Evaluation of Locally Available Feed Resources for Striped Catfish (*Pangasianodon hypophthalmus*)

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Abstract

This thesis investigated and compared inputs and outputs, economic factors and current feed use in small-scale farming systems producing striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta. The nutrient content of locally available natural feed resources for striped catfish was determined and growth performance, feed utilisation and body indices were analysed in pond-cultured striped catfish fed diets where fish meal protein was replaced with protein from local feed resources.

A survey showed that around 15 feed ingredients are used in striped catfish pond culture in the region. The combination of feed ingredients used in farm-made feeds varied among fish farms. The cost of producing 1 kg of fish using farm-made feeds was usually 8-10% lower than that of using commercial feeds. Digestibility trials on selected potential feedstuffs showed that the apparent digestibility (AD) of DM, CP, OM and energy was highest in soybean meal, groundnut cake, broken rice, shrimp head meal, golden apple snail and catfish by-product meal and earthworm meal, whilst the digestibility was in lower cassava leaf meal and sweet potato leaf meal. The average digestibility of most essential amino acids (EAA) in selected feed ingredients was high (range 70-92%), indicating high protein quality of these feedstuffs. In general, the AD of individual EAA was high for all diets except those with cassava leaf meal, rice bran and earthworm meal, where the AD of EAA was reduced. Two different growth experiments with the same diet (20-100% replacement of fish meal) were performed in an indoor and an outdoor culture system. A significant finding was that daily weight gain (DWG) was much higher (3.2- to 6-fold) in outdoor culture conditions compared with indoor. Feed conversion rate and feed utilisation were also 0.2-0.7 units (kg feed DM/kg weight gain) higher in the outdoor system. The results suggest that fish meal protein in feed for striped catfish fingerlings can be replaced with protein from locally available plant and animal ingredients without compromising growth performance, feed utilisation or carcass traits.

Keywords: striped catfish, local feed resources, dietary components, amino acids digestibility, alternative protein, growth performance.

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Dedication

To my family with my respectful gratitude,
My wife Thái Huỳnh Phương Lan,
My son Chau Thái Sơn, and
My son Chau Thái Bảo.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Da, C.T., Hung, L.T., Berg, H., Lindberg, J.E. and Lundh, T. (2011). Evaluation of potential feed sources, and technical and economic considerations of small-scale commercial striped catfish (*Pangasianodon hypophthalmus*) pond farming systems in the Mekong Delta of Vietnam. *Aquaculture Research* (doi:10.1111/j.1365-2109.2011.03048.x), 1–13

II Da, C.T., Lindberg, J.E. and Lundh, T. (2012). Digestibility of dietary components and amino acids in plant protein feed ingredients in striped catfish (*Pangasianodon hypophthalmus*) fingerlings. *Aquaculture Nutrition* (doi:10111/anu.12011), 1–10.

III Da, C.T., Lundh, T. and Lindberg, J.E. (2012). Digestibility of dietary components and amino acids in animal and plant protein feed ingredients in striped catfish (*Pangasianodon hypophthalmus*) fingerlings (Submitted to *Aquaculture Nutrition*).

IV Da, C.T., Lundh, T. and Lindberg, J.E. (2012). Evaluation of local feed resources as alternatives to fish meal in terms of growth performance, feed utilisation and biological indices of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. *Aquaculture* 364–365, 150–156.

V Da, C.T., Lundh, T., Berg H., and Lindberg, J.E. (2012). Growth performance, feed utilization and biological indices of pond-cultured striped catfish (*Pangasianodon hypophthalmus*) fed diets based on locally available feed resources (manuscript).

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Abbreviations

AD	Apparent digestibility
ADC	Apparent digestibility coefficient
AIA	Acid insoluble ash
BOD	Biochemical oxygen demand
BR	Broken rice
BW	Body weight
CF	Crude fibre
CFPM	Catfish by-product meal
CMC	Carboxymethyl cellulose
COD	Chemical oxygen demand
CP	Crude protein
CSLM	Cassava leaf meal
DM	Dry matter
DO	Dissolved oxygen
DWG	Daily weight gain
EAA	Essential amino acids
EE	Ether extract
EFA	Essential fatty acid
EWM	Earthworm meal
FAs	Fatty acids
FCR	Food conversion rate
FeM	Feather meal
FI	Feed intake (total) per fish
GAPS	Golden apple snail
GE	Gross energy
GNC	Groundnut cake
HCN	Hydrogen cyanide
HSI	Hepato-somatic index

IPF	Intra-peritoneal fat index
KI	Kidney index
N	Nitrogen
NDF	Neutral detergent fibre
OM	Organic matter
P	Phosphorus
PBM	Poultry by-product
PER	Protein efficiency ratio
PI	Protein intake
RB	Rice bran
SBM	Soybean meal
SFAs	Saturated fatty acids
SGR	Specific growth rate
SPLM	Sweet potato leaf meal
SR	Survival ratio
TAG	Triacylglycerols
TAN	Total ammonia nitrogen
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
VSI	Viscera somatic weight index
WG	Weight gain

1 Introduction

Diets for most farmed carnivorous and omnivorous fish, marine finfish and crustaceans are still largely based on fish meal from marine resources, especially low-value pelagic fish species. Fish meal is the major dietary protein source for aquafeeds, commonly making up between 20-60% of fish diets (FAO, 2012; Glencross *et al.*, 2007; Watanabe, 2002). It has been estimated that in 2008, the aquaculture sector used 60.8-71.0% of world fish meal production (FAO, 2012; Lim *et al.*, 2008; Tacon & Metian, 2008). Dietary protein is the major and most expensive component of formulated aquafeeds (Wilson, 2002) and feed costs have tended to increase with the rising price of fish meal. Thus, the cost of aquafeeds increased by 73% from 2005 to 2008 (FAO, 2012). Therefore, in order to reduce feed costs and the use of fish meal in aquafeeds, more extensive use of alternative feed ingredients is needed (Burr *et al.*, 2012; Hardy, 2010; Lim *et al.*, 2008; Glencross *et al.*, 2007).

Freshwater striped catfish (*Pangasianodon hypophthalmus*) is a Pangasiid species of high economic value for fish farming in South-East Asia (Hung *et al.*, 2004). This fish species has become an iconic success story of aquaculture production in Vietnam and has evolved into a global product (Silva & Phuong, 2011; Phuong & Oanh, 2010). Glencross *et al.* (2011) reported that improvement of the nutrition and feed management of the expanding local striped catfish industry in Vietnam has been identified as a key priority to improve production efficiency. Although soybean meal has been used in striped catfish feed as a replacement for fish meal, trash fish (marine origin) and fish meal are still the main dietary protein sources for striped catfish, comprising 20-60% of the feed (Da *et al.*, 2011; Phumee *et al.*, 2009; Hung *et al.*, 2007). However, using fish meal is not a sustainable long-term feeding strategy (FAO, 2010; Naylor *et al.*, 2009), and it will lead to the decline of some trash fish species and even to extinction (Edwards *et al.*, 2004). As the aquaculture industry is projected to continue expanding, fish meal must be used more strategically as the required aquafeed production volumes increase

(Güroy *et al.*, 2012). This will be a major challenge for thousands of small-scale striped catfish producers, as the feed is a major component of the total production costs and many fish farmers still rely heavily on trash fish and fish meal (Tacon & Metian, 2008). Increased use of cheap, locally available feed resources and more sustainable protein sources is considered a high priority in aquafeed industry and could provide a way to reduce the total production costs (Hardy, 2010; Edwards & Allan, 2004). Thus, development of feeding systems based on locally available feed resources for small-scale striped catfish farming in the Mekong Delta of Vietnam would be a way to improve the profitability of the industry and make the production more sustainable.

2 Objectives of the thesis

The overall aim of this thesis was to investigate the current feed use in small-scale farming systems for striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta in Vietnam, and to evaluate the potential of alternative locally available feed resources to replace trash fish and fish meal in striped catfish feed.

2.1 The specific aims

- To investigate and compare the detailed inputs and outputs of small-scale commercial striped catfish pond culture systems and to evaluate alternative feed formulations and feed ingredients.
- To provide baseline data on the nutrient contents of available natural feed resources that can be used to replace or reduce the use of trash fish or fish meal to a minimum.
- To assess technical and economic factors and feed usage aspects, and assess the availability of natural feed resources and their nutrient contents.
- To evaluate the potential nutritive value of some locally available plant and animal protein feed ingredients that have the potential to be used as feed ingredients in striped catfish feed.
- To evaluate the growth performance, feed utilisation and carcass traits of striped catfish fed diets in which fish meal protein has been replaced with protein from local feed resources.

2.2 Hypotheses examined in the thesis

- The nutrient content of available natural feed resources that can potentially be used to replace conventional protein sources in striped catfish feed varies considerably.

- The digestibility of nutrients in available natural feed resources that can potentially be used to replace conventional protein sources in striped catfish feed varies considerably.
- Growth performance of striped catfish is not negatively affected by partly or totally replacing trash fish or fish meal protein with protein from locally available protein and animal feed ingredients.

3 Background

3.1 The role of striped catfish farming systems in Vietnam

Freshwater striped catfish is primarily cultivated for household consumption and as a means of supplementary income in Vietnam (De Silva & Phuong, 2011). Commercial catfish production began to grow from 2000, since artificial mass seed production commenced and developed (Tuan *et al.*, 2003). Rapid growth of this aquaculture industry took place after 2002-2004, and reached a plateau between 2008 and 2010. The growth in striped catfish production relates to the change in production systems, particularly the rapid expansion of the predominant pond culture system (De Silva & Phuong, 2011).

During recent decades, the area of catfish farming has increased about 8- to 10-fold, whilst production has increased about 55-fold. Eighteen processing plants have been established, the production of catfish fillets has increased 60-fold and those products have been exported to over 136 countries and territories. In 2010, catfish production was estimated to be more than one million tonnes (Fisheries Directorate, 2010). It has triggered the development of a processing sector providing over 180,000 jobs, mostly for rural women, and many more in other associated service sectors (Phuong & Oanh, 2010). This fish species will continue to be the key species in Vietnamese aquaculture, and will have strong impact on the success of the whole aquaculture sector of the country (De Silva & Davy, 2010; Phuong & Oanh, 2010).

3.2 Feed and feeding practices in striped catfish farming

Feed is the single largest cost to farmers, accounting for 79-92% of the total production costs of striped catfish farming (Belton *et al.*, 2011; Da *et al.*, 2011; Phan *et al.*, 2009). In general, there are two types of feeds used for striped catfish, wet farm-made feeds and pelleted feeds, and these differ in formulation and quality (Phuong & Oanh, 2010; Phan *et al.*, 2009). According to Hung (2004), the traditional feeding of small-scale catfish farming is largely based on trash fish (marine origin) constituting approximately 50-70% of feed formulations. This is a protein source which has limited availability in Vietnam and is expensive. Therefore, more research is needed to help farmers replace trash fish with other protein sources. Soybean meal, groundnut meal, agriculture by-products, livestock by-products and other plant proteins have been suggested to be strong candidates for replacing fish meal and trash fish (Hung *et al.*, 2007).

3.3 Potential feed protein resources used for aquafeeds

The list of suitable feed protein sources to replace fish meal diets is relatively short, and includes products of the poultry and animal rendering industries, marine protein recovered from fish processing and by-catch, protein concentrates made from grains, oilseeds, and pulses, and novel proteins from marine invertebrates and single-cell proteins. Most of these protein sources have been studied in fish diets, and ranges of suitable replacement rates in fish meal for major fish species have been estimated (NRC, 2011; Hardy, 2008). According to Hardy & Barrows (2002) only three groups of ingredients have the potential to be used as crude protein (CP) resources in aquafeeds: a) wheat-germ meal and maize gluten meal in feeds with 20-30% CP in dry matter (DM); b) oilseed meals, crab meal and dried milk products in feeds with 30-50% CP in DM); and c) fish meal, blood meal, feather meal, tankage, meat and bone meal, yeast products, shrimp head meal, poultry by-product meal, soy protein concentrate, wheat gluten, maize gluten meal and casein in feeds with over 50% CP in DM.

3.4 Alternative protein sources to fish meal in aquaculture diets

In 2006, 45% of the fish meal produced for use in aquafeed was used for carnivorous fish species such as salmon, trout, sea bass, sea bream and yellowtail. However, at least 21% of the fish meal production was used in feeds for fry and fingerling carp, tilapia, catfish and other omnivorous species (Hardy, 2010). Alternatives to fishmeal and fish oil are now available from other sources, mainly grains/oilseeds and material recovered from livestock and poultry processing (rendered or slaughter by-products) (Sugiura *et al.*, 2000). Since 2006, many advances have been made in replacing part of the fish meal in aquafeeds with alternative protein sources (NRC, 2011). The proportion of fish meal in feeds for salmon, trout, sea bream, sea bass and all other carnivorous species has decreased by 25-50%, depending on species and life stage. A similar situation can be seen in feed for omnivorous fish species, especially in grow-out feeds (NRC, 2011; Hardy, 2010).

3.4.1 Terrestrial plant-based protein

Omnivorous fish species such as tilapia and *Pangasius* catfish have been demonstrated to have a capacity for utilising plant feedstuff carbohydrates for energy, but little research has been performed on these fish species with regard to alternative dietary selection (Hung, 2003). Using plant-based proteins in aquaculture feeds requires that the ingredients possess certain nutritional characteristics, such as low levels of fibre, starch and anti-nutritional compounds. They must also have a relatively high protein content, favourable amino acid profile, high nutrient digestibility and reasonable palatability (NRC, 2011; Lim *et al.*, 2008). A number of previous studies discuss the suitability of plant protein feeds and/or local agricultural by-products as an alternative protein source in fish feeds (Burr *et al.*, 2012; Bonaldo *et al.*, 2011; Brinker & Reiter, 2011; Cabral *et al.*, 2011; Nyina-Wamwiza *et al.*, 2010; Pratoomyot *et al.*, 2010; Garduño-Lugo & Olvera-Novoa, 2008; Olsen *et al.*, 2007).

3.4.2 Terrestrial animal by-products

Processed animal protein ingredients (often referred to as land animal products) such as blood meal, feather meal and poultry by-product meal, are comparable with many other protein sources used in fish feeds on a cost-per-unit protein basis (NRC, 2011). No effects on growth performance and feed utilisation were observed when fish meal protein in finfish diets was replaced with 60-80% of poultry by-products (PBM) or with 30-40% hydrolysed feather meal (FeM) (Yu, 2008). A number of published reports are available regarding the suitability of different animal protein feeds as alternatives to fish meal in fish feeds (Rossi Jr & Davis, 2012; Hernández *et al.*, 2010; El-Haroun *et al.*, 2009; Rawles *et al.*, 2009; Hu *et al.*, 2008; Saoud *et al.*, 2008; Wang *et al.*, 2008; El-Sayed, 1998).

3.5 Nutrient requirement of catfish

3.5.1 Protein requirements

Striped catfish is an omnivorous species and requires lower levels of dietary protein than carnivorous fish species (Cacot & Pariselle, 1999; Phuong, 1998). Cho *et al.* (1985) reported that the highest growth rate was achieved when striped catfish fry were fed diets containing 25, 30 and 35% CP in DM. The diet with the lowest CP content (20% in DM) and the diet containing 40% CP in DM supported similar growth rates, in both cases being significantly greater than that obtained with a 45% CP diet. The highest protein diet (50% CP in DM) resulted in significantly lower growth rates than any of the other experimental diets (Cho *et al.*, 1985). Hung *et al.* (2002) reported that the protein requirements for maximum growth of *P. bocurti, P. hypophthalmus* and *P. conchophilus* were approximately 27.8%, 32.5% and 26.6% CP in DM, respectively, when the energy content was fixed at 20 kJ gross energy/kg DM. Robinson *et al.* (2001) concluded that most estimates on the dietary protein requirements of channel catfish (*Ictalurus punctatus*) range from 25 to 55% CP in DM. However, a CP level as low as 16% in DM may be adequate for grow-out of channel catfish of food-size, when the fish are fed to satiety.

At present, the quality of commercial feeds used for striped catfish in the Mekong Delta in Vietnam is highly variable, with CP content ranging from 20-30% in DM, whilst that of farm-made feeds ranges from 17-26% CP in DM (Phan *et al.*, 2009). These levels of CP are comparable with dietary protein requirements (27-29% CP in DM) for normal growth of striped catfish fingerlings (Jantrarotai & Patanai, 1995), but they are higher than the level (15-26% CP in DM) suggested for grow-out fish by Paripatananont (2002). Hung *et al.* (2002) indicated that the lowest dietary CP levels could result in better protein efficiency and minimum feed costs, but the cycle of fish culture to achieve the 1.0-1.5 kg marketable size would be longer (12-16 months) than with high-protein feeding (8-10 months).

3.5.2 Essential amino acid requirements

Formulating cost-effective feeds meeting the essential amino acid (EAA) requirements of fish and shrimp can be a challenge (Kaushik & Seiliez, 2010) and will depend on relevant data on both EAA requirements of the fish species and the EAA supplied with the feed.

The maintenance requirement of EAA may account for a greater proportion of total requirement (maintenance + growth) because amino acids can be involved in a wide variety of other metabolic reactions beside protein synthesis and are subjected to significant endogenous losses (Rodehutscord *et al.*, 1997). Amino acids are also required as precursors for various metabolites, neurotransmitters, hormones and cofactors (NRC, 2011). Different approaches have been used to estimate the protein and EAA requirements of fish species (Pohlenz *et al.*, 2012; Grisdale-Helland *et al.*, 2011; Hua, 2011; Helland *et al.*, 2010; Richard *et al.*, 2010; Bodin *et al.*, 2009; Encarnação *et al.*, 2006; Encarnação *et al.*, 2004; Rodehutscord *et al.*, 1997).

Overall, the maintenance amino acid requirement of domesticated fish and shrimp represents a small proportion (generally between 5 and 20%) of their total amino acid requirements (Richard *et al.*, 2010; Abboudi *et al.*, 2007; Encarnação *et al.*, 2006; Rodehutscord *et al.*, 2000). Rodehutscord *et al.* (1997) estimated the maintenance EAA requirement of rainbow trout (live weight = 50 g/fish) to be (mg/kg$^{0.75}$/day): lysine, 4; tryptophan, 2; histidine, 2; valine, 5; leucine, 16, and isoleucine, 2. Bodin *et al.* (2009) obtained a markedly higher estimate of maintenance lysine requirement (24 mg/kg$^{0.75}$/day) for rainbow trout. Abboudi *et al.* (2006); Rollin *et al.* (2006) estimated the threonine maintenance requirement of Atlantic salmon fry (live weight = 1-2 g/fish) to be between 5-7 mg/kg$^{0.75}$/day.

NRC (2011) reported that the ideal amino acid patterns are usually stated as the ratio of each EAA to lysine, which is given the arbitrary value of 100. Most monogastric animals, including fish and shrimp, require the same 10 EAA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) (Table 1).

Table 1. *Estimated essential amino acid requirements (g 16/g N) of common fish and shrimp species*

	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Trp	Val
Channel catfish (*Ictalurus punctatus*)	4.3	1.5	2.6	3.5	5.1	2.3	2.1	2.2	0.5	3.0
Common carp (*Cyprius carpio*)	4.3	2.1	2.5	3.3	5.7	2.0	6.5	3.9	0.8	3.6
Nile tilapia (*Oreochromic niloticus*)	4.2	1.7	3.1	3.4	5.1	2.7	3.8	3.8	1.0	2.8
Mrigal carp (*Cirrhimus mrigala*)	4.6	2.1	3.2	3.9	5.8	3.0	3.3	4.5	1.0	3.8
Japanese eel (*Anguilla japonica*)	4.2	2.0	3.8	4.7	5.1	4.8	5.8	3.8	1.1	3.8
Rainbow trout (*Oncorhynchus mykiss*)	4.2	1.2	2.8	2.9	5.3	1.9	2.0	2.6	0.4	3.4
Black tiger shrimp (*Penaeus monodon*)	5.3	2.2	2.7	4.3	5.8	2.9	3.7	3.5	0.5	2.8

Data reported by NRC (2011): Nutrient requirements of fish and shrimp (National Academic Press, Washington, D.C). The response variable data was based on weight gain (WG).

Lysine is considered to be the first limiting amino acid for catfish species and if diets are formulated to meet the minimum lysine requirement, all other amino acids should be in excess (Robinson & Li, 2002).

According to Green & Hardy (2008), excess histidine, arginine, methionine and leucine had no negative effect in rainbow trout fed a diet with "balanced amino acid profile" according to the ideal protein concept. Fish have particularly high requirements for dietary arginine because it is one of the most versatile amino acids by serving as the precursor for the synthesis of nitric oxide, urea, polyamines, proline, glutamate and creatine in fish. Moreover, arginine is abundant in protein and tissue fluid (Li *et al.*, 2009; Wu & Morris, 1998). In contrast, with increasing use of plant-based proteins in shrimp feed as an alternative to marine protein sources (fish, shrimp or squid meal), lysine and methionine will be the first two limiting EAA (Gatlin *et al.*, 2007).

3.5.3 Lipid requirements

It has been shown that striped catfish fry are able to utilise dietary lipid energy efficiently and thereby reduce the use of protein as an energy source (Phumee *et al.*, 2009). The essential fatty acid (EFA) requirements of striped catfish are probably similar to those of other omnivorous fish species such as channel catfish, carp (*Cyprinus carpio*), tilapia (*Sarotherodon ziltii*) and African catfish (*Clarias gariepinus*) (NRC, 2011; Wilson & Moreau, 1996; Borlongan, 1992; Stickney & Hardy, 1989; Watanabe, 1982).

Increasing dietary lipids above the minimum level will support higher growth rates, possibly partly due to protein sparing (NRC, 2011). Robinson *et al.* (2001) reported that catfish have been fed diets containing up to 16% lipids without any negative effects on growth rate.

3.5.4 Carbohydrate and fibre requirements

In many fish species, a dietary carbohydrate supply appears to be necessary as it improves growth and especially protein utilisation (Hung *et al.*, 2003). It is important to provide the appropriate amounts of digestible carbohydrates in fish diets because carbohydrates are the least expensive energy source for aquatic animals (Pillay & Kutty, 2005; Robinson & Li, 2002). In omnivorous and warmwater fish such as channel catfish (*Ictalurus punctatus*), carp, Nile tilapia (*Oreochromis niloticus*) and *Pangasius* catfish, dietary carbohydrates are more important than lipids (Hung *et al.*, 2003; Wilson, 1994). Garling & Wilson (1977) reported that up to 25% dietary carbohydrates can be utilised as effectively as lipids as an energy source for channel catfish. *Pangasius* catfish species in the Mekong Delta of Vietnam are fed moist paste or dry pellets, traditionally containing a large amount of carbohydrate-rich feedstuffs such as rice bran, rice polishing, broken rice and vegetables. These feed resources can reach 60-80% of the total feed ration (Cacot, 1994). As a result, visceral fat accumulation in fish at harvest can be very high (Hung *et al.*, 2003). Moreover, Hien *et al.* (2010) reported that high carbohydrate and low protein diets result in low growth rates and longer time to reach marketable size of fish in striped catfish production.

3.5.5 Energy requirement

Feeding standards are often based on energy needs, and dietary energy in relation to dietary nutrient content is important when formulating catfish feeds (Robinson & Li, 2002). According to Hung *et al.* (2004), appropriate diets must be defined for each fish species and should be based on at least their requirements for dietary protein and protein to energy ratio. Glencross *et al.* (2011) estimated the maintenance energy requirement of striped catfish with a body weight of 40 g and at 32 °C to be approximately 9.56 Kcal/kg/day.

The energy requirements of fish depend on the species, water temperature and physiological stage of the animal itself (Guillaume *et al.*, 2001). For freshwater fish (10-250 g), the average daily energy expenditure is 25-45 KJ/kg (NRC, 1993). In general, the diet should provide at least 15-18 MJ DE/kg DM. In rainbow trout, this corresponds to about 15-16 MJ/kg gain in body mass at 8 °C and 17-19 MJ/kg gain in body mass between 15 and 18 °C. The values for energy growth requirements are similar for channel catfish, whilst they are higher for common carp and sea bream (NRC, 1993). As regards catfish, Hung *et al.* (2002) reported that the protein/energy ratio (P/E) for maximum growth of *P. hypophthalmus* is approximately 18.6 mg/KJ, which is higher than for *P. bocourti* (14.4 mg/KJ) or *P. conchophilus* (14.0 mg/KJ). However, it is low compared with that of other catfish species, which are reported to require a P/E ratio of 19-21 mg/KJ (Guillaume *et al.*, 1999).

3.6 Digestibility in fish

Modern aquaculture diets are routinely formulated based on the digestible nutrient and energy criteria (Cho & Kaushik, 1990). Diet design, feeding strategy, faecal collection method and method of calculation all have important implications for determination of the digestible value of nutrients from any ingredient (Glencross *et al.*, 2007).

3.6.1 Methods used in digestibility determination

Determining digestibility of food and feeds in animals requires collection of faecal material. In assessing diet digestibilities, the two key methodological approaches used are the direct and indirect assessment methods. Both involve feeding test feed ingredients singly or, more commonly, as a component of a diet (NRC, 2011).

3.6.1.1 Direct method

In the direct assessment method, a complete account of both feed inputs and faecal outputs is required (NRC, 2011). The digestibility value of the feeds is then determined on a mass-balance basis (Glencross *et al.*, 2007). The main advantage with the direct method is that faecal excretion is qualitatively collected, making it possible to determine the digestibility with high accuracy. In addition, this method allows the carbon and nitrogen balance to be determined, as well as digestible energy and metabolisable energy (NRC, 2011). The main problems with this method are related to the difficulty and the possible errors involved with collection of accurate data on feed intake and faecal production (NRC, 1993). Moreover, fish easily become stressed, which may affect digestive and metabolic processes and may result in digestibility values that are not credible (NRC, 2011).

3.6.1.2 Indirect method

The indirect method for digestibility determination is commonly used in most species of farmed fish and shrimp. This method relies on the collection of a representative sample of faeces that is free of uneaten feed particles and the use of an indigestible marker for calculation of digestibility (NRC, 2011). The marker can be added to the feed or it can be a component in the feed. The added marker should be non-toxic and inert and possible to include at low concentrations. Common indigestible markers added to the feed are chromic oxide (Cr_2O_3), yttrium oxide (Y_2O_3) and titanium dioxide (NRC, 2011). Acid insoluble ash (AIA) is a common and reliable feed-associated indigestible marker used to assess digestibility in pigs (McCarthy *et al.*, 1973) and fish (Montaño-Vargas *et al.*, 2002).

Digestibility of nutrients is estimated based on relative enrichment of marker in faeces compared with the level present in the feed (NRC, 2011). It is assumed that the amount of the marker in the feed and faeces remains constant throughout the experimental period and all ingested marker will appear in the faeces. The ratio of the marker in the feed and faeces determines the digestibility of dietary components and energy (Glencross *et al.*, 2007). According to NRC (2011), the indirect method has several advantages over the direct method. These include minimum stress on fish or shrimp associated with a rearing/holding tank environment and the fact that fish or shrimp can be used in a single replicate tank rather than a single fish or shrimp.

3.6.2 Factors affecting digestibility

Hepher (1988) reported that digestion in fish depends on three main factors: a) the ingested food and the extent to which it is susceptible to the effects of the digestive enzymes; b) the activity of the digestive enzymes; and c) the length of time the food is exposed to the action of the digestive enzymes. In addition, factors such as feed intake, fish size, age and water temperature are experimental variables that may affect digestibility (NRC, 2011). It has been reported that there is a linear increase of about 1% in the apparent digestibility of protein and energy with an increase in water temperature from 6 to 15 °C in rainbow trout (Azevedo *et al.*, 1998; Choubert *et al.*, 1982).

3.6.3 Protein and amino acid digestibility

Protein digestibility tends to be depressed when the concentration of dietary carbohydrates increases, and this affects the extent to which the protein can be hydrolysed to free amino acids (NRC, 1993). The digestion coefficient for CP in protein-rich feedstuffs is usually in the range 75-95%. Moreover, the digestibility of complex protein ingredients is the sum of the digestibility of the various proteins comprising the ingredient (NRC, 2011). Hence, processing of feed ingredients to partially break down or remove proteins that are difficult to digest improves overall protein digestibility. Moreover, increased amounts of dietary lipids result in increased protein digestibility (NRC, 1993).

Plant proteins present a different challenge for digestion in that they are associated with other plant structures that may prevent the action of digestive enzymes (NRC, 2011). Increasing the digestibility of plant proteins involves grinding the seeds or the biomass to release protein-surrounded plant structures. Heat treatment may enhance the digestibility of plant proteins, such as soybean meal and plant leaf meal protein, by reducing the activity of trypsin inhibitors and other anti-nutritional compounds.

Proteins are not absorbed as such, but rather the free amino acids and small peptides that make up proteins are absorbed. Thus, the digestibility of protein depends on the extent to which it can be hydrolysed to tri-peptides, di-peptides and free amino acids. Lysine, arginine, histidine and tryptophan contain reactive epsilon amino groups that form bonds that are not hydrolysed by digestive enzymes. The apparent digestibility (AD) values for proteins are the fractional sums of AD values for amino acids and other nitrogenous compounds in feed ingredients (NRC, 2011).

3.6.4 Carbohydrate and fibre digestibility

Carbohydrates are mixtures of sugar, starch and dietary fibre. In addition, the poly-phenolic compound lignin is associated with the dietary fibre fraction. The availability of carbohydrates differs and comprises highly digestible sugars, moderately digestible gelatinised starch, poorly digestible compounds such raw starch and chitin, and indigestible compounds such as insoluble carbohydrates (Stone, 2003). The negative effect of crude fibre (CF) has been reported for many fish species (Ferraris *et al.*, 1986). It has also been suggested that the AD of dietary components is negatively correlated to the fibre content in the diet (Khan, 1994; Anderson *et al.*, 1991). Carnivorous species, such as salmonids, derive very little energy from unprocessed plant starch. Omnivorous species, such as catfish, and herbivorous species, such as some carp species, derive a large amount of energy from starch, providing that it is cooked (NRC, 2011). Robinson *et al*. (2001) found that catfish can digest about 65% of uncooked maize starch when fed a diet containing 30% maize, while cooking increases the digestibility of maize starch to about 78%.

In addition to providing energy, starch is important in fish feed processing and is invaluable for obtaining an acceptable pelleting quality of the feed. Therefore, starch is included in most fish feeds. The dietary fibre fraction includes non-starch polysaccharides such as cellulose, pectins and gums. The fibre content of grains varies and is high in grains with a seed coat, such as oats, barley and rice, while it is low in grains without a seed coat such as wheat, rye and maize. The dietary fibre fraction is essentially indigestible to nearly all fish species, although there are exceptions such as grass carp (*Ctenopharyngodon idella*) (NRC, 2011). However, Hardy & Barrows (2002) found that the fibre fraction is indigestible in carnivorous fish, whilst omnivorous and herbivorous fish are able to digest fibre to varying, but limited, degrees.

The proportion of digestible carbohydrate that can be included in the diet has been reported to be 25-30% for channel catfish (Wilson & Moreau, 1996), 30-40% for common carp and 40% for Nile tilapia (Luquet, 1993), but 30-60% for the latter when cassava starch is used (Wee & Ng, 1986).

3.6.5 Energy digestibility

The digestibility of energy in a feedstuff is determined by its chemical composition and it affects the content of digestible energy (DE). The DE content corresponds to the gross energy (GE) ingested, less the GE excreted with the faeces. The faecal energy losses can vary considerably between feed ingredients, but generally comprise between 10 and 30% of the GE (Guillaume *et al.*, 2001).

The DE content of a diet can be calculated as the sum of the DE of each feed ingredient, under the assumption that there are no interactions between ingredients (NRC, 2011). Thus, if the interactions between ingredients and digestibility are negligible, DE in a diet can be considered to be additive (Guillaume *et al.*, 2001)

3.6.6 Digestibility of lipids

Evidence suggests that the AD decreases with increasing proportion of saturated fatty acids (SFA) in lipid sources in both warmwater and coldwater fish species (NRC, 2011). Lipids are almost completely digestible by fish and seem to be favoured over carbohydrates as an energy source (Cho *et al.*, 1985). Lipids are highly digestible sources of concentrated energy and contain about 2.25 times as much energy as an equivalent amount of carbohydrates (Robinson *et al.*, 2001).

The AD of lipids is 90-98% in Atlantic salmon when they are ingested as triacylglycerols (TAGs) or free fatty acids (FAs) (Guillaume *et al.*, 2001). The same type of response has been observed in other fish species, such as trout and carp. In turbot, a decrease in AD and reduction in growth rate has been observed when the food contains more than 15% lipids. In contrast, diets containing more than 30% lipids give excellent results for trout and Atlantic salmon, implying high utilisation (Guillaume *et al.*, 2001). The data also suggest important species differences in lipid utilisation.

3.7 Anti-nutrients present in feed ingredients

The use of plants or plant-derived feedstuffs such as legume seeds, different types of oilseed cake, leaf meal, leaf protein concentrates and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of anti-nutritional substances (Francis *et al.*, 2001). The effects of these substances on fish can include reduced palatability, altered nutrient balance of the diet, disturbance of digestive processes and growth, decreased feed efficiency, pancreatic hypertrophy, hypoglycaemia, liver dysfunction, goiterogenesis and immune suppression (NRC, 2011; Krogdahl *et al.*, 2010). Several anti-nutritional compounds are present in animal feed ingredients (Table 2). However, only a few are of major importance for fish feed formulation.

Hydrogen cyanide (HCN) and tannins are toxic compounds that are found in most plants such as cassava leaves and root, mango leaves and sweet potato leaves. HCN is toxic to humans and animals due to its binding to iron, manganese and copper ions, which are functional components of many enzymes involved in the reduction of oxygen in the cytochrome respiratory chain (Zagrobelny *et al.*, 2004). Acute HCN toxicity symptoms include saliva excretion, vomiting, excitement, staggering, paralysis, convulsions, coma and death (Zagrobelny & Møller, 2011). The amount of protein in the diet affects the degree of cyanide tolerance, particularly proteins high in cysteine, as they provide the sulphur essential for thiosulphate production (Gleadow & Woodrow, 2002).

Tannins are secondary compounds present in plants and comprise polyphenols of great diversity (Hoste *et al.*, 2006). The physical and chemical properties of tannins vary between plants, in different plant parts and between

Table 2. *Chemical substances of anti-nutrient compounds and general biological effects on animal*

Compound	Biological effects	References
Insoluble and soluble fibres	Interfere with digestion, absorption and utilisation of macro- and micro-nutrients	Van Der Kamp *et al*. (2004)
Phytic acid	Impairs mineral digestion and contains phosphorus in a form unavailable to monogastrics	Thompson (1993)
Protease inhibitors	Growth reduction, inhibition of proteolytic enzymes	Francis *et al.* (2001)
Enzyme inhibitors	Reduce the digestion of protein, carbohydrates and lipids	Thompson (1993); Krogdahl & Holm (1979)
Goitrogen	Growth reduction, thyroid hyperplasia, changes in T3 and T4 levels.	(Francis *et al.*, 2001)
Oestrogens	Growth reduction, induction of vitellogenin secretion	Francis *et al.* (2001)
Lectins	Binding to gut cell receptors, possibly stimulating intestinal growth, make the gut more permeable for increased influx of macromolecules and bacteria, stimulate insulin production and alter metabolism. Growth reduction, change in gut microflora.	Grant G (1991)
Saponins	Interfere with lipid and protein digestion and may increase the permeability of the gut mucosa. Growth and feed efficiency reduction, absorption of lipids, cholesterol, vitamin A and E.	Cuadrado *et al*. (1995)
Glucosinolates	Reduce the uptake of iodine into the thyroid gland and may lead to goitre the iodine level in the diet is increased accordingly.	Liener (1980)
Cyanogens	Respiratory failure. Inactivation of the enzymes that affect the release of cyanide from these glycosides. Growth and feed efficiency reduction.	Liener (1980)
Phytoestrogens	Interfere with endogenous oestrogen.	Price & Fenwick (1985)
Phytosterols	Interfere with cholesterol absorption and metabolism	Ostlund-Jr *et al.* (2003)
Quinolizidine alkaloids	Lupine alkaloids, may cause nervous system symptoms and intestinal disorders	Wink *et al*. (1998)
Oligosaccharides	Alter the microbiota in the gut and increase osmotic pressure in the intestine if not metabolised by the microbiota.	Cummings *et al*. (1986)
Alkaloids	Growth and reduced feed palatability, liver abnormalities.	Francis *et al.* (2001)
Anti-vitamins	Reduced vitamin availability	Melcion & Poel (1993)
Toxic fatty acids	Effect on reduction mortality	Liener (1980)

Source from: Hardy (2010); Krogdahl et al. (2010); Francis et al. (2001); Melcion & Poel (1993).

plants, in different plant parts and between seasons (Waghorn *et al.*, 1990). At high levels (above 50 g/kg DM), tannins in plant material can become an anti-nutritional factor and can result in reduced feed intake and digestibility in animal (Barry & McNabb, 1999). It has been observed that common processing techniques, such as cooking, soaking, drying and wet heating, as well as adding feed supplements, can reduce the concentration of anti-nutritional factors in plant feeds and improve the feed intake (Rehman & Shah, 2005; Francis *et al.*, 2001; Alonso *et al.*, 2000).

By-products of animal origin may also contain anti-nutritional compounds, especially if the products are not properly preserved or processed (NRC, 2011). However, whilst some anti-nutritional factors are easy to eliminate by processing, others may be more difficult to eliminate.

3.8 Environmental impact and water quality monitoring

3.8.1 Environmental impact assessment of intensive catfish farming

There is major concern regarding the impact of aquaculture on water quality and the environment related to the release of solid and dissolved phosphorus (P) and nitrogen (N) from feed wastes (Azevedo *et al.*, 2011). The waste may lead to water deterioration and environmental pollution, and is the major cause of eutrophication in aquatic ecosystems, bringing significant changes in ecosystem structure and functioning. The feed given to cultured fish is only partly transformed into fish biomass and is partly released into the water as suspended solids or dissolved matter such as carbon, nitrogen and phosphorus.

De Silva & Davy (2010) analysed a number of commercial feeds and farm-made feeds used on striped catfish farms and found that N discharge levels were similar for commercial feeds and farm-made feeds, with approximately 46.8 kg N/tonne fish. However, P discharge levels for farm-made feeds were considerably higher (26.6 kg P/tonne fish) than for commercial feeds (14.4 kg P/tonne fish).

It has been shown that grow-out catfish farming could have negative environmental effects on the Mekong River, such as eutrophication (Bosma *et al.*, 2009). The contribution of farming to these phenomena depends heavily on whether or not pond sludge is discharged into the river or used as fertiliser on nearby rice fields.

3.8.2 Water quality monitoring

Phuong *et al.* (2010) reported that most water parameters monitored in intensive catfish pond farming systems in the Mekong Delta of Vietnam are within an acceptable range and are within the limits of national standards of

Vietnam (TCVN 5942, 1995), except for total nitrogen (TN) and total phosphorus (TP). The concentrations of TN and TP, in particular TP, are considerably higher than the national standards.

Anh *et al.* (2010) reported that one tonne of frozen fillets releases 740 kg biochemical oxygen demand (BOD), 1020 kg chemical oxygen demand (COD), 2050 kg total suspended solids (TSS), 106 kg N and 27 kg P, of which wastewater from catfish ponds contributes 60-90% and sludge from fish ponds contributes the rest.

3.8.3 Phytoplankton and zooplankton monitoring

High plankton concentrations are strongly correlated with bio-available N and P in fish pond water. Phytoplankton correlate most strongly with PO_4-P concentrations and zooplankton most strongly with PO_4-P and DO concentrations (Rahman *et al.*, 2008).

Phuong *et al.* (2010) reported that five phyla [Chlorophyta (green-algae), Diatomeae (diatoms belonging to Ochrophyta), Euglenophyta (euglenoids), Cyanobacteria (blue-green algae) and Dinophyta (dinoflagellates)], containing about 176 species of algae, can be found in striped catfish ponds in the Mekong Delta of Vietnam. Most of the algae encountered are species indicating an eutrophic environment. They are *Actinastrum*, *Coelastrum*, *Pediastrum, Scenedesmus* (Chlorophyta), *Phacus*, *Euglena* (Euglenophyta), *Melosira*, *Cyclotella* (Diatomeae), *Spirulina* and *Oscilatoria* (Cyanobacteria). There are at least 99 zooplankton species that belong to four main groups, protozoa, rotifera, cladocera and copepoda, in fish ponds in catfish farming systems (Phuong *et al.*, 2010).

4 Materials and methods

4.1 Study site

In Paper I, a survey was carried out in 2009 in three provinces, namely An Giang, Can Tho and Dong Thap, in the Mekong Delta of Vietnam. Papers II & III (two experiments to investigate the digestibility of local feed sources) and Paper IV (indoor experiment on growth performance and feed utilization of striped catfish) were carried out in 2010 at the Laboratory of Aquaculture Nutrition, Faculty of Agriculture and Natural Resources, An Giang University, Vietnam. Paper V (outdoor experiment on growth performance and feed utilisation) was carried out in 2010 at a fish pond farm in Long Xuyen city of An Giang province in the Mekong Delta of Vietnam.

4.2 Field survey and feed samplings (Paper I)

The survey reported in Paper I focused on small-scale pond farming systems for striped catfish in An Giang, Can Tho and Dong Thap and 60 farmers (20 from each province) were selected randomly. Primary data on fish pond farming practices were collected through a structured questionnaire, observations and informal discussions. The questionnaire included questions on: a) socio-economic characteristics, b) details of pond culture practices, c) available feed resources and kinds of feed used, based on feed purchase records, and d) fish yield, investment costs and net returns for the last calendar year. Feed and feed ingredient samples (200-300 g) were collected randomly from the selected farms in each province and analysed for proximate chemical composition. A simple cost-benefit analysis was made, outlining total investment cost, total revenue, investment cost/kg fish, and loan and credit resources, in order to compare economic returns of the different pond farming practices in the three provinces (Sang, 1990).

4.3 Fish experiments (Papers II, III, IV & V)

4.3.1 Experimental design

Four experiments were set up as a substitution experimental design, with seven diets fed in triplicate for each experiment. At the beginning and end of the experiment, each acclimatised fish was individually weighed using a digital scale. Fifty homogeneous fish were distributed into each tank for each treatment in each experiment. The average initial body weight (BW) was 8.5 ± 0.3 and 25.3 ± 5.3 g/fish for Papers II & III (digestibility experiments), respectively. For Paper IV (indoor experiment), 30 homogeneous fish with an average initial BW of 16.3 ± 4.0 g/fish were selected and stocked into each tank. For Paper V (outdoor experiment), 200 homogeneous fish with an average initial BW of 16.5 ± 0.1 g/fish were selected and distributed into each hapa net cage for each treatment. The fish densities (fish/m^3) were equal in the indoor and outdoor experiments (Papers IV & V).

4.3.2 Experimental fish

All fingerlings of striped catfish used in experiments were bought from the Research Center of Aquaculture Seed Production of An Giang province. To eliminate ectoparasite infection, all fish were treated with a solution of 3% NaCl for 15 min at arrival. Fish fingerlings were reared and quarantined in composite tanks (3 m^3 water) for one month to acclimatise them to experimental conditions at the Laboratory of Aquaculture Nutrition (Papers II, III & IV). All fish fingerlings used in the outdoor experiment (Paper V) were reared and quarantined in a hapa net cage (4 m x 8 m x 2 m) in an earthen fish pond for one month to acclimatise to experimental conditions. The acclimatised fish were selected randomly, weighed and then transferred to each experimental tank (Papers II, III & IV) or to each hapa net cage (2 m x 3 m x 2 m) (Paper V) for one week before the experiment commenced for adaptation to experimental conditions.

4.3.3 Experimental diets

The experimental diets in the digestibility experiments (Papers II & III) constituted one reference diet and six test diets. The six test diets were made by mixing 70% of the reference diet and 30% of each test ingredient (Table 3). The reference diet used in the experiments described in Papers II & III was formulated to ensure that fish obtained all essential nutrient requirements for *Pangasius* species (Hien & Yen, 2005). In addition, 1% chromic oxide (Cr_2O_3) was incorporated as external marker for assessment of digestibility by the indicator method (Khan, 1994).

Table 3. *Ingredient composition of reference diet (RD) and test diets (g/kg) for striped catfish* (Pangasianodon hypophthalmus) *fingerlings in Papers II & III*

Ingredients	RD	Test diets of Paper II						Test diets of Paper III					
		Maize meal	Cassava leaf meal	Sweet potato leaf	Broken rice	Soybean meal	Duck-weed meal	Shrimp head meal	Golden apple snail	Earthworm meal	Catfish byproduct meal	Groundnut cake	Rice bran
Fish meal	260	182	182	182	182	182	182	182	182	182	182	182	182
Vegetable protein mix	480	336	336	336	336	336	336	336	336	336	336	336	336
Wheat flour	200	140	140	140	140	140	140	140	140	140	140	140	140
Squid liver oil	20	14	14	14	14	14	14	14	14	14	14	14	14
Vitamin premix	10	7	7	7	7	7	7	7	7	7	7	7	7
CMC[3]	20	14	14	14	14	14	14	14	14	14	14	14	14
Maize meal	–	300	–	–	–	–	–	–	–	–	–	–	–
Cassava leaf meal	–	–	300	–	–	–	–	–	–	–	–	–	–
Sweet potato leaf meal	–	–	–	300	–	–	–	–	–	–	–	–	–
Broken rice	–	–	–	–	300	–	–	–	–	–	–	–	–
Soybean meal	–	–	–	–	–	300	–	–	–	–	–	–	–
Duckweed meal	–	–	–	–	–	–	300	–	–	–	–	–	–
Shrimp head meal	–	–	–	–	–	–	–	300	–	–	–	–	–
Golden apple snail meal	–	–	–	–	–	–	–	–	300	–	–	–	–
Earthworm meal	–	–	–	–	–	–	–	–	–	300	–	–	–
Catfish by-product meal	–	–	–	–	–	–	–	–	–	–	300	–	–
Groundnut cake	–	–	–	–	–	–	–	–	–	–	–	300	–
Rice bran	–	–	–	–	–	–	–	–	–	–	–	–	300

However, due to problems with the Cr_2O_3 analysis, it was later decided to use acid-insoluble ash (AIA) as the indigestible marker for assessment of digestibility.

In the growth performance and feed utilisation experiments (Papers IV & V), the diets were composed of one reference diet and six test diets (Table 4).

Table 4. *Ingredient composition of the reference diet (RD) and test diets (g/kg) for striped catfish* (Pangasianodon hypophthalmus) *fingerlings in Papers IV & V*

	RD	Test diets						
		Ground-nut cake	Cassava leaf meal	Sweet potato leaf meal	Soybean meal	Golden apple snail meal	Shrimp head meal	Rice bran
Fish meal (FM)	260	195	195	208	0.0	0.0	0.0	0.0
Vegetable protein mix[1]	480	480	400	400	450	480	480	0.0
Wheat flour [2]	200	200	200	200	200	220	260	0.0
Squid liver oil [3]	20	20	20	20	20	20	20	20
Vitamin premix [4]	20	20	20	20	20	20	20	20
CMC [5]	20	20	20	20	20	20	20	20
Groundnut cake	–	65	–	–	–	–	–	–
Cassava leaf meal	–	–	145	–	–	–	–	–
Sweet potato leaf meal	–	–	–	132	–	–	–	–
Soybean meal	–	–	–	–	290	–	–	–
Golden apple snail meal	–	–	–	–	–	240	–	–
Shrimp head meal	–	–	–	–	–	–	200	–
Rice bran	–	–	–	–	–	–	–	940
Rate of replacement of fish meal (FM) (%)	0	25	25	20	100	100	100	100

[1] Commercial product used for striped catfish feed in the Mekong delta of Vietnam. The product is based on soybean and rice products; (% in DM), CP 21.2%, EE 7.6%, NDF 25.5%, ash 3.1%.

[2] Wheat flour: high wheat flour: content of protein: 9.10 g, fat: 1.32 g, cacbohydrate: 76.3g, calorie: 354 Kcal, Natri: 37 mg, ash: 0.55 % Max, moisture: 14% Max. Viet Nam Ky Nghe Bot Mi -VIKYBOMI JSC. Lot 32C/I, 2G Street, Vinh Loc, Binh Chanh Dist. Ho Chi Minh city.

[3] Squid liver oil: VIME-DAU GAN MUC, Vemedim Vietnam company, April 30th street, Can Tho city, Vietnam.

[4] Vitamin and mineral premix; content per kg: vitamin A, 4.000.000 UI; vitamin D3, 800.000 UI; vitamin E, 8.500 UI; vitamin K3, 750 UI; vitamin B1, 375 UI; vitamin C, 8.750 UI; vitamin B2, 1.600 mg; vitamin B6, 750 mg; folic acid, 200 mg; vitamin B12, 3.000 mcg; biotin, 20.000 mcg; methionine, 2.500 mg; Mn, Zn, Mg, K and Na, 10 mg.

[5] Carboxymethyl cellulose (CMC): Imported from Korea.

The reference diet contained fish meal as the main crude protein (CP) source, whilst in the six test diets 20-100% of the fish meal CP was replaced with CP from sweet potato leaf meal (20% replacement), cassava leaf meal (25% replacement), groundnut cake (25% replacement), soybean meal (100% replacement), golden apple snail meal (100% replacement) and shrimp head meal (100% replacement) (Table 4). The diets were formulated to meet the nutrient requirements of striped catfish (Hung *et al.*, 2002). In addition to the six diets tested, plain rice bran diet was also used in the outdoor study (Paper V), since rice bran without inclusion of fish meal is a traditional diet used for striped catfish (Table 4).

4.3.4 Experimental feed ingredients

Soybean meal and fish meal were purchased from the local market (AFIEX plant) in Long Xuyen city of An Giang province. Maize meal, rice bran and broken rice were purchased from My Long market in Long Xuyen city of An Giang province. Groundnut cake and shrimp head meal were bought from Vinh Long province and Kien Giang province, respectively. Golden apple snails were purchased from farmers in Cho Moi district of An Giang province and Tam Nong district of Dong Thap province. Only the meat of the golden apple snails was collected and it was cleaned by freshwater and sun-dried for three days before use. Earthworms were purchased from the Institution of Rice Research of the Mekong Delta in O Mon district of Can Tho city, cleaned by freshwater and then oven-dried at 60 ^{o}C for 24 h before use. Catfish by-product meal was bought from Thuy Thu Company, Binh Duc ward, Long Xuyen city of An Giang province. Leaves of cassava (*Manihot esculenta crantz*) and sweet potato (*Ipomoea batatas L.*) were collected during the harvest period from farms in Tri Ton and Cho Moi district of An Giang province, respectively. The leaves were cleaned with freshwater, sun-dried for 2-3 days and then ground to a meal. Duckweed (*Lemna polyrhiza*) was collected in earthen ponds from Chau Thanh and Thoai Son district of An Giang province, cleaned with freshwater, sun-dried for 2-3 days and then ground into meal.

4.3.5 Feeding and feed preparation

The feed was produced by careful mixing of the dry ingredients before adding squid oil and distilled water. The amount of distilled water was adjusted to get the mixture to form a stiff dough. The pellet feed was made using an electronic meat grinder (Quoc Hung Company, Vietnam) with diameter and length of pelleted feed in the range 1-2 mm. All diets were sun-dried for 2-3 days, and then weighed and stored in sealed plastic bags in small portions at 5 ºC until use. New batches of experimental feeds were made biweekly. The fish were fed daily manually to apparent satiety at 9.00 h and 14.00 h, at a fixed rate of 3-5% BW dry feed per day.

4.3.6 Experimental system and management

Three experiments, (Papers II, III & IV) were carried out in a closed re-circulation culture system in a series of 21 composite settlement tanks with a volume of about 500 L/tank. These settlement tanks were connected to a sedimentation tank, which contained sand and stone (1-2 mm) as a biological filter. Tap freshwater was aerated for 24 h to allow chlorine to evaporate and supplied into each tank at a flow rate of 3 L/min. In the digestibility experiments (Papers II & III), sedimentation tubes were connected with the funnel at the bottom of each tank where the fish faeces settled. The collection container was surrounded with ice, salt and rice husks to keep the temperature at 4-5 ºC in order to minimise microbial degradation of the fish faeces during collection.

In the outdoor experiment (Paper V), a series of 24 hapa net cages with 2.0 mm mesh size were placed in the pond and were used to hold the fish. The hapa net cages were rectangular (2 m x 3 m x 2 m deep) and were suspended by tying them to four melaleuca poles. One feeding sieve (feeding trap), 30 cm in diameter, was placed in each hapa net cage to retain feed and to prevent feed falling to the bottom. The feed was distributed to each feeding sieve using a small boat. About 20% of the water in the pond was replaced with new water from the river every second week during the experiment.

4.3.7 Sample collection and calculations

In digestibility trials (Papers II & III), fish faeces samples were collected twice a day for 30 days from the faecal settling tube (at 21.00 h and 8.00 h the next morning). Samples collected were pooled in sealed pots for each tank and kept frozen at -20 ºC until analysis. Digestibility calculation:

The apparent digestibility (AD) for dry matter (AD_{DM}), organic matter (AD_{OM}), crude protein (AD_{CP}), energy (AD_{E}) and EAA in the reference and test diets was calculated as described by Cho *et al.* (1982):

$AD_{diet}(\%) = 100-100 \times (\%M_{diet} / \%M_{faeces}) \times (\%Nutrient_{faeces} / \%Nutrient_{diet})$.
where %M = marker concentration (% in DM) and %N = nutrient content (% in DM).

The AD of test ingredient was calculated as described by Bureau & Hua (2006):

$AD_{test\ ingredient}(\%) = AD_{test\ diet}+[(AD_{test\ diet} - AD_{reference\ diet}) \times (0.7 \times D_{reference\ diet})/(0.3 \times D_{ingredient})]$.

where $D_{reference\ diet}$ = % nutrient of reference diet and $D_{ingredient}$ = % nutrient of test ingredient.

In the experiments on growth performance and feed utilisation (Papers IV & V), the following calculations on the growth performance, feed utilisation and biological indices were made:

Specific growth rate (SGR%) = $[(ln\ W_f - ln\ W_i) / T] \times 100$.

Daily weight gain (DWG) = $(W_f - W_i) / T$).

where W_f and W_i refer to the mean final weight and the mean initial weight, respectively, and T is the feeding trial period in days.

Survival rate [(SR%) = $(TF_f/TF_i) \times 100$].

where TF_f is total number of fish at finish (harvest) and TF_i is total number of fish at start.

Protein efficiency ratio (PER) = wet weight gain (g)/total protein intake (g).

Protein intake (PI) = feed intake (g) x per cent protein in diet.

Total feed intake per fish (FI) = [total feed intake (g)/number of fish].

Feed conversion ratio (FCR) = [total feed intake (g)/total wet weight gain (g)].

Hepato-somatic index (HSI%) = [100 x (liver weight (g)/body weight (g))].

Intra-peritoneal fat (IPF%) = [100 x (intra-peritoneal fat weight (g)/body weight (g))].

Viscera-somatic weight (VSI%) = [100 x (viscera-somatic weight (g)/body weight (g))].

Kidney index (KI) = [100 x (kidney weight (g)/body weight (g))].

4.3.8 Water quality monitoring

Water quality parameters in each experiment were recorded twice a month during the experiment. The pH was recorded by a pH meter (Schott Greate, Florida state, USA) and dissolved oxygen (DO mg/L) by Winkler titration (Stirling, 1985). Nitrite-nitrogen (mg/L), nitrate-nitrogen (mg/L) and total ammonia-nitrogen (mg/L), chemical oxygen demand (mg/L) and biochemical oxygen demand (mg/L) were measured using the Hach Lange cuvette test method (DR2800 visual spectrophotometer, Hach Lange Gmbh, Germany). Temperature (ºC) was recorded daily with a temperature meter at 8.00 h and 14.00 h.

The amount of plankton species in each tank (indoor experiment) and hapa net cage (outdoor experiment) (Papers IV & V) was monitored and determined twice a month as described by Bellinger & Sigee (2010). The density of plankton was calculated by the following formula:

$N = (P \times C \times 100)/V$

where N is the number of plankton per litre of water in the tank or hapa net cage, *P* is the number of planktonic organisms counted in different tanks or hapa net cages of different treatments, *C* is the volume of the plastic bottle holding the sample (100 mL) and *V* is the volume of water sample from each tank or hapa net cage. The identification of zooplankton and phytoplankton species was based on Suthers & Rissik (2008).

4.3.9 Chemical analysis

Samples of feed ingredients, diets and faeces, fish fillet, liver and kidney were analysed in duplicate using standard methods (AOAC, 1997). Acid-insoluble ash (AIA) in feed and faeces was analysed with the 4N-HCl procedure according to McCarthy *et al.* (1973) (Papers II & III).

All samples of experimental diets, feed ingredients, fish faeces and fish carcass were analysed for chemical composition (g kg DM), gross energy (MJ/kg DM) and amino acids (g kg DM). Dry matter was determined by drying samples in an oven at 105 ^{o}C for 24 h. Nitrogen (N) was determined by the Kjeldahl method and crude protein (CP) was calculated as N x 6.25. Crude fat (EE) content was analysed using the Soxhlet method after acid hydrolysis of the sample. Crude fibre (CF) content was determined by the standard method (AOAC, 1997) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Ash content was determined by incineration in a muffle furnace at 550 ºC for 12 h. Amino acid content of ingredients and diets was analysed by high-performance liquid chromatography according to Vázquez-Ortiz *et al.* (1995). Gross energy (MJ/kg) was determined with a bomb calorimeter (Calorimeter Parr 6300, Parr Instrument Company, Moline, IL, USA).

4.3.10 Statistical analysis

All digestibility data and all data on fish growth performance, feed utilisation and carcass traits were statistically analysed by one-way analysis of variance (ANOVA), using Tukey's post hoc ANOVA test for individual comparisons ($P \leq 0.05$ level of significance). All statistical analyses were carried out using the IBM SPSS STATISTIC (2011) program, version 19.

5 Summary of major results

5.1 Chemical composition of feed ingredients

Analyses of the nutrient content of feed ingredients used in Vietnamese fish farming showed that the CP content was highest in the feedstuffs of animal origin (artemia, moina, earthworms, golden apple snail, trash fish and fish meal) and in soybean meal. The highest lipid (EE) content was found in trash fish, moina and soybean meal. The NFE content ranged from 46 (g/kg DM) for trash fish to 882 (g/kg DM) for broken rice (Paper I).

The chemical composition (g/kg DM), gross energy (MJ/kg DM) and amino acid (g/kg DM) content of test ingredients used in Papers II-V are presented in Table 5. The CP content of test ingredients used in digestibility and growth performance trials was highest in catfish by-product meal, followed in descending order by shrimp head meal, golden apple snail meal, soybean meal, groundnut cake, cassava leaf meal and sweet potato leaf meal. The lowest CP content was found for broken rice, maize meal and rice bran (Table 5). The EE content was higher in catfish by-product meal, rice bran and groundnut cake than in the other test ingredients, whilst the highest content of NDF was found in sweet potato leaf meal, followed in descending order by groundnut cake, shrimp head meal, cassava leaf meal, duckweed meal, golden apple snail meal, rice bran and maize meal. The lowest ash content was found in broken rice and the highest in sweet potato leaf meal. The gross energy content of feed ingredients varied among feed ingredients within a range from 13.5 (MJ/kg DM) for duckweed meal to 23.3 (MJ/kg DM) for catfish by-product meal (Table 5). The EAA profile varied among feed ingredients, with the highest total amount in catfish by-product meal, shrimp head meal, golden apple snail meal, soybean meal and earthworm meal. In contrast, broken rice, maize meal, rice bran, duckweed meal and sweet potato leaf meal were lowest in most EAA (Table 5).

Table 5. *Chemical composition (g/kg DM), gross energy (MJ/kg DM) and amino acid (g/kg DM) content of test ingredients in Papers II-V*

	Maize meal	Cassava leaf meal	Sweet potato leaf meal	Broken rice	Soybean meal	Duck weed meal	Shrimp head meal	Golden apple snail	Earthworm meal	Catfish by-product meal	Groundnut cake	Rice bran
Crude protein	87	223	166	71	485	194	661	564	483	712	316	144
Lipid	27	65	24	1	10	4	36	16	40	161	100	126
NDF	153	339	397	25	198	242	342	239	25	20	344	217
Ash	48	97	170	3	73	219	143	118	92	45	55	79
Gross energy	15.9	18.9	14.6	15.7	17.8	13.5	16.6	16.3	16.8	23.3	19.5	18.2
						Essential amino acids						
Arginine	3.8	11.1	7.7	3.5	24.9	8.9	38.2	32.1	7.3	36.6	27.9	8.2
Histidine	1.1	3.4	1.9	0.7	4.8	3.5	11.1	11.9	5.9	15.0	5.0	1.9
Isoleucine	2.5	10.7	7.5	2.7	18.3	9.0	20.9	18.8	27.6	28.9	12.0	5.4
Lysine	1.2	6.7	2.6	1.3	15.1	4.7	37.2	36.2	43.5	45.8	19.8	8.7
Leucine	6.6	17.2	10.9	4.4	29.1	16.8	25.5	16.3	1.1	26.9	5.2	2.9
Methionine	1.8	5.2	4.3	1.6	6.4	4.6	14.5	10.9	11.6	18.7	4.0	2.8
Phenylalanine	3.0	10.6	7.6	2.5	18.6	10.5	19.0	16.4	20.2	18.7	13.8	5.9
Threonine	2.6	8.4	5.0	2.1	14.2	8.5	9.0	11.1	4.8	10.5	6.7	4.5
Valine	3.2	11.5	8.7	3.0	18.3	10.7	19.3	20.8	26.0	23.7	13.6	5.8
Total	25.8	84.8	56.2	21.8	149.7	77.2	194.7	174.5	148.0	224.8	108.0	46.1

5.2 Chemical composition of diets

The CP content of experimental diets used in Papers II & III ranged from 185 g/kg DM for the rice bran diet to 339 g/kg DM for the shrimp head meal diet (Table 6). The EE content was highest in the catfish by-product meal, groundnut cake, rice bran and soybean meal diets than in the other experimental diets. The highest NDF content was found in the vegetable diets based on groundnut cake, sweet potato leaf meal, soybean meal, rice bran and duckweed meal. For diets with feed ingredients of animal origin, the NDF content was higher in earthworm meal. The highest content of ash was found in broken rice and the lowest in duckweed meal, and the gross energy (GE) content was quite similar among all experimental diets and ranged between 16.2 and 17.9 MJ/kg DM. The EAA profile varied among experimental diets with a range of 69.7-108.1 g/kg DM in Paper II and 76.9-130.6 g/kg DM in Paper III (Table 6).

The chemical composition, gross energy content and EAA profiles in the growth performance trials (Papers IV & V) were similar among diets, with a CP content of 225-234 g/kg and a GE content of 16.2-17.2 MJ/kg (Table 7). Lipid content was highest in the rice bran diet, whilst the NDF content was higher in the rice bran, sweet potato leaf meal and cassava leaf meal diets than in the other test diets and the reference diet (Table 7). The highest Ca content was found in the groundnut cake, shrimp head meal and sweet potato leaf meal diets, whilst the rice bran, RD, groundnut cake and sweet potato leaf meal diets had a higher P content than the other experimental ingredients. In general, the content of Mg, K, Na and S was similar between the reference diet and test diets. Total EAA content ranged from 43.0 g/kg DM for the rice bran diet to 90.3 g/kg DM for the reference diet (Table 7).

5.3 Feed digestibility

5.3.1 Digestibility of diets

There were no differences in apparent digestibility (AD) between the reference diet and test diets ($P>0.05$) (Papers II & III). In general, the highest values of AD were found in the diets based on catfish by-product meal, shrimp head meal and soybean meal, followed in descending order by the diets based on groundnut cake, golden apple snail meal and sweet potato leaf meal (Table 8). However, the AD of the reference diet and test diets tended to differ for dry matter (AD_{DM}) and organic matter (AD_{OM}), within a range of 80.4-89.6% and 78.9-89.5%, respectively.

Table 6. *Chemical composition (g/kg DM), gross energy (MJ/kg DM) and amino acid (g/kg DM) content of reference diet (RD) and test diets in Papers II & III*

		Test diets in Paper II						Test diets in Paper III					
	RD	Maize meal	Cassava leaf meal	Sweet potato leaf meal	Broken rice	Soybean meal	Duckweed meal	Shrimp head meal	Golden apple snail	Earthworm meal	Catfish by-product meal	Groundnut cake	Rice bran
Crude protein	228	194	225	205	185	291	217	339	285	283	351	243	203
Lipid	46	42	43	42	34	51	37	45	48	52	74	56	66
NDF	189	166	153	249	109	227	225	185	157	214	147	289	224
Ash	101	79	84	109	67	90	123	108	99	98	78	76	88
Gross energy	16.2	16.2	17.0	15.5	16.2	16.5	15.5	16.8	16.7	16.2	17.9	17.0	16.5
AIA	13.2	15.9	11.1	23.2	10.7	17.5	32.7	12.85	15.01	22.21	12.6	14.12	12.67
Essential amino acids													
Arginine	14.8	11.5	13.7	12.7	11.4	17.9	13.0	21.8	20.2	12.6	21.3	18.8	12.8
Histidine	6.4	4.8	5.5	5.1	4.7	5.9	5.5	7.8	8.1	6.3	9.0	6.0	5.0
Isoleucine	9.7	7.5	10.0	9.0	7.6	12.3	9.5	13.0	12.4	15.1	15.4	10.4	8.4
Leucine	17.6	14.3	17.5	15.6	13.7	21.1	17.4	23.5	23.2	25.4	26.1	18.3	14.9
Lysine	9.3	6.8	8.5	7.3	6.9	11.0	7.9	14.1	11.4	6.8	14.6	8.1	7.4
Methionine	5.9	4.7	5.7	5.4	4.6	6.1	5.5	8.5	7.4	7.6	9.8	5.3	5.0
Phenylalanine	9.8	7.8	10.0	9.1	7.6	12.4	10.0	12.6	11.8	12.9	12.5	11.3	8.6
Threonine	6.3	5.2	6.9	5.9	5.0	8.6	6.9	7.1	7.7	5.8	7.5	6.4	5.7
Valine	10.5	8.3	10.8	9.9	8.2	12.8	10.5	13.1	13.6	15.1	14.4	11.4	9.1
Total	90.3	70.9	88.6	80.0	69.7	108.1	86.2	121.5	115.8	107.6	130.6	96.0	76.9

Table 7. *Chemical composition (g/kg DM), gross energy (MJ/kg DM), mineral (g/kg DM) and amino acid (g/kg DM) content of the reference diet (RD) and test diets in Papers IV & V*

	RD	Test diets (% of fish meal crude protein replaced)						
		Groundnut cake (25%)	Cassava leaf meal (25%)	Sweet potato leaf meal (20%)	Soybean meal (100%)	Golden apple snail meal (100%)	Shrimp head meal (100%)	Rice bran
Crude protein	225	230	227	223	234	227	225	124
Lipid	44	39	44	38	35	31	35	106
NDF	166	242	261	278	201	253	259	290
Ash	102	81	73	84	41	41	89	72
Gross energy	16.2	16.7	17.1	16.5	17.2	16.8	15.6	17.6
Ca	15.7	20.0	13.3	16.0	2.8	7.3	18.9	2.1
P	8.5	8.4	6.3	7.5	3.2	1.1	3.2	10.2
Mg	1.7	1.8	2.0	2.2	1.6	1.5	2.2	5.2
K	5.0	5.2	5.0	7.1	10.1	4.4	4.1	9.3
Na	4.8	4.0	3.5	5.1	1.4	1.8	6.0	1.5
S	2.7	3.1	2.5	3.0	2.3	2.0	2.1	1.6
Essential amino acids								
Arginine	14.8	14.7	13.4	13.2	13.7	14.6	14.7	7.7
Histidine	6.4	5.6	5.5	5.5	3.2	4.8	4.2	1.8
Isoleucine	9.7	9.3	9.3	9.0	10.2	9.7	9.5	5.0
Leucine	17.6	16.6	16.6	16.0	16.5	17.3	16.3	8.1
Lysine	9.3	8.0	8.2	7.9	6.9	6.6	7.8	2.7
Methionine	5.9	5.3	5.4	5.4	4.0	4.9	5.3	2.6
Phenylalanine	9.8	9.7	9.5	9.1	10.6	9.6	9.6	5.5
Threonine	6.3	6.1	6.3	5.8	7.7	6.6	5.7	4.2
Valine	10.5	10.1	10.1	9.8	10.5	10.5	9.5	5.4
Total	90.3	85.4	84.3	81.7	83.3	84.6	82.6	43.0

Apparent digestibility (AD) of gross energy (AD_{GE}) and crude protein (AD_{CP}) tended to differ between diets. The value of AD_{GE} ranged 78.7-90.0%, whilst AD_{CP} value ranged from 79.7% for the rice bran diet to 91.8% for the catfish by-product meal diet (Table 8). There were no differences ($P>0.05$) in AD of EAA between RD and test diets.

The average AD of individual EAA in Papers II & III was highest in the soybean meal, catfish by-product meal, golden apple snail meal and shrimp head meal diets, followed in descending order by the reference diet and the groundnut cake, duckweed meal and broken rice diets. In general, the AD values of individual EAA in the earthworm meal, rice bran and maize meal diets were lowest for arginine, histidine, lysine, methionine and valine (Table 9).

Table 8. *Apparent digestibility (%) of dry matter (ADDM), crude protein (ADCP), organic matter (ADOM) and gross energy (ADGE) in the reference diet (RD) and test diets in striped catfish* (Pangasianodon hypophthalmus) *fingerlings* [1]

Paper II	$AD_{(DM)}$	$AD_{(CP)}$	$AD_{(OM)}$	$AD_{(GE)}$
RD	87.2	86.8	89.5	84.3
Test diet ingredients				
Maize meal	85.9	83.9	82.6	89.8
Cassava leaf meal	81.1	80.0	81.6	78.7
Sweet potato leaf meal	85.0	83.3	80.5	81.9
Broken rice	88.1	84.9	81.5	89.9
Soybean meal	88.0	89.0	81.6	87.6
Duckweed meal	81.1	82.5	78.9	79.6
SEM	*0.94*	*1.11*	*1.28*	*1.77*
P-value	*0.064*	*0.095*	*0.051*	*0.053*
Paper III	$AD_{(DM)}$	$AD_{(CP)}$	$AD_{(OM)}$	$AD_{(GE)}$
RD	87.9	85.9	88.4	85.3
Test diet ingredients				
Shrimp head meal	88.7	89.2	86.9	89.0
Golden apple snail meal	85.9	83.2	85.0	87.7
Earthworm meal	84.6	81.8	83.3	82.8
Catfish by-product meal	89.6	91.8	87.9	90.0
Groundnut cake	85.3	86.2	83.5	87.2
Rice bran	80.4	79.7	79.3	83.1
SEM	*0.97*	*1.19*	*1.29*	*1.65*
P-value	*0.076*	*0.056*	*0.081*	*0.053*

SEM = Standard error of the mean; ($P > 0.05$).

Table 9. *Apparent digestibility (%) of essential amino acids in the reference diet (RD) and test diets in striped catfish* (Pangasianodon hypophthalmus) *fingerlings*

Paper II	RD	Test diets							
		MM	CSLM	SPLM	BR	SBM	DWM	*SEM*	*P-value*
Arginine	90.8	88.8	82.6	87.5	82.9	91.1	88.1	*1.42*	*0.16*
Histidine	87.1	81.9	78.3	87.5	90.3	89.9	86.2	*1.42*	*0.05*
Isoleucine	88.8	82.6	86.8	83.1	85.7	95.4	86.1	*2.51*	*0.26*
Lysine	90.3	78.2	84.9	87.6	81.8	91.9	88.4	*1.29*	*0.09*
Leucine	90.5	80.0	78.6	85.6	88.4	89.8	88.3	*1.54*	*0.08*
Methionine	87.4	82.2	78.4	84.1	88.2	96.9	87.2	*2.08*	*0.31*
Phenylalanine	88.3	80.7	84.8	82.8	86.3	87.7	87.0	*1.78*	*0.22*
Threonine	83.4	79.0	79.2	81.9	83.7	84.4	82.8	*1.32*	*0.06*
Valine	87.6	85.9	81.2	84.4	85.2	96.8	85.8	*2.28*	*0.10*
Average	88.8	80.3	78.3	85.1	87.9	91.5	86.9	*1.45*	*0.06*
Paper III	RD	Test diets							
		SHM	GAPS	EWM	CFPM	GNC	RB	SEM	*P-value*
Arginine	87.0	89.5	89.3	81.6	89.9	92.1	82.5	*1.50*	*0.06*
Histidine	88.2	88.0	90.5	84.6	91.8	91.2	88.2	*0.93*	*0.05*
Isoleucine	81.0	89.3	85.9	85.9	88.6	85.9	83.9	*1.06*	*0.22*
Lysine	87.2	90.4	88.5	75.3	89.9	86.7	84.3	*1.95*	*0.09*
Leucine	84.8	88.3	88.8	87.8	89.9	88.2	85.6	*0.68*	*0.08*
Methionine	79.8	85.1	84.8	84.7	88.4	84.9	82.3	*1.01*	*0.05*
Phenylalanine	81.5	84.4	84.2	82.0	84.8	85.2	82.5	*0.56*	*0.21*
Threonine	78.9	88.0	84.2	87.3	82.1	80.8	76.3	*1.62*	*0.07*
Valine	81.2	84.8	86.1	85.7	86.4	86.9	82.7	*0.79*	*0.06*
Average	81.7	87.8	87.3	84.6	88.4	87.6	80.3	*0.81*	*0.07*

MM, maize meal; CSLM, cassava leaf meal; SPLM, sweet potato leaf meal; BR, broken rice; SBM, soybean meal; DWM, duckweed meal. SHM, shrimp head meal; GAPS, golden apple snail meal; EWM, earth worm meal; CFPM, catfish-by product meal; GNC, groundnut cake; RB, rice bran.
SEM = Standard error of the mean; (P>0.05).

5.3.2 Digestibility of feed ingredients

Apparent digestibility of feed test ingredients (ADi) in Papers II & III differed significantly (P<0.05) and ranged from 66.2 to 89.8% for ADi_{DM}, from 63.6 to 91.3% for ADi_{CP}, from 65.4 to 86.9% for ADi_{OM} and from 69.8 to 89.3% for ADi_{GE} (Table 10). The highest ADi_{CP} values among test ingredients were obtained for soybean meal, catfish by-product meal and shrimp head meal, followed in descending order by golden apple snail meal, groundnut cake and duckweed meal. The highest ADi_{GE} was obtained for broken rice, catfish by-product meal, soybean meal, maize meal, golden apple snail meal and groundnut cake (Table 10). The highest ADi_{DM} and ADi_{OM} were found for soybean meal and catfish by-product meal, whilst duckweed meal, cassava leaf meal and sweet potato leaf meal had lower ADi_{DM} and ADi_{OM} values than the other feed test ingredients (Table 10).

Table 10. *Apparent digestibility (%) of dry matter (ADi_{DM}), crude protein (ADi_{CP}), organic matter (ADi_{OM}) and gross energy (ADi_{GE}) in test feed ingredients in striped catfish* (Pangasianodon hypophthalmus) *fingerlings in Papers II & III*

Paper II	$AD_{(DM)}$	$AD_{(CP)}$	$AD_{(OM)}$	$AD_{(GE)}$
Maize meal	82.0^{a}	66.0^{bc}	79.3^{ab}	86.4^{a}
Cassava leaf meal	79.4^{ab}	63.6^{c}	77.0^{ab}	76.7^{bc}
Sweet potato leaf meal	79.3^{b}	71.8^{b}	74.4^{b}	78.9^{b}
Broken rice	89.6^{a}	70.3^{b}	81.8^{a}	89.3^{a}
Soybean meal	88.9^{a}	91.3^{a}	85.3^{a}	87.3^{a}
Duckweed meal	66.2^{c}	81.7^{a}	65.4^{c}	69.8^{c}
SEM	*3.51*	*4.29*	*2.80*	*3.06*
P-value	*0.002*	*0.012*	*0.001*	*0.011*
Paper III	$AD_{(DM)}$	$AD_{(CP)}$	$AD_{(OM)}$	$AD_{(GE)}$
Shrimp head meal	84.9^{a}	89.4^{a}	80.4^{a}	81.8^{ab}
Golden apple snail meal	84.9^{ab}	88.1^{ab}	83.6^{bc}	86.3^{a}
Earth worm meal	81.1^{b}	84.0^{b}	81.2^{b}	79.0^{c}
Catfish by-product	89.8^{a}	90.0^{a}	86.9^{a}	87.8^{a}
Groundnut cake	85.8^{a}	87.6^{a}	84.3^{ab}	86.0^{ab}
Rice bran	82.2^{b}	76.3^{b}	78.7^{c}	80.5^{a}
SEM	*1.51*	*1.44*	*1.39*	*1.43*
P-value	*0.032*	*0.045*	*0.013*	*0.046*

SEM = Standard error of the mean.
Means within columns different superscript letters are significantly different ($P<0.05$).

The highest average AD values of individual EAA (in Papers II & III) were obtained for soybean meal, catfish by-product meal, golden apple snail meal, shrimp head meal and groundnut cake ($P<0.05$). The AD of individual EAA of test feed ingredients was lowest in maize meal and rice bran, followed by cassava leaf meal, sweet potato leaf meal and earthworm meal ($P<0.05$) (Table 11). In general, the AD value of arginine, histidine, isoleucine, lysine, methionine and valine was lower in maize meal, cassava leaf meal, sweet potato leaf meal and duckweed meal ($P<0.05$) than in the other feed ingredients (Table 11).

Table 11. *Apparent digestibility (%) of essential amino acids in test ingredients in striped catfish* (Pangasianodon hypophthalmus) *fingerlings*

Paper II	Maize meal	Cassava leaf meal	Sweet potato leaf meal	Broken rice	Soybean meal	Duckweed meal	*SEM*	*P-value*
Arginine	70.2[d]	82.5[abc]	92.0[a]	80.8[abc]	91.3[a]	77.7[c]	*3.39*	*0.031*
Histidine	86.8[a]	57.4[b]	90.6[a]	92.0[a]	98.4[a]	92.4[a]	*5.97*	*0.001*
Isoleucine	67.3[b]	76.5[b]	66.2[b]	78.2[ab]	88.0[a]	79.2[a]	*3.32*	*0.021*
Lysine	50.7[c]	78.6[b]	75.1[b]	92.1[a]	94.2[a]	79.5[ab]	*5.78*	*0.001*
Leucine	73.4[b]	78.5[a]	79.8[a]	69.4[bc]	89.3[a]	93.6[a]	*3.77*	*0.031*
Methionine	87.2[ab]	77.8[bc]	73.7[cd]	95.4[a]	88.9[a]	86.7[ab]	*3.22*	*0.012*
Phenylalanine	65.4[b]	68.5[b]	68.2[b]	57.3[c]	87.0[a]	80.4[a]	*4.39*	*0.004*
Threonine	80.1[a]	29.6[c]	77.6[ab]	89.5[a]	85.7[a]	89.3[a]	*6.35*	*0.001*
Valine	75.0[b]	76.0[b]	75.5[b]	79.8[b]	88.1[a]	81.8[ab]	*2.06*	*0.038*
Average	68.2[bc]	70.3[bc]	74.4[b]	81.3[a]	89.9[a]	82.6[a]	4.59	0.020
Paper III	Shrimp head meal	Golden apple snail meal	Earth-worm meal	Catfish by-product meal	Ground-nut cake	Rice bran	*SEM*	*P-value*
Arginine	89.5[b]	91.8[ab]	55.7[c]	92.6[ab]	98.3[a]	63.3[c]	*3.24*	*0.021*
Histidine	88.0[ab]	93.4[a]	75.5[b]	95.4[a]	91.5[a]	88.2[ab]	*2.89*	*0.031*
Isoleucine	89.4[ab]	91.7[a]	89.9[ab]	94.6[a]	89.8[ab]	95.9[a]	*1.13*	*0.052*
Lysine	90.4[a]	89.3[a]	72.6[b]	92.2[a]	86.7[a]	63.3[b]	*3.79*	*0.021*
Leucine	88.3[ab]	94.3[a]	90.6[a]	80.6[b]	88.3[ab]	97.4[a]	*2.36*	*0.041*
Methionine	85.2[b]	91.2[a]	84.6[bc]	94.7[a]	85.4[b]	82.5[c]	*1.88*	*0.032*
Phenylalanine	84.4[ab]	88.1[a]	82.4[b]	88.9[a]	85.4[ab]	86.6[a]	*0.98*	*0.040*
Threonine	95.1[a]	90.2[a]	91.7[a]	85.4[b]	90.1[a]	65.5[c]	*2.36*	*0.031*
Valine	84.8[b]	91.9[a]	89.9[ab]	91.9[a]	94.6[a]	87.6[ab]	*1.43*	*0.048*
Average	88.3[a]	90.7[a]	82.0[bc]	92.0[a]	84.5[bc]	81.0[c]	1.49	0.022

SEM = Standard error of the mean.
Means within rows different superscript letters are significantly different ($P<0.05$).

5.4 Growth performance and feed utilisation

Two different growth experiments with same diets were performed in an indoor culture system (300 L/tank) (Paper IV) and an outdoor culture system (earthen ponds) (Paper V). The most obvious findings were that the daily weight gain

(DWG) was much higher (3.2- to 6-fold) under outdoor culture conditions compared with indoor conditions, although the fish were fed the same diet. The feed conversion improved by 0.2 to 0.7 units (kg feed DM/kg weight gain) in the outdoor group (Table 12).

Table 12. *Growth performance and feed utilisation of striped catfish* (Pangasianodon hypophthalmus) *fingerlings fed the reference diet (RD) and the test diets*

		Test diets (% of fish meal crude protein replaced)								
Paper IV	RD	Groundnut cake (25%)	Cassava leaf meal (25%)	Sweet potato leaf meal (20%)	Soybean meal (100%)	Golden apple snail meal (100%)	Shrimp head meal (100%)	Rice bran	*SEM*	*P-value*
Final BW	51.9^{ab}	56.7^{a}	42.7^{b}	59.9^{a}	54.0^{a}	57.2^{a}	61.8^{a}	*	0.08	0.11
WG	35.5^{bc}	40.5^{ab}	26.2^{c}	43.8^{ab}	37.7^{ab}	41.0^{ab}	45.4^{a}	*	5.12	0.04
DWG	0.3	0.3	0.2	0.4	0.3	0.3	0.4	*	5.15	0.03
SGR	0.9	1.0	0.8	1.0	1.0	1.0	1.1	*	0.04	0.23
FCR	1.9	1.8	2.3	1.9	1.9	1.9	1.8	*	0.07	0.08
PER	1.5	1.4	1.2	1.4	2.2	2.1	2.1	*	0.20	0.22
PI	0.222^{ab}	0.224^{ab}	0.224^{a}	0.216^{b}	0.228^{a}	0.221^{ab}	0.221^{ab}	*	0.24	0.13
FI	98.6	97.6	98.9	96.8	97.7	97.3	97.3	*	0.002	0.16
SR (%)	94.4	97.2	93.3	100	96.7	97.8	97.8	*	0.77	0.52
Paper V										
Final BW	229.4^{ab}	219.0^{b}	177.9^{c}	204.9^{bc}	132.5^{d}	222.3^{b}	257.0^{a}	83.8^{e}	0.85	0.61
WG (g)	213.3^{ab}	202.1^{b}	161.7^{c}	188.8^{bc}	116.3^{d}	206.1^{b}	240.8^{a}	67.7^{e}	6.91	0.01
DWG (g)	1.78^{ab}	1.69^{b}	1.35^{c}	1.57^{bc}	0.97^{d}	1.72^{b}	2.01^{a}	0.56^{e}	0.07	0.02
SGR (%)	2.19^{ab}	2.13^{b}	1.96^{c}	2.06^{bc}	1.74^{d}	2.16^{ab}	2.29^{a}	1.38^{e}	0.04	0.03
FCR	1.3^{c}	1.6^{c}	1.6^{bc}	1.5^{c}	1.7^{bc}	1.4^{c}	1.3^{c}	4.9^{a}	0.05	0.02
PER	3.2^{a}	2.9^{a}	2.4^{a}	2.7^{a}	2.7^{a}	3.2^{a}	3.2^{a}	0.8^{b}	0.12	0.01
PI	0.59^{ab}	0.74^{a}	0.56^{ab}	0.56^{ab}	0.54^{b}	0.69^{a}	0.77^{a}	0.40^{c}	0.04	0.03
FI	326.1	320.8	347.3	351.7	359.1	305.5	340.0	293.2	1.22	0.14
SR (%)	95.2	93.2	90.5	96.3	92.5	93.5	95.2	92.2	0.74	0.62

BW (g): body weight; WG: weight gain; DWG: daily weight gain; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; PI: protein intake; FI: total feed intake per fish; SR: survival ratio.
SEM = Standard error of the mean.
Means within rows different superscript letters are significantly different (P<0.05).

In the indoor experiment (Paper IV), final body weight (BW) and total weight gain (WG) were lowest for the cassava leaf meal diet (P<0.05), whilst there were no differences in BW and WG between the other diets (Table 12).

In the outdoor experiment (Paper V), the final body weight (BW), total weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) differed between diets (*P=0.01-0.03*). The highest final BW, total WG, DWG and SGR were recorded for the shrimp head meal diet, followed in descending order by the golden apple snail meal and groundnut cake diets, the sweet potato leaf meal and cassava leaf meal diets, and finally the soybean meal diet (Table 12). There were no differences ($P>0.05$) in survival rate, feed utilisation (FCR), protein efficiency ratio (PER) and feed intake (FI) between the reference diet and the test diets, but protein intake (PI) differed between the diets in Papers IV & V. However, fish fed the traditional rice bran and soybean diet had lower protein intake (PI), in addition to poorer protein (PER) and feed utilisation (FCR), than fish on the other treatments (Paper V).

5.5 Carcass and body indices (Papers IV & V)

The proportion of fish fillet at the end of experiment ranged from 29.6 to 39.5% (wet weight basis), with the lowest value for the rice bran diet, whilst there were no differences between the reference diet and the other diets (Table 13).

Table 13. *Percentage of fillet, viscera-somatic index (VSI%), hepato-somatic index (HSI%), kidney and intra-peritoneal fat (IPF%) index in striped catfish* (Pangasianodon hypophthalmus) *fingerlings fed the reference diet (RD) and the test diets*

		Test diets (% of fish meal crude protein replaced)								
Paper IV	RD	Ground-nut cake (25%)	Cassava leaf meal (25%)	Sweet potato leaf meal (20%)	Soy-bean meal (100%)	Golden apple snail meal (100%)	Shrimp head meal (100%)	Rice bran	*SEM*	*P-value*
Fillet	39.5	38.6	36.2	38.3	36.2	37.0	37.2	*	*1.34*	*0.31*
VSI	9.0^{de}	10.0^{cd}	8.5^{e}	9.1^{de}	10.5^{bc}	11.8^{a}	11.2^{ab}	*	*0.64*	*0.02*
HIS	1.7^{c}	2.1^{ab}	1.7^{c}	1.7^{c}	1.8^{bc}	2.1^{a}	2.0^{ab}	*	*0.10*	*0.03*
Kidney	0.5	0.7	0.5	0.5	0.6	0.6	0.4	*	*0.08*	*0.07*
IPF	1.9^{cd}	1.7^{d}	1.3^{d}	1.9^{d}	2.6^{c}	4.5^{a}	3.8^{b}	*	*0.37*	*0.001*
Paper V										
Fillet	38.4^{a}	36.9^{a}	36.1^{a}	36.7^{a}	34.7^{a}	35.1^{a}	37.1^{a}	29.6^{b}	*0.95*	*0.052*
VSI	11.0^{c}	11.1^{c}	10.1^{c}	10.5^{c}	13.1^{b}	14.3^{ab}	13.3^{b}	14.7^{a}	*0.64*	*0.013*
HSI	1.9^{bc}	1.8^{c}	1.9^{bc}	1.7^{c}	1.8^{bc}	2.1^{a}	2.0^{ab}	2.2^{a}	*0.05*	*0.002*
Kidney	0.7	0.7	0.7	0.7	0.8	0.9	0.8	0.8	*0.08*	*0.274*
IPF	2.8^{c}	3.1^{bc}	1.8^{d}	2.2^{cd}	5.6^{a}	5.3^{a}	5.3^{a}	3.7^{b}	*0.25*	*0.001*

SEM = Standard error of the mean.
Means within rows different superscript letters are significantly different ($P<0.05$).

The viscera-somatic index (VSI%), hepato-somatic index (HSI%) and intra-peritoneal fat index (IPF%) index of Papers IV & V differed between treatments, with a range 9.0-13.3%, 1.7-2.1% and 1.3-5.6%, respectively. In general, the highest VSI, HSI and IPF were found for the golden apple snail meal, shrimp head meal, soybean meal and groundnut cake diets. The kidney index ranged from 0.4 to 0.9 and did not differ between treatments (Table 13).

The chemical composition of fillet, kidney and liver did not differ between the reference diet and the test diets ($P>0.05$). The highest CP content was found in fish fillet, followed in descending order by liver (range 131-159 g/kg) and kidney (range 111-155 g/kg). The highest lipid content was found in liver (range 52-207 g/kg) and the lowest in kidney (range 10-36 g/kg) ($P<0.05$). There were no differences ($P>0.05$) in ash content between fillet, kidney and liver.

5.6 Water quality and plankton monitoring

5.6.1 Water quality monitoring

There were no differences between treatments ($P>0.05$) in recorded water quality indicators. Water temperature was 26.6-28.8ºC, pH was 6.5-8.3 and dissolved oxygen (DO) content was 5.4-7.0 mg/L. Total ammonia (TAN), nitrite (NO_2) and nitrate (NO_3) concentrations were low during the experiment. TAN was 0.2-0.4 mg/L, NO_2 was 0.06-1.2 mg/L and NO_3 was 0.03-0.1 mg/L. The chemical oxygen demand (COD) was 2.2-4.0 mg/L and the biochemical oxygen demand (BOD) was 4.8-8.0 mg/L. The concentration of total suspended solids (TSS) was 49.2-71.0 mg/L (Papers II-V).

5.6.2 Plankton monitoring and assessment

Phytoplankton

Four phyla of algae, Chlorophyta (green-algae), Cyanophyta (blue-green algae), Bacillariophyta (diatom belonging to ochrophyta) and Euglenophyta (euglenoids), were recorded in the different experimental conditions of the indoor (Paper IV) and outdoor (Paper V) experiments. The highest incidence (individuals/L) was found for Chlorophyta, with a range from 512-17,622 individuals/L, followed by Bacillariophyta, with a range from 293-5,029 individuals/L. The lowest incidence in the indoor and outdoor experiment was found for Cyanophyta, with a range from 354-3,385 individuals/L, whilst Euglenophyta ranged from 79-3,625 individuals/L. At least six families in Chlorophyta, seven families in Cyanophyta, six families in Bacillariophyta and two families in Euglenophyta were recorded (Papers IV & V).

Zooplankton

Three main zooplankton groups, Copepoda, Cladocera and Rotifera, were recorded during the experiment. Rotifers were the most abundant group, accounting for 61.2% indoors and 65.1% outdoors (range 407-2,292 individuals/L) of the zooplankton, followed by 21.1% outdoors and 33.0% indoors of the Cladocera (range 220-749 individuals/L). The lowest density was found in Copepoda, with 5.8% indoors and 13.9% outdoors (range 38-489 individuals/L). The most common families were Mesocyclops (Copepoda), Moina (Cladocera) and Clolurella, Lecane, Lepadella, Trichocerca (Rotifera) (Papers IV & V).

6 General discussion

6.1 Feed and feeding in small-scale striped catfish farming

Of the catfish pond farms surveyed in An Giang, Dong Thap and Can Tho province, 40-90% used farm-made feeds (Paper I). This is comparable to data reported by Phan *et al.* (2009). The FCR of commercial feed and farm-made feeds ranged between 1.8-2.2 and 2.7-3.1, respectively (Paper I), which was similar to that found in earlier studies on the same fish species from the Mekong Delta (De Silva & Phuong, 2011; Phan *et al.*, 2009). Although the FCR of farm-made feeds was higher than that reported for commercial feeds, most small-scale farmers of striped catfish in pond culture who are using farm-made feeds have low production costs and therefore have higher net profits than if they had used commercial feeds. The cost of producing 1 kg of fish using farm-made feeds is usually 8-10% lower than using commercial feeds (Paper I; (Phan *et al.*, 2009; Hung *et al.*, 2007).

6.2 Potential feed ingredient resources for striped catfish

Feed ingredients for fish diets are chosen for a number of reasons already mentioned, including the nutrient content, cost, availability and physical properties (Hardy & Barrows, 2002).

Broken rice, rice bran, maize, cassava, groundnut (or peanut), sweet potato, soybean, duckweed, golden apple snail, catfish by-product and shrimp head meal are abundantly available throughout the year and have been used in diets for animals and fish in the Mekong Delta of Vietnam (Nguyen *et al.*, 2012; Da *et al.*, 2011; Tram *et al.*, 2011; Thuy, 2010; Khang, 2004; Ngoan, 2000). Broken rice, rice bran and maize meal are primarily useful as cheap energy feedstuffs for striped catfish (Hien *et al.*, 2009; Hung *et al.*, 2003), whereas cassava leaf meal, groundnut cake (or peanut cake), sweet potato leaf meal, soybean meal, duckweed meal, golden apple snail, catfish by-product meal and shrimp head meal constitute the main protein sources.

6.2.1 Plant feed ingredients

The inclusion of rice bran and broken rice in formulated striped catfish diets varies from 30 to 70% in farm-made feeds depending on the farmer's knowledge and fish growth stage (Hung *et al.*, 2007). Cassava, sweet potato, soybean, groundnut and maize are cultivated in the Delta. These crops are important human food crops after rice production and are also important for animal feeds (Paper I; Nguyen *et al.*, 2012; GSO, 2010). In addition, they produce a large and poorly utilised volume of green biomass and potentially useful co-products (from the tubers) that could be used as ingredients in fish feed and animal feed. Cassava leaf meal and sweet potato leaf meal are relatively rich in crude protein, minerals and vitamins and have been used successfully as feed for livestock (Nguyen *et al*., 2012; Hue *et al*., 2010; Phuc & Lindberg, 2001).

Maize is another important feedstuff in aquafeed and it is a good source of carbohydrate and energy for terrestrial farm animals and herbivorous and omnivorous fish species. Its utilisation by animals can be improved by thermal and hydro-thermal treatment (Hertrampf & Piedad-Pascual, 2000; Bombeo-Tuburan *et al.*, 1995). Duckweed (*Lemna* spp.) is an indigenous small aquatic macrophyte that grows rapidly in fish ponds, lakes and canals in the Mekong Delta, and is easy to harvest. It is also considered to be highly nutritious and digestible for livestock and fish due to its high protein, high EAA and low fibre content (Fasakin *et al.*, 1999; Hassan & Edwards, 1992; Culley *et al.*, 1981).

6.2.2 Animal feed ingredients

The golden apple snail is considered a pest in rice fields in Vietnam and can be found in large amounts in most waters in the Mekong Delta. For many years, golden apple snail meat has been used as the main protein source for tiger prawns (*Penaeus monodon*) (Bombeo-Tuburan *et al.*, 1995) and freshwater shrimp (*Macrobrachium* sp.) in An Giang and Dong Thap province, and has also been shown to be well-utilised by African catfish (Phonekhampheng, 2008).

The crustacean by-products from the shrimp industry, including shrimp head meal, crab meal and gammarid meal, are important low cost protein animal protein sources in Vietnam (GSO, 2010; Hardy & Barrows, 2002). They have been identified as good candidates to replace fish meal in diets for aquaculture (Tram *et al.*, 2011; Tibbetts *et al.*, 2006; Köprücü & Özdemir, 2005; Tibbetts *et al.*, 2004) and pigs (Ngoan, 2000).

The earthworm (vermiculture) industry has grown considerably with respect to its role in waste management and the production of earthworm biomass for potential use in the animal feed industry (Rawling *et al*., 2012). Preliminary evidence suggests that earthworm species (*Eisenia foetida* and *Eudrilus eugeniae*) can be used successfully at levels of 20-30% replacement of fish meal in the diet of a number of fish species (Sogbesan & Madu, 2008; Sayed, 1999; Stafford & Tacon, 1985).

Catfish by-product meal is a residue of catfish after filleting, with head and bone, broken meat and skin from the fillet processing factories accounting for 65% of the volume. It has been shown be an excellent source of protein for pigs (Thuy *et al.*, 2011; Tuan, 2010).

6.3 Nutrient digestibility of potential local feeds in striped catfish

Evaluation of the digestible protein and energy value of feed ingredients is critical to the cost-effective formulation of modern aquaculture diets and is also an important part of the process of establishing their nutritional value (Glencross *et al.*, 2007). However, the nutrient values of fish feed depend on the nutrient composition of the individual feed components and the ability of the animal to digest and absorb the nutrients (Falaye & Jauncey, 1999). Together with chemical analysis, digestibility determination may allow a more thorough estimation of the nutritive value of a particular protein and energy source in a complete diet for fish (Plakas & Katayama, 1981). The chemical composition and amino acid profile of test feed ingredients in Papers II & III were in good agreement with previously published data (Nguyen *et al.*, 2012; Tram *et al.*, 2011; Hien *et al.*, 2010; Hue *et al.*, 2010; Phuc & Lindberg, 2001; Hertrampf & Piedad-Pascual, 2000; Phuc *et al.*, 2000). The CP content of the diets studied ranged between 19.4-29.1% (Paper II) and 20.3-35.1% (Paper III)**,** and was within the range (19-30%) required for normal growth rate in *Pangasius* fingerlings (Hung *et al.*, 2002).

Stone (2003) indicated that despite major differences in the anatomy of the digestive tract of fish species, it appears that most fish are efficient in the digestion of protein and energy. The apparent digestibility of protein and energy for common feed ingredients used in aquaculture is relatively high for herbivorous, omnivorous and carnivorous fish species. Striped catfish has quite a high capacity to digest protein and energy from both animal feed ingredients and plant feed ingredients (Papers II & III). The highest AD of DM, CP, OM and energy was found in treatments with soybean meal, groundnut cake, broken rice, shrimp head meal, golden apple snail and catfish by-product meal and earthworm meal, whilst the AD was lower in cassava leaf meal and sweet potato leaf meal. This was in agreement with earlier studies showing that *Pangasius* catfish has the capacity to utilise a large number of alternative plant and animal protein sources (Asdari *et al.*, 2011; Glencross *et al.*, 2011; Phumee *et al.*, 2011; Hien *et al.*, 2010; Hung *et al.*, 2003; Hung *et al.*, 2002).

Earlier studies have reported that the AD of DM, OM and CP is lower in cassava leaf meal than in groundnut cake, soybean meal and shrimp head meal in hybrid catfish and Nile tilapia (Tram *et al.*, 2011; Degani & Revach, 1991). Francis *et al.* (2001) reported that a factor that could potentially reduce the digestibility of a feed ingredient is the presence of anti-nutritional factors. This could be an issue for cassava leaf meal, as cassava (root and foliage) is known to contain cyanogenic glycosides (Phuc *et al.*, 2000), whilst the content of anti-nutritional factors in sweet potato leaf meal and duckweed meal is considered negligible (Dongmeza *et al.*, 2009; Iqbal, 1999). However, it has been shown that the content of cyanogenic glycosides in fresh cassava leaves is reduced by more than 80% by sun-drying (Hue *et al.*, 2010; Phuc & Lindberg, 2001).

Earlier studies have shown that *Pangasius* catfish species have the capacity to digest and utilise diets with a high content of carbohydrates (Phumee *et al.*, 2011; Hung *et al.*, 2003). The high AD of DM and GE in broken rice, maize meal and rice bran (Papers II & III) confirm these findings and support their use as easy-available carbohydrate sources in feed formulation for striped catfish depending on availability and price.

According to Hien *et al.* (2010), striped catfish utilise DM and protein in feedstuffs of animal origin more efficiently than DM and CP in feedstuffs of plant origin, owing to a limited ability to digest non-starch polysaccharides. However, this could not be confirmed in the present study, as the AD_{DM}, AD_{CP}, and AD_{GE} in soybean meal and groundnut cake were quite similar to the AD in shrimp head meal, catfish fish meal and golden apple snail meal. Hanley (1987) reported a higher digestibility for soybean meal than for fish meal in omnivorous tilapia. Wilson & Poe (1985) reported that feedstuffs such as soybean and peanut meal were less digestible than fish meal in channel catfish, but the AD of CP of plant protein was much higher than that of animal protein.

In general, the apparent digestibility of DM, OM, CP and energy and EAA was high for all test ingredients (Papers II & III). The lower digestibility values observed for cassava leaf meal, sweet potato leaf meal, duckweed meal (Paper II) and earthworm meal (Paper III) may impose limitations in feed formulation for the possible replacement of fish meal in the diet of striped catfish. However, some earlier studies reported the successful use of duckweed as a feed ingredient for fish species such as tilapia, Thai silver barb, common carp and indigenous catla (Azim & Wahab, 2003; Fasakin *et al.*, 1999; Mbagwu & Adeniji, 1988). Earthworm meal has also been used to improve growth performance of tilapia, rainbow trout, wundu catfish and hybrid catfish (Rawling *et al.*, 2012; Sogbesan & Madu, 2008; Tram *et al.*, 2005; Sayed, 1999; Stafford & Tacon, 1985). Cassava leaf meal and sweet potato leaf meal have also been successfully used for livestock feeds (Nguyen *et al.*, 2012; Hue *et al.*, 2010; An, 2004; Khang, 2004; Phuc & Lindberg, 2000), as well as for Nile tilapia and hybrid catfish fish (Tram *et al.*, 2011). The lower digestibility of CP and energy may limit the possibility of using these feed ingredients as replacement for fish meal.

The average digestibility of most EAA in selected feed ingredients (Papers II & III) was high, with a range from 70 to 92% in striped catfish, indicating a high protein quality of these feedstuffs. The average AD of EAA and individual EAA was high for all dietary treatments and similar between diets, except for the diet with cassava leaf meal (Paper II), rice bran and earthworm meal (Paper III), where the AD of EAA was reduced. This suggests that striped catfish have a limited capacity to digest EAA in these feed ingredients. Low AD of individual EAA in cassava leaf meal has also been reported for Nile tilapia (Tram *et al.*, 2011). Low AD of EAA in rice bran has been reported for tilapia (Guimarães *et al.*, 2008) and rainbow trout (Gaylord *et al.*, 2010). The low AD of EAA in rice bran could be related to high content of fibre, lipids and anti-nutritional compounds (Guimarães *et al.*, 2008; Beyer *et al.*, 1983).

In general, the AD values of most individual EAA in the test ingredients were high compared with the AD values for EAA in common feed ingredients for channel catfish (Wilson *et al.*, 1981) and much higher than those reported for plant protein fed to the carnivorous fish species dourado (*Salminus brasiliensis*) (Borghesi *et al.*, 2009).

6.4 Replacing fish meal with locally available feed resources

The low profitability of the striped catfish industry demands that diet specifications be improved to reduce feed costs and increase productivity (Glencross *et al.*, 2011). In addressing this, some of the more unconventional feed ingredients in the Mekong Delta may have the potential to be used as aquafeeds for striped catfish and other omnivorous fish species. Of these, sweet potato leaf meal, cassava leaf meal, groundnut cake, soybean meal, shrimp head meal and golden apple snail meal appear to be potentially useful protein-rich, low cost feed ingredients that can be used to replace from 20% up to 100% of fish meal in the diet of growing striped catfish (Papers IV & V) without negative effects on fish growth performance and feed utilisation. However, in fish fed cassava leaf meal diets (20% fish meal replacement) there was a reduction in final BW, daily weight gain (DWG) and specific growth rate (SGR) and an increase in food conversion rate (FCR) compared with fish fed the other diets, whilst there were no differences in BW and WG between the other diets. This could be due to lower digestibility of CP and EAA in cassava leaf meal than in the other test ingredients (Paper II) and would be expected to have a negative impact on fish performance. Moreover, cassava leaf meal contains cyanogenic glycosides and although the content in fresh cassava leaves is reduced by more than 80% by sun-drying and cooking (Hue *et al.*, 2010; Phuc *et al.*, 2000), there may still be an anti-nutritional effect of feeding cassava leaf to striped catfish. In Nile tilapia (*O. niloticus*), cassava leaf meal significantly reduced growth performance, with the reduction increasing linearly with increasing cassava leaf meal inclusion (20, 40, 60 and 100%) (Ng Keong & Wee, 1989). However, Bureau *et al.* (1995) reported that depending on the carbohydrate sources in the diet (cassava-based or maize-based), up to 20% cassava leaf meal could be included in the diet of African catfish without negative impacts on growth performance.

In general (Papers IV & V), the final BW, WG, DWG and feed utilization were numerically highest for diets with shrimp head meal (100% replacement of fish meal) and golden apple snail meal (100% replacement of fish meal), followed by diets with groundnut cake (25% replacement of fish meal) and sweet potato leaf meal (20% replacement of fish meal). However, fish growth performance and feed utilisation on diets with soybean meal (100% replacement of fish meal) showed different responses indoors and outdoors. Whilst fish growth performance and feed utilisation of the soybean diet were good indoors compared with the other treatments (Paper IV), the performance outdoors (Paper V) was quite poor compared with the other treatments. Soybean meal has been shown to induce disturbed gut function in fish (Nordrum *et al.*, 2000) due to the presence of anti-nutritional factors. Moreover, soybean meal has a low content of sulphur-containing amino acids, which may also be a limiting factor for growth and can have negative impacts on feed utilisation. Phumee *et al.* (2011) also reported that the growth performance of juvenile striped catfish was decreased and that feed utilisation deteriorated with increasing soybean meal inclusion in the diet. The difference in response between indoor (Paper IV) and outdoor (Paper V) striped catfish in this thesis could be due to the markedly higher growth performance (5-fold higher) in outdoor conditions. This may have increased the sensitivity to disturbing factors in the feed, such as the anti-nutritional factors present in soybean meal. This indicates that inclusion of soybean meal has to be restricted to avoid negative impacts on fish performance.

The high growth performance and feed utilisation of shrimp head meal and golden apple snail meal could be due to their similar nutritional value as fish meal, as reflected in the quite similar digestibility of nutrient of DM, CP, OM, GE and EAA (Paper III). Earlier studies have shown high utilisation of shrimp head meal, shrimp processing by-product meal and shrimp head silage in mono-gastric animals and have indicated that these feed ingredients are potentially useful for pigs (Fanimo *et al.*, 2004; Ngoan, 2000) and ducks (Dong, 2005). Studies on African catfish and red tilapia (*Oreochromis niloticus* x *O. mossambicus*) found that replacing fish meal with 30% fermented shrimp head meal or 50% shrimp head meal in the diet did not adversely affect growth performance (Phonekhampheng, 2008; Nwanna *et al.*, 2004; Rachmansyah *et al.*, 2004; Nwanna, 2003). In the diet of Nile tilapia, it is reported to be possible to completely replace fish meal with shrimp head meal without negative impacts on growth performance (Oliveira Cavalheiro *et al.*, 2007). Moreover, golden apple snail meal has been used for many years as the main protein source for tiger shrimps (*Penaeus monodon*) (Bombeo-Tuburan *et al.*, 1995), giant freshwater prawns (Jintasataporn *et al.*, 2004) and tilapia (Catalma *et al.*, 1991).

Groundnut cake (groundnut by-product) has been successfully used as a feed ingredient for vundu (*Heterobranchus longifilis*) species at 10% replacement of fish meal in the diet (Ovie, 2010). Jackson *et al.* (1982) reported that groundnut cake can replace 50% of fish meal in the diet of tilapia (*Sarotherodon mossambicus*) without negative effects on growth performance, whilst at higher levels growth rate decreases rapidly. The poor performance of the groundnut cake diet in this thesis may be due to low levels of methionine and lysine. In addition, the presence of aflatoxin and anti-nutritional compounds (Ovie, 2010; Jackson *et al.*, 1982) are other factors that need to be considered.

One of the most striking findings in this thesis work was the marked difference in growth performance and feed utilisation between striped catfish fingerlings raised indoors and outdoors. The final BW and daily weight gain (DWG) were 3.2 to 6-fold higher under outdoor culture conditions (Paper V) than under indoor culture conditions (Paper IV), although the fish were fed the same diets. The feed conversion ratio was also better outdoors, resulting in about 0.2-0.7 kg less feed per kg body weight gain under the outdoor culture conditions. Similarly, Shahidul Islam (2002) reported that *Tor putitora* (Hamilton) fed the same diets had about 3-4 times higher net increase in length and final BW when raised outdoors compared with indoors. In this thesis, this effect was most likely related to the high content of phytoplankton and zooplankton in water, which were most likely utilised by the fish in addition to the feed provided.

7 General conclusions and applications

7.1 Conclusions

- Feed is the major cost in catfish fish production in the Mekong Delta, and small-scale fish farmers are searching for cheaper feeds as an alternative to expensive trash fish and fish meal and commercial feeds. Locally available plant and animal feed sources are utilised sub-optimally at present, but have the potential to provide nutrients and energy to fish cultivation if they are used in suitable combinations.
- Striped catfish appears to have a high capacity to digest dietary components and amino acids from locally available plant and animal feed resources.
- Selected feed ingredients such as shrimp head meal, golden apple snail meal, catfish by-product meal, groundnut cake and soybean meal could potentially be used to replace fish meal without any direct impact on diet digestibility of DM, crude protein, gross energy and essential amino acids.
- The apparent digestibility of dietary components was lower in duckweed meal, earthworm meal, cassava leaf meal and sweet potato leaf meal, which may impose limitations in feed formulation for the possible replacement of fish meal.
- Rice bran, broken rice and maize meal contain easily available carbohydrates, in addition to fibre, and are well utilised in striped catfish. They are potential energy sources in feed formulation, and could be used alone or in combination, depending on availability and price.
- Shrimp head meal, golden apple snail meal, catfish by-product meal, groundnut cake, cassava leaf meal and sweet potato leaf meal appear to be well-utilised protein-rich feed ingredients and could potentially be used to replace fish meal protein without compromising growth, feed utilisation and carcass traits in fish. However, the degree of replacement will depend on the properties of the individual feed ingredient.

7.2 Implications and further research

7.2.1 Implications

Sustainable small-scale striped catfish farming systems should require minimal external inputs and should efficiently use locally available feed resources to improve fish production and reduce feed costs. This thesis shows that there is great potential for using low cost supplementary feeds, including non-conventional feeds and agricultural by-products such as shrimp head meal, golden apple snail meal, catfish by-product meal, soybean meal, groundnut cake, cassava leaf meal and sweet potato leaf meal, to replace expensive fish meal and/or trash fish in diet formulations.

It is suggested that an acceptable nutrient balance in striped catfish diets can be achieved with respect to protein and amino acid requirements by replacing or reducing the proportion of fish meal/trash fish, by reducing the proportions of energy-rich feed ingredients (such as rice bran, broken rice and maize mail) and by increasing the proportion of available low-cost protein-rich feed ingredients, such as shrimp head meal, golden apple snail meal, catfish by-product meal, groundnut cake, cassava leaf meal and sweet potato leaf meal.

7.2.2 Future research

As the striped catfish industry continues to develop, there will be an increasing need to use alternative raw materials in catfish diets. In order to gain acceptance in the industry for the use of alternative feed resources to trash fish and fish meal, and to implement new feeding into practice, the economic benefits have to be demonstrated and communicated. Thus, studies should be performed on grow-out fish to confirm the present findings and to quantify the impact on fish performance and product (fillet) quality traits, on the economics of production and on environmental water quality.

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