ab}	21.7 ±0.3^{ab}	24.2 ±0.6^{b}	23.8 ±1.9^{b}	26.2 ±1.8^{b}	22.1 ±1.3^{b}
Phosphatidyl-serine	3.7 ±0.8	2.2 ±0.1	3.8 ±0.5	4.0 ±0.3	2.2 ±0.4	3.2 ±0.4	2.8 ±0.2	2.5 ±0.2
Phosphatidyl-inositol	4.3 ±0.1^{b}	3.0 ±0.0^{a}	4.5 ±0.8^{abcc}	4.8 ±0.1^{c}	3.6 ±1.3^{bc}	4.7 ±0.4^{bc}	4.5 ±0.7^{bc}	3.7 ±0.5^{bc}
Phosphatidyl-ethanolamine	9.7±1.7^{a}	8.9 ±0.2^{a}	10.0 ±1.3^{a}	11.4 ±0.6^{a}	9.4 ±0.3^{a}	12.3 ±0.2^{a}	10.2 ±0.8^{a}	22.6 ±2.4^{b}
Cardiolipine	nd	nd	1.2 ±0.4^{a}	1.0 ±0.0^{a}	1.1 ±0.1^{a}	1.5 ±0.0^{a}	1.1 ±0.4^{a}	12.2 ±0.5^{b}

Values are means ±SD of three and four replications for Farm A,B,C,D,E,F and wild fish).

Different superscript letters within rows represent significant differences ($p<0.05$)

nd; not detected.

Table 11 Total lipid, and lipid classes (% of total lipid) of wild and cultured fish liver samples collected from different farms

	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F	Farm G	Wild fish
Total lipid (TL) (g /100g wet sample)	10.79 ±0.18^{a}	38.29 ±0.95^{b}	34.91 ±1.03^{b}	33.33 ±0.20^{b}	34.71 ±3.62^{b}	36.42 ±1.37^{b}	10.22 ±1.77^{a}	12.37 ±1.82^{a}
Lipid classes								
Total neutral lipids (% of TL)	82.3 ±1.6^{a}	98.1 ±0.0^{b}	91.8 ±1.3^{ab}	96.5 ±1.9^{ab}	96.8 ±0.1^{b}	92.5 ±1.8^{ab}	83.7 ±1.5^{a}	92.1 ±5.2^{b}
Cholesterol	14.6 ±1.3^{c}	8.2 ±0.1^{b}	6.0 ±0.0^{a}	7.2 ±3.3^{ab}	8.7 ±1.2^{a}	12.3 ±0.9^{c}	11.7 ±0.6^{c}	6.5 ±4.2^{ab}
Free fatty acids	36.2 ±1.5^{c}	11.1 ±0.6^{a}	17.1 ±2.0^{b}	12.9 ±2.6^{a}	11.3 ±1.2^{a}	18.4 ±0.6^{b}	19.1 ±2.2^{b}	17.9 ±1.2^{b}
Triacylglycerols	14.9 ±2.3^{a}	51.2 ±0.8^{bc}	31.3 ±0.5^{b}	41.5 ±13.9^{b}	51.4 ±4.4^{b}	37.6 ±6.1^{b}	34.1 ±3.0^{b}	62.2 ±21.9^{c}
Sterol ester	16.6 ±4.1^{a}	27.6 ±0.0^{bc}	37.4 ±0.5^{ab}	34.9 ±6.1^{c}	25.3 ±6.9^{c}	24.3 ±7.7^{ab}	18.9 ±1.3^{ab}	19.7 ±1.7^{ab}
Total polar lipids (% of TL)	17.7 ±1.6^{b}	1.9 ±0.0^{a}	10.2 ±2.1^{ab}	3.5 ±1.9^{a}	3.2 ±0.1^{a}	7.5 ±1.8^{ab}	16.3 ±1.5^{b}	7.9 ±5.2^{a}
Springomyelin	1.6 ±0.1^{b}	nd	nd	nd	nd	nd	0.9 ±0.3	4.0 ±2.9
Phosphatidyl-choline	7.5 ±0.2^{b}	1.9 ±0.0^{a}	2.6 ±0.2^{ab}	2.9 ±1.0^{ab}	1.8 ±0.0^{a}	5.9 ±1.6^{b}	9.6 ±0.5^{b}	1.4 ±0.3^{a}
Phosphatidyl-serine	nd	nd	nd	nd	nd	nd	nd	nd
Phosphatidyl-inositol	3.0 ±0.0^{b}	nd	nd	nd	nd	nd	1.4 ±0.6^{b}	0.8 ±0.7^{a}
Phosphatidyl-ethanolamine	5.5 ±1.3^{b}	nd	5.0 ±1.5^{b}	0.6 ±0.9^{ab}	nd	1.6 ±0.2^{a}	4.0 ±0.4^{b}	1.7 ±1.3^{ab}
Cardiolipine	nd	nd	nd	nd	1.4 ±0.0^{b}	nd	0.45 ±0.6^{a}	nd

Values are means ±SD of three and four replications for Farm A,B,C,D,E,F and wild fish).
Different superscript letters within rows represent significant differences ($p<0.05$)
nd; not detected.

5.4 Discussion

5.4.1 Fish nutritional composition and slaughter indices

Diet significantly affected lipid and moisture contents whilst crude protein and ash contents of both muscle and whole fish showed no marked differences. Moisture content in whole fish was inversely related to lipid content as in other fishes (Arzel et al. 1994; Alvarez et al. 1998; Peres and Oliva-Teles, 1999b; Vergara et al. 1999; Alvarez et al. 1998; Peres and Oliva-Teles, 1999b; Vergara et al. 1999). Increasing in HSI and VSI seemed to be associated with an increase in dietary lipid as seen for groups of cage fish fed largely on freshwater trash fish; the Thai river sprat (Table 5.1-5.4 and Table 7).

Higher liver lipid contents in most of the farmed fish fed trash fish, as compared to wild fish that relied mainly on natural foods suggest differences in fish energy budgets (Marshall and James, 1990) and also reflected dietary differences. (Mnari et al. 2007). Farmed fish appears to gain in energy intake rather than energy expenditure (especially on searching for foods and escaping from predators) that leads to deposition of liver lipid reserves than of wild fish. The similar results were reported by Rueda et al (2001), fat content of muscle, mesenteric and liver in reared sharpsnout sea bream *Diplodus puntazzo* were higher than of wild one. In general, fish that are fed commercial diets do exhibit a greater body fat content than wild specimens (Shearer, 1994). However, the liver lipid storage is influenced by factors such as season and/or developmental state (Henderson and Tocher, 1987).

Although marble goby liver lipid contents in this study were high, especially in fish fed with higher dietary lipid levels (freshwater trash fish), muscle lipid content was not affected by dietary lipid level in all samples. Moreover, muscle lipid contents were not significantly different and remained consistently low (<1%). This may have been due to

species specific differences in the adipogenic capacity of different tissues (Henderson and Tocher, 1987; Shearer, 1994). Marble goby show a greater tendency to deposit lipid in the liver than in the muscle. Similar patterns of lipid storage have been reported in Atlantic cod *Gadus morhua* (Henderson and Sargent, 1985a), haddock *Melanogrammus aeglefinus* (Supis, 2005) and Murray cod *Macculochella peelii peelii* (Giorgio et al. 2007). A contrasting situation, however, has been reported in cobia *Rachycentron candum* (Ji-Teng et al. 2005)where muscle was a primary site of body fat deposition. However, lipid contents in fish tissues show temporal changes during development, growth, maturation and spawning season (James and Randal, 1990; Shirai et al. 2001b; Sabates et al. 2003).

In terms of fillet quality, the lower levels of lipid contained in marble goby muscle (both farmed and wild) suggest that this species can be referred to as a lean fish. From a nutritional viewpoint, fish are often classified into groups according to their lipid contents: lean (<2%); low-fat (2-4%); medium-fat (4-8%) and high-fat (>8%) (Cowey, 1993). Additionally, Huss (1995) categorised fish as lean or fatty species depending on how they store lipids for energy: lean fish use the liver as their energy depot, while fatty species store lipids in fat cells throughout the body. Moreover, the lipid contents of fillets from lean fish are low and stable whereas the lipid contents in fillets of fatty species vary considerably and the accumulated amount is generally influenced by level of dietary lipid that the fish consume (Jorge et al. 1993).

In farm-A a higher liver glycogen level seemed to be related to increased levels of liver glycogen deposition, possibly induced by excess energy in the diet especially energy from rice bran in which carbohydrate is the main constituent (Du et al. 2005).

5.4.2 Fatty acid composition and lipid class

In these investigations the fatty acid compositions of lipids found in marble goby tended to be influenced by dietary inputs as reported in other fish species (Almansa et al. 1999; Turchini et al. 2003; Rodriguez et al. 2004; Nielsen et al. 2005). The fatty acid profile of lipid in wild fish reflects the availability of fatty acids in the aquatic food chain (Sargent et al. 2002).

16:0, 18:0, 16:1 and 18:1 are the predominant saturated fatty acids (SFA) and monosaturated fatty acids (MUFA) in marble goby. This observation was not surprising as Andrade et al (Andrade et al. 2003) have pointed out that these fatty acids are preferred substrates for mitochondrial β-oxidation and heavily catabolized via the TCA cycle to generate metabolic energy in fish (Henderson and Sargent, 1985b). The highest levels of all these fatty acids are consumed during fish growth and development (Henderson, 1996).

The SFA and MUFA can be synthesized *de novo* by all known organisms, including fish, which can convert SFA produced de novo to monounsaturated (MUFA). This action is completed by the action of microsomal fatty acid Δ9 desaturase on 16:0 and 18:0 to yield 16:1n-7 and 18:1n-9, respectively (Henderson, 1996). The ratio of SFA:MUFA in fish muscle in Farm A was opposite to that of their diet indicating the fish SFA was distributed partly from the synthesis *de novo* of dietary non-lipid precursors especially of carbohydrate obtained from rice bran. This claim is supported by Henderson (1987) who revealed that fatty acids in fish can arise from either synthesis *de novo* from non-lipid precursors, or directly from dietary lipid. *De novo* fatty acid production increases when diets are high in carbohydrate and protein, and protein is the preferred carbon source for energy provision in fish (Sargent et al. 2002).

Moreover, SFA and MUFA are storage lipids preferentially used as energy sources (Daniela, 2005). In the present study all fish contained almost identical levels of SFA ($p>0.05$) although fish were fed with different dietary levels of SFA (7.9-40 %). This suggests that the marble goby has a mechanism that can regulate and maintain certain levels of body lipid saturation. This observation agrees with that of James and Randal (1990), who found that the proportional distribution of SFA in fillets of channel catfish *Ictalurus punctatus* did not differ over growth periods between groups of fish fed on practical feeds with and without a top-dressing of oil. Yu et al (1977) also reported similar results in their feeding experiment with rainbow trout *Onchrhynchus mykiss* fed on feeds containing different levels of herring oil and also feed without adding the herring oil. A similar trend was also reported in Murray cod *Maccullochella peelii peelii* fillets in which fish were fed on crude oil extracted from trout offal (Turchini et al. 2003). The higher level of SFA than of MUFA in all groups fish probably also means that marble goby may be unable to use SFA as an energy source efficiently when compared to MUFA and as result indicate, SFA tend to be more accumulated in their tissues. This is consistent with several previous studies that claimed MUFA are generally good substrates for β-oxidation in fish (Henderson and Sargent, 1985b; Sidell et al. 1995; Stubhaug et al. 2005).

Fish are unable to synthesise PUFA completely *de novo* from non-lipid precursors (Henderson, 1996). Freshwater fish are believed to contain Δ6, Δ5 and Δ4 desaturases (Henderson and Tocher, 1987)but lack both the Δ12 desaturase enzyme which can desaturate 18:1n-9 to 18:2n-6 and the Δ15 desaturase which can convert 18:2n-6 to 18:3n-3.These PUFA must therefore be preformed in the diet (Sargent et al. 2002). In this present study, total PUFA in fish muscle is the majority group of fatty acids found in all fish. PUFA represents 48- 56% of total fatty acids and $C:_{20}$ and $C:_{22}$

PUFA are present in higher proportions than $C:_{18}$ PUFA. This is an indication that marble goby has the capability to either produce $C:_{20}$ and $C:_{22}$ PUFA from dietary $C:_{18}$ PUFA or directly obtain from the final end product $C:_{22}$ PUFA from their diets.

The ratio of total n-6 PUFA to n-3 PUFA in fish muscle of all groups reflected those of the diets. A study of wild fish that are living in their natural ecosystems, under natural feeding and physical regimes, is recognised as an important tool in providing information about qualitative dietary lipid requirements with respect to fatty acids in several fish species (Rodriguez et al. 2004; Rueda et al. 1997; Bo-young et al. 2000; Cejas et al. 2004). In the present study, the higher concentration of n-6 PUFA (33%) compared to n-3 PUFA (18%) in wild fish indicates the preferential incorporation of n-6 PUFA, supporting the contention that n-6 fatty acids are essential for marble goby as a freshwater fish. According to Ackman (1994), freshwater and tropical oceanic fish commonly contain large percentages of n-6 PUFA, especially arachidonic acid (20:4n-6). This is particularly true for marble goby since the fatty acid compositions of all fish were characterized by higher proportions of n-6 PUFA, especially arachidonic acid (20:4n-6, AA), docosapentaenoic acid (22:5n-6, DPA) and linoleic acid (18:2n-6). Both 18:2n-6 and 20:4n-6 have a significant biological role; especially with respect to eicosanoids derived from 20:4n-6 that are physiologically active in fish and are essential for reproduction and cellular signal transduction in fish (Bell and Sargent, 2003). Elevation of 20:4n-6 can improve growth and survival (Bessonart et al. 1999) and resistance to handling stress (Koven et al. 2001). The concentration of 20:4n-6 was higher in wild fish than in farmed fish, probably reflecting the greater consumption of aquatic insects by wild fish (Carole et al. 2005). Even though species types and fatty acid composition of natural foods for marble goby were excluded in this study, 18:2n-6 and 18:3n-3 are frequently the major PUFA in many freshwater invertebrate groups

(Bell et al. 1994). Tropical brine shrimp *Artemia* sps. contains a high proportion of 18:2n-6 and total n-6 HUFA (Chinavenmeni and Natesan, 2007) and is commonly deficient in 18:3n-3 (Lian et al. 2003). Additionally, Chinavenmeni and Natesan (2007) also reported high concentrations of 18:2n-6 and 20:4n-6 in a freshwater benthic prey fairy shrimp *Streptocephalus dichotomous*. In fish, the fatty acid composition of several tropical freshwater fish, such as eel *Monopterus albus*, bighead carp *Aristichthys nobillis*, rohu *Labeo rohita* (Suriah et al. 1995) and Japanese catfish, *Silurus asotus* (Nobuya et al. 2002) collected from their natural habitats, also showed deposition of higher levels of 18:2n-6 and 20:4n-6 than n-3 PUFA.

The lower levels of arachidonic acid (20:4n-6) in farmed fish diets and the higher conversion of linoleic acid (18:2n-6) to the longer chain polyenoic derivative; arachidonic acid (20:4n-6) in fish flesh than in their diets indicates that marble goby probably has an innate ability to convert 18:2n-6 to 20: 4n-6 as almost all freshwater fish do (Turchini et al. 2006).

Similarly, the profiles of n-3 fatty acids found all farmed fish are generally similar to those found in their diets. The levels of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) in the diets were correlated with the levels of 20:5n-3 and 22:6n-3 accumulated by marble farmed goby.

The 22:6n-3 level was highest in farmed fish fed on marine trash fish (Farm-E) causing a significantly higher ratio of total n-3 PUFA to n-6 PUFA. This result indicates that the percentage of 22:6n-3 in the muscle of marble goby is influenced by dietary 22:6n-3. In a similar feeding trial with freshwater silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobillis* by Steffens et al (1993), a diet containing 10% mackerel oil led to high levels of EPA and DHA in the fish muscle. However, most freshwater fish do not require long chain HUFA but often require 18:3n-3 to

manufacture the highly biologically active 20:5n-3 and 22:6n-3 (Henderson, 1996; Sargent et al. 2002). With the exception of the above general ability, in those species that are unable to synthesize 22:6n-3 from its precursor linolenic acid (18:3n-3), it must be supplied in the diet i.e., it is a dietary essential fatty acid (Bell et al. 2001). Some adults of freshwater carnivorous fish species such as channel catfish *Ictalurus punctatus* (Satoh et al. 1989a) and pike, *Esox lucius* (Henderson et al. 1995) have very limited abilities to convert 18:2n-6 to 20:4n-6, and 18:3n-3 to EPA and DHA as result of adaptation to a diet naturally rich in n-3 and n-6 HUFA (Sargent et al. 2002). A similar result was also found in rainbow trout by Bell et al (2001) where the amount of 22:6n-3 synthesized from 18:3n-3 was only about 5% of that obtained directly from the fishmeal in the diet.

The ability of marble goby to bioconvert linolenic acid (18:3n-3) to 20:5n-3 and 22:6n-3 is still in question and needs more research. All of the feeds contained minimal amounts of linolenic acid (18:3n-3) and also fish muscles contained less of this fatty acid. In addition, the apparent conversion ratios (18:3n-3/22:6n-3) varied among farmed fish groups and most of them showed much lower convertion rates than of their diets, especially in groups of fish fed on freshwater trash fish (including wild fish).

From a field observation, reported by a farmer, it should be noted that the significantly better survival rate of fish fed mainly on marine trash fish could perhaps, at least point to the positive effect of n-3 HUFA, particularly 22:6n-3, on the health of this species. It would therefore be interesting to conduct further research to investigate the capacity of marble goby to bioconvert 18:3n-3 to 22:6n-3.

Knowledge of the significance of long-chain EPA and DHA to human health has increased considerably in the last four decades (Maurice E. Stanby et al. 1990). EPA and DHA found in fish possess extremely beneficial properties for the prevention of

human artherosclerosis, heart attack and depression (Simopoulos, 1989; Sargent, 1997; Logan, 2003). They are particularly effective in preventing obesity-related disease (Browning, 2003), reducing inflammation (Simopoulos, 2002) and the risk of developing breast and prostate cancer (Greenwald, 2001). They are also essential for early retina and brain development (Uauy et al. 2001) and in the maintenance of cognitive function in the elderly (Scott, 2003; Ruxton et al. 2005). Therefore, when fish are suggested as a means of improving health for humans, both the fat content and the HUFA distribution must be considered.

Since both farmed and wild marble goby contain less fat in their fresh muscle and appear to have the ability to deposit dietary n-3 HUFA, especially DHA, into their muscle consumption of either wild or cultured marble goby will contribute to dietary n-3 PUFA intake, with benefits to human health. Additionally, the ratios of n-6/n-3 found in this study were less than the value (4.0 maximum) recommended by the UK department of health. A value higher than the maximum value is considered harmful to health and may promote cardiovascular diseases (HMSO, 1994).

It should be noted that beneficial effects of silver carp oil (containing 2.8% and 8.4% of AA and DHA respectively) on blood pressure, serum lipids and platelet function are more pronounced than the effects of mackerel oil (containing 0.2% and 16.0% of AA and DHA respectively) (Steffens, 1997). These results suggest that a decrease in dietary AA is not necessary for lowering blood pressure and that a balanced n-6/n-3 PUFA supply provided by a freshwater fish diet might be clinically even more efficient than isolated n-3 supplementation.

5.4.3 Lipid class composition

Total lipid content is a common measure of energy storage in fish (Tocher and Harvie, 1988; Bo-young et al. 2000; Cejas et al. 2004; Bo-young et al. 2000; Cejas et al. 2004). However, information on lipid class composition could give more precise and detailed information on fish energy status (Finstad et al. 2004b; Naesje et al. 2006; Naesje et al. 2006).

Distribution of lipid within the muscle and liver in marble goby showed the same trend; the higher proportion of neutral lipids over polar lipids in both organs was due to an increase mainly in triacylglycerol (TAG), cholesterol and sterol ester. This is not surprising because TAG is the most abundant single lipid class in fish lipid and plays an important role as an energy storage substrate (Cejas et al. 2004). Additionally, TAG is primarily metabolized during fish starvation (Henderson and Sargent, 1985a). High liver TAG in marble goby is in agreement with Jacquot (1961) and Osman et al (2001) who stated that the lean fish stores 50-80% of its fat in the form of triacylglycerol in liver (Jacquot, 1961). Excess accumulation of liver lipid in cultured fish has been a concern to nutritionists as it is thought to reflect excess energy in diets, which are not efficiently utilized for production, therefore, the high levels of neutral liver lipid in cultured fish are reflected in a comparatively higher dietary energy intake. Moreover, the neutral liver lipid in wild fish was also high, while muscle neutral lipid was lower compared to cultured groups, This supports the earlier statement that marble goby has a limited ability to transport the large amount of deposited fat out of the liver to the muscle for storage. In other words the hepatic uptake of lipids exceeds the oxidation and secretion of lipid by liver. Additionally, the higher ratios of neutral lipids to polar lipids (NL/PL) in liver than in muscle in cultured fish also support this

observation. This pattern of lipid deposition, however, perhaps limits feeding this fish with high-energy, lipid-rich diets - a point nutritionists should take into account.

Sterols are essential components of the membranes of all eukaryotic organisms (Phillips et al. 2002)and also play an important role in controlling membrane fluidity and permeability (Piironen et al. 2000). It is well known that cholesterol is the main sterol in animal cell membranes including fish (Morris et al. 1982; Graeme et al. 1996; Copeman and Parrish, 2004; Graeme et al. 1996; Copeman and Parrish, 2004). This is also apparently true in marble goby because muscle cholesterol was the dominant sterol in the present study. This result is consistent with muscle cholesterol compositions obtained from mud skipper *Boleophthalmus boddaerti* (Dipankar et al. 1997), eel *Anguilla anguilla* (Nikolaus et al. 1994) and tilapia *Oreochromis niloticus* (Karapanagiotidis, 2004). Furthermore, as suggested by Banerjee et al (1997) in response to increase in water temperature fish normally adjust their cell membranes by increasing the cholesterol content in relation to the environmental temperature. Therefore, the high level of cholesterol in this present study was perhaps consistent this statement since sampled fish were collected during high water temperatures (25-32 ºC).

The lower muscle and liver cholesterol levels in wild fish than in cultured fish in the present study are in agreement with the liver and muscle cholesterol patterns found in cultured and wild counterparts of black sea bream *Spondyliosoma cantharus* (Rodriguez et al. 2004) and tilapia *Oreochromis niloticus* (Karapanagiotidis, 2004). A contrasting result was, however, reported in muscle cholesterol contents of wild fish that were higher than of farmed sea bass *Dicentrarchus labrax* (Orban et al. 2003). Addition, Coz et al (2002) reported the occurrence of ‘‘fatty liver’’ (fatty infiltration and degeneration) in cultured sea bass *Dicentrarchus labrax* L. that was associated with higher levels of TAG and cholesterol compared to the healthy wild sea bass. Therefore,

based on field observation of fatty liver in cultured marble goby together with lower levels of TAG and cholesterol in wild fish than farmed fish in this study, this supports the healthier status of wild fish. It would therefore be of interest to investigate effects of these two neutral lipids on health status of marble goby in further studies because high mortality caused by diseases has been one of the major constraints in farming this fish (Somkiat, 1997; Lin and Kaewpaitoon, 2000; Prasankok et al. 2002).

The high concentrations of free fatty acids observed in fish indicate that the TAG levels may be underestimated. Free fatty acids are the products of enzymatic hydrolysis of phospholipids and TAG, and free fatty acids tend to accumulate in frozen samples (Refsgaard et al. 1998). Increases in free fatty acid levels are usually attributed to the hydrolysis of phospholipids (Hardy et al. 1979; Kaneniwa et al. 2000; Ron et al. 2004; Kaneniwa et al. 2000; Ron et al. 2004), suggesting that present study estimates of TAG are less likely to be influenced by hydrolyzation than phospholipids. In the present study the total phospholipid fraction was not saponified separately and only the most abundant types of naturally occurring phosphoglycerides (PC, PI, PE, PS and cardiolipin) and sphingomyelins are available. In addition, estimates of total lipid are unaffected by hydrolyzation, and variation in TAG (Ron et al. 2004). However, all samples were kept in the same condition until analysis.

Of total muscle lipid, a relatively high proportion (44% on average for cultured fish) was polar lipid in relatively stable amounts. This implies that differences in total muscle lipid are likely to be influenced by neutral lipid content. Even though the fatty acid compositions of neutral and polar lipids were not available in the present study in general, polar lipid contains considerably more polyunsaturated fatty acids than neutral lipid (Sargent et al. 2002).

Generally, long chain $C:_{20}$+$C:_{22}$ PUFA to $C:_{18}$PUFA ratio is 4.3-9.8, greater in polar than in neutral lipids (Ackman, 1994; Kozlova and Khotimchenko, 2000; Kozlova and Khotimchenko, 2000). Therefore, the higher concentration of polar lipids in wild fish are likely to reflect their ability to convert C_{18} PUFA in their natural foods to $C:_{20}$ +$C:_{22}$ PUFA which were then deposited in the phosphoglycerides (Ghioni et al. 1996). Besides, with respect to their natural feeding habit they consume largely freshwater zooplankton that comprises mainly polar lipids with low levels of neutral storage lipids (Wainman et al. 1993). In addition, a contribution of 61% - 90% of phospholipids to the total lipid content was reported in freshwater cladocerans (Macedo and Pinto-Corlho, 2001).

The dominant phospholipids in muscle and liver in both cultured and wild marble goby were PC and PE. This finding is similar to polar lipids analysed in muscle and liver of 27 fish species by Takama et al (1999). This is also in accordance with the common opinion that PC and PE are important for maintaining normal functioning of cell membranes. PC and PE have been frequently assessed as the main phospholipids classes in fish (Henderson and Tocher, 1987; Dipankar et al. 1997). Moreover, PC is the most abundant of phospholipids, comprising approximately 50% of total phospholipids in fish tissue (Lie et al. 1992; Medina et al. 1995; Medina et al. 1995). The phospholipids of white muscle of freshwater species have a noteworthy nutritional value, supplying substantial amounts of PUFA (Isabel et al. 1995). Additionally, $C:_{20}$+$C:_{22}$ PUFA are generally found to be abundant in polar lipids and in major membrane phosphoglycerides, PC and PE in particular (Ghioni et al. 1996). Therefore, a characteristic property of phospholipids found in this present study showed some general similarities to those revealed in other freshwater species (Henderson and Tocher, 1987; Erickson, 1993; Kozlova and Khotimchenko, 2000; Nobuya et al. 2002).

5.5 Conclusion

1.) The compositions of lipid found in the cultured fish were closely related to their diets. The significantly higher EPA and DHA levels were found in fish fed marine trash fish than other groups of cultured fish and also wild fish. This suggests that cultivation of marble goby on a diet containing large amount of EPA and DHA could remarkably benefit in increasing the content of these fatty acids in the fish muscle. However, the ability of marble goby to bioconvert linolenic acid (18:3n-3) to EPA and DHA is still in question and needs more research. However, detrimental effects such as the higher liver lipid deposition, higher HSI and VSI and also higher ratios of liver NL/PL in cultured fish may be indicative that the diet fed to the cultured fish might not be ideal for optimal performance thus balance of dietary lipid and energy needs to be improved.

2.) Distribution of fatty acids in total lipid and lipid class did not show marked differences between wild and cultured fish fed freshwater trash fish based diets. This suggest that the lipid composition of feed supplied to the cultured fish did not differ greatly from that of the diets consumed by the fish in the wild, which hypothetically contains the desirable composition for the lipid nutrition of this species.

3.) Even though fish fed feed of marine origin had a higher nutritional value for humans in terms of n-3 supply than wild fish, a difference exists between the two groups of fish in the patterns of eicosanoid produced; AA which was found to be higher in wild fish. It has been documented that AA is the major eicosanoid precursor in fish cells and these components are important in the control of many fish physiological functions (Bell and Sargent, 2003). Thus, dietary ratios of AA/EPA and also DHA/EPA should be considered important factors to the requirements of this species in promoting their growth and maintaining their health.

Chapter 6 A Comparison of Combinations of Rice Bran and Trash Fish in Rearing of Marble Goby (*O. marmoratus*) With Reference to Digestibility, Feed Intake and Growth

6.1 Introduction

Feeding fish with compound feeds has the most advantages in culture practice in the context of environmental and balanced nutritional approaches. However, adaptation to artificial feeds by farmers is often not straight forward due to feed cost and availability constraints. Use of on-farm feeds has some advantages over commercially produced feed, in particular they can be more economical and efficiently utilize locally available raw materials (FAO, 1993).

Thailand has the potential to expand aquaculture production for both local consumption and export (FAO , 2005a). The country has a net surplus of high quality feedstuffs after domestic demands are met, except soybean (FAO, 1979; Edwards and Allan, 2004). Within the SE Asian region, Thailand is considered the most self-sufficient country in terms of feed ingredient supplies with minimal importation of fish meal which is destined mainly for the shrimp feed industry (Supis, 2005). About 90-95% of raw materials used in animal feeds are available locally (Nanthiya, 1989). Although, there are reports on potential use of some feed ingredients in preparing complete diets for certain economic freshwater species (Jantrarotai and Jantrarothai, 1993; Somsueb, 1993; Suraniranat, 1998), until recently, Thailand has however been confronted with a lack of nutritional research about on-farm feed use and its effects on fish nutritional composition.

A consistently high price and large demand for the carnivorous marble goby (*O. marmoratus*) makes it one of the most attractive candidates for aquaculture in Thailand (Ausyfish, 2006). Until recently feeding of marble goby still predominantly relied upon the traditional method of using trash fish from both marine and freshwater sources. However, the rapid deterioration in quality accompanied by loss of nutrients that occurs with time in unfrozen trash fish is a common problem in using trash fish (Supis, 2005). Raw chopped or minced trash fish is highly unstable in water causing rapid deterioration of water quality in surrounding areas (Edwards et al. 2004). In addition overfeeding can lead to excessive lipid accumulation (Erfanullah and A. K. Jafri, 1998). Additionally, prolonged feeding using a single feed of thiaminase-containing raw trash fish causes thiamin deficiency in fish (Royes B. Juli-Anne and Chapman, 2003).

However, when they are available trash fish are relatively less expensive for aquaculture than fish meal (FAO, 2005c). The problems of water quality deterioration, nutrient deficiency and health may, however, be partially overcome by careful management of feeding strategies (Suchart et al. 2005). Proper processing of feedstuffs by mixing trash fish with dry components such as rice bran and compound feed in the correct proportions can make feed more economical, provide adequate nutrition and water stability (Yakupitiyage, 1993). In particular, rice bran is recognised as one of the most widely available feed ingredient and has potential for use by fish farmers throughout the country (Edwards and Allan, 2004). Besides being an excellent source of nutrients, especially vitamins B and E, rice bran is also an effective feed binder (Joachim and Felicitas, 2000). An adequate supplement of vitamins combined with a protein source, such as fish meal and trash fish, potentially makes rice bran an excellent feed stuff for the aquaculture of many species (Yakupitiyage, 1993; Jantrarotai and Jantrarothai, 1993) including marble goby (Survey result this study). The use of rice

bran for aquaculture feed in the tropical rice-growing countries of the region would therefore have a comparative advantage in production of both feeds and fish.

Despite the recognised and successful rearing of many economically attractive carnivorous species, including marble goby, using traditional on-farm aquafeeds (Supis, 2005), there is little available information to date concerning effects of those diets on nutrient digestibility, feed intake and growth performance of marble goby.

The objectives of the present experiments were:

1) To determine the effects of diets on feed intake, fish growth performance and survival rate of marble goby
2) To determine the effects of various diets on fish nutrient composition, glycogen content and hepatosomatic index
3) To compare apparent protein and dry matter digestibility in fish fed various diets

6.2 Material and methods

6.2.1 Experiment to evaluation the use of different feed types on fish digestibility

6.2.1.1 Experimental system and design

The digestibility trial was carried out in an indoor aquarium tank system (Figure 4). The trial was set up under ambient conditions in the hatchery of the Asian Institute of Technology (AIT), Pathum Thani, Central Thailand. The experimental period was 60 days; 24th January – 25th March 2004. A Completely Randomized Design (CRD) with four replicates was employed. Six different diets were assigned as treatments (see also Table 12 and 13):

Treatment 1 (T-1) = Mackerel trash fish

Treatment 2 (T-2) = Complete diet

Treatment 3 (T- 3) = Mackerel trash fish + Rice bran

Treatment 4 (T -4) = Mackerel trash fish + Complete diet

Treatment 5 (T -5) = Mackerel trash fish + Rice bran + Complete diet

Table 12 Formulation (g kg^{-1} air dry) of experimental diets

Ingredient	**Diet**				
	T-1	T-2	T-3	T-4	T-5
Mackerel	334	-	164	16	50
Rice bran	-	-	54	-	12
Complete feed [a]	-	111	-	102	80
Vitamin premix [b]	2	-	2	2	2
Mineral premix [c]	1	-	1	1	1

[a] Juvenile catfish feed is composted of fish meal, soybean meal, broken rice, rice bran, maize, coconut meal, mineral & vitamin premix and feed preservative.

[b] At 20 g kg^{-1} feed inclusion level, provided in 1 kg of final diet: retinol; 192 mg, α-tocopherol actetate (50%); 272 mg, thiamine; 30 mg, riboflavin (96%); 60 mg, pyridoxine (98%); 20 mg, cobalamin (1%); 12 mg, menadione (98%); 8 mg, ascorbic acid (96%); 160 mg, D-calcium pantothenate (98%); 100 mg, niacin (99%); 80 mg, Folic acid (80%); 4 mg, biotin (2%); 20 mg, inositol (97%); 200 mg, choline choride (50%); 1.12 g, Yeast; 1.6 g ethoxyquin (20%); 12 mg, alpha starch ; 36.11 mg

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

Note: Vitamin premixed ([b]) and mineral premixed ([c]) were purchased from the White crane company, Bangkok, Thailand. Products details [a] and [b] above were attached along with the products.

Table 13 Chemical compositions of experimental feeds and feed ingredients

Experimental feeds	Moisture (%)	Crude protein (%dm.)	Crude lipid (%dm.)	Ash (%dm.)	Crude fibre (%dm.)	Gross energy (GE) (kJ g^{-1}diet)	P:GE ratio (mg protein $kcal^{-1}$)
Diet 1 (T- 1)	76.9 ±0.7	69.4 ±0.2	15.74 ±1.3	13.89 ±0.4	0.27 ±0.1	20.92	138.8
Diet 2 (T- 2)	31.2 ±0.2	39.7 ±0.1	4.76 ±0.1	15.22 ±0.3	1.14 ±0.1	18.25	91.0
Diet 3 (T- 3)	30.1 ±0.6	39.4 ±0.1	9.26 ±0.2	14.73 ±0.1	2.49 ±0.1	20.28	81.3
Diet 4 (T- 4)	30.9 ±0.6	39.5 ±0.1	5.11 ±0.1	15.62 ±0.2	1.07 ±0.1	17.81	92.8
Diet 5 (T -5)	28.3 ±0.6	39.5 ±0.1	5.58 ±0.2	15.98 ±0.1	1.26 ±0.2	18.45	89.6
Feed ingredients							
Mackerel	71.0±4.9	67.2 ±0.4	13.86 ±1.3	14.74 ±3.8	0.27 ±0.1	20.49	
Rice bran	9.0 ±0.0	17.1 ±0.0	15.02 ±0.1	3.39 ±0.1	4.57 ±0.5	17.73	
Complete diet	9.7 ±0.0	40.4 ±1.1	6.31 ±0.1	11.21 ±0.0	2.15 ±0.4	1,785.0	

Note: dm: dry matter, Mean ±SD of triplicates

6.2.1.2 Experimental feeds

The spoiled Indian mackerel (unpreferable for human consumption) was purchased from 'Talad Thai' fish wholesale market which was located nearby the experimental site (1.5 km distance). Whole fish were passed twice through a mincer to ensure all fish bones were finely crushed. A purchased juvenile catfish diet from a private feed company; Charaen Pokhaporn (CP) Ltd, Bangkok, Thailand was used as the complete feed. Chromic oxide (Cr_2O_3) was included as a digestibility marker at an inclusion rate of 1g /100g. In all feed formulas (except in T-1) chromic oxide was first mixed with dried ingredients for 15- 20 minutes at a low speed in a food mixer (Kenwood KM336, 4.6L capacity) to prevent dust. Water was then added to the mixture to bring 30% moisture to the feed and was mixed again for another 20 minutes. Feeds were pelleted by extruding the semi-moist mixture (dough) twice through a 3-mm diameter die plate fitted to a screw mincer attached to mincer (Hobart 4146). Extruded feeds in form of 'spaghetti' of 3-mm diameter were air-flow dried in a drying oven

(Heraeus, TU 60/60) at 60 °C for 5-6 hours and reduced to pellets size of approximately 3 mm by manual cutting, which were stored at -20°C and transferred to 4°C a day before they were used for the test fish.

6.2.1.3 Experimental fish and culture conditions

Wild juvenile marble goby at an average size of 25±2 g were acclimatised to circular cement tanks (1.20 m diameter, 0.51 m^3 water volume) at a temperature of 23-25 °C for 30 days. During this acclimatization period fish were fed twice a day at about 0830 and 1600h using the prescribed diets. Complete water exchange was performed every 3-4 days. Subsequently, seven experimental fish were selected according to size uniformity and absence of physical defects and stocked into individual rectangular glass aquaria (45 ×60 ×45 cm) supplied with 24 hour diffuse aeration. A thermostatically controled submersible heater was inserted into each aquarium when water temperatures were below 25 °C. Aquarium glass was covered using darkened plastic sheet to reduce fish stress by limiting fish visual contact with outside disturbances (Figure 4). During the experimental period in the aquarium system, fish were fed their prescribed diets twice a day to satiety at 0830 and 1600 hours. Partial water exchange was performed every 3-4 days. Uneaten feed in the system was siphoned out 2 hours after feeding. Water temperature (to ensure the heater thermostat worked properly) and pH were monitored weekly.

6.2.1.4 Fish faeces collection and digestibility measurement

Fish faeces were collected 2 hours after feeding by siphoning the bottom of the tank. The separation between an eaten and uneaten feed was done at the same time by gravity filtering; the siphoned faeces was filtered through a fine plankton mesh screen

and hand sorting. Faeces were then pooled and stored at –20°C. After termination of the collection period, frozen faecal sample were dried and thoroughly mixed, and kept frozen at –20°C until analysed.

Figure 4 The experimental digestibility set up at AIT indoor culture unit.

6.2.2 Experiment to evaluation the use of different feed types on fish nutrient composition and feed intake

6.2.2.1 Experimental design

The fish feed intake experiment was carried out in an indoor cement tank system at the Asian Institute of Technology, Pathum Thani, Thailand (AIT). The experiment was conducted for 74 days; 23rd December 2003 to 7th March 2004. A complete

randomized design (CRD) was employed with six replicates and an individual fish was presented as a replicate. The experimental feeds used and design treatments were the same as in the fish digestibility experiment (see also Table 12 and Table 13) but without the addition of chromic oxide.

6.2.2.2 Experimental fish and culture system

Six juvenile fish at an average size of 25±2 g were stocked individually into each cement compartment (25 x 60 x 45 cm) which was set up in a rectangular cement tank (1.2 ×4.0×0.50 m). Each compartment was separated by a wooden frame stretched with a polyethylene net to allow water to flow horizontally throughout the whole tank (Figure 5). Diffuse aeration was supplied to each compartment to maintain a dissolved oxygen level above 4 mg L^{-1}. Submersible heaters were switched on when the temperature dropped below 25°C. Experimental fish were fed the prescribed experimental feeds for 4 weeks in conditioning tanks and 2 weeks in the culture system before starting the experiment. During the experimental period fish were hand-fed to satiation twice a day at 0900 and 1600 h respectively.

Fish faeces and uneaten feeds were removed by siphoning every 2 days after the second daily feeding. Water was replaced to the same volume as the water lost due to siphoning. The experimental tank was covered using a polyethylene net and a plastic sheet to prevent the fish escaping and limiting fish visual contact with outside disturbances.

6.2.2.3 Fish feed intake evaluation

At the end of experiment, after 45 minutes of feeding, fish were anaesthetized using a concentration of 4%benzocaine (4g of benzocaine powder in 100 ml of methanol) (Davies, 2000). As anaesthesia progressed, fish weight and length were

recorded. Subsequently, before recovery from anaesthesia, fish were killed and the digestive system was removed for the amount of feed consumed determined following the method of Malcolm et al (2004), an abdominal incision was made from the buccal cavity to the anus. Ingested food was collected from the fish mouth, oesophagus and stomach. Collected feed was then weighed before and after drying in an oven at 70°C overnight.

Feed intake as % of biomass was calculated according to the equation below;

% feed intake = ((amount of feed consumed (g) / fish body weight (g wet weight)) x 100

Figure 5 The experimental unit used for feed intake experiment.

6.3 Results

6.3.1 Fish growth performance, feed and fish chemical composition

The formulation of experimental diets, chemical compositions of diets and feed ingredients used are shown in Table 12 and Table 13, respectively.

Final weights were significantly ($P<0.05$) highest in fish fed single trash fish (T-1) ($p<0.05$) with no marked differences between the other diets (Table 14). A similar trend was also observed in fish daily weight gain (DWG). The Feed Conversion Ratio (FCR) for fish fed single trash fish (T-1) was significantly higher than for T-4 and T-5 ($p<0.05$) but, not for T-2 and T-3. The survival rate was 100% in fish fed single trash fish (T-1) followed by T-2 (83%) and T-3, T-4 and T-5 with the same percentage (67%). Similarly, values obtained for feed intake were highest for T-1 followed by T-2, T-3, T-4 and T-5 respectively.

Final proximate body composition showed that there was no significant difference in whole fish moisture content ($p>0.05$). There were no significant differences in the ash content between treatments though T-1 was slightly higher. In terms of protein content, fish fed T-2 had a higher protein content than for other groups of fish, whilst the lowest value was observed in fish fed only trash fish, T-1 ($p<0.05$). Lipid content in whole fish and liver were highest in fish fed trash fish ($p<0.05$), whilst there was no marked difference in lipid content in fish muscle between fish fed the different diets (Table 15).

6.3.2 Fish glycogen content, hepatosomatic index (HSI) and viscerosomatic index (VSI)

Glycogen levels in fish muscle differed little between treatments. However, the glycogen level of fish muscle for T-3 was higher than that of T-5 ($p<0.05$). A similar

trend was found in glycogen content of fish liver where T-1 showed the highest value. The value of liver glycogen observed in T-1 was approximately 3 times higher than of T-3 ($p<0.05$) (Table 15).

Viscerosomatic index (VSI) and hepatosomatic indices (HSI) showed the same trends; significantly higher VSI and HSI were observed in T-1 ($p<0.05$). However, there was no statistical difference in VSI and HSI between the other groups of fish ($p>0.05$).

The apparent dry matter digestibility coefficients (ADC_{dm}) and the apparent protein digestibility coefficients (ADC_{cp}) of formulated diets used are shown in Table 16. ADC_{dm} and ADC_{cp} showed the same trends across treatments, that the T-1 diet was significantly higher for both ADC_{dm} and ADC_{cp} than T-2, T-3, T-4 and T-5, respectively.

Table 14 Feed intake and growth performance of marble goby fed different experimental diets for 74 days

	T-1	T-2	T-3	T-4	T-5
Initial weight (g)	25.2 ±3.9	24.8 ±4.0	27.2 ±2.0	26.5 ±2.0	22.9 ±2.2
Final weight	64.1 ±8.4^{b}	42.8 ±7.6^{a}	44.8 ±5.9^{a}	42.9 ±3.8^{a}	38.7 ±7.2^{a}
DWG (g fish^{-1}day^{-1})[1]	0.53 ±0.11^{b}	0.24 ±0.10^{a}	0.24 ±0.08^{a}	0.22 ±0.05^{a}	0.21 ±0.10^{a}
FCR[1]	4.99 ±1.10^{b}	2.07 ±0.96^{ab}	2.10 ±0.74^{ab}	0.94 ±0.22^{a}	0.99 ±0.50^{a}
PER[1]	0.32 ±0.07^{a}	1.51 ±0.64^{ab}	2.77 ±0.93^{b}	3.12 ±0.72^{b}	3.17 ±1.45^{b}
Survival rate (%)	100	83	67	67	67
Feed intake (g^{-1}day)[1] (max-min)	2.54 ±0.33^{c} (2.20-2.86)	0.43 ±0.03^{ab} (0.40-0.46)	0.23 ±0.02^{ab} (0.42-0.48)	0.19 ±0.01^{a} (0.18-0.20)	0.18 ±0.01^{a} (0.16-0.18)
Feed intake(g^{-1}day)[2] (max-min)	5.49 ±1.00^{c} (9.56-12.4)	0.31 ±0.04^{ab} (0.58-0.68)	0.33 ±0.03^{ab} (0.58-0.70)	0.14 ±0.02^{a} (0.24-0.30)	0.12 ±0.02^{a} (0.18-0.26)

Values (Mean ±SD of triplicates) are in the same row not sharing a common superscript are significantly different ($p<0.05$). Fish feed intake data were given in median

[1]as dry weight of feed [2]as wet weight of feed

DWG = daily weight gain = fish weight gain/culture period (day)

FCR = dry feed weight intake (g)/fish wet weight (g).

PER = (final body weight × final body protein) – (initial body weight × initial body protein)/total protein intake (g)

Table 15 Chemical composition (% wet weight), visceral indices and glycogen contents of marble goby

	T-1	T-2	T-3	T-4	T-5
Moisture (%)	77.9 ±2.0	76.7 ±1.2	77.5 ±1.0	78.5 ±1.4	78.2 ±0.1
Crude protein	15.93 ±0.39^{a}	17.93 ±0.05^{c}	16.98 ±0.02^{a}	17.17 ±0.07^{b}	16.07 ±0.07^{a}
Ash (%)	5.43 ±0.11^{b}	4.93 ±0.16^{a}	4.66 ±0.18^{a}	4.76 ±0.18^{a}	4.77 ±0.20^{a}
Crude lipid					
- whole fish	0.75 ±0.13^{b}	0.62 ±0.12^{ab}	0.44 ±0.01^{a}	0.56 ±0.02^{ab}	0.73 ±0.04^{b}
- muscle	0.33 ±0.04^{a}	0.34 ±0.33^{bc}	0.42 ±0.18^{b}	0.35 ±0.02^{a}	0.63 ±0.01^{c}
- liver	21.90 ±2.27^{c}	8.03 ±0.33^{b}	6.96 ±1.23^{b}	7.01 ±0.96^{b}	2.90 ±0.52^{a}
Glycogen (%)					
- Muscle	0.91 ±0.11^{b}	0.66 ±0.11^{a}	0.70 ±0.12^{a}	0.75 ±0.22^{ab}	0.54 ±0.05^{a}
- Liver	14.15 ±2.24^{c}	5.37 ±0.88^{b}	4.93 ±0.54^{ab}	3.75 ±1.01^{a}	3.47 ±0.18^{a}
VSI (%)	13.06 ±1.25^{b}	4.95 ±1.06^{a}	4.88 ±1.12^{a}	2.82 ±3.31^{a}	3.22 ±1.17^{a}
HSI (%)	3.47 ±0.59^{b}	1.35 ±0.51^{a}	0.95 ±0.05^{a}	0.89 ±0.58^{a}	0.74 ±0.07^{a}

Values (Mean ±SD of triplicates) in rows followed by different superscripts are significantly different ($p<0.05$). Moisture, crude protein and ash are from whole fish samples
%Viscerosomatic index (VSI) = (internal organs wet weight (g)/fish body weight (g)) ×100
%Hepatosomatic index (HSI) = (liver wet weight (g)/fish body weight (g)) ×100

Table 16 Apparent digestibility coefficients (%) for dry mater (ADC_{dm}) and crude protein (ADC_{cp}) of the diets used.

Treatment	ADC_{dm}	ADC_{cp}
Trash fish (T-1)	72.6 ±1.1^{d}	86.8 ±0.5^{d}
Compound feed (T-2)	54.4 ±2.9^{c}	75.9 ±1.5^{c}
Trash fish +rice bran (T-3)	30.4 ±0.4^{b}	60.0 ±0.2^{b}
Trash fish +compound feed (T-4)	24.6 ±0.1^{a}	51.5 ±0.0^{a}
Trash fish +rice bran +compound feed (T-5)	23.5 ±0.6^{a}	49.4 ±0.9^{a}

Values (Mean ±SD of triplicates) in columns followed by different superscripts are significantly different ($p<0.05$)

6.4 Discussion

Marble goby fed the compound and combination feeds had lower final weight, daily weight gain (DWG) and survival rate than fish fed raw mackerel in the present study. This is possibly because the protein contents in the compound and combination feeds were suboptimal for this species. The quantitative dietary protein levels recommended for carnivorous fingerlings (0.5-10g) and juvenile fish (10-50g) are 49% and 47% respectively (FAO, 1987). However, the isonitrogenous diets (approximately 40% protein level) in the present study were initially formulated according to the recommended protein requirement for carnivorous species in Thailand (Van Weerd, 1995; Geoff et al. 2000). In addition a dietary protein level of 40% has been claimed to be optimal for satisfactory growth of tropical carnivorous species raised in 'clear water' systems such as snakehead *Channa striata* fingerlings (12g individual size) (Samantaray and Mohanty, 1997), African catfish *Clarias gariepinus* (Ng et al. 2001) and Asian catfish *Clarias macrocephalus* fingerlings (Evangelista et al. 2005). However, protein requirements within the same species can vary with culture environment, fish size, dietary amino acids and protein-energy profiles (Jauncey, 1982; Wilson, 2002).

Even though fish fed on mackerel trash fish (T-1) showed significantly higher growth and survival rate, they also had higher feed intake, hepatosomatic index (HSI) and viscerosomatic index (VSI). This suggests that marble goby may deposit lipid in viscera and liver as a result of higher dietary energy consumption in the mackerel trash fish. Body protein concentration generally appears to be stable, even when there are fluctuations in protein intake the highest being in fish fed T-1. As opposed to lipid, body protein content is less influenced by dietary protein levels. In accordance with this view

Kim et al (1991) failed to observe any correlation between whole body protein content in rainbow trout and dietary protein concentrations. In catfish *Mystus nemurus*, body protein increased with dietary protein level up to 42%. Beyond 42% no body protein gain was observed also, protein efficiency ratio (PER) decreased with increasing protein level (Khan et al. 1993). Moreover, Rasmussen (2001) also revealed that salmonid carcass protein variations are caused mainly by changes in proportions of water and lipid rather than actual differences in body protein content.

However, prolonged feeding with mackerel trash fish may lead marble goby to develop fatty liver and thiamin deficiency as reported in other fish (Royes B. Juli-Anne and Chapman, 2003; Santosh and Dominic, 2003).

It is evident from better fish growth, feed intake and survival data in the present study that mackerel trash fish (T-1) is more effectively utilised by marble goby than the artificial diet. This phenomenon is perhaps due to the presence of feeding-stimulants contained in the mackerel which contribute a feeding-enhancing effect accounted for in palatability to marble goby. Studies by Kohbara et al (2000) claimed that inosine-5′-monophosphate (IMP) and amino acids plus nucleotides were major stimulants in mackerel muscle extract. The supplementation of these stimulants into diets resulted in improving feed intake, growth performance and protein digestion in yellowtail *Seriola quinqueradiata* (Hidaka et al. 2000; Patricia et al. 2006).

The poor acceptability of artificial diets by marble goby has commonly been reported by farmers (personal communication during the field survey in 2003). High fish mortality associated with feeding on artificial diet was reported by Pham (2001) who claimed this was because of its poor attractiveness to marble goby. The promising survival rate (83%) of marble goby given an artificial diet in the present study indicates to some extent the level of acceptability by fish. In fact, the artificial diet used in the

present study was re-extruded and water was added to form a 30% moisture diet. It is believed typical semi-moist feeds are more palatable for marble goby similar to other carnivorous species in Thailand (Thanom, 1989; Nanthiya, 1989; Jantrarotai and Jantrarothai, 1993; Boonyaratpalin, 1997). However this claim needs more support by further experiments where proper comparisons are made.

The lower protein and dry matter digestibility, poor growth and survival rate associated with rice bran based feeds indicate that the protein was not well digested by marble goby. Similar results were found in humpback grouper *Cromileptes altivelis* (Laining et al. 2003), red drum *Sciaenops ocellatus* (Bruce and Robert, 1996)and bullfrog *Rana catesbeiana* (Secco et al. 2005). Opposite results, however, were reported for silver barb *Puntius gonionotus* (Mohanta et al. 2006)and Indian major carp *Labeo rohita* (Usmani and Jafri, 2002). Besides, the low feed intake of fish fed T-3 diet may be because the diet contained a supra-optimal amount of rice bran that limited palatability (Joachim and Felicitas, 2000).

In terms of dietary fibre content, in our case it is possible that the high fibre content of the rice bran used in the diet T-3 contributed to a reduction in protein digestibility. This is in agreement with an earlier study by Bruce and Robert (1996) who reported that protein digestibility in red drum was highest for feeds with less than 2% fibre, but beyond this level reduced protein digestibility was observed. Additionally, Tacon (1987) suggested that for carnivorous fish the cellulose cell wall within plant protein sources may render the protein present within the cell inaccessible to digestive enzymes. Under such conditions much of the protein may be unavailable for digestion.

Furthermore, the differences in fibre content between T-3 and the other diets tested (2.49 vs. 0.27-1.26 %) were not large. It is unlikely, therefore, that differences in fibre content were the primary reason for the observed differences in digestibility.

Particularly, reduced protein and dry matter digestibility in fish fed trash fish based diets appeared to be associated with reduced levels of trash fish in diets. This perhaps reflected the imbalanced ratio of animal protein to plant protein or in these diets. Studies have shown that carnivorous fish tend to utilize the dry matter and energy in animal products better than that in plant products (Wilson, 1991; Reigh and Ellis, 1992; Sullivan and Reigh, 1995; Boonyaratpalin, 1997).

Moreover, the poor growth of fish fed rice bran based diets may be attributed to deficiency of some essential amino acids as most plant-based diets are linked to an amino acid imbalance (Wilson, 2002). Unfortunately, none of the diets used in this study were subjected to amino acid analysis. Moreover, the phytic acid content in rice bran may limit utilisation of rice bran by marble goby. According to Samli et al (2006) the phytic acid concentration in rice bran is particularly high, typically 9.5% to 14.5%. Phytic acid in diets markedly reduced biological availability and impaired utilization of dietary minerals such as phosphorus, calcium, iron, zinc, copper and cobalt (Usmani and Jafri, 2002). Phytic acid also had a negative effect on protein digestibility and growth as well as other essential body functions (Hendricks, 2002; Francis et al. 2001; Francis et al. 2001). Additionally, since rice bran contains a relatively high proportion of unsaturated fatty acids, this promotes the development of rancidity substances, particularly endogenous lipase and peroxidase enzymes that were released rapidly during the milling process (Lam and Proctor, 2003). Therefore, endogenous rancidity occurring in rice bran in the present study may have resulted in poor acceptability; low feed intake and growth depression in marble goby.

Overall, a more promising growth and survival rate compared to a previous study by Tawee and Panida (Tawee and Panida, 1978) may be due to individual stocking of experimental fish in the present study which prevented cannibalism.

Cannibalistic behaviour is recognised as common in marble goby from larvae and continuing until the juvenile stage (Anusorn and Sujittra, 1989).

In conclusion based on the results of the present study;

1) Prepared diets are possible for growing of marble goby. However, further research to establish precise nutritional requirements of marble goby are required. Moreover, more studies on suitable texture and forms of diets need to be carried out.
2) The incorporation of rice bran should not exceed 25% of diet. The previous study by Anusorn and Sujittra (1989), also found that inclusion of 32% of rice bran in marine trash fish based diet for adult marble goby affected growth and survival of fish.
3) There is a need to conduct long-term studies in order to evaluate the effects of body lipid deposition on health and growth of marble goby.
4) In terms of using trash fish, information is need on nutritional composition, cost and seasonal availability of each of the species used. This will allow more efficient use of such fish in compound diets and will permit adjustment of dry components to compensate for any significant nutritional differences in particular species of trash fish used. However, it should be emphasised here that the use of fresh/wet feeds can result in deteriorating water quality. Therefore, aspects of on-farm management (feed application method and feeding regime in particular) need to be optimised to maximise feed efficiency and minimise feed wastage.

It should also be stated here that as the demand for marine fishery products for aquaculture increases, while their availability decreases, the cost is expected to rise. A

dependable supply of cost-effective, non-marine, alternative sources of protein must be provided for the marble goby farming to be sustainable.

Chapter 7 Experiments to Evaluate The Effects of Vitamin E Supplementation of Oxidised Trash Fish Based Diets on Growth Performance, Biochemical Composition and Haematology of Marble Goby (*O. marmoratus*)

7.1 Introduction

One concern with respect to using feedstuffs rich in PUFA is that they are highly prone to auto-oxidation on exposure to atmospheric oxygen (Stephan et al. 1995; Sargent et al. 2002). During the process of lipid auto-oxidation chemical degradation products are formed, including free radicals, peroxides, hydroperoxides, aldehydes and ketones, which in turn react with other dietary ingredients (vitamins, protein and other lipids) reducing their biological value and availability (Tacon, 1992). The free radicals and peroxides that are produced from lipid oxidation can also lead to the development of undesirable rancidity (Freeman and Hearnsberger, 1994) and potentially toxic reaction products (Shuze et al. 2001). Dietary lipid oxidation can cause oxidative damage to biological membranes and organelles (Chinabut, 2002). Moreover, there have been reports of adverse health effects as result of consumption of oxidised dietary lipid in trout (Cowey et al. 1984; Sage et al. 2003; Sage et al. 2003), sea bass (Stephan et al. 1993) African catfish (Baker and Davies, 1996b) and hybrid catfish (Chinabut, 2002). Dietary oxidation also depletes antioxidants in both diets and fish tissues (Tacon, 1992; Gatta et al. 2000; Gatta et al. 2000). The absence of suitable antioxidant protection in feedstuffs rich in PUFA can result in accelerating oxidation rate (Stephan et al. 1993; Chen-Hei and Sue-Lan, 2004).

Vitamin E, in the form of α-tocopherol acetate, is widely used as a natural alternative to the synthetic antioxidants such as bytylated hydroxytoluen (BHT) and butylated hydroxyanisole (BHA). It is an important antioxidant in protecting biomembranes. Its main function is to protect unsaturated lipids in living tissues against free-radical mediated oxidation. It has beneficial effects in preventing growth depression, reduced peroxidation stress (Tocher et al. 2002) and enhancing immune responses of farmed fish (Pearce et al. 2003). Supplementation of oxidised diets with DL-α-tocopherol has been shown to reduce liver lipoid degeneration (Smith, 1979)and prevent the occurrence of pathological effects of oxidised lipids in fish (Tocher et al. 2002).

According to the earlier farm survey (Chapter 4), nutritional pathologies associated with lipid oxidation i.e. the swollen pale fatty liver and an excessive visceral fat, were observed in marble goby fed oxidised dietary lipid, particular marine trash fish and rice bran based diets, for prolonged periods. The first indication was that farmers observed reduced appetite, inactive fish that were susceptible to stress and subsequently mortality rate was increased. Moreover, fish tended to die just before they got to the marketable size.

Two experiments were therefore conducted to

1) Investigate the effects of supplementation of α-tocopherol acetate in oxidised diets on growth performance, biochemical composition and haematology of marble goby (*O. marmoratus*).
2) Evaluate the use of culture-based freshwater trash fish, tilapia, as an alternative feed both as single ingredient and in combination for culturing marble goby.

7.2 Material and Methods

7.2.1 Experimental stock and facilities

The 2 experiments were carried out in situ in a cage system. The cages were set up in a rectangular earthen pond of dimension 150 ×20 ×1.8m located at the Asian institute of Technology (AIT), Pathum Thani, Thailand. The experiment was carried out from 15th December – 15th May 2005 and total culture period was 5 months.

Wild marble goby juveniles at an average size of 26±5g were used as experimental fish. Before commencing the experiment fish were acclimatised to the cage system and the prescribed feeds for one week. After this 30 fish were randomly stocked into each square cage (1 ×1 ×1.2m, 6mm mesh). The cage frame was constructed from steel painted with an epoxy resin as a water resistant substance. Cages were suspended in water by supporting them on bamboo poles. Cages were installed at 50 cm above the pond bottom to allow sufficient flow of water underneath the cage. To prevent fish escaping and predation by birds and reptiles, the cage lip was positioned 20 cm above the water surface and each cage was covered by applying a net lid. The installation distance between each cage was 1m (Figure 6). A rectangular feeding tray (30 ×45 ×5cm, 2 mm mesh) was provided in the middle of each cage.

The water level of the pond was on average 1.50m in depth. Water was added whenever the water level was lower than 1.50m. The pond was fertilised every 2 weeks using inorganic fertilizers; triple superphosphate (TSP) and urea at a rate of 7 kg P ha^{-1} and 28 kg N ha^{-1} respectively (Yi et al. 2002). Cages were cleaned by brushing every 2 weeks to clear out debris that could potentially obstruct water movement through the cage.

7.2.2 Experimental design

7.2.2.1 Experimental 1

A complete Randomised Design (CRD) which consisted of 5 treatments and 4 replicates of each treatment was applied as follows;

Treatment 1 (F-1) = Oxidised mackerel

Treatment 2 (F-2) = Oxidised tilapia

Treatment 3 (F-3) = Un-oxidised tilapia

Treatment 4 (F-4) = Oxidised mackerel + vitamin E

Treatment 5 (F-5) = Oxidised tilapia + vitamin E

7.2.2.2 Experimental 2

A complete Randomised Design (CRD) which consisted of 5 treatments and 4 replicates of each treatment was applied as follows;

Treatment 1 (D-1) = Oxidised mackerel + rice bran

Treatment 2 (D-2) = Oxidised tilapia + rice bran

Treatment 3 (D-3) = Oxidised mackerel + rice bran + vitamin E

Treatment 4 (D-4) = Oxidised tilapia + rice bran + vitamin E

Treatment 5 (D-5) = Complete diet (Juvenile catfish diet)

7.2.3 Experimental feeds

Experimental feed formulations, proximate analyses and vitamin E contents are given in Table 17 and Table 20 for experiment 1 and experiment 2, respectively. ‘‘Un-oxidized tilapia’’ was described as live tilapia with individual sizes between 50-100 g. Tilapia were raised in phytoplankton rich ponds and fed occasionally using a commercial catfish feed. Fish were freshly killed using ice. Whole fish were ground twice using a meat grinder (Hobart 4146) to ensure that fish bones were finely crushed.

Tilapia mince was then packed into a plastic bag and preserved in a freezer at -20 °C prior to use.

To prepare 'oxidised tilapia', pooled dead tilapia were left indoors at ambient temperatures to allow lipid oxidation to occur until the TBARS reached 100-150 µmols MDA g^{-1}sample. The progress of lipid oxidation in dead tilapia was monitored every hour until the value reached 100-150 µmols MDA g^{-1} sample. This level was recorded 5-7 hrs after leaving fish at ambient temperatures ranging between 28-33°C. Fish guts were removed before grinding. Fish mince was then packed into a plastic bag and stored in a freezer at -20 °C.

Mackerel were purchased from contracted fish vendors at a local market near AIT. Whole fish were ground and the samples of fish paste were collected for TBARS analysis before storage in a freezer at –20 °C. However, fish were already prone to lipid oxidation since purchase. The average TBARs value for mackerel at purchase was 250-300 µmols MDA g^{-1} (Table 17).

Vitamin E, in the form of dl-α-tocopherol acetate purchased from Sigma, was added to relevant feeds at an inclusion rate of 200 mg kg^{-1} feed. Sodium selenite (Se) (0.25 mg Se kg^{-1}feed) (Gatlin and Wilson, 1984)was also supplemented to each vitamin E based feed. A Hobart4146 food mixer with 5 stainless steel bowls was used for mixing the feed ingredients. Mixing was performed for at least 30 minutes to ensure that all ingredients were thoroughly mixed before the final feed was put into a small plastic bag according to the daily feeding ration in each cage. Food bags were then stored in a freezer at -40°C. To prevent floating of the given feeds they were de-frosted in a 4°C refrigerator overnight before feeding.

Figure 6 The layout of experimental cages installed in an ethern pond located at Asian Institute of Technology, Thailand

7.2.4 Feeding method, feeding rate and feeding frequency

Feed was formed into small balls then put into a feeding tray (30 ×45 ×5cm, 2 mm mesh) that was attached at the middle of the cage bottom. Fish were fed manually to satiation twice a day at 0830 and 1500h. Feed ration was adjusted regularly to ensure sufficient feed was provided. Any uneaten feed was collected 2 hours after the evening feeding session.

7.2.5 Water quality measurement

In situ measurement of water temperature and pH was done at dawn every two weeks. Water quality parameters; total alkalinity, NH_3, NO_2, NO_3, total N and total P were also monitored in a laboratory monthly. Water quality analyses were carried out according to APHA (APHA, 1985).

7.2.6 Growth performance, Survival rate, Carcass composition

At the beginning of the experiments, 100 fish were randomly selected for weight and length measurements. Fish weight sampling was carried out monthly. At the end of the experiments the total numbers of fish in each cage were counted and individual weight and length were measured. Six fish from each cage/replicate were sacrificed for muscle and liver tissue samples. The same fish tissues were pooled together according to treatment. The biochemical parameters were examined as follows;

1) Proximate composition of whole fish and muscle

2) Degree of lipid oxidation of fish muscle and liver (TBARS)

3) Glycogen content of fish liver and muscle

4) Vitamin E content in fish muscle, liver, blood and plasma

Analysis methods for these parameters are given in Chapter 3

7.2.7 Haematological measurement

7.2.7.1 Fish blood sampling

A total of 8 fish (80-90% of remaining fish after taking samples for the analyses above) from each treatment were taken for blood sampling except for treatment D-5 that had low survival rate so all 5 remaining fish were used. These fish were transferred live to an AIT laboratory. Subsequently fish were tranquilised using a concentration of 4% benzocaine (4g of benzocaine powder in 100 ml of methanol) (Davies, 2000). Before recovery from anaesthesia fish were sacrificed for blood collection. Blood was collected by puncture of the caudal vein and caudal severance (Houston, 1990; Svobodova and Vykusova, 1991).

Using the caudal vein method a blood sample was taken at the midline just posterior to the anal fin by inserting the needle into the musculature perpendicular to the ventral surface until the spine was reached or blood entered the syringe. Immediately, blood samples were transferred into a 1 ml labelled vial containing an anticoagulant; 0.5M EDTA (186.1 g disodium EDTA from Sigma to 800 ml of distilled water, adjusted to pH 8 using NaOH) at 0.1 mL mL^{-1}blood sample (Yale and Esnouf, 1973; Bouyai, 2004). The sample containing anti-coagulated blood was transferred to non-heparinized microhaematocrit capillary tubes for haematocrit analysis. The remaining blood was then preserved in crushed ice prior to haemoglobin analysis as described below.

In the event that the caudal vein method failed then the caudal severance method was used. Following this method, the caudal peduncle was wrapped in a towel to absorb epidermal moisture and then the tail was completely severed posterior to the

anal fin. The first few drops of blood were discarded to prevent contamination and the rest was collected using the same method detailed above.

Immediately after the fish blood sample was mixed with EDTA, the sample was then centrifuged (centrifuge haematocrit, Sigma 1-15, UK) at 10,000 rpm for 5 minutes (Houston, 1990).

7.2.7.2 Haemoglobin determination

Determination of haemoglobin concentration in the blood can indicate a disease condition in fish, including anaemia (AMC, 2006).

Fish haemoglobin was determined using the cyanmethaemoglobin method. The cyanmethaemoblobin method is the most widely used procedure in determining haemoglobin concentration in fish (AMC, 2006). The principle of this method is that when blood is mixed with a solution containing potassium ferricyanide and potassium cyanide the potassium ferricyanide oxidizes iron to form methaemoglobin. The potassium cyanide then combines with methaemoglobin to form cyanmethaemoglobin. The cyanmethaemoglobin is a stable colour pigment read photometrically at a wavelength of 540nm (Houston, 1990; Salakit, 2004).

7.2.7.3 Haematocrit /Packed Cell Volume (PCV) measurement

Haematocrit is the amount of red blood cells in a given volume of blood, also referred to as packed cell volume. The haematocrit will vary, depending on the health and physiological conditions of the individual fish (AMC, 2006). The haemtocrit is an indicator of stage of disease development in fish especially, anaemia (Yngvar and Knutalk, 1992)

The percentage of red blood cells in the blood was determined using a modified microhaematocrit method (Houston, 1990; Salakit, 2004). A capillary tube filled with blood and sealed at one end was centrifuged at high speed in a microhaematocrit centrifuge. The height of the resulting column of red blood cells was measured and calculated as a fraction of the height of the entire column of blood.

7.3 Results of experiment 1

The formulation and chemical compositions of experimental feeds are shown in Table 17

7.3.1 Fish growth performance

Individual fish final weight and daily weight gain (DWG) revealed no significant differences between treatments ($p>0.05$) except that F-5 had the lowest value ($p<0.05$) (Table 18). Fish survival rate in F-1 was significantly higher than that of F-5 and F-2 ($p<0.05$). However, there were no significant differences in fish final weight, DWG and survival rate between treatments when compared within groups of fish fed the same trash fish.

7.3.2 Visceral index and biochemical composition

Fish fed mackerel (F-1 and F-4) showed higher hepatosomatic indexes than groups of fish fed tilapia (F-2, F-3, F5). Lower values for hepatosomatic index (HSI) were observed in fish fed un-oxidised (F-3) and oxidised feeds supplied α-tocopherol (F-4 and F-5) ($p>0.05$). Moreover, adding α-tocopherol to the oxidised feeds resulted in significantly lower ($p<0.05$) values of HSI in fish fed the oxidised mackerel supplemented with α-tocopherol than of fish fed without α-tocopherol. Similarly, fish fed the oxidised tilapia with α-tocopherol gave lower values for HSI, even though they were not significantly different between treatments (Table 18).

Lipid contents in fish muscle were not significantly different between the five treatments. The lower lipid content in fish liver was found in fish fed the oxidised tilapia supplemented with α-tocopherol (F-4) ($p<0.05$). However, liver lipid content was not significantly different when compared to fish fed un-oxidised tilapia (Table 18). Supplementation of α-tocopherol to the oxidised feeds was shown to reduce the

level of TBARS of fish muscle in fish fed both oxidised tilapia and mackerel based feeds ($p<0.05$). Lower liver TBARS were found in fish fed un-oxidised tilapia (F-3) and oxidised tilapia with α-tocopherol (F-5) than in fish fed oxidised tilapia without α-tocopherol (F-2) ($p<0.05$). In contrast, TBARS appeared to be higher in fish fed oxidised mackerel without α-tocopherol (F-4) than in fish fed the same feed but with α-tocopherol added.

Table 17 Feed formulation and proximate composition of feeds of experiment 1

Components (g /100 g dry weight)	**Feed**				
	F-1	F-2	F-3	F-4	F-5
Tilapia trash fish	-	97.0	97.0	-	97.0
Indian mackerel	97.0	-	-	97.0	-
Rice bran	-	-	-	-	-
Commercial pellet	-	-	-	-	-
Mineral premix	1.0	1.0	1.0	1.0	1.0
Vitamin premix	2.0	2.0	2.0	2.0	2.0
Chemical composition (% dry matter)					
Crude protein	55.0	50.0	49.9	55.0	51.2
Crude lipids	10.9	22.1	22.1	9.6	22.6
Ash	22.1	21.9	21.9	26.25	20.73
Crude Fibre	0.4	0.30	0.3	0.4	0.4
Moisture (%)	69	70	70	69	70
Nitrogen Free Extract (NFE)	11.7	2.60	2.7	8.7	5.0
Gross Energy (kJg^{-1}diet)	17.6	19.7	19.7	17.6	19.7
Measured α-tocopherol acetate ($\mu g\ g^{-1}$sample)	43.8	59.9	45.5	258.9	268.5
Measured total vitamin E ($\mu g\ g^{-1}$sample)	65.0	65.3	53.2	270.0	275.5
Thiobarbituric acid reactive substances (TBARS) (μmols MDAmg^{-1}wet sample)	269 ±54	256 ±15	45 ±9	256 ±53	266 ±38

Note: Measured α-tocopherol acetate and total vitamin E contents in rice bran were 91.0 and 174.5 $\mu g\ g^{-1}$sample, respectively. The profiles of vitamin and mineral premix used were the same as described in the fish digestibility experiment (Chapter 6).

Table 18 Fish growth, hepatosomatic index, biochemical composition and haematological results for marble goby in experiment 1

	Feed					Feed	Feed		
	F-1	F-2	F-3	F-4	F-5	F1 × F4	F-3	F-2	F-5
Mean initial weight (g)	20.9 ±1.0	23.4 ±3.7	21.0 ±2.0	21.2 ±2.3	24.0 ±6.8	ns	21.0 ±2.0	23.4 ±3.7	24.0 ±6.8
Mean final weight (g)	90.2 ±6.3^{b}	72.9 ±2.5^{ab}	73.5 ±1.6^{ab}	78.0 ±8.4^{ab}	69.9 ±3.0^{a}	ns	73.5 ±1.6	72.9 ±2.5	69.9 ±3.0
Daily weight gain (g)	0.5 ±0.1^{b}	0.4 ±0.0^{ab}	0.4 ±0.0^{ab}	0.4 ±0.1^{ab}	0.3 ±0.1^{a}	ns	0.4 ±0.0	0.4 ±0.0	0.3 ±0.1
Survival rate (%)	75 ±6.3^{b}	49 ±7^{a}	61 ±12^{ab}	67 ±17^{ab}	51 ±8^{a}	ns	61 ±12	67 ±1.7	51 ±8
Hepatosomatic index (%HSI)	3.6 ±0.7^{b}	1.7 ±0.6^{a}	1.4 ±0.7^{a}	2.2 ±0.4^{a}	1.4 ±0.2^{a}	*	1.4 ±0.7	1.7 ±0.6	1.4 ±0.2
Lipids (% of fresh tissue)[1]									
Muscle	0.7 ±0.0	0.6 ±0.1	0.6 ±0.0	0.6 ±0.2	0.6 ±0.0	ns	0.6 ±0.0	0.6 ±0.1	0.6 ±0.0
Liver	40.9 ±2.3^{c}	32.5 ±2.5^{b}	7.5 ±0.8^{a}	46.8 ±1.4^{d}	29.5 ±3.0^{ab}	*	27.5 ±0.8^{a}	32.5 ±2.5^{b}	29.5 ±3.0^{a}
TBARS (µmols MDA mg^{-1}wet sample) [1]									
Muscle	18.9. ±1.3^{b}	19.9 ±1.2^{b}	5.0 ±07.2^{a}	10.8 ±2.2^{a}	10.2 ±2.6^{a}	*	5.0 ±07.2^{a}	19.9 ±1.2^{b}	10.2 ±2.6^{a}
Liver	419.6 ±14.7^{c}	667.8 ±25.3^{d}	173.5±22.7^{a}	599.9 ±27.5^{d}	240.4 ±30.6^{b}	*	173.5 ±22^{a}	667.8 ±25.3^{c}	240.4b ±30.6^{b}
Haematocrit (%)[2]	15.4 ±1.9^{a}	18.6 ±2.8^{b}	15.6 ±1.7^{ab}	15.0 ±2.1^{a}	18.3 ±2.3^{b}	ns	15.6 ±1.7	18.6 ±2.8	18.3 ±2.3
Haemoglobin conc. (g dl^{-1}) [2]	5.6 ±0.8	4.8 ±0.7	5.4 ±0.7	5.1 ±0.8	5.0 ±0.8	ns	5.4 ±0.7	4.8 ±0.7	5.0 ±0.8

Values are means ±SD of 4 replicates of pooled sample from 6 fish ([1]), and 8 replicates which an individual fish was represented as a replicate ([2])
Values in the same line with different superscripts are significantly different ($p<0.05$)

Table 19.1 α-tocopherol acetate concentrations in liver, muscle and whole blood from marble goby fed different feeds of experiment 1

Feed	Liver (µg g^{-1})	Muscle (µg g^{-1})	Whole blood (µg ml^{-1})
F-1	148.4 ±55.9^{a}	5.9 ±1.3^{bc}	146.9 ±0.4^{b}
F-2	143.4 ±49.3^{a}	3.1 ±0.6^{a}	146.3 ±0.8^{b}
F-3	689.2 ±16.2^{d}	8.0 ±1.0^{c}	182.1 ±0.6^{b}
F-4	565.9 ±81.3^{c}	7.2 ±2.2^{c}	73.5 ±1.7^{a}
F-5	418.5 ±58.4^{b}	3.9 ±0.6^{ab}	76.9 ±0.2^{a}

Values are means ±SD of 4 replicates; liver and muscle were pooled from 6 fish and whole blood was from individual samples of 4 fish
Values in the same column with different superscripts were significantly different ($p<0.05$)

Table 19.2 α-tocopherol acetate concentrations in liver, muscle and whole blood from marble goby fed different feeds of experiment 1 (continued)

	Oxidised mackerel feed[1]	Tilapia trash fish feed[2]		
	F-1 × F-4	F-3	F-2	F-5
Liver (µg g^{-1})	*	689.2 ±16.2^{c}	143.4 ±49.3^{a}	418.5 ±58.4^{b}
Muscle (µg g^{-1})	ns	8.0 ±1.0^{b}	3.1 ±0.6^{a}	3.9 ±0.6^{a}
Whole blood (µg ml^{-1})	*	182.1 ±0.6^{b}	146.3 ±0.8^{b}	76.9 ±0.2^{a}

Values are means ±SD of 4 replicates; liver and muscle were pooled from 6 fish and whole blood was from individual samples of 4 fish
[1]Analysis of Independent-samples t-test. * means there were significant different between 2 cases ($p<0.05$), and no significant differences was given as ns ($p>0.05$).
[2]Analysis of variance which values in the same row with different superscripts were significantly different ($p<0.05$)

7.3.3 Haematology and α-tocopherol contents

Overall there were minor differences haematocrit between fish fed the five different feeds. However, there were no significant differences in haematocrit between treatments for both the tilapia and oxidised mackerel feeds. Similarly, the haemoglobin concentrations of fish were not significantly different between treatments ($p>0.05$) (Table 18).

With respect to muscle and liver levels of α-tocopherol (Table 19.1 and Table 19.2) in all cases lower levels of α-tocopherol were associated with feeding oxidised feeds with no added α-tocopherol. However, the group of fish fed un-oxidised tilapia appeared to have the highest level of α-tocopherol deposited in muscle and liver ($p<0.05$).

7.4 Results of experiment 2

Table 20 Feed formulation and proximate compositions of diets of experiment 2

Components (g /100 g dry weight)	**Diet**				
	D-1	D-2	D-3	D-4	D-5*
Tilapia trash fish	-	68.7	-	68.7	-
Indian Mackerel	59.8	-	59.8	-	-
Rice bran	37.2	28.3	37.2	28.3	-
Commercial pellet	-	-	-	-	100.0
Mineral premix	1.0	1.0	1.0	1.0	-
Vitamin premix	2.0	2.0	2.0	2.0	-
Chemical composition (% dry matter)					
Crude protein	40.0	40.0	39.9	40.2	40
Crude lipids	12.6	20.0	12.4	20.2	6.7
Ash	17.3	17.6	15.2	17.3	11.2
Crude Fibre	2.7	2.5	3.0	2.5	2.2
Moisture (%)	47	52	46	52	40
Nitrogen Free Extract (NFE)	27.5	19.8	29.5	19.8	39.9
Gross Energy (kJg^{-1}diet)	17.8	19.2	17.8	19.2	17.9
Measured α-tocopherol acetate ($\mu g\ g^{-1}$sample)	62.4	68.0	266.0	274.5	78.1
Measured total vitamin E ($\mu g\ g^{-1}$sample)	74.6	82.1	279.7	282.5	86.6
Thiobarbituric acid reactive substances (TBARS) (μmols MDA mg^{-1}wet sample)	202 ±14	207 ±36	194 ±19	200 ±24	60 ±7

Note: Measured α-tocopherol acetate and total vitamin E contents in Rice bran were 91.0 and 174.5 $\mu g\ g^{-1}$sample, respectively. The profiles of vitamin and mineral premix used were the same as described in the fish digestibility experiment (Chapter 6)

*water was added to make moist diet

7.4.1 Fish growth performance

The mean final weight and daily weight gain of fish (DWG) given the mackerel based-diet; D-1 was significant higher than of D-3 and lowest in fish fed completed diet; D-5 ($p<0.05$). Survival rates of fish were not significantly different between fish fed the same trash fish based-diets (Table 21).

7.4.2 Viscerosomatic index and biochemical composition

TBARS of fish muscle were lower in fish fed tilapia ($p>0.05$) and mackerel ($p<0.05$) based-diets with α-tocopherol added. However, there were no significant differences in TBARS of fish liver in all treatments.

In terms of hepatosomatic index (HSI), fish fed the oxidised mackerel-based diet with α-tocopherol added had significantly lower HSI than fish without added α-tocopherol ($p<0.05$). Similarly, fish fed oxidised tilapia supplemented with α-tocopherol gave slightly lower HSI ($p>0.05$) than fish fed oxidised tilapia without tocopherol added. Similarly, relatively high values of fish liver lipid were found in fish fed the oxidised trash fish, both the tilapia and mackerel-based diets, without supplemented α-tocopherol ($p<0.05$).

Glycogen contents of fish muscle in fish were significantly higher ($p<0.05$) in fish fed diets supplied with α-tocopherol in both the oxidised mackeral and tilapia-based diets. In contrast, glycogen contents in fish liver were significanly higher in fish fed diets without α-tocopherol supplementation ($p<0.05$).

7.4.3 Haematology and α-tocopherol acetate content

The haematocrit and haemoglobin concentrations were no different in all treatments for fish fed the oxidised tilapia based diets. However, feeding fish with

oxidised mackerel-based diet with α-tocopherol supplemented resulted in higher haematocrit and haemoglobin levels than for fish fed diets without supplementation ($p<0.05$).

α-tocopherol concentrations in fish muscle, liver and whole blood are presented in Table 22.1 and Table 22.2. Regarding the same trash fish-based diet, there were no significant differences in α-tocopherol concentration of fish muscle, liver and whole blood between treatments.

Even though statistically there were no differences, there were slightly higher α-tocopherol concentrations in muscle and liver of fish fed the oxidised mackerel-based diet with α-tocopherol supplemented. In the group of fish fed the oxidised tilapia-based diet with α-tocopherol added there was a slight increase in the level of α-tocopherol in fish blood than in fish fed the diet without α-tocopherol.

7.4.4 Water quality

Results of monthly water quality measurement are summarised in Table 23. In general the water quality was within the range considered acceptable for culturing warm water species (Boyd, 1979), though low values of dissolved oxygen were observed during the morning.

Table 21 Growth, hepatosomatic index, biochemical composition and haematological results of marble goby fed different diets of experiment 2

	Diet					Diet	Diet
	D-1	D-2	D-3	D-4	D-5	D-1 × D-3	D-2 × D-4
Mean initial weight (g)	20.5 ±2.5	21.4 ±3.1	20.9 ±1.7	21.3 ±0.8	22.3 ±0.9	ns	ns
Mean final weight (g)	86.9 ±6.3^{c}	74.2 ±4.8^{b}	73.3 ±3.9^{b}	67.9 ±3.8^{b}	48.7 ±2.3^{a}	*	ns
Daily weight gain (g)	0.5 ±0.1^{c}	0.4 ±0.1^{b}	0.4 ±0.0^{b}	0.4 ±0.0^{b}	0.1 ±0.1^{a}	*	*
Survival rate (%)	84 ±13^{b}	73 ±11^{b}	63 ±13^{ab}	59 ±18^{ab}	36 ±16^{a}	ns	ns
Hepatosomatic index (%HSI)	4.65 ±0.90^{c}	1.81 ±0.31^{b}	1.68 ±0.55^{b}	1.39 ±0.31^{b}	0.7 ±0.2^{a}	*	ns
Lipids (% of fresh tissue)[1]							
Muscle	0.81 ±0.10^{b}	0.56 ±0.03^{ab}	0.53 ±0.02^{ab}	0.52 ±0.05^{ab}	0.47 ±0.01^{a}	*	ns
Liver	36.78 ±1.33^{d}	25.55 ±3.22^{c}	30.07 ±1.25^{c}	17.06 ±2.69^{b}	4.80 ±1.11^{a}	*	*
TBARS (μmols MDA mg^{-1}wet sample) [1]							
Muscle	19.5 ±4.1^{b}	15.7 ±1.9^{b}	15.4 ±3.1^{b}	2.5 ±0.3^{a}	2.2 ±0.6^{a}	*	*
Liver	168.6 ±19.3	145.4 ±6.1	160.5 ±26.1	174.1 ±23.9	138.8 ±3.6	ns	ns
Glycogen content (% wet weight) [1]							
Muscle	0.42 ±0.04^{a}	0.51 ±0.07^{a}	0.50 ±0.06^{a}	0.66 ±0.11^{b}	0.54 ±0.04^{ab}	*	*
Liver	4.84 ±1.13^{c}	3.35 ±0.59^{b}	2.98 ±0.30^{b}	1.69 ±0.28^{a}	1.72 ±0.11^{a}	*	*
Haematocrit (%)[2]	15.3 ±2.1	15.6 ±5.0	12.7 ±1.2	18.4 ±1.6	15.3 ±2.3	*	ns
Haemoglobin conc. (g dl^{-1}) [2]	5.4 ±0.8^{c}	3.5 ±0.5^{a}	3.7 ±0.8^{a}	4.5 ±1.2^{b}	3.1 ±0.6^{a}	*	ns

Values are means ±SD of 4 replicates of pooled samples from 6 fish ([1]), and 8 replicates from 8 individual fish ([2]).
Values in the same line with different superscripts were significantly different ($p<0.05$)

Table 22.1 Concentrations of α-tocopherol acetate in liver, muscle and whole blood from marble goby fed different diets of experiment 2

Diets	Liver (µg g^{-1})	Muscle (µg g^{-1})	Whole blood (µg ml^{-1})
D-1	83.1 ±33.7[a]	4.1 ±0.8[bc]	196.0 ±2.5[e]
D-2	528.9 ±4.1[d]	3.4 ±0.2[ab]	30.7 ±0.2[a]
D-3	578.5 ±29.5[d]	4.9 ±0.8[c]	90.6 ±1.4[d]
D-4	305.0 ±8.1[c]	2.8 ±0.1[a]	81.7 ±1.7[c]
D-5	180.7 ±6.5[b]	2.4 ±0.3[a]	136.5 ±1.3[b]

Values are means ±SD of 4 replicates; liver and muscle were pooled from 6 fish and whole blood was from individual samples of 4 fish.
Values in the same column with different superscripts were significantly different ($p<0.05$)

Table 22.2 Concentrations of α-tocopherol acetate in liver, muscle and whole blood from marble goby fed different diets of experiment 2 (continued)

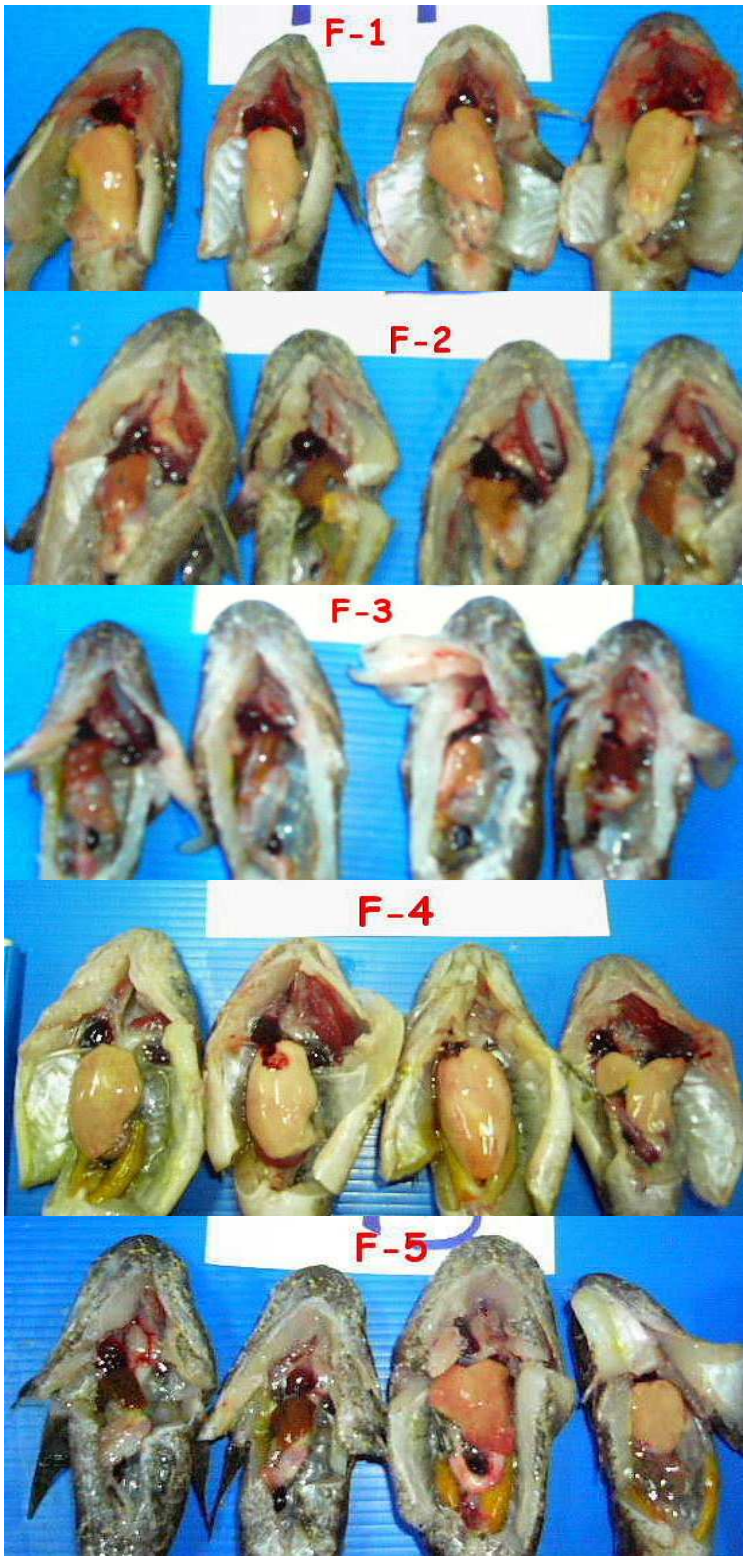
	Oxidised mackerel-based diet[1]	Oxidised tilapia-based diet[1]
	D-1 × D-3	D-2 × D-4
Liver (µg g^{-1})	*	*
Muscle (µg g^{-1})	ns	*
Whole blood (µg ml^{-1})	*	*

Values are means ±SD of 4 replicates; liver and muscle were pooled from 6 fish and whole blood was from individual samples of 4 fish. [1]Analysis of Independent-samples t-test. * means there were significant different between two cases ($p<0.05$), and no significant differences was given as ns ($p>0.05$).

Table 23 Results of monthly water quality measurement in experimental pond and cages

Parameter	Duration					
	January	February	March	April	May	June
Temperature (°C)	24.8 ±0.2	29.0 ±0.2	31.5 ±0.1	31.5 ±0.1	32.4 ±0.0	31.2 ±0.0
pH	6.9 ±0.0	7.6 ±0.1	7.44 ±0.0	6.9 ±0.0	7.43 ±0.0	7.19 ±0.1
DO (mg L^{-1})	4.0 ±0.2	2.50 ±0.1	2.40 ±0.0	3.6 ±0.0	2.50 ±0.1	2.00 ±0.0
Total alkalinity (mg L^{-1} of $CaCO_3$)	152.1 ±3.3	164.1 ±2.3	108.5 ±4.0	114.4 ±5.0	68.0 ±2.0	150.1 ±1.0
TAN (ppm.)	1.88 ±0.04	1.30 ±0.07	1.60 ±0.01	1.20 ±0.0	5.12 ±0.02	5.12 ±0.02
Nitrite (ppm.)	0.04 ±0.0	0.08 ±0.01	0.09 ±0.01	0.07 ±0.0	0.08 ±0.01	0.08 ±0.00
Nitrate (ppm.)	0.78 ±0.01	0.86 ±0.01	1.73 ±0.02	1.62 ±0.02	0.66 ±0.02	1.74 ±0.04

Values are means ±SD of triplicates. Values of the water quality parameters between cages and open water were not significantly different ($p>0.05$).



F-1 = Oxidised mackerel

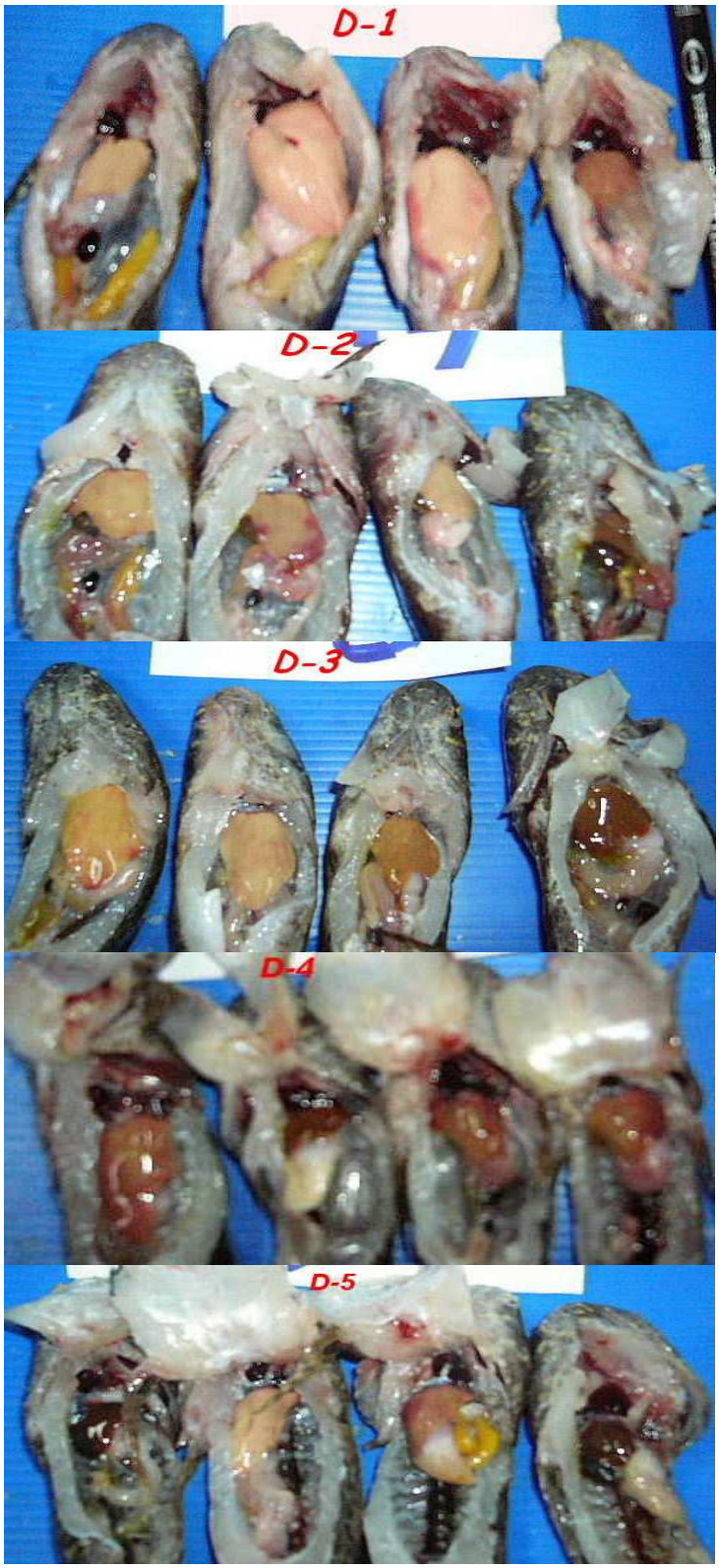
F-2 = Oxidised tilapia

F-3 = Un- oxidised tilapia

F-4 = Oxidised mackerel + vit E

F-5 = Oxidised tilapia + vit E

Figure 7 Physical characteristics of livers of fish fed different feeds for 5 months in experiment 1



D-1 = Oxidised mackerel +rice bran

D-2 = Oxidised tilapia +rice bran

D-3 = Oxidised mackerel +rice bran +vit E

D-4 = Oxidised tilapia +rice bran + vit E

D-5 = Complete diet

Figure 8 Physical characteristics of livers of fish fed different feeds for 5 months in experiment 2

7.5 Discussion

7.5.1 Effects of α-tocopherol supplementation on fish growth performance

Overall, fish in all treatments showed higher growth and survival rate than in a previous study by Tawee and Pannida (1978), who reported an average 35% survival rate and 0.01 g of DWG obtained from marble goby fed marine trash fish combined with rice bran for a culture period of 24 days.

In the present study supplementation with vitamin E of oxidised diets did not give a significant beneficial effect in reducing mortality or improving growth in marble goby. Similar results were claimed by Stephan et al (1993) who observed no differences in mortality and fish final weight in sea bass *Dicentrarchus labrax* fed either fresh or oxidised oil diets. In contrast, Tocher et al (2002) claimed that vitamin E supplementation improved survival rate in turbot *Scophthalmus maximus*, halibut *Hippoglossus hippoglossus* and gilthead sea bream *Sparus aurata* fed oxidised diets. Furthermore, the same authors also inferred that supplementation of vitamin E improved growth in sea bream fed oxidised oil but not in turbot or halibut. However, the degree of oxidation of diets used in their experiment (TBARS of 6.2 µmol mg^{-1} dry mass) was far lower than that of the present study (TBARS of 250 and 200 µmol mg^{-1} wet weight in experiment 1 and 2 respectively). However, HSI appeared to be influenced by supplementation with vitamin E. Lower HSI were observed in fish fed oxidised diets with α-tocopherol supplemented than in fish fed oxidised diets without adding α-tocopherol in both experiments. This finding was in agreement with previous results from Baker and Davies (Baker and Davies, 1996b) and Tocher et al (Tocher et al. 2002).

Although actual amount of feed intake was not measured in the present study, slightly higher growth in fish fed oxidised mackerel and higher accumulated liver TBARS for oxidised feeds with no α-tocopherol supplemented in experiment 1 were observed. Besides, growth was not different between fish fed oxidised tilapia and fish fed un-oxidised tilapia. These results indicate that marble goby did not seem to discriminate against oxidised diets in terms of feed intake. Additionally, over the given culture period of 74 days juvenile marble goby seems to tolerate quite high levels of oxidation products since the experimental oxidised diet had a high TBARS content of approximately 250 µmol mg^{-1}.

7.5.2 Effects of α-tocopherol supplementation on fish liver and muscle α-tocopherol and TBARS levels

Significantly higher accumulation of liver α-tocopherol in fish fed diets supplemented with α-tocopherol was observed in this species. This is similar to most fish species as liver is the main site of deposition of supplemented dietary vitamin E (Stephan et al. 1995; Hamre and Lie, 1997; Tocher et al. 2002; Hamre and Lie, 1997; Tocher et al. 2002) and is a sensitive indicator of the antioxidant status in fish (Baker and Davies, 1996a; Tocher et al. 2003; Tocher et al. 2003).

Although no α-tocopherol was added to the un-oxidised tilapia feed (F-3), fish maintained on this feed appeared to have remarkably the lowest muscle and liver TBARS whereas significantly higher muscle and liver α-tocopherol concentrations were also observed. This possibly suggests that the inclusion of vitamin E might not be necessary in a situation where the practically fresh/un-oxidised tilapia is being used for feeding juvenile marble goby. In other words a dietary α-tocopherol concentration of 45.5 µg g^{-1} is a safe level to maintain health and growth of fish. This correlates well

with findings by Hung et al (1981) who observed no deficiency signs in rainbow trout fed a practical diet containing fresh herring oil without supplementation of synthetic vitamin E. The same author suggested that no vitamin E or antioxidants supplementation was needed to prevent a deficiency of vitamin E in experimental fish. However, it should be noted that the fresh tilapia used in this present study had a measured TBARS of 45 µmol MDA mg^{-1}. This was presumably caused during feed preparation. Feed was prepared under tropical conditions where lipid might become rapidly oxidized even though it was immediately preserved under refrigeration. However, where a cold room is not available freshly preparing just before feeding to fish could be an alternative feeding method for farmers.

Interestingly, supplementation of α-tocopherol to oxidised diets resulted in increasing fish muscle and liver α-tocopherol contents for both fish fed mackerel and tilapia as single feed. However, when rice bran was combined supplementation with α-tocopherol to the mackerel based diet resulted in an increase in tissue α-tocopherol levels but the tilapia based diet did not. This variation in α-tocopherol response appeared to be associated with increased dietary n-6 PUFA from rice bran (the ratio of n-6:n-3 of 31:2 of % total lipid) and also from tilapia. Although analysis of fatty acid profile was excluded in the present study, Karapanagiotidis (2000; 2004) had shown that cultured tilapia grown in freshwater under practical systems and conditions in Thailand had n-6 levels of 45-70 % of total PUFA of their fillets. These findings are similar to those in African catfish that the rate of muscle α-tocopherol accumulation was affected by increased dietary n-6 PUFA in partial replacement of dietary cod-liver oil by palm oil and its by-products (Ng et al. 2003). Similarly, rat liver α-tocopherol concentration dramatically decreased as the n-6/n-3 decreased in the diet (Chuantan et al. 1990). However these studies were done on fresh lipid in basic form.

In the present study the reduction of liver and muscle α-tocopherol in fish fed combined diet with α-tocopherol supplemented it is difficult to interpret whether oxidised oil leads to a decreased rate of liver and muscle α-tocopherol replenishment or if oxidation products are rapidly taken into other body tissues such as spleen (Baker, 1997), intestinal lumen (Izaki et al. 1984) and heart (Chuantan et al. 1990). These tissues initiate lipid oxidation and thereby induce increased consumption of vitamin E *in vivo*. In fact blood α-tocopherol showed an opposite trend to tissues and was highest in fish fed fresh tilapia. This may caused a lesser amount of α-tocopherol returned to the liver. More research is required on modulation of tissue α-tocopherol and mechanisms responsible for transferring α-tocopherol to those tissues of marble goby in response to oxidised oils and vitamin E supplement.

In terms of TBARS, in experiment 1, lower liver TBARS appeared to be influenced by supplementation of α- tocopherol. However, in fish fed mackerel with α-tocopherol supplement, higher liver TBARS was observed whereas their liver α-tocopherol were also high. This indicates the nature of dietary lipid has significant influence on liver TBARS in marble goby especially a higher quantity n-3 PUFA in the diet. The higher n-3 PUFA in mackerel diets could potentially cause higher liver TBARS from *in vivo* lipid peroxidation. According to Stephan et al (1995) who claimed that muscle and liver TBARS were significantly higher in turbot fed diets containing cod liver oil than fish fed diets containing peanut oil whereas higher muscle TBARS were not accompanied by lower muscle α-tocopherol content. Moreover, higher TBARS were found in Atlantic salmon fed a diet rich in n-3 HUFA fish oil than in fish fed diets formulated with fish oils that were less rich in n-3 fatty acids (Menoyo et al. 2002).

In experiment 2, liver TBARS were consistent for all treatments whereas muscle TBARS were significantly lower in fish fed diets supplemented with α-tocopherol. This was probably due to the fact that rice bran may contribute some biologically active forms of vitamin E to the diets that induce synergistic effects on potentially reducing TBARS. Rice bran is a naturally rich in E vitamers and provides a relatively high level of tocotrienols (an analog of tocopherol) when compared with other vegetable oils (Shin et al. 1997). A study by Kim (2005) showed that the E vitamer fraction (α-tocopherol, α-tocotrienol, γ-tocopherol and γ-tocotrienol) extracted from rice bran was most effective in inhibiting the autooxidation of cholesterol and emulsified linoleic acid compared to all rac-α-tocopherol at the same concentrations. Extracts of milled-rice co-products showed potential in inhibiting lipid oxidation in beef and thus prolonged storage stability (Shih and Daigle, 2003). In addition, the protective ability of tocotrienols distributed from palm oil was reported to be significantly higher compared to α-tocopherol as effective inhibitors of lipid peroxidation in rat liver microsomes with γ-tocotrienol being the most effective (Kamat et al. 1997). A similar conclusion was reached by Ng et al (2004) who claimed that muscle TBARS decreased as the proportion of dietary tocotrienols increased in African catfish fed palm oil. Furthermore, muscle TBARS responded more favourably to dietary α-tocopherol supplementation than liver. In addition increased TBARS in response to feeding oxidised diets were generally more pronounced in liver than in fish muscle. These remarks lead us to believe that lipid may be more protected from peroxidation in marble goby muscle than in liver.

7.5.3 Effects of α- tocopherol supplementation on fish haematocrit and haemoglobin

The haematological responses of fish in both experiments resulted in haematocrit and haemoglobin values that fell within the normal ranges for healthy fish as reported in other species; African snakehead *Parachanna obscura* (Kori-Siakpere et al. 2005), African bony tongue fish *Heterotis nitoticus* (Fagbenro et al. 2000) and *O. niloticus* (Nilza et al. 2003). They were, however, lower than values recorded in carp (Svobodova and Vykusova, 1991) and hybrid catfish (*H. longifilis x C. gariepinus*) (Osuigwe et al. 2005). In fact, the normal ranges in individual species can be varied as they are influenced by nutritional status, stress, season, growth stage, sex and genetic variation (Clarks et al. 1979; Fagbenro et al. 2000; Jawad et al. 2004; Kumari et al. 2006).

Haematocrit and haemoglobin values were not significantly influenced by supplementation with α- tocopherol in both experiments. This response is similar to a study by Leah and Santosh (2007) who reported that supplementation of vitamin E to oxidised diets had no significant effect on haematocrit in Atlantic halibut. However, a reduction in haematocrit and haemoglobin values was associated with increasing tissue TBARS. This haematological change may originate from a decrease in the protective power that vitamin E has against peroxidation of cell membrane phospholipids induced by the presence of oxidised oil in the diets (Smith, 1979; Hung et al. 1981; Cowey et al. 1984; Messager et al. 1992; Hung et al. 1981; Cowey et al. 1984; Messager et al. 1992). Moreover, it is well documented that the lipid peroxidation can cause oxidative stress in fish (Leah and Santosh, 2007). Despite the fact that external pathological symptoms were not observed in the present study, decreased haematocrit and haemoglobin levels

suggest a mechanism developed in response to oxidative stress. This was especially apparent in fish fed oxidised oil regardless of dietary antioxidant status.

7.6 Conclusion

This study therefore highlights the fact that no deficiency signs were observed in the present study in fish based diets with or without α- tocopherol supplements. In addition there was no significant beneficial effect of supplementing with vitamin E on either fish growth or mortality in the present study. However, with prolonged feeding using oxidised diets, the serious deleterious effects on health and well-being of fish would perhaps become evident. According to Tocher et al (2002; 2003), duration of feeding was an important additional factor to consider in determining the biochemical responses of gilthead sea bream to oxidative stress. Additionally, Mourent et al (2002) claimed that the activities of liver antioxidant defence enzymes were significantly affected by time of the exposure to the extremely oxidised oils whereas the activities of radical scavenging enzymes were improved by supplementation with dietary vitamin E (Tocher et al. 2002).

Based on results from the present study, when fish were fed the fresh diet (fresh tilapia), the amount of α- tocopherol naturally contained in diet (45.5 $\mu g\ g^{-1}$) appeared to be sufficient to reduce fish tissue peroxidation. However, when fish were fed the oxidised diets containing a similar amount of measured α- tocopherol, fish tissues showed more susceptibility to peroxidation. Similarly, when oxidised diets contained higher levels of n-3 PUFA, dietary α- tocopherol supplemented up to the level of 200 mg kg^{-1}diet appeared insufficient to prevent increases in TBARS. Therefore, the actual benefits of supplementing vitamin E to oxidised diets in terms of fish health needs more

experimental research. Further study is also required on *in vivo* defence mechanisms of marble goby in response to higher degrees of peroxidation.

Moreover, inclusion of rice bran with trash fish showed reduced tissue peroxidation, especially of fish muscle. However, in terms of health, fish fed mackerel combined with rice bran showed adverse effects in the context of fish haematological changes. Incorporation of vegetable oil may induce cardiovascular lesions in salmon and depression of immune functions in turbot (Bell et al. 1991; Stephan et al. 1995; Stephan et al. 1995). Therefore, substitution of dietary n-3 with n-6 fatty acids may lead to α- tocopherol decrease in certain vital tissues as partially evident in the present study. The latter can potentially cause adverse effects on fish growth and health later on. Therefore, many additional criteria remain to be evaluated on the effects of n-3 replacement with n-6 from vegetable oil on fish growth, health and also the quality of the final product for human consumption.

Chapter 8- Conclusions and Future Perspectives

8.1 Farm-based feeds for marble goby; current practices and constraints

Farmer-to-farmer contracts coupled with experiences from learning by doing have been the key means of gaining fish culturing skills among fish farmers in Thailand. Seed supply is still largely sourced from wild and the quantity of seed collected has been unpredictable which has made production planning difficult. At the same time, the quality of seed varies depending on the skills of collectors in seed collecting, conditioning and transporting. The number of cage farmers using marine trash fish has declined since a heavy disease outbreak in the year 2000 (Prasankok et al. 2002; Edwards et al. 2004) (Edwards et al. 2004)and because of a steady decline in marine fisheries resources (Pakjuta et al. 2005). Meanwhile cage culture feeding employing freshwater by-catch has increased.

Feeds and feeding strategies for marble goby varied little across the regions surveyed. Both marine and freshwater trash fish were majority ingredients in farm based feeds for marble goby both as a single and combination moist feed with rice bran whilst commercial feed is not yet available for marble goby. Nevertheless, the use of trash fish is species and area-specific. In eastern and central Thailand where local trash fish is available at nearby ports and when delivery of trash fish from cold storage room to culture site is facilitated, the use of marine trash fish still exists. In other areas, especially where cage culture is practiced, the on-site bycatch trash fish is used exclusively. It should be noted here that the demand for bycatch trash fish may increase

fishing pressure on over-exploited wild stocks and also restrict future expansion of marble goby aquaculture.

In terms of nutrition, fish farmers lack knowledge on fish nutrition management and ways of improving the quality of farm-made feeds. This lack of knowledge resulted in fish lipid-related nutritional pathology, high mortality and limited production. It may well be possible that the problems farmers face could be overcome by making better nutritional information available to them. Unfortunately, this is not currently possible since nutritional information for marble goby is almost completely lacking. In addition, there has as yet been no alternative feed to convince farmers to withdraw from using trash fish. Hence, using trash fish, especially bycatch, will continue to be a major feed source for marble goby for the foreseeable future.

To reduce the threat to the fisheries in which trash fish are caught, nutritional research to develop improved feeds that match the needs of marble goby whilst minimising the use of bycatch, and encouraging more environmentally friendly practices is imperative.

8.2 Information on lipid nutrition of marble goby gained from analysis of fish fed different farm–made feeds

1. This study suggests that marble goby is classified as a lean fish with less than 2% of lipid contained in fillet found in all groups of fish.

2. The higher HSI, VSI values and liver lipid levels indicate liver is a primary storage site for lipid in marble goby. Most of the cultured fish in this study had a range of total liver lipid contents of 36-38%. The liver is an important organ

controlling lipid metabolism in animals including fish (Sargent et al. 2002). Under normal nutritional and environmental conditions, lipid-rich livers are not common in freshwater fish (Henderson and Tocher, 1987). Additionally, higher liver lipid oxidation (TBARS) levels were observed in fish fed marine trash fish that contained higher TBARS. These circumstances may indicate that the nutritional lipid quality of farm-based feeds needs to be improved since these adverse alterations in liver may increase susceptibility to stress and infectious disease which has been one of the primary culture problems for marble goby.

3. The significantly higher levels of muscle SFA than MUFA (2-3 times) in all fish groups indicates marble goby are unlikely to use SFA as an energy source efficiently when compared to MUFA. This finding suggests further diet formulation modification to consider using a lipid source that has more MUFA than SFA in order to maintain a better energy balance in marble goby.

4. The pattern of lipid deposition; the significantly higher ratios of liver neutral lipids to polar lipids (NL/PL) (in which triacylglycerol (TAG) is a majority lipid in NL) than of muscle of cultured fish perhaps limits feeding this fish with a high-energy diet- a point nutritionists should take into account.

5. Fatty acid composition of marble goby was influenced by dietary lipid inputs. In all groups of cultured fish the ratio of n-6/n-3 of fish muscle reflected the ratio of their diets. Marble goby muscle was characterised by higher proportions of n-6 PUFA, especially arachidonic acid (20:4n-6) and docosapentaenoic acid (22:5n-6), supporting the contention that n-6 fatty acids are essential for marble goby as a freshwater fish. The lower levels of arachidonic acid (20:4n-6) in farmed fish diets and the higher conversion of linolenic acid (18:2n-6) to

arachidonic acid in fish flesh than in their diets indicate that marble goby probably has an innate ability to convert 18:2n-6 to 20:4n-6 as almost all freshwater fish do. This ability might give an advantage for future diet development on the use of oils from vegetable sources to replace oils from marine resources. (This would however need more research before actual benefit can be claimed).

6. Even though feeding this fish a diet containing large amounts of EPA and DHA could remarkably benefit in increasing the contents of these fatty acids in the fish muscle, there has not been enough evidence to prove whether marble goby has the ability to bioconvert linolenic acid to the long chain derivatives; EPA and DHA. This ability needs more research to explore and such an exploration must consider not only nutritional values to the consumer but also health and welfare the fish themselves.

However, the present study is considered by the author to be a first attempt to provide some useful information on the lipid nutrition of this species under captive conditions, and therefore additional samples of fish from different seasons and developmental states should also be analysed in the future.

8.3 Fish composition, nutritional status, feed intake and digestibility of practical diets: a comparison of combinations of rice bran and trash fish

The results from this study showed that fish fed a single mackerel trash fish diet gave significantly higher growth and survival rates than any combination diets ($p<0.05$). It

should be noted here that even through feeding fish with single mackerel trash fish could satisfy fish growth and survival, significantly higher HSI and VSI ($p<0.05$) values were also observed. This causes concern regarding fish health since prolonged feeding with mackerel trash fish may lead to marble goby developing nutritional pathologies; a pale lipid-rich liver as observed in fish fed the same diet made on-farm as described in Chapter 4 and Chapter 5.

The lower protein and dry matter digestibility and poor growth associated with rice bran based feeds indicate an imbalanced ratio of animal protein to plant protein in the diet. This could contribute to deficiency of essential amino acids in diets (Wilson, 2002). Moreover, the lower feed intake of fish fed rice bran based diets could be because the diet contained a supra-optimal amount of rice bran that limited palatability (Joachim and Felicitas, 2000).

Based on growth and digestibility results the present study suggests that the incorporation of rice bran for marble goby should not exceed 25% of a practical diet. However, the optimal amount utilized by marble goby is not yet known and requires further research.

Even the growth of fish fed prepared semi-moist diets was significantly lower than fish fed raw trash fish alone, the prepared diet was accepted by marble goby to some extent and gave higher survival rates than in a previous study reported by Tawee and Panida (Tawee and Panida, 1978). Moreover, the findings of experiments in Chapter 7 suggest that inclusion of rice bran in trash fish resulted in decreased tissue peroxidation, especially in fish muscle. Thus, feeding a complete diet for marble goby could be

developed as an opportunity feed for farmers, especially when it is nutritionally balanced and feed acceptability by fish is met.

Moreover, inclusion of rice bran in marble goby feed would not only make feed more economical, but would also be more environmentally friendly compared to feeding raw trash fish alone. Therefore, strategies to maximise the use of rice bran as feed ingredient compromised with fish growth and health aspects should be beneficial to fish farmers in tropical rice-growing countries.

8.4 Farm-made feeds and their improvement through using supplemental vitamin E its effects on fish growth, survival and fish quality

The present study demonstrates that supplementation of α-tocopherol to all oxidised diets, both mackerel and tilapia based, did not show a significant beneficial effect in reducing mortality and improving growth in marble goby. The haematocrit and haemoglobin values were not significantly influenced by supplementation with α-tocopherol in both experiments with the exception of fish fed combination mackerel based diets. A significant reduction in haemotocrit and haemoglobin of fish fed this diet supplemented with α-tocopherol was observed ($p<0.05$).

Supplementation of α- tocopherol in diet did not help in reducing liver TBARS of fish fed either combination mackerel or tilapia based diets. The supplementation of α-tocopherol in both single tilapia and mackerel based diets showed a positive effect in reducing fish liver TBARS.

In terms of muscle supplementation with α- tocopherol helped in reducing fish muscle TBARS in both experiments. However, decreased fish muscle TBARS ($p<0.05$) appeared not to be related to muscle α- tocopherol levels ($p>0.05$). In fact, decreased muscle TBARS and α- tocopherol levels ($p<0.05$) were observed in fish fed combination tilapia with and α- tocopherol supplemented diet.

The findings of experiment 1 suggest that in diets with higher quantities of n-3 PUFA present, dietary α- tocopherol supplemented up to the level of 200 mg kg^{-1} diet appeared insufficient to retard the progress of development of tissue TBARS. Another meaning is that when feeding an n-3 rich diet containing lipid peroxidation up to 250-300 μmols MDA g^{-1}, use of vitamin E appeared not to help in reducing tissue peroxidation, especially in lipid-rich tissue.

The results of experiment 2 suggest that rice bran may contribute some biologically active antioxidant vitamin E isoforms to diets that help in reducing muscle TBARS. However, the increased n-6 fatty acids in tilapia based diet with α-tocopherol supplement led to the reduction of fish liver and muscle α-tocopherol levels in the present study.

High dietary lipid peroxidation could depress biological functions of synthesized α-tocopherol in immunological protection in marble goby. Therefore the use of marine trash fish in practical diets for marble goby, preventing the occurrence of lipid peroxidation, would be a more worthwhile practice than supplementation of α-tocopherol into diets when in vivo oxidation has already occurred.

8.5 Using culture-based tilapia as an alternative feed for marble goby

It should be noted here that the tilapia and mackerel are different in terms of being marine and freshwater lipid sources. The original peroxidation value of oxidised tilapia (100-150 µmols MDA g^{-1}) was also lower than that of mackerel (250-300 µmols MDA g^{-1}) at the start of the experiments. Therefore any comparison made in the present study has taken these differences into account.

Fish fed fresh tilapia contained intrinsic α- tocopherol appeared to be sufficient to reduce fish tissues peroxidation. However when fish fed the oxidised tilapia contained a similar amount of measured α- tocopherol, fish tissues in the latter group of fish showed more susceptibility to peroxidation. These results suggest an advantage of using fresh tilapia in practical diets, especially where farming bait tilapia is practiced and a cold room is not available, freshly preparing just before feeding to fish could be an alternative feeding method for farmers.

Supplementation of α- tocopherol to tilapia based diets in both experiments did not give statistically different growth or survival rate. In fact, positive responses of fish tissue TBARS to tissue α- tocopherol levels were observed in fish fed single oxidised tilapia.
In combination diets the lower TBARS and higher haematological values appeared to be the result of synergic effects on antioxidant activities between α- tocopherol supplement and natural E vitamer contained in rice bran. Thus, the addition of E vitamer from rice bran in marble goby diet may be an alternative to replace/reduce the use of synthetic α-tocopherol in which more work is required.

The experimental results presented above suggest the potential use of tilapia as alternative animal ingredient for growing marble goby. However, the socio-economic and environmental costs reflected in using tilapia must also be closely examined before actual benefits of using tilapia can be claimed. Particularly, before any recommendations has been made to farmers.

8.6 Future perspectives

Several specific areas that require further research are mentioned in each of the chapter conclusions above. Below are indicated some remaining points that should also be considered for future work:

1. Growth rate of marble goby in all growth experiments appeared relatively low. One reason may be due to the genetic variation within the stocking since they were collected from the wild. Thus, the use of hatchery-based seed should be investigated for further research. Moreover, the longer weaning period to manufactured diets and the extent of the culture period should also be taken into account.

2. Inclusion of rice bran in marble goby diet would certainly make feed more economical for fish farmers in rice-growing countries. However, excessive use of rice bran appeared to cause adverse effects on fish growth. Besides having high biological values rice bran also contains anti-nutritional factors; phytic acid for instance which can be harmful to fish. Thus, further research on improving quality and quantity of rice bran for marble goby is required.

3. The present study explored some fish nutritional lipid problems as reflected from consuming practical diets. Further studies may be required to elucidate the interactions between protein/lipid level and the oil source on nutrients and fatty acid digestibility and also the sparing effects on fish growth and health.

4. To promote the use of on-farm feed by farmers and to increase fish production, further studies on nutrient requirement by marble goby are needed in which information can be use in developing and improving of on-farm feed formulation.

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