Evaluation of Local Feed Resources for Hybrid Catfish (*Clarias macrocephalus* x *C. gariepinus*) in Smallholder Fish Farming Systems in Central Vietnam

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Cover: Cassava leaf and shrimp by-product as feed for hybrid catfish
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Abstract

The aim of this thesis was to examine the current feeding situation for fresh water fish in Central Vietnam, to evaluate the potential nutritive value of locally available feed resources for hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and Nile tilapia, to determine the dietary requirement of lysine for hybrid catfish, to estimate the requirements for the other essential amino acids (EAA) by using ideal protein concept, and finally to evaluate the suitability of cassava leaf meal and shrimp head meal as a partial replacement for fish meal in the diet, without or with lysine supplementation, for hybrid catfish fingerlings.

The survey indicated that, in total, 22 feed ingredients were used by the farmers. The main ingredients were cassava root meal, rice bran, cassava residue, groundnut meal, soybean meal and fish meal. Furthermore, several more unconventional feedstuffs were also commonly used, such as cassava leaves, coconut meal, shrimp head meal, sesame husk and squid by-product. The combination of ingredients used in farm-made fish feeds varied among farms and districts leading to a large variation of nutrient composition and energy content. The fish yield varied among districts and ranged from 0.8 to 6.5 t ha^{-1}. The digestibility trial showed that the apparent digestibility (AD) of dry matter (DM), organic matter (OM) and crude protein (CP) in cassava leaf meal was significantly lower than in groundnut meal, soybean meal, sesame husk meal and shrimp head meal in both hybrid catfish and Nile tilapia. The AD of DM and OM in cassava leaf meal was higher in hybrid catfish than in Nile tilapia. Most EAA in the selected feedstuffs were equally well utilized by the two fish species. In the third experiment, the dietary lysine requirement of hybrid catfish fingerlings was found to be 56 g kg^{-1} of CP, corresponding to 16.8 g kg^{-1} of dry diet. In the feeding trial with hybrid catfish, replacing fish meal with shrimp head meal had no effect on final weight (FW) and specific growth rate (SGR), while replacing fish meal with cassava leaf meal led to impaired FW and SGR. Supplementing cassava leaf meal and shrimp head meal diets with lysine improved FW and SGR.

Keywords: amino acids, cassava leaf meal, *Clarias macrocephalus* x *C. gariepinus*, digestibility, fish meal, ideal protein, lysine, Nile tilapia, shrimp head meal, smallholdings.

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Dedication

To my parents
My husband Nguyễn Khoa Huy Sơn
My son Nguyễn Khoa Gia Cát

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 Nguyen Duy Quynh Tram, Le Duc Ngoan, Le Thanh Hung, Ogle, B. and Lindberg, J. E. (2010). Feeding and production of fresh water fish in smallholdings in Central Vietnam. (Submitted).

II Nguyen Duy Quynh Tram, Le Duc Ngoan, Le Thanh Hung, Lindberg, J. E. (2010). A comparative study on the apparent digestibility of selected feedstuffs in hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* (doi: 10.1111/j.1365-2095.2010.00813.x).

III Nguyen Duy Quynh Tram, Le Duc Ngoan, Lindberg, J. E. (2010). Dietary amino acid requirements of fingerling hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*). (Submitted).

IV Nguyen Duy Quynh Tram, Le Duc Ngoan, Lindberg, J. E. (2010). Influence of partial replacement of fish meal with shrimp head meal or cassava leaf meal, without or with lysine supplementation, on growth performance of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) (manuscript).

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Abbreviations

A/E	Essential amino acid content / total essential amino acid content, including cysteine and tyrosine
AA	Amino acids
AD	Apparent digestibility
BW	Body weight
CMC	Carboxymethyl cellulose
CF	Crude fibre
CP	Crude protein
DM	Dry matter
DO	Dissolved oxygen
EAA	Essential amino acids
EE	Ether extract
FER	Feed efficiency ratio
GE	Gross energy
HCN	Hydrogen cyanide
LWG	Live weight gain
NDF	Neutral detergent fibre
OM	Organic matter
PER	Protein efficiency ratio
SGR	Specific growth rate

1 Introduction

Worldwide, aquaculture is developing, expanding, and intensifying. In Southeast Asia, aquaculture output has been increasing rapidly, especially during the last 15 years. Recently, the production from capture fisheries has leveled off, and most of the main fishing areas have reached their maximum potential, and therefore, in order to meet the growing global demand for aquatic food, aquaculture appears to have the potential to make a significant contribution to this increasing demand (FAO, 2006).

Vietnam is a tropical country with a high rainfall and with around 1.7 million hectares of inland water suitable for aquaculture development. Production of fresh water aquaculture has been rapidly increasing in recent years, reaching around 1.86 million tonnes in 2008, contributing to about 75 % of the total aquaculture production (GSO, 2010). The growth rate of fresh water aquaculture production was 8 % per year in the period 1985-1998 and the plan of the Government is to achieve two million tonnes by 2010. This growth of fresh water aquaculture production is mainly expected to come from small-scale aquaculture (Hung, 2004; Tung, 2000).

However, in order to achieve this target, the sector will face significant challenges (FAO, 2006). Among these, the quantity and quality of feed is a major constraint. Feed is the principle cost in the cultivation of most fish species and this cost has tended to increase with the rising price of fish meal. The feed cost had increased by 73 % in 2008 compared with the price in 2005 (Hishamunda *et al.*, 2009; Rana *et al.*, 2009; Edwards *et al.*, 2004). It was reported that during late 2008, feed prices had increased by over 30 % on average in many Asian countries, while the prices of aquaculture products had remained the same. This is a challenge for thousands of small-scale producers that form the backbone of the aquaculture sector (Rana *et al.*, 2009).

In Vietnam, small-scale aquaculture is an important sub-sector in the rural economy that contributes significantly to the nation's food security, family nutrition, economy and employment, especially in the rural areas. Fish is a traditional food of the Vietnamese people (Tu & Giang, 2002), and about 30 % of the total animal protein intake of the Vietnamese people comes from fish. Small-scale aquaculture, which presently contributes over 70 % of the national aquaculture production, is a potential resource for improving household food security and supplementary family income for the rural poor (Tung, 2000). Therefore, an improved feeding system incorporating locally available feed resources for smallholder fish farming would be very useful for the farmers in Central Vietnam.

Objectives of the study:

- To examine and evaluate the current feeding situation for fresh water fish in Central Vietnam and to provide a database on locally available feed resources for fish.
- To evaluate the potential nutritive value of local feed resources, readily available in Central Vietnam, as feed ingredients for hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*).
- To determine the dietary requirement of lysine for hybrid catfish and to estimate the requirements for the other essential amino acids (EAA) by using the ideal protein concept.
- To evaluate the suitability of cassava leaf meal and shrimp head meal as a partial substitute for fish meal in the diet, without or with lysine supplementation, for hybrid catfish fingerlings.

Hypotheses of the study:

- Nile tilapia is superior to hybrid catfish in digesting unconventional feedstuffs.
- The dietary requirement of lysine and other EAA of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) is comparable with that of the African catfish (*C. gariepinus*).
- Growth performance of hybrid catfish fingerlings will not be negatively affected by partially replacing fish meal with shrimp head meal.
- Supplemental lysine in diets where fish meal is replaced by cassava leaf meal or shrimp head meal will improve growth performance of hybrid catfish fingerlings.

2 Background

2.1 The role of fresh water aquaculture in food supply and rural development in Vietnam

Vietnam remains a predominantly agrarian country with 70.4 % of the total population living in rural areas and two-thirds of them depending on farming for a living (GSO, 2010).

Rural aquaculture is an important sub-sector of Vietnam that contributes significantly to national and local food security, family nutrition, economy and rural employment, especially in remote areas. About 30 % of the total animal protein intake by the Vietnamese people comes from fish. Aquaculture smallholdings, which contribute to over 70 % of national aquaculture production, are a potential resource for improving household food security and supplementing family income of rural poor communities (Luu, 1999).

In 2001, more than half a million people were employed in aquaculture in Vietnam. Aquaculture thus is promoted by Vietnamese policy-makers because it provides rural employment, thereby diversifying rural economies and discouraging rural-urban migration. Vietnamese policy-markers, with plans to double aquaculture output by 2010 (to two million tonnes), expected that by 2010 three million people (at least 50 % of them women) will be employed in aquaculture. This is also a sector with promising export potential. Vietnam forecasts that the value of aquaculture exports will increase and earn US $3 billion by 2010. Aquaculture is also a sector for the poor, who have few alternatives and no resources. In Vietnam, aquaculture does not typically attract the wealthy, who perceive aquaculture risks as high and with high investment required. The wealthy prefer offshore fishing and trading. Aquaculture therefore is attractive to policy-makers because it absorbs the poor (FAO, 2009a).

2.2 Development of fresh water aquaculture in Vietnam

With a total of 1 990 000 ha of water surface, including 127 000 ha of small size ponds, 340 000 ha of reservoirs, 580 000 ha of low land and flood plains, 619 000 ha of tidal flats and 350 000 ha of bays and lagoons, Vietnam is considered as a country with enormous potential for aquaculture development (Luu, 1999).

There are various farming systems of fresh water fish culture existing in Vietnam, including household pond culture, rice cum fish, the integration of fish culture with livestock farming and cropping - VAC system (V-Garden, A-pond and C-piggery), reservoir fish culture and wastewater aquaculture. Rice cum fish is seen mainly in low-land areas of coastal provinces, with the cultured area being relative large, and the minimum area being about 1000 m^2. The pond size at household level is relatively small, and usually varies from 200 to 500 m^2. Fish stocking density is relatively low and varies from 0.5 to 1.5 fish per m^2, and the average fish productivity of fish pond culture is about 1.5 tonnes per ha and varies from region to region. The VAC is a very popular farming practice in the northern and central parts of Vietnam. The pig sties are usually built in pond edges, with each sty having at least 5-6 pigs. Besides the waste matter discharged into the pond, fish are usually also fed with vegetables and commercial feed. Productivity of this model is relatively high with a mean fish yield of about 3 tonnes per ha (Ministry of Fisheries & World Bank, 2006).

According to the General Statistics Office of Vietnam (GSO, 2010), in 2008, total fresh water aquaculture area of Vietnam was 338 800 ha, which was 10.2 % higher than that in 2007. The total fresh water culture production was about 1.86 million tonnes in 2008 and increased to about 1.95 million tonnes by 2009 (Figure 1).

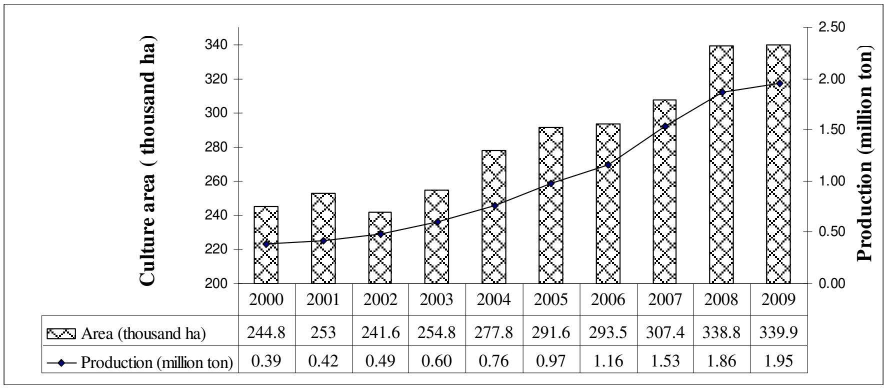

Figure 1. Production and area of fresh water fish culture from 2000-2009 (Source: General Statistics Office of Vietnam, 2010)

2.3 Fish species used in smallholder fish farming

Vietnam has about 30 fish species from six families cultured in inland aquaculture (Table 1). Fish species that have been selected for aquaculture are highly tolerant of confined conditions in ponds, cages, reservoirs, and are resistant to diseases at high stocking density (Hung, 2004).

Table 1. *Number of fish species cultured in Vietnam grouped under families (Source: Hung, 2004)*

Family	Number of species	Indigenous species	Introduced species
Cyprinidae	12	03	09
Pangasiidae	04	04	0
Ophicephalidae	03	03	0
Cichlidae	03	0	03
Anabantidae	04	02	02
Clariidae	03	02	01
Total	29	14	15

In general, fish are divided into four groups with different feeding habits: herbivorous, filter feeders, omnivorous and carnivorous (Table 2). The most favoured cultured fish species in Vietnam are omnivorous, with more than 70 % of the total, since they can feed on a large range of materials such as rice bran, fresh manure, cooked rice, trash fish, vegetables, restaurant leftovers, etc. Moreover, the fish are highly adaptable to changes in feed and feeding. The carnivorous cultured species are all indigenous species and have a high market value. The filter feeders are nearly all exotic species.

Table 2. *Feeding behavior of cultured fish species in Vietnam (Source: Hung, 2004)*

Feeding behavior	Fish species
Herbivorous	Giant gourami (*Osphronemus gorami*), grass carp (*Ctenopharyngodon idellus*), silver barb (*Barbodes gonionotus*) and *Spinibarbus denticulatus*
Filter feeders	Bighead carp (*Aristichthys nobolis*), silver carp (*Hypophthalmichthys molitrix*), Nile tilapia (*Oreochromis niloticus*) and red tilapia (*O.* sp.)
Omnivorous	Rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), red finned barb (*Barbodes altus*), kissing gourami (*Helostoma temminckii*), river catfish (*Pangasius bocourti*, *P. hypophthalmus*) and walking catfish (*Clarias gariepinus*, *C. macrocephalus*)
Carnivorous	Sand goby (*Oxyeleotris marmoratus*), spotted featherback (*Notopterus notoptarus*) and snakeheads (*Channa gachua*, *C. micropeltes*, *C. striata*)

Asian catfish

The most important aquaculture species of Asian catfish is *Clarias batrachus* (family claridae). Another important *Clarias* species is *C. macrocephalus*, which is preferred by the consumer for its appearance and the quality. The ability to adapt to poor environmental conditions, makes *Clarias* valuable for small and large-scale rural fish farming (Pillay & Kutty, 2005).

African catfish

Clarias gariepinus is classified as omnivorous and has the ability to feed on a variety of feedstuffs. It has good growth and survival in poorly dissolved oxygen water, and is thus an attractive fish for rural aquaculture (Pillay & Kutty, 2005). It has been suggested that the African catfish can digest a high animal protein diet more efficiently than a plant protein diet (Degani & Revach, 1991).

Hybrid catfish (Clarias macrocephalus x *C. gariepinus)*

The hybrid Asian-African catfish is a cross between a female Asian catfish, *Clarias macrocephalus*, and a male African catfish, *C. gariepinus*. The culture of this fish is rapidly gaining in popularity in Southeast Asia due to its rapid growth, resistance to disease, the possibility for high stocking density and excellent meat quality (Ng & Chen, 2002; Jantrarotai *et al.*, 1998).

Little is known about the nutrient requirements of this hybrid catfish. The fish can perform well on diets containing raw carbohydrates (CHO) from broken rice, ranging from 37 to 50 % of the diet, with a lipid (L) content ranging from 4.4 to 9.6 or a CHO/L ratio of 3.8-11.2 (Jantrarotai *et al.*, 1994). Diets containing 35 % protein and 13.6 MJ DE kg^{-1} gave the best performance (Jantrarotai *et al.*, 1998).

Tilapia

Tilapia are farmed in at least 85 countries because of many desirable qualities, such as the ability to survive and grow in shallow and turbid water, performs well in low-input extensive systems as well as in high-input intensive systems, is highly resistant to disease and parasites in comparison with the other cultured fish species (De Silva & Davy, 2010). Tilapia is regarded as an opportunistic omnivorous and herbivorous feeder. Nile tilapia is the most important farmed species within the 16 tilapia species in the world (El-Sayed, 2006). Practical diets for grow-out tilapia usually contain 25-30 % CP. However, for fish cultured in ponds access to natural foods that are rich in protein will allow dietary protein levels as low as 20-25 % without negative impact on performance (Shiau, 2002).

2.4 Current status and constraints of feed and feeding for inland aquaculture in Vietnam

Feed and feeding in inland aquaculture vary due to differences in feeding behaviour of the cultured fish species, and also depend on culture system, from extensive systems on a small scale to intensive floating cage culture. In the extensive system, farmers tend to feed their fish according to the availability of feeds, using raw materials or home made feeds.

The omnivorous fish are fed on rice bran, broken rice, maize, cassava root meal, trash fish and fish meal. Of these, rice bran usually comprises two-thirds of the diet at the grow-out stage. The high carbohydrate and low protein diets give a low growth rate. Home-made feeds are often made in a wet form, giving a food conversion ratio of about 3 to 4. Feeds for carnivorous fish are heavily dependent on trash fish, which is a limited resource and supply is unstable.

The strategies in feed and feeding for inland aquaculture in Vietnam are (i) alternative feeds for carnivorous species to replace or reduce the dependence on trash fish; (ii) development of supplementary feed for herbivorous fish; (iii) identification of locally available ingredients from which home-made feed can be prepared for omnivorous and carnivorous fish (Hung, 2004; Luu, 1993).

2.5 Amino acid requirements in fish

2.5.1 Qualitative amino acid requirements

Traditionally, absolute amino acid requirements in terrestrial animals have been measured in dose-response studies. Although alternative methods have been applied, in general recommendations have been based on body weight gain response. Amino acid requirements for a number of fish species have been measured by similar methods (Cowey & Luquet, 1983).

All studies on finfish to date have shown that they need the same ten EAA as most other animals: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (NRC, 1993).

2.5.2 Lysine requirement

Lysine requirement values for fish are shown in Table 3. In general, lysine appears to be the first limiting amino acid in feedstuffs commonly used in formulating feeds for fish. Therefore, more requirement values have been reported for this amino acid. The requirement appears to range from 4 to 5 % of protein for most fish species.

2.5.3 A/E ratios and the ideal protein concept

Arai (1981) introduced the concept of using A/E ratios [(essential amino acid content / total essential amino acid content, including cysteine and tyrosine) x 1000] of whole-body coho salmon fry to formulate test diets for this fish. Fish fed casein diets supplemented with AA to simulate the A/E ratios of whole-body tissue showed much improved growth and feed efficiency. The same growth rates were observed with a 33 % CP diet containing casein plus AA and a 40 % CP diet containing casein alone. Ogata *et al.* (1983) have also used the A/E ratios concept to design test diets for cherry and amago salmon fry and obtained similar results as Arai (1981) for both species.

Cowey & Tacon (1983) observed a strong correlation when the essential amino acid requirement pattern was regressed against the essential amino acid composition of whole body protein. Cowey & Luquet (1983) also discussed the apparent relationship between dietary amino acid requirements of fish and the essential amino acid composition of fish muscle tissue. A/E ratios for channel catfish whole body protein were also calculated by the method of Arai (1981) and found to be highly correlated (r=0.96) with the essential amino acid requirement pattern for channel catfish. These data indicated that the whole body essential amino acid patterns can serve as a valuable index to confirm amino acid requirement data as determined by growth studies and to formulate test diets for those species where requirement data are not available (Wilson, 1985).

Table 3. *Lysine requirement of some fish species (Adapted from Wilson, 2002)*

Fish species	Requirement[a]	Based on
African catfish	5.7	Growth studies
Atlantic salmon	3.2-6.1	Growth studies
Blue tilapia	4.3	Growth studies
Catla	6.2	Growth studies
Channel catfish	5.0-5.1	Growth studies
Chinook salmon	5.0	Growth studies
Clarias hybrid	4.8	Growth studies
Coho salmon	3.8	Growth studies
Common carp	5.7	Growth studies
	5.3	Protein accretion
European sea bass	4.8	Growth studies
Gilthead sea bream	5.0	Growth studies
Hybrid striped bass	4.0	Growth studies
Japanese eel	5.3	Growth studies
Japanese flounder	4.6	Growth studies
Milkfish	4.0	Growth studies
Mozambique tilapia	4.1	Growth studies
Nile tilapia	5.1	Growth studies
Rainbow trout	3.7-6.1	Growth studies
	5.5	Protein accretion
Red drum	4.4-5.7	Growth studies
	5.7	A/E ratio[b]
Red sea bream	4.4	Growth studies
Rohu	5.7-5.9	Growth studies

[a] requirements are expressed as percentage of protein

[b] (Essential amino acid content / total essential amino acid content including cysteine and tyrosine x 1000)

A/E ratios have been used as a means of estimating the requirements of all EAA when only one is known by relating the A/E ratio of each essential amino acid to that of the A/E ratio of the known amino acid times the requirement value for the known amino acid (Moon & Gatlin III, 1991). This technique has also been used by Forster & Ogata (1998) to estimate the AA requirements of the Japanese flounder and red sea bream.

The ideal protein concept uses lysine as a reference amino acid, with the requirements for all other EAA expressed as a percentage of lysine. Lysine was chosen because lysine is normally the first limiting amino acid in most feedstuffs. Lysine analysis in feedstuffs is straightforward and dietary lysine is used only for protein accretion (Emmert & Baker, 1997). Thus if one knows the dietary lysine requirement and the whole-body amino acid composition of an animal, then one should be able to estimate the dietary requirement for the remaining EAA of the animal relative to the lysine requirement. This procedure is much less time-consuming and less costly than determining the amino acid requirements of the fish by conventional methods (Wilson, 2002).

2.6 Alternative protein sources for fish

The rapid expansion of aquaculture, along with improvements in fish culture techniques have increased the demand for fish feeds that depend on fish meal and fish oil as the major dietary components because of their ideal nutritional quality. The use of various alternative proteins as a replacement for fish meal has been practiced for many years (Watanabe, 2002). To be a viable alternative for fish meal, a candidate ingredient must possess certain characteristics, including nutritional suitability, ready availability, and ease to handle, and store and be affordable (Naylor *et al.*, 2009).

2.6.1 Terrestrial plant-based proteins

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, favorable amino acid profile, high nutrient digestibility, and reasonable palatability.

A number of published reports are available regarding the suitability of plant protein feeds as alternative protein sources in fish feeds (Nyina-Wamwiza *et al.*, 2010; Fournier *et al.*, 2004; Kaushik *et al.*, 2004; Bureau *et al.*, 1995; Gomes *et al.*, 1995; Kaushik *et al.*, 1995; Hossain & Jauncey, 1989b; Hossain & Jauncey, 1989a; Ng & Wee, 1989). The range of plant feedstuffs in aqua-feeds currently include barley, canola, corn gluten, cottonseed, peas, soybean, sunflower meal, rapeseed meal and leaf protein (Naylor *et al.*, 2009).

Inclusion of plant protein above 25-50 % of the total diet frequently results in reduced growth and/or high mortalities attributed to an improper balance of EAA, a reduction in the digestibility of lipid and energy, the presence of anti-nutritional factors and/or poor palatability (Francis *et al.*, 2001; Fagbenro, 1999; Balogun & Ologhobo, 1989).

However, it has been observed that common processing techniques, such as different cooking methods, soaking, drying, wet heating and adding feed supplements, reduce the concentration of anti-nutritional factors in plant feeds and improve the feed intake (Rehman & Shah, 2005; Francis *et al.*, 2001; Gomes da Silva & Oliva-Teles, 1998; Balogun & Ologhobo, 1989).

Relative to fishmeal, plant feedstuffs generally have more indigestible organic matter (OM), in the form of insoluble carbohydrate and fibre, leading to higher levels of excreta and waste (Naylor *et al.*, 2009).

2.6.2 Rendered terrestrial animal products

Rendered animal protein, such as meat and bone meals, poultry by-product meal, and blood meal are readily available, economical sources of protein. Compared with plant protein, animal by-product meals have a more complete amino acid profile, and some of them contain high levels of available lysine and phosphorus. The digestibility of these products has increased over the last 30 years to 80-90 % because of improved processing techniques. Moreover, they are significantly less expensive per kg of CP than fish meal (Naylor *et al.*, 2009).

2.6.3 Seafood by-products

The use of seafood by-products is another avenue for reducing aquaculture's dependence on fish meal. With the exception of fish silage, little attention has been given to the commercial potential of fisheries by-products, including fish protein concentrate and hydrolysates, shrimp meal, krill meal and squid meal as partial or total protein sources for fish (El-Sayed, 1999). El-Sayed (1998) found that shrimp meal (51.7 % CP) can be used as a total fish meal alternative for fingerling tilapia (*O. niloticus* L.) without significant loss in weight gain and feed efficiency. On the other hand, shrimp meal is not well digested by humpback grouper (*Cromileptes* *altivelis*) and can not replace more than 10 % of the fish meal in the diet (Rachmansyah *et al.*, 2004).

2.7 Digestibility in fish

2.7.1 Nutrient digestion

Digestion is the process by which food in the digestive tract is split into simpler compounds capable of passing through the intestinal wall to be absorbed in the blood stream. This task is performed primarily by the digestive enzymes. The digestive enzymes, which are secreted into the lumen of the alimentary canal, originate from the oesophageal, gastric, pyloric caecal and intestinal mucosa and from the pancreas (De Silva & Anderson, 1995).

Protein digestibility

The digestion coefficients for protein in protein-rich feedstuffs are usually in the range of 75 to 95 %. Protein digestibility tends to be depressed as the concentration of dietary carbohydrates increases (NRC, 1993). Increased amounts of dietary lipids have produced increased protein digestibility (Takeuchi *et al.*, 1978). The negative effect of CF has been reported for many fish species (Ferraris *et al.*, 1986; Wang *et al.*, 1985; Hilton *et al.*, 1983).

Lipid digestibility

Digestibility of lipid ranges from 85 to 95 % in most fish species (NRC, 1993). Long-chain fatty acids exhibit a higher digestibility than short-chain fatty acids. Polyunsaturated fatty acids such as 20:5 or 22:6 acids are up to 100 % digested by rainbow trout (Austreng *et al.*, 1980). The lipid digestibility coefficients of carp range between 53 and 90 % (Kirchgessner *et al.*, 1986), and between 64 and 94 % for channel catfish (Andrews *et al.*, 1978).

Carbohydrate digestibility

Warm-water fish are able to utilize much higher levels of dietary carbohydrates than coldwater or marine fish. This difference may be related to the relative amylase activity present in the digestive system of the various fish. Source, dietary level, and heat treatment affect the digestibility of carbohydrates in fish (Wilson, 1994). Channel catfish were found to digest over 70 % of the energy of raw corn starch. Cooked starch was 12.1 % more digestible than raw starch when fed at 30 % dietary level to channel catfish (Wilson, 1994).

2.7.2 Determination of digestibility

The nutritive value of a feedstuff is determined by its chemical composition and the availability of dietary components. The bioavailability of nutrients or energy in feedstuffs for fish may be defined mainly in terms of digestibility or in the case of energy, metabolizability. Digestibility describes the fraction of nutrient or energy in the ingested feedstuff that is not excreted in the faeces (NRC, 1993).

Methods for determining digestibility involve either a direct or an indirect measurement of the amount of nutrient or energy ingested and subsequently excreted.

Indirect method

This method uses a non-digestible marker, such as chromic oxide (Cr_2O_3) included in the diet at a concentration of 0.5 to 1.0 %. 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 digestibility of the nutrient can be determined by assessing the difference between the feed and faecal concentration of the marker and the nutrient or energy. The indirect method has the advantages that it eliminates the need to quantitatively collect all of the excreta, and the test fish can eat voluntarily (NRC, 1993).

Direct method

The direct method involves measuring all the feed consumed by the fish and all of the fish excreta. The fish are force-fed a measured amount of feed and the excrements are subsequently collected and analyzed for their nutrient or energy content. The amounts of the nutrients or energy in the excrements are then subtracted from those in the feed to determine the amounts retained. This method allows for determining carbon and nitrogen balances as well as digestible energy and metabolizable energy values. Also, the problem of faecal leaching is eliminated because all of the water in the chamber is included in the analysis. However, this method is open to criticism because the fish are immobilized and so stressed that the utilization of the feed may be compromised (NRC, 1993).

2.7.3 Factors influencing digestibility

Digestion of food in fish depends on three main factors: (1) the ingested food and the extent to which it is susceptible to the effects of the digestive enzymes; (2) the activity of the digestive enzymes; (3) the length of time the food is exposed to the action of the digestive enzymes. Each of these main factors is affected by a multitude of secondary factors, some of which are associated with the fish itself, such as fish species, age, size and physiological condition; some are associated with the environmental conditions, such as water temperature, and some are related to the food itself, i.e., its composition, particle size and amount eaten (Hepher, 1988).

Fish species

Nutrient digestibility may vary among fish species, due to differences in the digestive system and its digestive enzymes and to the different foods consumed. Despite these differences and the lack of pepsin in fish without a stomach, variations in the digestibility of proteins and lipids among species are small. Much more pronounced are variations in digestibility of carbohydrates, especially starch. Carnivorous fish digest starch to a much lesser degree than omnivorous and herbivorous fish (Hepher, 1988). Differences in digestibility of carbohydrates can also occur among different families of carnivorous fish within the same species, as was demonstrated by Refstie & Austreng (1981) in rainbow trout. With respect to amylase activity, the omnivorous species show higher activity than the carnivorous species. The ratio of total amylase : total proteolytic activity was higher in omnivorous fish species (Hidalgo *et al.*, 1999). A comparative study on digestive capabilities of the three fish species, carp, tilapia and African catfish, by Degani & Revach (1991) has shown that, protein from poultry sources was digested better by tilapia than carp or catfish, while carp showed the best ability to digest fats, followed by catfish, and with tilapia having the least ability. However, tilapia digested carbohydrate better than the other two species and there was no difference in digestibility of energy among the three fish species.

Fish age and size

Digestibility can increase with size in omnivorous and herbivorous fish due to a relative increase in intestinal length, thereby prolonging digestion and assimilation time (Ferraris *et al.*, 1986). Enzymatic activity may vary with fish age and size, and proteolytic and amylolytic activities are usually lower during the first development stage of fish than in the later stages.

This may affect the digestibility of nutrients (Kolkovski, 2001; Hepher, 1988).

Physiological condition

Stressed fish, due to either excessive handling or to disease, may have a disturbed digestibility. A long period of starvation may also affect enzyme secretion and digestibility. Parallel to the seasonal variations in digestive enzyme activities, seasonal variations in digestibility may also occur (Hepher, 1988).

Water temperature

Fish are poikilotherms, and therefore, their metabolism, including the activity of digestive enzymes, is reduced at low temperature. This may reduce the rate of nutrient utilization. Increasing water temperature may increase both enzyme secretions and enzyme activity (Smit, 1967; Nordlie, 1966). Temperature may also affect the rate of absorption of digested nutrients through the intestinal wall (Smith, 1970). However, the higher the temperature, the more rapid is the transport of food and the shorter its exposure time to the digestive enzymes (Elliott, 1975). In trout and carp, starch and CP digestibility decreased with a decrease of water temperature (Yamamoto *et al.*, 2007).

Food composition

The proportion of nutrients in the diet may affect the digestibility. Increased amounts of dietary lipids are known to support increased protein, carbohydrate, lipid and energy digestibility (Takeuchi *et al.*, 1978). High carbohydrate content in the diet has been reported to reduce protein digestibility (Kaushik *et al.*, 1989). The explanation for this is that the undigested portion of the carbohydrates passes more rapidly through the alimentary canal, carrying with it some of the proteins (Hepher, 1988). Digestibility of protein was found to be negatively correlated with fibre content (Ferraris *et al.*, 1986). Moreover, some feeds may contain digestive enzyme inhibitors which reduce digestibility (NRC, 1993).

Feeding level and frequency

The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed utilization and body chemical composition (Bureau *et al.*, 2006; Juell & Lekang, 2001; Reddy & Katre, 1979). In addition, feeding levels have been reported to influence body composition and morphometry of rainbow trout, and fish on a lower ration level contained less fat and were smaller in size (Kiessling *et al.*, 1991). Lei (2006) found that digestibility of DM and protein was significantly affected by feeding level but not feeding frequency when the feeding level was similar. However, the feeding frequency affected the apparent digestibility when turtles were fed to satiation. Similar results were obtained by Yuan *et al.* (2010), who showed that apparent digestibility coefficients of DM, protein and energy were significantly affected by feeding level in juvenile Chinese sucker, *Myxocyprinus asiaticus*.

3 Materials and Methods

3.1 The survey

3.1.1 Site and household selection

The survey was conducted in three districts (Phong Dien, Huong Thuy and Phu Vang), out of eight in Thua Thien Hue Province, Vietnam, to describe the current situation in smallholdings involved in aquaculture in this region of the country (Paper I). Phong Dien district is located to the North West of Hue City, Phu Vang district is to the East of Hue City and Huong Thuy district is to the South of Hue City.

In total 90 households were interviewed to acquire information on the use of feed resources, feeding practices and fish production. In selecting households, a consensus meeting was organized at the Department of Fisheries of the province to select the three districts that had the most experience in fish culture. Follow-up discussions were conducted with the district level authorities to select the communes that best corresponded with the above mentioned criteria, and households were randomly selected within the selected communes, to give a total of 30 households per district.

3.1.2 Interviews

The farmers were interviewed using a structured questionnaire that focused on stocking strategies and feeding practices. The questionnaire was tested on ten households before it was used in the actual interviews. After this test, some parts were adjusted to improve the questionnaire.

3.1.3 Sample collection

Samples of feed ingredients were collected randomly from households in the study and were sent to the laboratory the same day. They were stored at -20 ^{o}C until analysis of proximate chemical composition.

3.1.4 Chemical analysis

Samples of feed ingredients were subjected to proximate analysis according to standard AOAC methods (AOAC, 1990).

3.1.5 Statistical analysis

Data and information collected were coded and incorporated into computerized databases using SPSS (Statistical Package for Social Science) software, version 13.3.

3.2 Fish experiments

3.2.1 Location

The digestibility and amino acid requirements experiments were conducted at the Faculty of Fisheries of the Hue University of Agriculture and Forestry in Vietnam (Paper II and III).

The experiment in Paper IV was carried out in a fish farm in Thuy Van, Huong Thuy, Thua Thien Hue, Vietnam.

3.2.2 Experimental design

In Paper II, a digestibility study was conducted in two parallel experiments, in each of two species, designed as 6 x 6 Latin-square, with six experimental diets (one reference diet and five test ingredient diets) and six collection periods. Each collection period was 14 days, comprising 7 days of adaptation to the new diet and 7 days for collection of faeces.

In Paper III, 360 fish were distributed into groups of thirty into each of 12 glass aquaria with 130 L capacity. Each aquarium was then randomly assigned to one of three replicates of the four dietary treatments. In order to study the EAA profile and E/A ratio of hybrid catfish fingerling, samples of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) of varying size classes obtained from the Provincial Breeding Centre (Thua Thien Hue, Vietnam) and from local farms were classified into five groups based on their body weight (BW): less than 20, 20-50, 50-100, 100-200 and more than 200 g. The fillet proportion, fillet protein and AA were analyzed.

In Paper IV, the experiment was arranged as a completely randomized design with five treatments and three replications. The control diet (Ctrl) was based on fish meal, and in the four experimental diets 50 % fish meal was replaced either by cassava leaf meal or shrimp head meal without or with lysine supplementation (CLM, CLM-Lys, SHM, SHM-Lys). The experiment lasted for 60 days.

3.2.3 Experimental fish and facilities

Sex-reversed male Nile tilapia of BW 100 (±1.5) g and hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) with a BW of 200 (±3.9) g were used as experimental fish in the digestibility study (Paper II). Hybrid catfish fingerlings with average initial BW of 4.0 (±0.7) and 3.8 (±0.5) g were used in Experiment III and IV, respectively. The experiments in Paper II and III were conducted indoors in a re-circulating culture system consisting of 12 glass aquaria with 130 L capacity and containing about 90 L of water, and equipped with a biological filter. Compressed air was supplied to each tank, and the biological filter via air stones was connected to an air compressor. Each tank was continuously supplied with fresh water at a mean rate of 6 L per min. Tanks were covered with a polyethylene sheet (5 mm thick) to prevent the fish from escaping. A constant photoperiod was maintained of 12 h light and 12 h dark. The fish in experiment IV were reared in 15 net cages (2.0 m x 1.0 m x 1.4 m), at a density of 15 fish per m^3, and the cages were placed in a fish pond.

3.2.4 Fish management

In Paper II, hybrid catfish and Nile tilapia were acclimatized in a composite tank for 2 weeks prior to the digestibility study, while being fed a commercial pelleted feed (28 % CP; Con Co Feed Company, Vietnam). All fish were fed twice daily, at a fixed rate of 3 % BW of dry feed per day. Before the start of the experiment, the fish were treated with a solution of 3 % NaCl for 15-20 minutes to eliminate ectoparasites and to prevent fungal infection (Hoa *et al.*, 2004). The fish were distributed randomly between tanks at a stocking density of 10 fish per tank. During all handling procedures the fish were anaesthetized in a bath of 0.05 ml L^{-1} FA 100 (4 allyl-2-methoxyphenol) for 5 minutes. During the experiment, fish showing symptoms of disease or fish that died were replaced with acclimatized fish from the composite tank.

In Paper III, the fish were acclimatized to the experimental conditions and their respective diets for one week prior to the start of the study and were fed the experimental diets for eight weeks.

In Paper IV, the fish were adapted to the experimental conditions for one week before the start of the experiment. During this period they were fed a commercial diet (Lai Thieu, DT02, 35 % CP) twice a day. During the experimental period, all fish were fed with their respective diets at a level of 5 % of BW. The daily feed was divided into two equal amounts and given to the fish at 08:00 and 16:00 h. Random samples of fish from each experimental treatment were weighed every 15 days and the feed ration was adjusted accordingly.

3.2.5 Feed ingredients and diet formulation

The five ingredients (4 plant and 1 animal-based) evaluated in the digestibility study were cassava leaf meal, groundnut meal, soybean meal, sesame husk meal and shrimp head meal (Paper II), in which cassava leaf meal and shrimp head meal were selected for a later study (Paper IV). Shrimp head meal was prepared from fresh shrimp by-products that were purchased at the local market. The fresh shrimp by-products were first heated at approximately 65 ^{o}C for about 10-15 minutes, and then sun-dried for 3 days, and ground through a 1 mm screen. Cassava leaves were collected from farms in Thua Thien Hue Province. They were harvested from the lower parts of the cassava plants after about 3-4 months of growth. The cassava leaves were sun-dried for two or three days and then ground to a meal with a particle size less than 1 mm. The other feedstuffs were purchased from local feed shops. All the feedstuffs were carefully packed in plastic bags and kept in a cool dry place before being incorporated into the diets.

In Paper II, the reference diet was composed of rice bran, cassava root meal, fish meal and oil. The five test diets were formulated by mixing 70 % of the reference diet with 30 % of each of the test ingredients on an air-dry basis. Chromic oxide was used as an inert marker at a concentration of 0.5 % in the reference and test diets.

In Paper III, four iso-nitrogenous (300 g kg^{-1} CP) and iso-caloric (18.3 MJ kg^{-1} gross energy; GE) diets were formulated using casein and gelatin as protein sources. The diets were supplemented with mixtures of crystalline AA to simulate the reference AA profile of hybrid catfish fillet protein, with the exception of lysine. The diets were made iso-nitrogenous by decreasing the non EAA content as the level of lysine increased. The level of lysine in the four diets was 45, 50, 55 and 60 g kg^{-1} CP, respectively. The other components were dextrin as a source of carbohydrate, fish oil and vegetable oil as essential fatty acid source, carboxymethyl cellulose (CMC) as a binder, vitamin and mineral premixes and cellulose. All the dry ingredients, except for CMC, were thoroughly mixed, then oil and water added. The pH of all diets was adjusted to 7.0 by using a 6 N NaOH solution as described by Ngamsnae *et al.* (1999). Carboxymethyl cellulose was gelatinized with hot water at 80 ^{o}C and was added to the pH-adjusted mixture.

In Paper IV, five experimental diets were formulated on a dry weight basis to contain 30 % CP and 19 MJ kg^{-1}GE. The diets were formulated to be iso-nitrogenous and iso-caloric by adjusting the proportion of cassava root meal, fish oil and vegetable oil. The control diet was formulated based on fish meal. In the test diets 50 % of the fish meal was replaced with either cassava leaf meal or shrimp head meal, without or with lysine supplementation, to meet the dietary lysine requirement of hybrid catfish.

All the diets were pelleted to 3 mm diameter using a mill (Sheng Kiang, China). The pellets were oven dried at either 45 or 60 ^{o}C for 16 to 24 h and stored in airtight plastic bags at 4 ^{o}C until fed (Paper II, III and IV).

3.2.6 Sample collection, measurements and calculations

Water quality parameters, such as water temperature, pH, dissolved oxygen (DO), and total ammonia were recorded. Water temperature was recorded daily using a mercury thermometer. Dissolved oxygen, pH and ammonia were measured by a test kit (Advance Pharma Co Ltd., Thailand) (Paper II, III and IV).

In the digestibility trial (Paper II), the faecal samples were collected twice per day before feeding, and then frozen, and at the end of each collection period, all the faecal samples were pooled for each tank and stored at -20 ^{o}C until analysis.

In Paper II, the apparent digestibility (AD) of the reference diet and test diets was calculated as described by Cho *et al.* (1982):

$$AD\ (\%) = 100-100*(\%Cr_{Feed}/\%Cr_{Faeces})*(\%Nutrient_{Faeces}/\%Nutrient_{Feed})$$

The AD was calculated for dry matter (AD_{DM}), organic matter (AD_{OM}), crude protein (AD_{CP}), and essential amino acids (AD_{EAA}).

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

$$AD_{test\ ingredient} = AD_{test\ diet} + [(AD_{test\ diet} - AD_{ref.diet})*(0.7*D_{ref}/0.3*D_{ingr})]$$

Where D_{ref} = % nutrient of reference diet; D_{ingr} = % nutrient of test ingredient.

The growth performance, feed efficiency and survival of the fish in Paper III and IV were calculated according to Hepher (1988), as follows : Specific growth rate (SGR) = 100 x ($\mathrm{Ln}W_t$-$\mathrm{Ln}W_o$)/t, where W_t is final body weight, W_o is initial body weight, t is experimental duration in days;

Percentage live weight gain (LWG) = [(final weight – initial weight)/initial weight] x100; Protein efficiency ratio (PER) = wet weight gain (g)/protein fed (g); Feed efficiency ratio (FER) = wet weight gain (g)/dry feed fed (g); Survival rate = [(final number of fish)/(initial number of fish) x 100].

In Paper III, the A/E ratio (individual EAA to total EAA ratio) = (individual EAA/total EAA, including cysteine and tyrosine) x 1000 (Arai, 1981).

The EAA requirement was calculated as described by Fagbenro (2000): Individual AA requirement = lysine requirement x (A/E ratio/100) (Paper III).

3.2.7 Chemical analysis

Samples of feed ingredients in Paper II, III and IV were subjected to proximate analysis according to AOAC (AOAC, 1990) and AA analysis was performed using high-pressure liquid chromatography (Amino Quant, 1990) (Paper II and III), and using liquid chromatography tandem mass spectrometry (Phenomenex, 2005) (Paper IV). Samples were analyzed for DM by drying fresh samples at 105 ^{o}C for 24 hours. Total nitrogen (N) was determined by the Kjeldhal method (Kjeltec system, Tecator, Sweden) and CP content was calculated as N*6.25. Ether extract (EE) was determined by Soxhlet extraction without acid hydrolysis. Ash was the residue after ashing the samples at 550-600 ^{o}C. Crude fibre (CF) content was determined according to AOAC (1990) and neutral detergent fibre (NDF) according to Robertson & Van Soet (1981). Gross energy of feed ingredients and the diets was measured using an automated bomb-calorimeter (Calorimeter Parr 6300, USA) (Paper I, II, III and IV). Chromic oxide was determined according to Fenton & Fenton (1979).

3.2.8 Statistical analysis

The General Linear Models of the Minitab Reference Manual Release 15.1 was used. Least Square means were compared statistically using Tukey's pairwise comparison procedures Test ($P<0.05$) (Paper II, III and IV).

The SAS package version 9 was applied for the preliminary statistical test of carry-over effects (Paper II).

In Paper III, the lysine requirement was estimated based on LWG, SGR, PER and FER data using the second-order polynomial model ($Y=a+bX+cX^2$), as described by Zeitoun *et al.* (1976).

4 Summary of results

4.1 Potential feed resources and diet formulation at farm level (Paper I)

The practice of fish poly-culture was dominant in the surveyed area (78.9 %). The most common fish species was Nile tilapia (*Oreochromis niloticus*), and other commonly cultured fish species were grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio),* hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*), mrigal (*Cirrhinus mrigala*), pangasius (*Pangasius hypophthalmus*) and redfin pacu (*Colossoma brachypomum*).

In total 22 feed ingredients were used by the farmers. The main ingredients were traditional feedstuffs such as cassava root meal, rice bran, cassava residue, groundnut cake, soybean meal and fish meal. Furthermore, several more unconventional feedstuffs were also commonly used as fish feed, such as cassava leaves, coconut meal, shrimp head meal, sesame husk and squid by-product. The combination of ingredients used in farm-made fish feeds varied among farms and districts, leading to a large variation in terms of nutrient composition and energy content.

Feed supplementation ranged from single feed ingredients, such as rice bran (in Phong Dien), cassava root meal (Huong Thuy) or cassava residue (in Phu Vang), to the use of many different combinations of ingredients. There were large differences between farms and within farm depending on the availability of feed ingredients in each region.

An attempt was made to estimate the nutrient content in a typical fish feed used in three different districts and the results showed that the estimated average CP content was highest in Phong Dien district, followed by Phu Vang and Huong Thuy districts (257, 215 and 162 g CP kg^{-1} DM, respectively). The average crude fat content ranged from 97 to 133 g kg^{-1} DM and the average CF content from 90 to 111 g kg^{-1} DM, both with large variation among farms in the three districts. The average GE content was similar among districts (20 MJ kg^{-1} DM) but there was variation among farms within districts.

4.2 Fish yield (Paper I)

The estimated average annual yield of cultured fish ranged from 0.8 to 6.5 t ha^{-1}, with differences in yield between districts. Farmers in Phong Dien district achieved higher fish yields than in the other two districts, with approximately 50 % of the famers getting a yield of 3 to 4 t ha^{-1}, and 30 % a yield of 4 to 5 t ha^{-1}. In Phu Vang district more than 50 % of farmers got a yield of less than 2 t ha^{-1}

4.3 Digestibility of nutrients and amino acids of selected feedstuffs in hybrid catfish and Nile tilapia (Paper II)

There were no differences in the calculated AD_{DM} between groundnut meal, soybean meal, sesame husk meal and shrimp head meal ($P>0.05$). A difference in calculated AD_{DM} between Nile tilapia and hybrid catfish was only detected for cassava leaf meal, with higher values in hybrid catfish ($P<0.05$). There were no differences ($P>0.05$) in calculated AD_{CP} and AD_{OM} between Nile tilapia and hybrid catfish. The highest calculated AD_{CP} was found for soybean meal in tilapia (96.3 %), which was not different ($P>0.05$) from either groundnut meal or shrimp head meal.

Within species, among the ingredients, the calculated AD_{DM} for cassava leaf meal (63.0-76.2 %) was lower ($P<0.05$) than that of groundnut meal, soybean meal, sesame husk meal and shrimp head meal. In Nile tilapia, the calculated AD_{DM} increased numerically and progressively ($P>0.05$) in the order shrimp head meal, sesame husk meal, soybean meal and groundnut meal. Similarly, the calculated AD_{DM} in hybrid catfish was not different ($P>0.05$) among the four ingredients and increased progressively in the order soybean meal, groundnut meal, sesame husk meal and shrimp head meal.

The average calculated AD_{EAA} ranged from 82.2 to 93.0 % and from 86.1 to 94.8 %, for Nile tilapia and hybrid catfish, respectively. There was no difference ($P>0.05$) between fish species in the calculated AD_{EAA}, except for threonine in shrimp head meal, which had a higher AD in hybrid catfish than in Nile tilapia (98.6 and 96.2 %, respectively).

Among the five feed ingredients, sesame husk meal had the highest ($P<0.05$) calculated AD for arginine and lysine in Nile tilapia and for arginine, lysine and valine in hybrid catfish. However, no difference ($P>0.05$) was observed between cassava leaf meal, groundnut meal, soybean meal and shrimp head meal for most EAA, except for lysine, threonine and valine, which were lowest ($P<0.05$) in cassava leaf meal in Nile tilapia. There was no difference ($P>0.05$) among cassava leaf meal, groundnut meal, soybean meal and shrimp head meal for all EAA in hybrid catfish.

Within ingredient, the lowest calculated AD value was obtained for histidine in both fish species, except for the AD of histidine in sesame husk meal and shrimp head meal in hybrid catfish.

4.4 Essential amino acids requirements of hybrid catfish (Paper III)

There was an increase of LWG and SGR when dietary lysine concentration increased from 45 to 50 g kg^{-1} of CP ($P<0.05$). However, increasing dietary lysine concentration above 50 g kg^{-1} of CP did not further improve LWG and SGR ($P>0.05$). Fish fed the lowest level of lysine presented the worst LWG and SGR. Maximum LWG (403 %) was observed in fish fed 16.5 g kg^{-1} dietary lysine (55 g kg^{-1} of CP). Protein efficiency ratio and FER followed the same tendency as LWG and SGR. The highest SGR (2.89 %), PER (4.00) and FER (1.20) were found at 16.5 g kg^{-1} dietary lysine. On subjecting the data on LWG, SGR, PER, FER and lysine to second-order polynomial regression analysis, the break point was in the range from 55.3 to 55.8 g kg^{-1} of CP, corresponding to 16.6 to 16.7 g kg^{-1} lysine of the diet.

The dietary lysine levels did not ($P>0.05$) influence carcass chemical composition.

Using the A/E ratios in fillet protein and the experimentally determined lysine requirement of 56 g kg^{-1} of CP (corresponding to 16.8 g kg^{-1} diet), the requirements for the other EAA were estimated. This resulted in systematically low requirement values compared with other published estimates, which were due to the high lysine content in fillet protein used as reference.

4.5 Growth performance of hybrid catfish fingerlings fed diets with partial replacement of fish meal by cassava leaf meal or shrimp head meal, without or with lysine supplementation (Paper IV)

Final weight (FW) and SGR differed between diets ($P<0.001$), while there were no treatment effects on FER, PER and survival. Replacing fish meal with shrimp head meal had no effect on FW and SGR, while replacing fish meal with cassava leaf meal led to impaired ($P<0.05$) FW and SGR.

Supplementing diets CLM and SHM with lysine, resulted in improved ($P<0.05$) FW and SGR compared with non supplemented diets.

Fish survival did not differ ($P>0.05$) between diets and was higher than 93% for all treatments. There were no treatment effects on carcass moisture content and gross chemical composition.

5 General discussion

5.1 Potential feed resources for fresh water fish in Central Vietnam

5.1.1 Energy feeds

Rice bran, cassava root meal, cassava residue, maize and rice are commonly used as fish feed in small-scale fish farming. They are all characterized by high starch content and can be classified as energy feedstuffs.

Rice bran is a by-product from rice production, which is abundant and an important feedstuff for livestock and aquaculture in Vietnam. The national yearly production of paddy is about 33-34 million tonnes (GSO, 2010), with a conversion rate of paddy rice into rice bran of 10 %. Thus, the annual production of rice bran is 3.3-3.4 million tonnes. The inclusion of rice bran in formulated catfish and tilapia diets varies from 20 to 50 % and from 40 to 70 % in farm-made feed depending on growth stage (Hung & Huy, 2007). In Central Vietnam, rice bran was found to be the principle feedstuff at farm level, with more than 70 % of farmers using rice bran as main feedstuff with inclusion levels from 20 to 77 %. Moreover, many farmers fed rice bran only to the fish (Paper I)

Maize is another important feedstuff in aqua-feed and it is a good source of carbohydrate and energy for herbivorous and omnivorous fish (Law, 1986; Chiou & Ogino, 1975). The inclusion rate of maize in aqua-feeds varies depending on the kind of product, and it can be included with up to 30 % for maize grain meal and with up to 60 % for maize bran in omnivorous fish feeds (Hertrampf & Piedad-Pascual, 2000). Cooked whole grains were fed to fish at levels of up to 67 % in Central Vietnam (Paper I).

Cassava (*Manihot esculenta Crantz*) is the second most important food crop after rice in terms of total production in Vietnam (GSO, 2009). The total production of fresh cassava root was 2.6 million tonnes in 2009 (GSO, 2010). Recent studies have shown that cassava root meal can be used to completely replace maize in the diet of hybrid catfish (*Clarias* x *Heterobranchus*) without any adverse effects (Abu *et al.*, 2010a; Abu *et al.*, 2010b). In Central Vietnam, cassava root meal was included in fish feed with up to 57 % of diet dry weight (Paper I).

5.1.2 Protein-rich feedstuffs

The commonly used protein-rich feedstuffs in smallholdings are both conventional (trash fish, fish meal, soybean meal and groundnut meal) and unconventional feedstuffs (shrimp head meal, cassava leaves and squid by-product) (Paper I).

In Vietnam, trash fish used is either from by-catch of marine or inland capture fisheries. Traditionally trash fish is used for carnivorous and omnivorous fish (Hung & Huy, 2007). Farm-made feeds contain between 20-30 % of trash fish (Edwards *et al.*, 2004) or between 14-32 % (Paper I). The supply of trash fish is highly seasonal, causing unstable supply and price. Therefore, its availability is the most serious constraint for aquaculture development in Vietnam (Hung & Huy, 2007).

Crustacean by-products, including shrimp head meal, crab meal, and gammarid meal have been identified as good candidates to replace fish meal in diets for aquaculture (Tibbetts *et al.*, 2006; Köprücü & Özdemir, 2005; Tibbetts *et al.*, 2004). In Vietnam, shrimp by-products from the shrimp industries are an important low-cost animal protein source. It was estimated in 2008 that over 194 thousand tonnes of shrimp by-product were produced (GSO, 2010). Shrimp head meal, with high protein content, is considered to be a valuable protein source for livestock (Evers & Carroll, 1996; Balogun & Akegbejo-Samsons, 1992). Recent studies have shown that shrimp head meal could be a potential protein source for fish (Oliveira Cavalheiro *et al.*, 2007; Chimsumg *et al.*, 2006). At the same time, crustacean by-products meals are usually high in ash content (> 20 %), which can adversely affect digestibility of fish feed (NRC, 1993). Shrimp head meal had a high CP content (Paper I, II and IV) and was well digested by hybrid catfish and Nile tilapia, and the AD_{CP} were 96.0 and 89.2 % in hybrid catfish and Nile tilapia, respectively (Paper II). With respect to the CP content as well as amino acid pattern, this protein feed source could be used to replace trash fish or fish meal in fish diets, and the high ash content should be taken into account when formulating the diet.

Plant materials commonly used as fish feed includes cassava leaves, groundnut meal and soybean meal, of which cassava leaves are abundantly available in Central Vietnam (Paper I) as well as in other parts of the country (Dongmeza *et al.*, 2009). Cassava leaves can be harvested at root harvest time or from 3-4 months of age in a cycle of 60-75 days (Phuc & Lindberg, 2001). Cassava leaves have a high level of CP (20-30 % in DM) and are a good source of protein for animals, but have a high content of cyanide that limits their use as an animal feed (Ravindran *et al.*, 1987). Drying or ensiling cassava leaves will markedly reduce the HCN content. However, cassava leaves are low in both lysine and methionine (Phuc & Lindberg, 2001). The proximate and amino acid composition of cassava leaves, soybean meal and groundnut cake in this study (Paper I, II) was comparable to the other published data (NIAH, 2001; NRC, 1993). The AD of nutrients and AA of cassava leaf meal in hybrid catfish and Nile tilapia were somewhat lower than in other feed ingredients (Paper II).

Cassava leaves are often fed in the fresh form to the fish in ponds, and can be used as feed for grass carp-a typical herbivorous fish. It has been shown that cassava leaf meal made into pellets can be included in the feed at up to 20 % (Bureau *et al.*, 1995) for African catfish, and up to 50 % for hybrid catfish and Nile tilapia (Paper IV).

Soybean meal is a widely used plant protein in aqua-feeds because of its high protein content, satisfactory essential amino acid composition, competitive price and consistent quality (Metts *et al.*, 2010). Soybean meal was reported to be highly digestible in tilapia, with an average CP digestibility of 85 % (Ogunji, 2004) and in hybrid catfish, the AD_{CP} was 94.1 % (Paper II). This is also a potential plant feed for cultured fish.

Due to the low lysine content in shrimp head meal and plant protein feedstuffs compared with fish meal (FAO, 2009b), dietary lysine supplementation has been shown to have positive effects on the nutrient utilization and performance of fish. Bai & Gatlin (1994) found that the CP level in the diet could be reduced with lysine supplementation in channel catfish. In diets for rainbow trout, where fish meal was replaced by plant proteins, supplementation with lysine increased CP and lysine, and reduced fat levels in whole trout body (Cheng *et al.*, 2003). Supplemental lysine in the diet may lead to a better utilization of the plant feedstuffs and can improve the growth performance of the fish (Paper IV).

5.2 Apparent digestibility of nutrients in hybrid catfish and Nile tilapia

Knowledge of the digestibility of the various nutritional components of formulated fish diets is necessary for the determination of the most appropriate diet for each fish species (Degani & Revach, 1991). The determination of digestibility, together with chemical analysis, allows more exact estimation of nutritional value of a particular feed source (Hossain & Jauncey, 1989b). The digestibility of a feed ingredient depends primarily on its chemical composition and the digestive capabilities of the species to which it is fed. However, factors unrelated to diet formulation, such as environmental conditions in the production system, feeding practices and diet manufacturing techniques affect the digestibility. Thus, digestibility coefficients are not constant (McGoogan & Reigh, 1996). Stone (2003) stated 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 the AD of protein for common feedstuffs used in aquaculture is relatively high for herbivorous, omnivorous and carnivorous fish. The current study (Paper II) shows that hybrid catfish and Nile tilapia, despite different feeding habits, have similar capacity to digest plant and animal protein. The calculated AD of DM, OM, and CP in groundnut meal, soybean meal, sesame husk meal, and shrimp head meal was high in both hybrid catfish and Nile tilapia. This is in accordance with earlier studies on tilapia, which have shown that they have the capacity to utilize a large number of alternative plant and animal protein sources (Ogunji, 2004; Degani & Revach, 1991). Tilapia has a digestive tract that is relatively long, as in herbivorous fish (Anderson *et al.*, 1991), and feeds at all levels of the water body, with the result that the tilapia's food contains a higher percentage of plant matter. The *Clarias* catfish has been considered as a carnivorous (Degani & Revach, 1991) or an opportunistic omnivorous (Jantrarotai *et al.*, 1994) fish and thus may have a limited capacity to digest a range of different feed sources for its growth. Degani & Revach (1991) showed that African catfish better digested a high animal protein diet than a plant protein diet. The present study also confirms this: the AD_{CP} was higher in shrimp head meal than in the plant feedstuffs (96 % vs. 91.4 to 94.1 %, respectively). On the other hand, it was found that hybrid catfish has a better capability of digesting cassava leaf meal than Nile tilapia. This suggests that the Nile tilapia may have some limitation in its capacity to digest the dietary components in cassava leaf meal.

It has been found that the AD of dietary components is negatively correlated with increasing content of dietary fibre (Hilton *et al.*, 1983).

However, this could not be confirmed in the current study, where the AD values for dietary components in sesame husk meal with extremely high fibre content (52.7 %) were comparable to soybean meal and groundnut meal with much lower fibre content.

The high protein digestibility of feedstuffs of plant origin, in comparison to feedstuffs of animal origin in the current study (Paper II) may be related to the omnivorous/herbivorous feeding habit of the two fish species (Jafri & Anwar, 1995). Hanley (1987) has also reported a higher digestibility coefficient for soybean than fish meal in omnivorous tilapia, *Oreochromis niloticus,* whereas Lorico-Querijero & Chiu (1989) recorded high digestibility of both plant and animal proteins for this fish species. In channel catfish, *Ictalurus punctatus*, it was reported that although feedstuffs like soybean and peanut meal were less digestible than fish meal, the mean AD_{CP} of plant protein was much higher than that of animal protein (Wilson & Poe, 1985a).

The average digestibility of most AA in selected feedstuffs was higher than 82 % in both hybrid catfish and Nile tilapia, indicating a high protein quality of these feedstuffs (Paper II).

5.3 Dietary essential amino acids requirements

In practical fish culture, protein is the most important component in fish feed, with AA as essential material for structural elements in the body (Akiyama *et al.*, 1997). Moreover, protein is also the most expensive component in fish feed (De Silva & Anderson, 1995). Therefore, dietary protein must be assessed in terms of quality and quantity (Wilson, 2002).

Lysine is an amino acid of particular interest because it is the first limiting EAA in most grain by-products and plant protein materials used to produce commercial feed (Montes-Girao & Fracalossi, 2006; Akiyama *et al.*, 1997).

The requirement of lysine appears to range from 4 to 5 % of protein for most fish species (Wilson, 2002). A higher value (6.2 %) was reported for catla (Ravi & Devaraj, 1991), while lower values were reported for rainbow trout (3.7 %) and coho salmon (3.8 %) (Wilson, 2002; Kim *et al.*, 1992).

The estimated requirement of dietary lysine for fingerling hybrid *Clarias* (Paper III) was 56 g kg^{-1} of CP, corresponding to 16.8 g kg^{-1} of the dry diet. This was based on the maximal growth (LWG and SGR) and feed utilization (PER and FER) in response to increasing dietary lysine content.

The result obtained from the current study was within the same range as discussed above.

Among the techniques used to determine AA requirements of fish species, the one relating the body amino acid composition to dietary lysine requirement, measured through dose-response experiments, seems to be the fastest and the most cost efficient (Meyer & Fracalossi, 2005). The use of roe, muscle or whole body protein EAA pattern as reference protein has been suggested (Cowey & Luquet, 1983; Ogata *et al.*, 1983; Ketola, 1982). In the current study (Paper III) fillet protein AA pattern was used as reference protein to estimate the EAA requirement of hybrid catfish. The results obtained were systematically lower than estimates suggested for hybrid *Clarias* (Wilson, 2002) and (except for threonine) than those suggested for channel catfish (Wilson & Poe, 1985b). The reason was the high lysine content in hybrid *Clarias* fillet protein in the current study as compared with other published data (Osibona *et al.*, 2009; Montes-Girao & Fracalossi, 2006; Meyer & Fracalossi, 2005; Oellermann & Hecht, 2000; Wilson & Poe, 1985b). However, by applying the EAA profile in body protein obtained from other published data on different catfish species, all with lower lysine content, the estimated EAA requirements became more comparable to earlier published data on hybrid *Clarias* and channel catfish.

5.4 Replacing fish meal by local protein-rich feed sources

Some of the more unconventional feedstuffs in Central Vietnam may have potential to be used as fish feeds in fresh water aquaculture (Paper I, II, IV). Of these, shrimp head meal appears to be a potentially useful protein-rich, low cost feedstuff that can be used to replace up to 50 % of fish meal in the diet for growing hybrid catfish without any negative affect (Paper IV). This could be due to similar nutritional value of shrimp head meal and fish meal, as the fish meal based control diet and diet SHM showed comparable digestibility of OM, CP and AA (Paper II). An earlier study on African catfish (*C. gariepinus*) reported that substituting fish meal with 30 % fermented shrimp head waste meal in the diet did not adversely affect growth performance (Nwanna, 2003). In another study on humpback grouper (*Cromileptes altivelis*), replacing fish meal with shrimp head meal adversely affected growth performance and feed utilization if the inclusion rates exceeded 10 % (Rachmansyah *et al.*, 2004). In contrast, in diets for red tilapia (*Oreochromis niloticus* x *O. mossambicus*), shrimp head meal could be included at 50 % in the diet without any differences in growth performance compared with the control diet. Moreover, in the diets for

Nile tilapia (*Oreochromis niloticus*) it was possible to completely replace fish meal with shrimp head meal without a negative impact on performance (Oliveira Cavalheiro *et al.*, 2007).

Feed utilization (FER and PER) was unaffected when fish meal was replaced with cassava leaf meal, while the growth performance of hybrid fingerlings was impaired. The impaired growth performance could be due to high fibre content in the diet (8.2 %). Increasing content of dietary fibre in fish feed may impair growth performance (NRC, 1993). This can be related to decreased digestibility of dietary components and energy, and thereby reduced availability of nutrients and energy (Hilton *et al.*, 1983; Spannhof & Plantikow, 1983; Paper II).

In Nile tilapia (*O. niloticus*), cassava leaf meal significantly reduced growth performance with growth reduction increasing linearly with increasing leaf meal inclusion (20, 40, 60 & 100 %) (Ng & Wee, 1989). Depending on the carbohydrate source in the diet (cassava-based or corn-based), up to 20 % cassava leaf meal could be included in the diet for African catfish (*C. gariepinus*) without negative impact on growth performance (Bureau *et al.*, 1995).

The present study (Paper IV) indicated that, hybrid catfish fingerlings fed diets with cassava leaf meal or shrimp head meal supplemented with lysine improved final weight and SGR compared with those fed the non supplemented diets. These results are supported by growth performance responses obtained in channel catfish (*Ictalurus punctatus*) (Bai & Gatlin, 1994), in rainbow trout (*Oncorhynchus mykiss*) (Cheng *et al.*, 2003) and in Atlantic cod (*Gadus morhua*) (Hansen *et al.*, 2010) when the diets were supplemented with lysine.

6 General conclusions and implications

6.1 Conclusions

- Smallholder fish farmers in Central Vietnam utilize available feed resources sub-optimally, leading to poor nutrient supply, in particular with regards to protein content. Nevertheless, the local feed resources have potential to provide nutrients and energy to culture fish if they are used in proper combination.
- Hybrid catfish and Nile tilapia appear to have similar capacity to digest nutrient components and AA in groundnut meal, soybean meal, sesame husk meal and shrimp head meal. The exception was cassava leaf meal, which was better utilized by hybrid catfish than by Nile tilapia.
- A dietary lysine content of 56 g kg^{-1} CP, corresponding to 16.8 g kg^{-1} of the dry diet, appears to give maximal growth performance and feed utilization in fingerling hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*). It is recommended that the requirements for the other EAA in the diet for hybrid catfish should be calculated from an EAA profile in body protein based on an average of published data on *Clarias*.
- Shrimp head meal appears to be a potentially useful protein-rich low cost by-product that can be used to replace up to 50 % of fish meal in the diet for growing fingerling hybrid catfish. In contrast, cassava leaf meal can replace less than 50 % of fish meal if the growth performance of hybrid catfish should be unaffected.
- Supplementation of lysine to the diet appears to be an effective way of improving the growth performance when fish meal has been replaced by shrimp head meal or cassava leaf meal.

6.2 Implication and further study

6.2.1 Implication

There appears to be potential for improving fish productivity and reducing feed costs in smallholder fish farming systems in Central Vietnam, by improving the existing feeding practices. By reducing the proportions of energy-rich feedstuffs (such as rice bran) and increasing the proportion of available low-cost protein-rich feedstuffs (such as shrimp head meal, groundnut meal and cassava leaf meal) in the diet, a better balance can be achieved with respect to protein requirements for cultured fish species. Supplementation of low-cost diets, based on locally available feedstuffs, with lysine can be a simple and cheap way of meeting fish lysine requirements and thereby improving fish growth performance.

6.2.2 Further study

Studies are needed on the impact on fish growth performance and carcass quality in cultured fish, and on the economic viability, when diets with different combinations of locally available feed resources are used to replace fish meal.

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