On-Farm Feed Resources for Catfish (*Clarias gariepinus*) Production in Laos

Evaluation of Some Local Feed Resources

Oudom Phonekhampheng

Faculty of Veterinary Medicine and Animal Science Department of Animal Nutrition and Management Uppsala

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Abstract

The aim of the thesis was to collected information African catfish (*Clarias gariepinus*) production systems, and the chemical composition and nutritive value of potentially available feedstuffs. Moreover, the apparent digestibility (AD) of nutrient and amino acids (AA) in raw, sun-dried and ensiled Golden Apple snails (GAS) was evaluated, as well as the potential of GAS to replace fish meal in the diet for growing African catfish fingerlings.

The farmers used traditional feedstuffs such as maize, broken rice, cassava root meal, rice bran, soybean, soy waste, dried fish and fish meal. In addition, some more unconventional feedstuffs such as leucaena meal, earthworms, termites and GAS were used. The fish diets were low (150-220 g kg⁻¹ dry matter (DM)) in crude protein and had to low protein to energy (P/E) ratio. Catfish pond areas per farm ranged from 100 to 5,000 m² with a fish yield that ranged from 1.7 to 3.0 tons ha⁻¹ 6months⁻¹. The average AD of crude protein (CP), crude fat, total carbohydrates and energy in potentially available feedstuffs was 90.6 % (SD 2.4), 87.0 %, (SD 3.4), 77.7 % (SD 3.7) and 84.5 % (SD 2.3), respectively. Only a few of the currently available and used feedstuffs have the potential to provide optimum P/E ratios in the diet for growing African catfish (25-30 g CP/MJ digestible energy (DE)). The content of DE ranged from 13.2 MJ kg DM⁻¹ for rice bran to 18.1 MJ kg DM⁻¹ for frogs. The AD of CP, AA and energy was highest in sun-dried, followed by raw and ensiled GAS. The CP to DE ratio that ranged from 17.8 g M_{J}^{-1} in raw GAS to 24.8 MJ^{-1} in ensiled GAS. It was concluded that sun-drying, rather than ensiling should be the preferred preservation method of GAS. High addition of sugar cane molasses (>15 % in DM) was needed to maintain an acceptable quality of GAS ensiled for 28 days. Finally, it was shown that protein from GAS can completely replace fish meal in diets for growing African catfish fingerlings without negative effects on growth performance and feed utilization.

Keywords: Golden Apple snail, catfish, digestibility, nutritive value, sun-drying, ensiling, amino acid, growth performance, nutrition, feed resources.

Author's address: Oudom Phonekhampheng, Department of Livestock and Fisheries, Faculty of Agriculture, National University of Lao, Vientiane, Lao PDR. *E-mail: oudomp@yahoo.com*

Dedication

To my parents My wife Khambay Vorasane My daughter Boutsady Phonekhampheng My son Piyaphong Phonekhampheng

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List of Publications

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

- I Phonekhampheng, O., Hung, L.T. and Lindberg, J.E. (2008a) A survey on African catfish (*Clarias gariepinus*) feeding and production in Laos. *Journal of Asian Fisheries Science* 21, 21-33.
- II Phonekhampheng, O., Hung, L.T. and Lindberg, J.E. (2008b) Nutritive value of potential feed resources used in Laos for African catfish (*Clarias* gariepinus) production. *Livestock Research for Rural Development* (In Press).
- III Phonekhampheng, O., Hung, L.T. and Lindberg, J.E. (2008c) Nutritive value of raw, sun-dried and ensiled Golden Apple snail (*Pomacea canaculata*) for African catfish (*Clarias gariepinus*) fingerlings (In manuscript).
- IV Phonekhampheng, O., Hung, L.T. and Lindberg, J.E. (2008d) Ensiling of Golden Apple snail (*Pomacea canaculata*) and growth performance of African catfish (*Clarias gariepinus*) fingerlings fed diets with raw and ensiled Golden Apple snail as protein source. *Livestock Research for Rural Development* (In Press).

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Abbreviations

| AA | Amino acid |
|---------|--|
| ADCP | Aquaculture Development and Coordination Program |
| B+GAS-D | Basal feed + Golden Apple Snail-dried |
| B+GAS-R | Basal feed + Golden Apple Snail-raw |
| B+GAS-E | Basal feed + Golden Apple Snail-ensiled |
| CF | Crude fiber |
| СР | Crude protein |
| DE | Digestible energy |
| DM | Dry matter |
| EE | Ether extract |
| EAAI | Essential amino acid index |
| FAO | Food and Agriculture Organization |
| FCR | Feed conversion ratio |
| GE | Gross energy |
| GAS | Golden Apple Snail |
| kJ | Kilo-joules |
| Lao PDR | Lao People's Democratic Republic |
| LARReC | Living Aquatic Resources Research Center |
| MJ | Mega-joules |
| ME | Metabolisable energy |
| MAF | Ministry of Agriculture and Forestry |
| MRC | Mekong River Commission |
| NRC | National Research Council |
| Р | Phosphorus |
| PER | Protein efficiency ratio |
| ppm | Parts per million |
| SD | Standard deviation |

1 Introduction

The Lao PDR is situated in the centre of the Mekong region. It is a small land-locked country in Southeast Asia, bordered by China to the North, Thailand to the West, Myanmar to the North West, Vietnam to the East and Cambodia to the South. The total population of the country is 6.5 million, of which 23 % are urban and 77 % are rural and ethnically diverse. The total area of the country is 236,800 km², and the topography is predominantly mountainous with cultivated floodplains along some reaches of the Mekong River and larger tributaries. The climate is tropical monsoon with a rainy season (May to November) and a dry season (November to April).

Laos is one of the poorest and least developed countries in South-East Asia. In 1998, the estimated per capita income was US \$ 320 per year, and about 73.2 % of its population lived on less than US \$ 2 per day. Since 2000, however, the Laotian economy has developed rapidly owing to government policies that opened the economy and stimulated innovation. The economic strategy is known as the New Economic Mechanism and was aimed at exposing the economy to market forces, opening trade and foreign investment opportunities, strengthening the role of the private sector and improving macro-economic management. Growth averaged 6 % in 1998-2004 (after the Asian financial crisis in 1997) but Laos remains an economy burdened by bureaucratic regulation, dependent on aid, and vulnerable to external shocks (World Bank, 2006).

As the majority of the population depends on farming in rural areas as their main livelihood, economic development is strongly linked to rural development. Agriculture (including forestry and fisheries) is the most important sector in the economy, accounting for over 50 % of GDP, though its relative share has been declining since the beginning of the 1990s. The predominant crop is glutinous rice. In non-drought years, Laos as a whole is

self-sufficient in food, but each year flooding, pests, and localized drought cause shortages in various parts of the country. The aquaculture sector has expanded and diversified in the last twenty years. Many exotic fish species, including Nile tilapia, common carp, Chinese carp and African catfish have become popular for aquaculture as they grow quickly and are easy to manage. However, there is limited knowledge within the country on nutrition and management issues under Laotian conditions related to these imported fish species. Therefore, research is needed in order to avoid problems and to optimize the production systems. The African catfish (*Clarias gariepinus*) was chosen for more detailed studies, related to feed resource utilization and feed formulation, in the current thesis.

Objective of the study

- To survey the availability of natural feed resources for aquaculture and to study their nutrient content.
- To study the possibilities to preserve Golden Apple snails to be used as protein-rich feed resource.
- To evaluate the nutrient availability in Golden Apple snails fed either fresh or in a preserved state to African catfish (*Clarias gariepinus*).
- To evaluate the influence of replacing fish meal with Golden Apple snails on the performance and yield of cultivated African catfish (*Clarias gariepinus*).

Hypotheses of the study

- The nutrient content of natural feed resources and their availability varies considerably between sources.
- The total tract digestibility of carbohydrates in African catfish (Clarias gariepinus) is high and is not influenced by feed source.
- Natural feed resources can replace conventional protein sources in nutritionally balanced diets without any negative effects on performance in African catfish (*Clarias gariepinus*).

2 Background

2.1 Importance and status of aquaculture in Laos

Presently aquaculture is the main an important economic sector in Laos, and contributes more than half of the animal protein to the human population (FAO, 2008). However, the overall importance of captured fish from rivers has declined, due to serious environmental and social issues. These are linked to the exploitation of timber resources, with deforestation proceeding at a quite alarming rate, together with soil loss, siltation, and loss of biodiversity, pressure on tribal people to change traditional land use practices, and hydro-power development with dams likely to displace whole communities and impact on river flow regimes and potentially wild fisheries (Rigg and Jerndal, 1996).

The economic outlook for Lao PDR remains favorable, but rising inflation poses a risk, and has been climbing from 4.5 % in 2007 to approximately 6.5 % in January 2008 (East Asia, 2008). Food security at household level derives principally from forestry, livestock and fisheries, with freshwater fish being the principal source of animal protein for the rural population. Estimates of average annual per capita consumption vary widely, from 7 to over 57 kg, depending on the area, but an overall average for most of the provinces lies between 15 and 25 kg/head/yr. Surveys carried out by the MRC Fisheries Program suggest that, in Lao PDR and Cambodia, the consumption of traditionally dried and fermented fish products alone amounts to 10 to 14 kg/head/yr (FAO, 2006). Consumption of fish and aquatic products was estimated to be between 13-48 kg/capita/yr, representing between 22 to 55 % of animal product consumption (Funge-Smith and Dubeau, 2002).

The Government of Lao PDR is endeavoring to increase the annual fish consumption to 23 kg per capita by year 2010 (MAF, 2005). The gap between supply and demand must be met by aquaculture related developments, which are still in their infancy and currently account for less than 250 t per year (MRC, 2007). In this regard, it has been recognized that substantial increases in fish production could be obtained through effective and optimal utilization of seasonal water bodies, such as flood plain depressions and reservoir coves for culture based fisheries. This is a practice that requires little or no capital inputs and harnesses natural productivity of these water bodies for augmenting fish production. As fish provide a great part of the animal protein intake, the demand for fish is stable year-round. Demand, and therefore prices, peaks only during the Lao New Year festival (mid-April), which is in the dry season, when fresh fish is in short supply. Aquaculture provides fish during that season, allowing farmers to benefit from a good price for what is often a relatively low quality product (FAO, 2006).

In addition to eating fish, rural Lao people eat many other aquatic resources such as eels, frogs, tadpoles, crabs, insects and shrimps, and many of these are foraged from water bodies and make up a considerable part of their daily diet. Increases in demand for animal protein are likely to occur throughout Laos over the coming decade because of the increasing population. In addition to protein and essential amino acids, and fat and essential fatty acids, fish are an important source of thiamine and riboflavin of the vitamin B complex. The health and nutritional status of young children and lactating women could be enhanced through more regular consumption of fish (FAO, 1998). Aquaculture is making a significant contribution to improving livelihoods and alleviating poverty, and can in addition play a social role, as it may contribute in maintaining populations in their native places.

2.2 African catfish production systems

Production systems for culture of African catfish will vary with the geographical conditions and the investment capacity of the farmer. Based on the intensity of the production, the production systems used for fish culturing in general can be classified as extensive, semi-intensive and intensive.

Extensive system: In these systems the ambient environment provides the total feed requirement of the cultured fish. These systems use low stocking densities (e.g. 5,000-10,000 fingerlings/ha/crop) and no supplemental

feeding (Ravagnan, 1978), although fertilization may be applied to stimulate the production of natural food in the water. Water change is effected through tidal means, i.e. new water is let in only during high tide and the pond can be drained only at low tide. The ponds used for extensive culture are usually large (more than two ha) and may be shallow and not fully cleared of tree stumps. Production is generally low, amounting to less than 1 t/ha/year. In China, lakes are classified into three categories according to their surface area (ADCP, 1979).

Semi-intensive systems: These systems use supplementary feeding and they are managed by the application of inputs and the manipulation of the environment, primarily by way of water management through the use of pumps and aerators (Ravagnan, 1978). These systems use higher densities than extensive systems (e.g. 50,000-100,000 fingerlings/ha/crop). In Africa semi-intensive pond culture of catfish is limited to very specific areas, where the climate is warm enough for good growth, and where there is an ample source of water. In southern Africa such locations are usually found in regions situated far from the market and where riparian land is expensive. Site selection is therefore very important. Semi-intensive systems are very suitable for pond culture conditions and under small-scale rural farming conditions (FAO, 1998)

Intensive systems: Intensive culture uses very high densities of fish (e.g., 200,000-300,000 fingerlings/ha/crop), and well balanced feed that supplies all the energy and nutrient needs of the cultured fish. The computed daily feed ration is given in equal doses from as low as three to as high as six times a day (Ravagnan, 1978). The stocking rate depends on the marketable size desired and varies from 2 to 10 fingerlings per square meter, corresponding to a marketable size fish of approximately 500 and 200 g, respectively, after 6 months (de Kimpe and Micher, 1974; Richter, 1976). Higher stocking densities are not recommended as adverse water conditions at the end of the production cycle are difficult to manage. Monoculture of the African catfish is possible when suitable feed, with a high protein content (including animal protein), is available. Feeding with a balanced feed is a prerequisite for intensive monoculture of the African catfish, and the feed should contain 30 to 35 % digestible protein (about 40 to 50 % crude protein) and 10.5 to 14.5 MJ digestible energy/kg dry feed.

2.3 Digestive capacity of catfish

Digestibility of the feed is a function of feed composition and is directly related to the digestive capacity. A digestibility value is a relative measure of

the extent to which ingested food and its nutrient components have been digested and metabolized by the fish. The ability of fish and vertebrates to use nutrients depends on many factors, such as the synthesis of appropriate enzymes, production of digestive enzymes in suitable amounts and their distribution along the gut lumen (Kuz'mina, 1990). In carnivorous fish or those with a meat-orientated omnivorous diet there is normally a definite stomach (foregut) whilst herbivorous or plant-orientated omnivores normally have no stomach but rely on a much extended mid gut area for the digestion of the food (Chou *et al.*, 2001).

The African catfish (*Clarias gariepinus*), together with the walking catfish and the hybrid catfish, are classified as omnivores, and can be expected to utilize dietary carbohydrates more efficiently than other fish (Jantrarotai *et al.*, 1994; Nematipour *et al.*, 1992a, Chow and Halver, 1980). Previous studies on omnivorous fish such as common carp, tilapia and African catfish, have shown that they are able to digest carbohydrates efficiently. Moreover, tilapia appears to digest animal protein better than carp, probably because most of the carp food originates from benthic source, while most of the tilapia food originates from plants (Anderson *et al.*, 1984; Degani and Revach, 1991). Studies on African catfish (*Clarias gariepinus*) have shown that they can perform equally well on isonitrogenous and isocaloric diets (40% protein, 20 kJ GE g⁻¹) with a dietary carbohydrate content ranging from 27 to 38% and with a carbohydrate to lipid ratio of 1.7 to 3.4 (Ali and Jauncey, 2004).

Protein digestibility: The total tract apparent digestibility values for protein in protein-rich feedstuffs in channel catfish are usually in the range of 75-95 % (NRC, 1993). According to Fagbenro (1996), the apparent digestibility of protein in feedstuffs of animal origin or of plant origin in catfish (*Clarias isiriensis*) is similar. It has been reported that the protein digestibility of cottonseed meal in catfish is about 84%, but the lysine availability is only about 66% (Fagbenro *et al.*, 1998). A similar situation may exist for other feedstuffs. Therefore, if feeds are formulated on a protein basis, a lysine deficiency may result. A major problem in formulating catfish feeds on an available amino acid basis is the lack of sufficient data (Robinson *et al.*, 2001)

Lipid digestibility: The total tract apparent digestibility values for lipids in channel catfish are high and vary between feedstuffs (NRC, 1993). Lipids are a highly digestible source of concentrated energy for catfish feed and contain more than twice as much energy as does an equivalent amount of carbohydrates (Robinson *et al.*, 2001). Total tract apparent digestion values for lipids in channel catfish fed uncooked corn, meat and bone meal, cotton seed, soybean meal, poultry feather meal, wheat grain, fish oil and anchovy

meal were 76, 77, 81, 81, 83, 96, 97 and 97%, respectively (Wilson and Poe, 1985).

Carbohydrate digestibility: Starch is an important dietary non-protein energy source for catfish, and which has to be included at suitable levels to maximize the use of dietary protein for growth (Ali and Jauncey, 2004). The ability to digest starch depends on the secretion of α -amylase to the gastrointestinal tract. It is believed that all species of fish have the ability to secrete α -amylase. In carnivores such as the rainbow trout and sea perch, amylase is primarily of pancreatic origin, whereas in herbivores the enzyme is widespread throughout the entire digestive tract (Chow and Halver, 1980). Average total tract apparent digestibility values of starch in channel catfish fed wheat grain, uncooked corn (30 percent of diet), uncooked corn (60 percent of diet), cooked corn (30 percent of diet) and cooked corn (60 percent of diet) were 59, 66, 59, 78 and 62 %, respectively (Wilson and Poe, 1985).

2.4 Potential feed resources for catfish production in Laos

2.4.1 Conventional

African catfish culture in Laos makes a significant positive contribution to food security in extensive and semi-intensive systems of production in which natural feed resources predominate. It has been estimated that a total of 13 different feed resources are available, the most important being cassava roots, cassava leaves, maize, broken rice, rice bran, dried brewers yeast, sugar wastes, leucaena leaves, soybeans, water hyacinth and dried fish (*Clupeichthys aesamensis*; PaKeo) (Thailand/Laos, 1996). Most of the raw materials used in African catfish feed is produced in the country, but the domestic production of some of the important ingredients such as soybeans, cassava and maize is insufficient because they are used also for manufacturing poultry feed. The protein sources of animal origin for African catfish feed are mainly from small fish, such as PaKeo.

In the Nam Ngum reservoir (90 km North of Vientiane, the capital city; maximum area of 45 000 ha), PaKeo makes up 28 % of the total catch, but only about 10 % of the total value. Most of the PaKeo catch, approaching 80 %, is dried immediately and sold to fish traders. Some PaKeo (13.9 %) is also consumed locally, and is a cheap source of protein. Only 2.5 % (48 tons) of the PaKeo catch is used for aquaculture as feed in cages (Mattson *et al.*, 2001). Most of the catfish farms are found in the Naxaythong, Sikhotabong and Xaythani districts (LARReC, 2000). These districts have

suitable conditions for small-scale on farm feed formulation, due to the availability of PaKeo as a good protein source. Moreover, the fish farmers in these districts can also make use of agro-industrial residues such as dried brewers yeast from the beer factory in Vientiane municipality and carbohydrate-rich by-products such as rice bran and broken rice from the glutinous rice industry.

2.4.2 Non-conventional

A wide range of non-conventional feed ingredients could potentially be used to prepare fish feed (Sogbesan and Ugwumba, 2008). Nonconventional feed resources include aquatic and terrestrial plants such as duckweed, azalea and water hyacinth, aquatic animals such as snails, clams and frogs, and terrestrial animals such as larvae, earthworms and termites. They may be incorporated into animal and/or fish feed as protein supplements so as to replace the high-cost conventional protein feedstuffs (Chen and Meyers, 1983).

Rural Lao African catfish culture requires a relatively low entry cost (selfconstruction of pond, fingerlings for stocking and occasional feeding or fertilization). Non-conventional feed resources are low cost inputs that are affordable to poorer farmers and because the cost of production is low, catfish can be sold at a reasonable and affordable price to poor consumers.

Snails are potential feed resources for on farm feed preparation of aquaculture feed (Basa, 1988; New and Csava, 1993; Pascual, 1989). In Laos, the Golden Apple snail (GAS) is an interesting unconventional feed resource, characterized by a high protein and fat content (Kaensombath, 2003), that potentially may be used as a replacement for fish meal in catfish feed. In Southeast Asia, the live GAS are collected manually from rice fields, where it is considered to be a pest that causes great damage to the growing rice plants (Duagbupha and Khamphoukeo, 1998; Dancel and Joshi, 2000). Today, GAS have spread to 10 of 17 provinces in Laos, mainly by way of connecting waterways, such as irrigation canals and rivers, as well as by people. GAS does most damage to young rice seedlings (seedbeds up to 20 days after transplanting), and consequently fields infested with GAS have to be replanted several times in order to replace the missing seedlings. Moreover, GAS also consume other plants of direct economical importance, such as taro (Colocasia esculenta L.), morning glory (Ipomoea aquatica Forska° l), lotus (Nelumbo nucifera Gaertner) mat rush (Juncus decipiens Buchenau), Chinese mat grass (Cyperus monophyllus Vahl), wild rice (Zizania latifolia Turczaninov), Japanese parsley (Oenanthe stolonifera Wall), water chestnut (Trapa bicornis Osbeck), and azolla (Azolla spp.).



Table 1. Chemical composition (% in dry matter) of snail meal

| Parameters | Flesh meal | Shell meal | Whole meal | Whole meal | Whole meal |
|----------------|------------|------------|------------|------------|------------|
| Moisture | - | - | - | 4.7 | 7.0 |
| Crude protein | 60.9 | 2.8 | 16.1 | 13.7 | 52.8 |
| Crude fat | 6.1 | 1.0 | 2.0 | 1.7 | 1.8 |
| Ash | 9.6 | 54.5 | 46.0 | 73.8 | 20.9 |
| Crude fiber | 4.5 | - | - | 1.0 | 1.9 |
| N-free extract | 18.9 | - | - | 9.8 | 22.6 |
| Calcium | 2.0 | 36.1 | 31.1 | - | - |
| Phosphorus | 0.84 | 0.41 | 0.32 | - | - |

Source: Handbook on ingredients for aquaculture feeds, chapter 40 (Herthampf and Pascual, 2000).

2.5 Preservation of potential feed resources

The increasing use of indigenous feed resources involves searching, not only for new sources of aqua-feed, but also for many ways in which the supply of existing resources may be extended to cover a longer period. This may involve drying or ensiling, so that surplus feed from seasons of abundance will still be available many months later when feed is scarce. In the monsoon climate common in Asian countries, it is usually the dry season which is the time when livestock are most in need of a supplementary feed supply (FAO, 2008).

2.5.1 Drying

Under tropical conditions, sun-drying is an attractive simple low-cost method to remove water from fresh feed resources and thereby allow longterm storage. Drying is basically a simple operation, however, when applying additional heat to speed up the drying process considerable skill is required to get the drying conditions just right. There are two main types of dryers, direct and indirect. In the direct dryer very hot air at a temperature of up to 500 °C is passed over the material as it is tumbled rapidly in a cylindrical drum; this is the quicker method, but heat damage is much more likely if the process is not carefully controlled (Windsor, 2001). In the indirect dryers, less air is used and the hot air does not come in direct contact with the feed material. This reduces the risk for reaching to high temperatures in the material and causing heat damage.

2.5.2 Ensiling

Ensiling is a suitable method to preserve different feed resources for longterm storage. The fresher the raw materials used for silage making, the better the quality of the end product (Hertrampt and Pascual, 2000).

Fish silage: Organic acids commonly used for fish silage making, are formic and propionic acids. Moreover, inorganic acids such as hydrochloric and sulphuric acid have also been used (New, 1987; Raa, 1994). Common for both acid sources is that they help to quickly lower the pH to 4.0 or less at a low inclusion level. Fish offal, including the gut and whole pelagic fish, will liquefy very fast, while processing waste without the guts hardly will autolyse (Backhoff, 1976; Gildberg, 1982; Raa and Gildberg, 1976).

Fermented fish silage: It is also possible to produce fish silage without acid addition provided that a microbial fermentation process can be initiated. It has been shown, that fermented fish silage can be prepared by mixing fish with molasses at inclusion levels of 10 to 12 % (New, 1987; Raa, 1994; Raa and Gildberg, 1976). The starting temperature was 21 to 25 °C and the pH 6.0 to 6.2, then decreasing to 4.5 during the first 48 hours (New, 1987; Raa, 1994). Provided that there are sufficient lactic acid bacteria available, they will quickly convert the sugar to lactic acid with a reduction in pH as a result.

Crustacean silage: Fresh shrimp waste (comprising mainly heads and carapace) can be used as a feed resource. The method for preparing shrimp waste silage is similar to that used for making fish silage. However, the content of $CaCO_3$ in shrimp shells is higher than in fish and therefore more acid is needed for stabilizing the silage (Meyers and Benjamin, 1987; Chen and Meyers, 1983). The liquefaction process is completed after a few days. The liquid can be separated from the solid sediment by centrifugation. The protein content in the wet weight of the liquid ranges from 60 to 70 %, and more than 85 % of the chitin remains in the sediment. If the insoluble chitin is removed the silage can be processed to a pure end-product (Hall and Silva, 1994).

Fermented crustacean silage: The technology used is similar to that used for fermented fish silage. In addition to the use of molasses as a source of sugar for lactic acid bacteria, additives such as fermented rice, cooked rice and cassava has been used (Hall and Silva, 1994). Shrimp head silage has successfully been used as replacement for fish meal in diets to cultured African catfish (Nwanna, 2003).

Squid viscera silage: The technology used is similar to that used for shrimp head silage. The protein content of squid viscera is lower than in the fresh material. On the other hand the dry matter content of the silage is higher

than in the raw material (Carver *et al.*, 1989). However, the squid viscera have a higher content of most essential amino acids than shrimp head silage (Meyer and Benjamin, 1987).

| Parameters | Fish silage | Shrimp head silage | Squid viscera silage |
|-----------------------|-------------|--------------------|----------------------|
| Dry matter | 24.9 | 21.0 | 19.9 |
| Crude protein | 58.3 | 45.6 | 60.8 |
| Crude fibre | 3.6 | - | - |
| Crude fat | 16.0 | 4.8 | 7.3 |
| Ash | 14.7 | 24.3 | 9.5 |
| Nitrogen free extract | 28.9 | - | - |
| Calcium | 31 | 6.0 | 0.4 |
| Phosphorus | 1.9 | 1.6 | 1.0 |

Table 2. Chemical composition (% in dry matter) of fish silage, shrimp head silage and squid viscera silage

Source: Handbook on ingredients for aquaculture feeds, chapter 20 (Herthampf and Pascual, 2000).

2.6 Nutrient requirements of African catfish

Energy requirements: Energy is one of the most important parts of the diet and feeding standards for many animals are based on energy needs. Feed intake for catfish may be more a function of how much feed they are allowed to have rather than energy concentration in the feed. Although catfish feed intake may not be strictly regulated by dietary energy, balance of dietary energy is important when formulating catfish feeds (Jantrarotai et al., 1994). Moreover, if the dietary energy content is too high, catfish may not eat as much as expected, resulting in too low intake of essential nutrients. The absolute energy requirements for catfish are unknown. The estimates that are available have been made by measuring weight gain or protein gain of catfish fed diets with known content of energy (Hossain et al., 1998; Henken et al., 1985). Energy requirements for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein (DE/P), range from 31.0 to 50.2 kJ g⁻¹. Based on current knowledge, a DE/P ratio from 35.6 to 39.8 kJ g^{-1} is adequate for use in commercial catfish feeds. Increasing the DE/P ratios of catfish diets above this range will increase fat deposition and reduce processed yield, and in contrast if the energy value is too low the fish will grow slowly (Nematipour *et al.*, 1992a).

Protein and amino acid requirements: Protein comprises about 65-70 % of the dry weight of fish muscle (Wilson and Halver, 1986). A continual supply

of protein is needed throughout life for maintenance and growth. African catfish (*Clarias gariepinus*) has a relatively high dietary protein requirement. The best growth rates and food conversions are achieved with diets containing 35-42% crude protein and a calculated digestible energy level of 12 kJ g⁻¹ (ADCP, 1983). Catfish, like other animals, need nitrogen and certain amino acids rather than protein as such. Usually the most economical source of these elements is a mixture of proteins in feedstuffs. Using protein for energy is expensive, so catfish feeds should be balanced to ensure adequate levels of nonspecific nitrogen, amino acids, and non-protein energy are supplied in the right amounts.

| i able 5. Amino acia requirement of juvenile channel catilish | | | | | | |
|---|---------------------------|---------------------------|--|--|--|--|
| Amino acid | Requirement as percentage | Requirement as percentage | | | | |
| | of dietary protein | of dry diet | | | | |
| Arginine | 4.3 | 1.0 | | | | |
| Histidine | 1.5 | 0.4 | | | | |
| Isoleucine | 2.6 | 0.6 | | | | |
| Leucine | 3.5 | 0.8 | | | | |
| Lysine | 5.1 | 1.2 | | | | |
| Methionine | 2.3 | 0.6 | | | | |
| Phenylalanine | 5.0 | 1.2 | | | | |
| Threonine | 2.0 | 0.5 | | | | |
| Tryptophan | 0.5 | 0.12 | | | | |
| Valine | 3.0 | 0.71 | | | | |

Table 3. Amino acid requirement of juvenile channel catfish

Source: NRC, 1993.

Economical gain may differ as the cost of feed ingredients varies. Moreover, it is difficult to set a level of protein that is best for all situations because of the factors that affect the dietary protein requirement of catfish. These include water temperature, feed allowance, fish size, amount of non-protein energy in the diet, protein quality, natural food available and management practices (Robinson, 1989). To obtain the best profit, the optimum dietary protein level should be changed as fish and feed prices change. However, in practice, most catfish producers feed a diet with the same amount of protein throughout the growing period. Commercial catfish feeds used for growing food fish typically contain 28 to 32 % protein (Beem *et al.*, 1988). Diets containing lower levels of protein are adequate for maximum growth but may increase body fat. Catfish fry and small fingerlings require diets with more protein. Fry diets used in the hatchery

should contain 45 to 50 % protein, and fingerlings (less than 40 kg /1,000) could be fed a 35 % protein diet (Reis *et al.*, 1989).

Table 4. Recommended dietary nutrient levels (in DM) for African catfish (Clarias gariepinus)

| Component | Fry and Fingerlings | Growers | Broodstock |
|-----------------------------|------------------------|-----------|------------|
| Digestible protein,% | 35-40 | 30-35 | 35-40 |
| Digestible energy(kJ/g) | 12.5-16.7 | 10.5-14.6 | 12.5-16.7 |
| Ca (min-max),% | 0.8-1.5 | 0.5-1.8 | 0.8-1.5 |
| P (min-max),% | 0.6-1.0 | 0.5-1.0 | 0.6-1.0 |
| Methionine + Cystine(min),% | 1.2 | 0.9 | 1.0 |
| Lysine (min),% | 2.0 | 1.6 | 1.8 |

Source: ADCP, 1983.

Table 5. Dietary protein and energy requirements for African catfish (Clarias gariepinus)

| Protein (%) | Energy (kJ/g) ^a | $P/E (mg/kJ)^{b}$ | References |
|-------------|----------------------------|-------------------|----------------------------|
| >40 | 13 ME | 31 | Machiels and Henken (1985) |
| 40-42 | 14-16 DE | 26-29 | Uys (1989) |
| 40 | 11-13 GE | 31-36 | Degani et al. (1989) |

^a GE = gross energy; ME= metabolizable energy; DE= digestible energy

^bP/E= protein-energy ratio

Carbohydrate requirements: Carbohydrates are the main form of energy stored in seeds, roots and tubers. Carbohydrates have several functions in animals. Although catfish do not have a specific need for carbohydrates in their diet (Raj *et al.*, 2008), catfish feeds contain considerable amounts of carbohydrates that are supplied from grains or grain by-products that are rich in starch. Starch is not only an inexpensive energy source but also aids in feed manufacture. Normally catfish feed contains 25 % or more soluble carbohydrates plus 3 to 6 % less soluble carbohydrates that are in general present as crude fiber (Hogendoorn *et al.*, 1983). African catfish cannot digest crude fiber well, so it should be kept at as low a level as possible. Commercial catfish feeds typically contain less than 5 % crude fiber.

Lipid requirements: Lipids play several important roles in an animal's metabolism, including supplying essential fatty acids (EFA), and helping to absorb fat-soluble vitamins. Moreover, including lipids in the diet may increase food intake (Murai *et al.*, 1985). Catfish apparently require 0.5 to 0.75 % omega-3 fatty acids in the diet (Robinson and Li, 1996). Lipid levels in commercial feeds for food-sized catfish rarely exceed 5 to 6 %. About 3

to 4 % of the lipids are naturally found in the feed ingredients, with the remaining 1 to 2 % being sprayed onto the finished pellets. Spraying feed pellets with lipid increases dietary energy and helps reduce feed dust. Essential fatty acids can be supplied by marine fish oil such as menhaden oil.

Vitamin requirements: Vitamins vary greatly in structure and function. They are generally defined as organic compounds that animals require in small amounts in their diets for normal growth, health, and reproduction (Robinson *et al.*, 2003). Some vitamins may be made in the body and thus are not required in the diet. The amounts and quality of vitamins that catfish need have been fairly well determined in laboratory and pond studies (NRC, 1993). Commercial catfish feeds are generally supplemented with a vitamin premix that contains enough of all essential vitamins to meet the requirement and make up for losses from feed processing and storage. Vitamin loss during storage is not a major factor if the feed is stored for a short time (Robinson, 1989).

Mineral requirements: Although mineral studies with fish are difficult to do, mineral needs of catfish have been suggested, and the signs of mineral deficiency have been described (Robinson and Li, 1996). Phosphorus is particularly important in fish feeds because fish require a large amount of it. Feedstuffs, especially those from plants, are poor sources of phosphorus, and fish do not get enough phosphorus from pond water. As a result, African catfish feeds are usually supplemented with phosphorus. Dicalcium and defluorinated phosphates are commonly used as phosphorus supplements in catfish feeds. African catfish feeds are typically supplemented with a trace mineral premix with enough of all essential trace minerals to meet or exceed dietary requirements of catfish. A trace mineral premix may not be needed in catfish feeds that contain 4 % or more animal protein (Yan *et al.*, 2007; Robinson, 1989).

| Vitamin | Deficiency signs | Units | Require- |
|------------------|---|-------|----------|
| | | | Ment |
| Thiamin | Dark skin color, neurological disorders | ppm | 1.0 |
| Riboflavin | Short-body dwarfism | ppm | 9.0 |
| Pyridoxine | Greenish blue coloration, tetany, nervous disorders | ppm | 3.0 |
| Pantothenic acid | Clubbed gills, anemia, eroded skin, lower jaw, fins and barbells | ppm | 15 |
| Niacin | Anemia, lesions of skin and fins, exophthalmia | ppm | 14 |
| Biotin | Anemia, skin depigmentation, reduced liver pyruvate carboxylase activity | ppm | R |
| Folic acid | Reduced hematocrit | ppm | 1.5 |
| B12 | Reduced hematocrit | ppm | R |
| Choline | Hemorrhagic kidney and intestine, fatty liver | ppm | 400 |
| Inositol | None demonstrated | ppm | NR |
| Ascorbic acid | Reduced hematocrit, scoliosis, lordosis, increased susceptibility to bacterial infections, reduced bone collagen formation, internal and external hemorrhage | ppm | 60 |

 Table 6. Water soluble vitamin deficiency signs and minimum levels required to prevent signs of deficiency in catfish

Source: NRC, 1993. Notes: R: required; NR: Not required

 Table 7. Fat soluble vitamin deficiency signs and minimum levels required to prevent signs of deficiency in catfish

| Vitamin | Deficiency signs | Units | Require- |
|---------|--|-------|----------|
| | | | ment |
| А | Exophthalmia, edema, acities | IU | 450-900 |
| D | Low bone ash | IU | 110-220 |
| Е | Skin pigmentation, exudative diathesis, muscle dystrophy, erythrocyte hemolysis, splenic and pancreatic hemosiderosis. | IU | 23 |
| К | Skin hemorrhage, prolonged clotting time | ppm | R |

Source: NRC, 1993. Notes: R: required

| Mineral | Deficiency signs | Requirement |
|---------|--|----------------|
| Ca | Reduced bone ash | <0.1 %, 0.45 % |
| Р | Reduced bone mineralization | 0.40 % |
| Mn | Muscle flaccidity, sluggishness, reduced bone, serum, and whole body | 0.04 % |
| Na | ND | ND |
| К | ND | 0.26 % |
| Cl | ND | ND |
| S | ND | ND |

Table 8. Macro mineral deficiency signs and minimum levels required to prevent signs of deficiency in catfish

Source: Robinson, 1989. ND: not determined

Table 9. Micro mineral deficiency signs and minimum levels required to prevent signs of deficiency in catfish

| Mineral | Deficiency signs | Requirement |
|---------|---|-------------|
| Со | ND | ND |
| Ι | ND | ND |
| Zn | Reduced serum zinc and serum alkaline phosphatase activity, reduced bone zinc and calcium concentrations. | 20 ppm |
| Se | Reduced liver and plasma selenium-dependent glutathione peroxidase activities. | 0.25 ppm |
| Mg | None | 2.4 ppm |
| Fe | Reduced hemoglobin,hematocrit anderythrocyte count. Reduced serum iron and transferring saturation levels. | 20 ppm |
| Cu | Reduced hepatic copper-zinc superoxide dismutase, reduced heart cytochrome oxidase activities. | 4.8 ppm |

Source: Robinson, 1989. ND: not determined

| Descriptions | Contents |
|-------------------------|---------------------|
| Thiamin, g | 11 |
| Riboflavin, g | 13 |
| Pyridoxine, g | 11 |
| Pantothenic acid, g | 35 |
| Nicotinic acid, g | 88 |
| Folic acid, g | 22 |
| Vitamin B 12, g | 0.09 |
| Choline, g | 550 |
| Ascorbic acid, g | 350 |
| Vitamin A, IU | 4400 x 1000 |
| Vitamin D, IU | 22 00 x 1000 |
| Vitamin E, IU | 55 x 1000 |
| Vitamin K, IU | 11 x 1000 |
| Filler (maize meal), kg | 2.0 |

Table 10. Composition of a dietary vitamin premix for African catfish (Clarias gariepinus)

Source: Hecht et al., 1988.

2.7 Feeding of African catfish

2.7.1 Potential feed resources

Feed cost is a major operating cost in fish farming mainly due to lack of cheap sources of protein (FAO, 2006). It has been estimated that feed costs account for 60-70 % of the total production costs in African catfish culture in large fish farms in Laos. In principle, the production conditions for catfish farming are similar to any type of livestock production system in that there is a need to provide enough feed composed of good quality feed ingredients (Beem and Anderson, 1990). There is considerable potential to expand African catfish farming in Africa and Asia to improve food security. In order to develop a sustainable and resource efficient production of African catfish in Laos there is a need to make better use of potentially available local feed resources, including livestock waste, industrial and agricultural by-products, as well as non-conventional feed resources (Kapetsky, 1994; Adikwu, 1991; Ayinla and Akande, 1988; Alegbelege *et al.*, 2001; Yang *et al.*, 2003).

There are several examples of successful partial or complete replacement of fish meal protein in catfish diets with other protein-rich feedstuffs, such as soybean meal and poultry by-product (Goda *et al.*, 2007), full-fat winged bean (*Psophocarpus* tetragonolobus) seed meal (Fagbenro *et al.*, 1999) and fermented shrimp head waste meal (Nwanna, 2003).

2.7.2 Possible constraints

Time may be a limiting factor for the farmer, and may be a constraint for preparing large quantities of farm-made feeds. Moreover, in some rural areas there is no electricity supply, which means that the farmers have to use manpower to process the feed (Luu, 1993).

A more extensive use of non-conventional feed resources may lead to poor fish performance due to the use of nutritionally unbalanced diets. It can be suspected that the quality and quantity of available feed resources in many cases are unknown, which will result in unpredictable production (Somsueb, 1993). Moreover, there is a potential risk for the occurrence of antinutritional factors in non-conventional feed resources not previously used for fish culturing (Francis *et al.*, 2001a). This is a well-known problem associated with leguminous seeds, such as soybeans, which may be a reason for poor performance in catfish (Spinelli, 1980).

Fat is a concentrated source of energy and is less expensive than protein, and therefore some fat is normally included in catfish diets (Nematipour *et al.*, 1992b). However, lipids are stored in body tissues and excess dietary lipids may result in too much fat deposited in the body. This may affect processed yield, product quality, and shelf-life of processed products.



3 Summary of materials and methods

3.1 Location and climate of the study area (Paper I, II, III and IV)

The survey (Paper I) was conducted in three of ten districts in Vientiane municipality (Paper I), namely Naxaythong (N), Sikhotabong (S) and Xaythani (X), which are located about 40 km to the north, 12 km to the north and 20 km to the south of Vientiane City, respectively. The area has an altitude of around 150 m above sea level. The climate in the area can be divided into two main seasons: wet and dry. The wet season lasts from May to October and the dry season from November to April. Annual rainfall averages about 1600 mm and the peak rainfall occurs in the period July to August. The average minimum and maximum temperatures are about 16 and 32 °C, respectively.

The experiments (Paper II, III and IV) were carried out in facilities of the Faculty of Fisheries at Nong Lam University, Ho Chi Minh City, Vietnam (Paper II, III and IV). The feedstuffs studied were collected and prepared in Laos, then transported to Vietnam for determination of the nutritive value of potential feed resources (Paper II), nutritive value of Golden Apple snails (GAS) (Paper III), and ensiling GAS and growth performance for African catfish fed raw and ensiled GAS. The mean ambient temperatures recorded were 31-34 °C in the middle of the day during the trial, which ran from February to June 2007.

3.2 Experimental design (Paper I, II, III and IV)

Paper I: A survey was performed which initially involved the selection of the research site with three categories of farms selected according to size, namely: small, medium and large. Rapid rural appraisal methods (Melville,

1993) were designed to collect essential information relating to the availability of catfish feed resources and the utilisation of agricultural by-products.

Paper II, III and IV: In all experiments the dietary treatments were randomized to three replicate fish tanks per treatment.

Paper II: A digestibility trial was carried out on 10 diets with African catfish juveniles. A basal diet (B), composed of fish meal, soybean meal, rice bran, cassava root meal, fish oil, soybean oil, vitamins and minerals, a binder (carboxy-methyl cellulose) and Cr_2O_3 as an indigestible marker was formulated. Four test diets were formulated to determine the digestibility of dietary components in the following selected carbohydrate–rich feedstuffs; rice bran, broken rice, maize and cassava root meal. Five test diets were formulated to determine the digestibility of dietary components in the following selected protein–rich feedstuffs; raw Golden Apple snail (*Pomacea spp*) (GAS), boiled GAS, dried fish, earthworms, frogs and termites (*Coptotermes curvignathus*).

Faeces were pooled within each treatment to obtain sufficient material for analysis, which meant that no statistical analysis was possible to perform on the data presented

Paper III: Three test diets were formulated to determine the digestibility of dietary components in raw (R), sun-dried (D) and ensiled (E) GAS. The test diets were composed of raw, sun-dried or ensiled GAS plus Cr_2O_3 , making up 30% of the dietary dry matter (DM), and diet B making up 70% of the DM. A basal diet (B), composed of fish meal, soybean meal, rice bran, cassava root meal, fish oil, soybean oil, vitamins and minerals, a binder (carboxy-methyl cellulose) and Cr_2O_3 as an indigestible marker was formulated.

Paper IV: Ground GAS was cleaned then mixed with additives (citric acid or molasses) in the raw form and placed in sealed plastic containers for ensiling for a total of 28 days. Citric acid (CA) was added at 1 (CA1) and 5 (CA5) % of dry matter (DM). Sugar cane molasses (M) was added at 5 (M5), 10 (M10), 15 (M15) and 20 (M20) % of DM.

Growth performance was studied in African catfish (*Clarias gariepinus*) fingerlings fed diets with raw and ensiled GAS as protein source.

3.3 Feed ingredients and diets (Paper I, II, III and IV)

The test diets (Paper II and III) were prepared by mixing the ingredients in the basal diet with the raw, sun-dried and ensiled GAS, respectively. After mixing the dietary ingredients the feed was cold pelleted using a 5 mm dye and then dried at 60°C for 24 hours.

To prepare the raw GAS (Paper II, III and IV), the shell was broken and removed, and the remaining cover and flesh cleaned with water before chopping and grinding. To prepare the boiled GAS (Paper II), snails were immersed in boiling water for 3 minutes, before shell removal and further preparation as described above. Both raw and boiled GAS (Paper II) was sun-dried outdoors for 2 days before milling.

Paper II: Termite nests, comprising mainly termites but also some of the nest, were broken up into small pieces before drying and milling. Dried fish, frogs and earthworms were cleaned with water before sun-drying and milling. After preparation, all tested feedstuffs were dried, milled through a 1 mm sieve and stored at room temperature until use.

Paper III: The ensiled GAS was ensiled with 15 % sugar cane molasses (in DM) and stored for 28 days before use

Paper IV: A control diet (C), composed of fish meal, rice bran, vitamins and minerals, and a binder (carboxy-methyl cellulose) was formulated. In addition, three experimental diets where fish meal was replaced with GAS were formulated. Fish meal was replaced with GAS ensiled with 5 % citric acid (diet GAS-CA5), with GAS ensiled with 20 % molasses (diet GAS-M20) and with raw GAS (diet GAS-Raw). The ensiled GAS was ensiled with 5% of citric acid and 20 % sugar cane molasses (in DM) and was allowed to ferment for 14 days before use. The test diets were prepared by mixing the raw and ensiled GAS with the other feed ingredients. After mixing the dietary ingredients the feed was cold pelleted using a 5 mm dye and then dried at 60°C for 24 hours before use.

3.4 Measurements (Paper I)

Paper I: In connection with farm visits, samples of feedstuffs used by the farmers were collected from each farm in each district. This was performed over a period of two weeks per district. Data for each farm were collected with respect to catfish yield, number of workers employed and available feed resources.

3.5 Fish management (Paper II, III and IV)

Paper II and III: African catfish (*Clarias gariepinus*) juveniles were acclimatised to the experimental conditions and cultured in concrete tanks to be familiar with artificial feed for at least 10 days before faeces collection

started. The digestibility studies were performed in 120 L cylindrical tanks with stagnant water. Each tank was stocked with 16-20 fingerlings of mixed sex. The digestibility study lasted for 14 days, comprising an adaptation period of 3-4 days followed by total faeces collection. Water quality parameters (temperature, pH, dissolved oxygen) were controlled during the experiment.

Fish were fed twice daily at 08.00h and 14.00h with a daily feed allowance of 5 % of body weight. Diets were placed in the water for 1 h, after which the tanks were cleaned from residual feed and faeces. The faeces were collected daily at 07.00 h and at 13.00 h from each tank and were stored at -20°C in a freezer. At the end of the collection period faeces were dried in an oven at 60°C, ground and preserved in airtight containers until analysis.

Paper IV: Fingerlings of African catfish (*Clarias gariepinus*) (360 in total) with an average body weight of 15 ± 0.4 g were randomly allocated to 12 net-cages (0.5 m x 2 m x 2 m). Each cage was stocked with 30 fingerlings. The fish were then acclimated to the experimental conditions and cultured in the net-cages to be familiar with artificial feed for 15 days before starting the experiment. The growth performance and each of the diets was fed to the fishes in triplicate cages at 5 % body weight twice daily (09.00 h and 16.00 h) for 42 days. The weight of each group of fish was taken fortnightly using a triple beam balance, and the feed adjusted accordingly. Dissolved oxygen and pH in the pond water were monitored every day (06.30 h) by using a digital/electronic pH meter.

3.6 Chemical analysis and calculations (Paper I, II, III and IV)

Analyses of dry matter (DM), ash, crude protein (CP) and crude fat (EE) were performed according to standard methods of AOAC (1990). Amino acids were determined with the performed acid oxidation with acid hydrolysis-sodium metabisulfite method described in Llames and Fontaine (1994), not applicable to determine tyrosine and tryptophan. Chromic oxide content in diets and faeces was analyzed according to Furukawa and Tsukahara (1966). The content of gross energy (GE) in feeds and faeces was determined with an adiabatic bomb calorimeter.

Paper I: The content (g/kg) of nitrogen-free extracts (NFE) in DM was calculated as; 1000-(ash + CP + EE + CF). The content (kJ/kg DM) of gross energy (GE) was calculated from the content of CP, EE and ash as described by Ewan (1989).

Paper II and III: The content (g/kg) of total carbohydrates (CHO_T) in DM was calculated as; 1000-(ash + CP + EE).

The apparent digestibility (AD) of the diets was calculated using the indicator technique according the equation

 $\mathrm{AD}_{\mathrm{D}} = 1 - (\mathrm{DC}_{\mathrm{F}} \times \mathrm{I}_{\mathrm{D}}) / (\mathrm{DC}_{\mathrm{D}} \times \mathrm{I}_{\mathrm{F}})$

 AD_{D} is the apparent digestibility of the dietary component; DC_{F} is the dietary component concentration in faeces, while I_{D} is the indicator concentration in diet, DC_{D} is the dietary component concentration in diet and I_{F} the indicator concentration in faeces.

The digestibility of tested ingredient (AD_T) was calculated from the AD_D of each dietary component in the basal diet and in the test diet (Bureau and Hua, 2006), as follows:

 $AD_{T} = AD_{D}$ test diet + [(AD_{D} test diet - AD_{D} basal diet) x (0.7 x DC_{D} basal diet/0.3 x DC_{D} ingredient)]

Paper IV: The following calculations were made on collected data to describe and evaluate fish performance.

(i) Mean weight gain (MWG) = Wf - Wi

Where, Wf is the mean final fish weight and Wi the mean initial fish weight.

- (ii) Percentage body weight gain $(PWG) = (MWG \ge 100)/Wi$
- (iii) Specific growth rate (SGR, %) (Brown 1957) = [(log Wf log Wi) x 100)]/D
- (iv) Protein efficiency ratio (PER)=MWG/protein consumed
- (v) Net Protein Utilization (NPU, %) = 100 x (Fish protein gain/protein consumed)
- (vi) Feed conversion ratio (FCR) = feed consumed (dry weight)/fish weight gain

3.7 Statistical analysis (Paper I, III and IV)

Paper I: Descriptive statistics was used to present general data while Minitab (2000) was used to calculate mean values and standard deviations (SD) on data from the proximate analysis of samples and for linear regression analysis.

Paper III and IV: The data were subjected to analysis of variance (ANOVA) by using the General Linear Model (GLM) procedure of Minitab version 14 (Minitab, 2000). When the F test was significant (p<0.05) the Tukey test for paired comparisons was used to compare means.

4 Summary of results

4.1 Potential feed resources (Paper I)

The main feedstuffs used by the farmers were traditional feedstuffs such as maize, broken rice, cassava root meal, rice bran, soybean, soy waste, dried fish and fish meal. In addition, some more unconventional feedstuffs such as leucaena meal, earthworms, termites and Golden Apple snails were used.

The highest CP content was found in Golden Apple snail, and dried fish, followed by soy waste, soybean, termites and earthworms. Soybean, termites and dried fish were highest in EE and leaucaena was highest in CF. Highest ash content was found in fish meal, earthworms and Golden Apple snails.

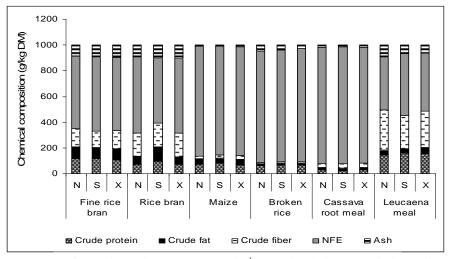


Figure 1. Analyzed chemical composition (g kg⁻¹DM) of carbohydrate-rich feedstuffs in different agro-ecological zones in Laos. Notes: N=Naxaythong district; S= Sikhotabong district; X= Xaythani district

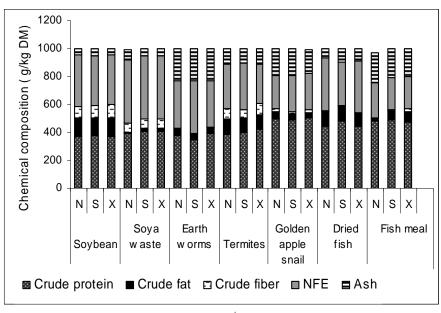


Figure 2. Analyzed chemical composition (g kg⁻¹DM) of protein-rich feedstuffs in different agro-ecological zones in Laos. Notes: N=Naxaythong district; S= Sikhotabong district; X= Xaythani district

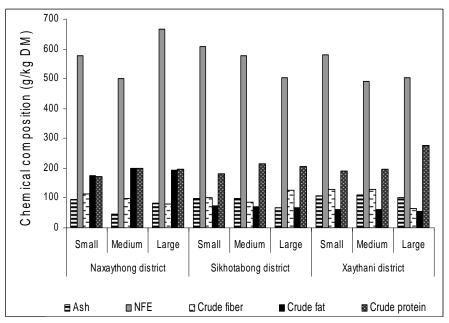


Figure 3. Analyzed chemical composition (g kg⁻¹DM) of catfish feed used in different agroecological zones in Laos.

4.2 Fish yield (Paper I)

The survey of fish production was performed in three districts with three level of farm size (large, medium and small). The estimated maximum fish yield from ponds in Naxaythong district was about 2.5 tons ha⁻¹ 6 months⁻¹, while in Sikhotabong district the corresponding yield was about 2.4 tons ha⁻¹ 6 months⁻¹ and in the Xaythani district it was about 3.0 tons ha⁻¹ 6 months⁻¹. In rain-fed, wet-land and irrigated areas the estimated catfish yield (per 6 months and ha pond area) ranged from 1.5 to 2.5, 1.8 to 2.4, and 1.7 to 3.0 tons ha⁻¹ 6 months⁻¹, respectively.

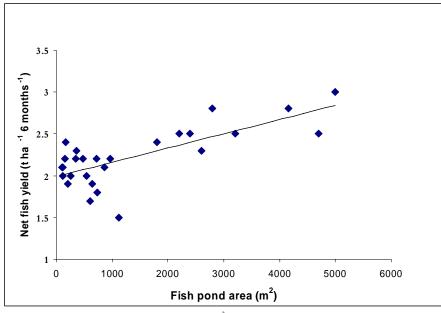


Figure 4. Relationship (y=0.0002 X + 1.99; $R^2 = 0.55$; P<0.0001) between fish pond area in catfish farms in three districts in Vientiane municipality.

4.3 Digestibility of potential feed resources (Paper II)

The apparent digestibility (AD) of CP in the test diets ranged from 86.6 to 93.2 %, and was on average 90.6 % (SD 2.4). The latter was only slightly lower than the AD of CP in the basal diet and was also reflected in high AD of CP in most feed ingredients.

However, on average the carbohydrate-rich ingredients showed lower AD-values than the protein-rich ingredients (54.8 vs. 82.7 %). This could

partly be due to low CP content in the carbohydrate-rich feeds as compared with the protein-rich feeds.

The AD of EE in the test diets ranged from 79.9 to 91.2 %, and was on average 87.0 % (SD 3.4). Although the range for the AD of EE was higher than for CP, the data indicates a high utilization of EE in most of the feed ingredients studied. Due to unexpectedly low AD of EE in fresh and boiled Golden Apple snails (GAS), the average AD of EE in the protein-rich feeds was lower than in the carbohydrate-rich feeds.

The AD of the carbohydrate components (NFE and CHO_{T}) in the diets and ingredients was lower than the AD for CP and EE. However, despite a larger variation in the AD between diets and ingredients, on average 72.2 % (SD 6.7) of the NFE and 77.7 % of the CHO_{T} (SD 3.7) were digested

The AD of energy for the feed ingredients showed a wider range than that of test diets (68.1-86.4 %; SD 6.9).

4.4 Influence of preservation method on the nutritive value of Golden Apple snails (Paper III)

The AD of CP was lower (P<0.05) in diet B+GAS-R and B+GAS-E than in diet B, while the AD of CP for diet B+GAS-D was higher (p<0.05). The lowest AD of CP was found for diet B+GAS-E. The AD of EE was higher in all test diets (p<0.05) than diet B. The highest value was found for diet B+GAS-D. There were no differences in the AD of EE between diets B+GAS-R and B+GAS-E. The AD of CHO_T was similar in diet R and B+GAS D, and these values were higher than in diets B and B+GAS-E.

There were no differences between diets (p>0.05) in the AD of CHO_T. The AD of ash was higher (p<0.05) in diet B+GAS-R than in diet B+GAS-D, but lower (p<0.05) in diets B+GAS-E and B. The lowest AD of GE was found for diet B+GAS-E. The AD of GE was higher (p<0.05) in diets B+GAS-R than diet B. The highest value was found for diet B+GAS-D.

The AD of CP was highest (p<0.05) in sun-dried, followed by raw and ensiled GAS (Table 4). The AD of ash and energy was highest (p<0.05) in sun-dried, followed by raw and ensiled GAS. There were no differences (p>0.05) between raw, sun-dried and ensiled GAS in the AD of EE and CHO_T.

The AD of essential (EAA) and non-essential amino acids (NEAA) was high and similar (p>0.05) between the basal diet and diet B+GAS-D. The lowest (p<0.05) AD for all EAA and most NEAA was found for diet B+GAS-E. The AD for most EAA and NEAA in diet B+GAS-R were intermediate between diets B+GAS-D and B+GAS-E.



The sun-dried GAS showed the highest (p<0.05) AD of EAA and NEAA, followed in decreasing order by raw and ensiled GAS. The AD of the EAA argininine, methionine and histidine, and the NEAA cystine and glutamic acid, did not differ (p>0.05) between raw and sun-dried GAS.

4.5 Energy content and protein supply from potential feed resources (Paper II and III)

Paper II: The estimated content of digestible energy (DE) ranged from 13.2 MJ/kg DM for rice bran to 18.1 MJ/kg DM for frogs. On average, the DE content of the carbohydrate-rich feed ingredients was lower than that of the protein-rich feed ingredients. This was mainly due to the lower AD for energy, as the content of gross energy differed less between feed ingredients.

The protein: energy ratio (P/E ratios) ranged from 2.2 to 6.4 g CP/MJ DE for the carbohydrate-rich feed ingredients, and from 17.4 to 26.1 g CP/MJ DE for the protein-rich feed ingredients.

Paper III: The estimated content of digestible energy (DE) in GAS (per kg DM) ranged from 14.9 MJ in sun-dried GAS to 10.0 MJ in ensiled GAS. The CP to DE ratio ranged from 17.8 g MJ^{-1} in raw GAS to 24.8 MJ^{-1} in ensiled GAS. The digestible CP (DCP) to DE ratio was higher in sun-dried GAS than in raw GAS, and the lowest value was found in ensiled GAS.

4.6 Growth performance of African catfish fingerlings fed diets with raw and ensiled Golden Apple snails (Paper IV)

There were no differences (P>0.05) in growth performance of African catfish fingerlings, expressed as specific growth rate (SGR) or percentage body weight gain (PWG), between the control diet and the diets with ensiled and raw GAS. Moreover, feed utilization, expressed as the feed conversion ratio (FCR), was similar (P>0.05) between diets. The dietary protein utilization, expressed as the protein efficiency ratio (PER) and as net protein utilization (NPU), was numerically higher on diets with GAS inclusion than on the control diet. However, these differences were not significantly different.

There were no treatment differences (P>0.05) in whole body moisture content and in chemical composition on a fresh weight basis. However, whole body protein content on a dry matter basis was lower (P<0.05) in fish fed diet GAS-Raw (41.2 %) than in those fed the other diets (50.9 to 52.2 %).

5 General discussion

5.1 Nutrient supply from potential feed resources

In intensive catfish production systems a nutritionally complete diet has to be provided to ensure that nutrients and energy needs are fulfilled. This may also be necessary in a semi-intensive production system, as the quantity of nutrients from natural food organisms in the pond may be too small to support a high growth rate. In the feed industry, a range of feed ingredients and additives is used to formulate nutritionally adequate fish feeds. In smallscale fish farms, the range of available feedstuffs is more limited and may be one of the factors contributing to impaired production results.

Commercial catfish feeds contain grain or grain by-products that are rich in starch. In addition to providing an inexpensive energy source, starch helps bind feed ingredients together and increases expansion of extruded feeds so that the feed pellets are water stable and float in the water. A typical catfish feed contains 25 % or more of digestible carbohydrates (Robinson, 1989). There are several starch-rich feedstuffs available and used at farm level in Laos (Paper I) that will contribute with digestible carbohydrates to the fish feed. Maize, rice bran and broken rice were the most commonly used starch-rich feedstuffs in catfish feed (Paper I), while cassava root meal was less commonly used. Rice bran had a low digestibility of organic matter, crude protein, and total carbohydrates compared with maize, broken rice and cassava root meal (Paper II), suggesting that the inclusion rate of this feedstuff may have to be limited in order not to reduce nutrient availability of the feed.

The most commonly used conventional protein-rich feedstuffs in catfish feed are fish meal and soybean meal. Fish meal is prepared from dried, ground tissues of whole fish and/or fish cuttings, and the quality depends on

the fish included, on its processing and the salt content. It has high energy and protein content and is rich in essential amino acids (lysine, methionine, cystine and trytophan), essential fatty acids and minerals. Fish meal is highly palatable to catfish and since it is a good source of essential amino acids, it is often used to supplement feeds containing plant proteins. In tropical areas fish meal is used in catfish feed at levels up to 50% in fry feeds, up to 42% in fingerling feeds, and up to 40% in grow-out feeds (Unprasert, 1988).

Soybean meal is prepared by grinding the flakes after solvent extraction to remove the oil from the dehulled soybeans. Dehulled, solvent-extracted soybean meal contains around 48% crude protein and is the predominant plant protein source used in catfish feeds. It has a good amino acid profile compared with other common plant protein sources, is highly palatable and has a high digestibility in catfish. Antinutritional factors are destroyed or reduced to insignificant levels with heat that is applied during the extraction process. Levels of soybean meal up to 50% have been used in commercial catfish feeds without detrimental effect (NRC, 1993). Andrews and Page (1974) reported that growth and feed efficiency of channel catfish were significantly reduced when soybean meal was substituted on an isonitrogenous basis for menhaden fish meal but the reason for this reduction was not identified. Properly processed soybean meal has been found to have less available lysine for carp than for poultry (Viola et al., 1982; Viola et al., 1983). Moreover, lysine supplementation of a diet with 25% CP from soybeans did not improve performance to the level obtained by fish fed 30% CP from soybeans without lysine supplementation. Animal protein sources have been found to be beneficial in catfish feeds for reasons not explained on the basis of improving essential amino acid profile or increasing digestible energy content of the diet (Mohsen and Lovell, 1990).

The non-conventional feedstuffs of animal origin are often high quality feed ingredients that can compare with the conventional feed ingredients. Non-conventional feedstuffs are cheaper then conventional animal feedstuffs, such as fish meal, and in addition there is no competition for human consumption. In most parts of Africa and Asia, non-conventional feedstuffs such as tadpole meal, maggots, earthworm meal, housefly larvae and snails (Paper I, II, II & IV) are available in small quantities, and their production is inconsistent and sporadic in nature (Anunne, 1990; Ugwumba and Abumoye, 1998). Thus, these feedstuffs can fit well into small-scale fish production systems but are of limited value for large scale aquaculture production systems due to the small quantities and sporadic availability. However, the sporadic availability of non-conventional feed resources can be overcome by introducing preservation techniques to utilize a temporal

abundance and which allows long-term storage (Paper III and IV). In this context, sun-drying is a cheap and simple option in tropical countries during the dry season but is not applicable throughout the year. As an alternative, the fermentation technology is an affordable low-level preservation technique ideally suitable for tropical developing countries (Lee, 1990; Han-Ching *et al.*, 1992). This technology is more weather independent and has previously been successfully applied to fish and shrimp offal (Fagbenro 1996; Ngoan *et al.*, 2000).

Aquatic animals are common features in many fresh and marine bodies in the tropics (Fasakin, 2002) and can constitute natural feed resources from where dietary animal protein can be sourced (Mercer, 1992). In addition to fish, aquatic animals include crayfish, shrimp, crabs, squid and toads. Byproducts such as crayfish waste meal, shrimp waste meal and frog waste meal among others are prepared by the local people and are incorporated into animal feed as protein supplements as a replacement for high cost conventional protein feedstuffs.

Another non-conventional protein source that should be considered are the arthropods. Anthropods are insect groups that are known to be rich in crude protein and minerals (Aduku, 1993; Ojewola *et al.*, 2003; Ojewola and Annah, 2005). Some research has been carried out with some of these products and their by-products (Oduguwa *et al.*, 2004; Agunbiade *et al.*, 2004; Ojewola *et al.*, 2003; Ojewola and Annah, 2005). There is a need for further identification and exploitation of these and other novel animal protein supplements that may have the potential to replace more expensive conventional protein sources.

Catfish feeds are generally supplemented with a vitamin premix, phosphorus and a trace mineral premix to meet dietary requirements. However, this may not be necessary if feedstuffs of animal origin are used in combination with suitable feedstuffs of plant origin. It has been suggested that it should not be necessary to give supplemental trace minerals in diets using animal protein sources (Wilson *et al.*, 2000).

5.2 Apparent digestibility of potential feed resources

The bioavailability of nutrients and energy in feedstuffs for catfish may be defined mainly in terms of digestibility or, in the case of energy, metabolizability. Digestibility describes the fraction of the nutrient or energy in the ingested feedstuff that is not excreted in the faeces. Metabolizability describes the fraction of the digested energy that is not excreted in the urine and through the gills. Both digestible energy and metabolizable energy have

been used to express feedstuff energy values for fish, but many researchers use and report only digestible energy values because of difficulties in obtaining metabolizable energy values for fish (NRC, 1983).

The present results (Paper II and III) indicate that the nutrient components and energy in most of the potential feed ingredients available in Laos were well digested in the African catfish (*Clarias gariepinus*). This is in line with the view that the African catfish should be classified as an omnivorous fish that has the potential to utilize all dietary components, including carbohydrates, more efficiently than many other fish species (Jantrarotai *et al.*, 1994; Nematipour *et al.*, 1992a; Chow and Halver, 1980). Since the digestibility values are adequate, choice of these feed ingredients could therefore primarily be based upon their protein content, amino acid composition and palatability.

Raw and sun-dried Golden Apple snails (GAS) had a high apparent digestibility (AD) of dietary components, energy and amino acids, while the AD of CP and energy was markedly reduced in ensiled GAS (Paper III). Apparently, the ensiling process had a negative impact on the digestion of GAS which merits to be studied further.

It has been reported that the gastric evacuation rate increases with increased feeding rate (Pandian and Vivekanandan, 1985). This may result in increased rate of passage of dietary material through the digestive tract, resulting in less material digested, and lower apparent protein digestibility values. This has been noted in *Clarias batrachus* and *Clarias gariepinus*, and it has therefore been suggested that these fish should be fed two times per day when they are past the fingerling stage, in order to utilize dietary protein more efficiently (Pandian and Vivekanandan, 1985).

5.3 Replacing fish meal with other potential protein-rich feed resources

Using locally available low-cost feed ingredients in fish feeding is important for the development of African catfish production in Laos. One good example is the Golden Apple snail (GAS) that can be used as a valuable protein source for fish production (Paper I, II, III and IV).

Replacement of fish meal with raw or ensiled GAS did not negatively affect the growth performance, feed utilization and protein utilization of African catfish fingerlings (Paper IV). This indicates that GAS has a high nutritive value and could be used to completely replace fish meal in African catfish production (Paper II and III). Similar findings were reported by Sogbesan *et al.*, (2006) for African catfish (*Clarias gariepinus*) fingerlings fed diets where fish meal was replaced by garden snail (Limicolaria aurora) meal. GAS meal has also successfully been used to replace fish meal in diets for tilapia (Catalma et al., 1991b), and giant freshwater prawns (Macrobrachium rosenbergi) (Tabthipwon et al., 2004). Trash fish has been replaced by earthworms in diets for growing hybrid catfish (*Clarias macrocephalus x Clarias*) gariepinus) without any negative effects on performance (Tram et al., 2005). Moreover, worm meal replaced fish meal as the dietary protein source for freshwater crayfish without any reduction in either growth rate or intake (Dynes, 2003). Earthworms are easy to culture and can be used as a means to utilize farm and household waste, and thereby recycle organic matter and nutrients within the farm (Bay, 2002). Fagbenro et al. (1993) reviewed a number of feeding studies with catfish and concluded that there were no differences in growth performance when fish meal was replaced for frog meal. Frog meal has a high content of both fat and protein, and is considered as a suitable supplemental protein source for feeding shrimp fry (Ranu et al., 1993). Moreover, termites is also and interesting protein source for catfish feed (Fagbenro et al., 1992), but may be limiting in essential amino acids (Ugwumba et al., 2001; Sogbesan et al., 2005). Termite meal has also been reported to have a poor mineral content (Barker et al., 1998).

5.4 Dietary protein and energy content

According to Ali (2001), the optimum dietary protein to energy ratio (P/E) found for African catfish (*Clarias gariepinus*) was 20.54 mg protein kJ⁻¹ of GE, for a diet containing crude protein over 40% and gross energy content of more than 20 kJ g⁻¹. Based on current knowledge (NRC, 1993), a digestible energy to crude protein (DE: P) ratio of 35.6 to 41.8 kJ g⁻¹ (8.5 to 10 kcal g⁻¹) is adequate for use in commercial catfish feeds. This corresponds to 10.7 to 12.5 MJ DE for a catfish feed containing 30% CP. The estimated content (MJ kg⁻¹ DM) of digestible energy (DE) ranged from 13.2 for rice bran to 18.1 for frogs (II), and from 10.0 to 14.9 for GAS (Paper III). On average, the DE content of the carbohydrate-rich feed ingredients was lower than that of the protein-rich feed ingredients. This was mainly due to the lower AD for energy, as the content of gross energy differed less between feed ingredients.

A DE:P ratio higher than 41.8 kJ g⁻¹ may lead to increased fat deposition and if the energy ratio is too low, the fish will grow slowly (Hogendoorn *et al.*, 1983). The increased fattiness in channel catfish fed lower protein diets appears to be caused by an imbalance that occurs between dietary energy and protein when dietary protein is reduced and energy is not adjusted

accordingly. If the digestible energy of the diet can be reduced to within the optimum range of 35.6 to 41.8 kJ g^{-1} protein, without adverse effects on fish growth, then fattiness may be maintained at an acceptable level in fish fed low-protein diets.

Robinson and Li (1997) fed channel catfish in ponds with diets in which protein concentrations ranged from 16 to 32% and DE:P ratios from 37.2 to 67.8 kJ g⁻¹ protein (8.9 to 16.2 kcal g⁻¹ protein) and found that a diet containing 16% protein and a DE:P ratio of 67.8 kJ g⁻¹ protein (16.2 kcal g⁻¹ protein) can be fed to channel catfish with only a slight reduction in weight gain. However, body fat increased dramatically in fish fed 16 and 20% protein diets, respectively. Fattiness in channel catfish is a concern because of possible adverse effects on dressed yield and on shelf-life of processed fish. In addition, the public perception that fat is "bad" diminishes the value of products containing significant levels of fat (Machiels and Henken, 1985; Degani *et al.*, 1989).

5.5 Feeding and catfish yield

Feed is one of the major production inputs in semi-intensive and intensive catfish cultivation. Moreover, the economic efficiency of catfish production is not only dependent on productivity but also on the relationship between inputs and outputs. Feed inputs, such as protein supplements, are usually expensive, especially for rural catfish producers in developing countries, because of an increasing human demand for protein, and the relative high cost of imported ingredients (Chuapoehuk, 1987).

Feed formulation is a central operation in intensive catfish production, ensuring that feed ingredients are economically used for optimum growth (Robinson *et al.*, 2001). This requires a good knowledge of both catfish nutrient requirements and the potential utilization of feed ingredients. Most large-scale catfish farmers depend on commercial feed mills for their feeds. However, irrespective of intensity of production and farm size it is essential that formulations are accurate, to ensure that fish farmers are not adversely affected.

Available feed resources in Laos (Paper I) include both carbohydrate-rich and protein-rich feedstuffs. Therefore, it should be possible to formulate catfish feeds which fulfil the nutrient requirements and can support high fish growth rate. Feed formulation in African catfish farms of different size, within district, were similar, while there were differences between different agro-ecological areas (Paper I). However, the level of crude protein was comparable in all areas, with the exception of one farm, and was considerably lower as compared with commercial catfish feed (Robinson, 1989). Moreover, there were large differences in crude fat content between Naxaythong and the other two districts (Paper I). However, there is no good explanation for these differences.

The potential nutrient and energy supply for catfish from different feed resources were estimated by analyzing nutrient content and estimating the energy value (Paper I) and by performing feeding trials (Paper II, III and IV). The survey on feed formulation for African catfish cultivation in three districts in Vientiane municipality (Paper I) resulted in values that ranged from approximately 150 to 200 g CP kg⁻¹ DM. This is well below the protein levels required for high growth rates in grow-out catfish. The best growth rates and feed conversion for African catfish (*Clarias gariepinus*) are achieved with diets containing 350-420 g CP kg⁻¹ DM (ADCP, 1983; Janssen, 1985c; Uys, 1989). Similarly, the CP requirement of *Clarias batrachus* Linnaeus is in the range 350-400 g CP kg⁻¹ DM (Chuapoehuk, 1987; Khan and Jafri, 1990; Erfanullah and Jafri, 1998a).

The net production of catfish from pond culture was in the range 1.5 to 3.0 tons ha⁻¹ half year⁻¹ irrespective of farm size (Paper I). African catfish (Clarias gariepinus) fingerlings were stocked at densities of 8 fish m³ in traditional fish ponds and showed production data that ranged from 3.1 to 22.8 tons hai year in South Benin, West Africa (Toko et al., 2007). In Ghana, the growing of African catfish (Clarias gariepinus) with stocking density of 20 000 to 80 000 fingerlings ha-1, gave a net yield of approximately 2 to 7 tons ha⁻¹ after a half year rearing period (Micha, 1976). In the Central African Republic static ponds were stocked at density of 100 000 fingerlings ha⁻¹ and were harvested after 6 months when the standing biomass reached 10 tons ha⁻¹ but the water quality was difficult to manage (Janssen, 1985c). However, in South Africa and Zambia standing crops of 40 to 100 tons ha⁻¹ were attained in ponds with a water exchange of 25% day⁻¹ (Hecht et al., 1988). In polyculture systems of Nile tilapia (Oreochromis niloticus) and African catfish (Clarias gariepinus) production levels of 7 to 8 tons ha⁻¹ year ⁻¹ have been obtained (ADCP, 1983). In Thailand, polyculture of hybrid catfish (Clarias macrocephalus × Clarias gariepinus) and Nile tilapia (Oreochromis niloticus) in an integrated pen-cum-pond system, production levels of up to 84 tons ha⁻¹ year⁻¹ has been reported (Yi et al., 2003). Moreover, in Southern Vietnam many small companies are involved in Tra catfish (Pangasius hypophthalmus) and Basa catfish (Pangasius bocourti) production, almost all located in the Mekong Delta. They can produce on average 100 tons ha⁻¹ year⁻¹ (Thuy et al., 2007). Studies on channel catfish cultured for 250 days have reported an estimated yield of 18 to 22 tons ha⁻¹

(Mc Gee and Lazur, 1987). These data compare favorably to those reported for channel catfish by Robinson and Rushing (1994), but they are somewhat higher than those observed by Li *et al.* (2004). In Florida, common stocking rates of channel catfish for commercial growers are generally around 12,500 to 17,500 fish ha⁻¹, and 20,000 to 25,000 fish ha⁻¹ for producing smaller catfish of 250 to 300 g live weight. Production yields average 5 tons ha⁻¹ for larger fish and yields for producing small channel catfish average 7.5 tons ha⁻¹ . Maximum feeding rates range between 75 to 100 kg ha⁻¹ day⁻¹ but can reach as high as 375 kg ha⁻¹ day⁻¹. Fish are stocked at a fingerling size of 10 to 15 cm, and grown to the required market size. The average grow-out period is 4 to 5 months for small fish and 6 to 8 months for large fish (Mc Gee and Lazur, 1987).

The fish yield (Paper I) was positively related to fish pond area. Possibly, this could be a reflection of a more intensive production, with a higher nutrient input to the pond with increasing fish pond area. Raising African catfish without supplemental food resulted in fish weights at harvest of 135 g after a six months growth period (Bok and Jongbloed, 1984). This should be compared with fish weights of 200 to 300 g for African catfish obtained after the same growth period (Hogendoorn and Koops, 1983). De Graaf *et al.* (1996) recorded fish weights of 200 to 300 g for pond cultured African catfish after a 6 months growth period, which gave a total yield of 7 to 8 tons ha⁻¹ year⁻¹ in polyculture with Nile tilapia (*Oreochromis niloticus*).

In general, most small scale catfish farmers have poor education and consider fish production to be a very simple undertaking. They put manure and any kind of feed (made from agriculture by-products and/or commercial feed) into the fish pond, and do not calculate the exact nutrient supply from the diets properly. Moreover, they simply guess the total weight of fish at time of harvest as well as the price for the fish at the time of sale. This is not a good practice and may lead to considerable errors. There are several examples of failed catfish farms in developing countries, , such as in Congo (de Graff and Janssen, 1996), Ghana (Halver and Hardy, 1973), Nigeria (Okafar, 2005), Myanmar (Keong Ng, 2007), Sri Lanka (Weerakoon, 1979), Bangladesh (Islam et al., 2005), Vietnam (Luu, 1993) and Laos (LARReC, 2000). According to Tacon et al. (1995), this is due to many issues, such as poor planning, poor knowledge of fish culture, both in theory and in practice, lack of a nutritionist to assist in optimization in the feed formulation to reduce feed costs and limited capital to invest in fish farming.

6 General conclusions and implications

6.1 Conclusions

- Fish farmers in Laos use a wide range of both conventional and nonconventional feedstuffs for African catfish feeding. The use of feedstuffs can be expected to vary between different agro-ecological areas and between farms of different size within each area. There is a need to further evaluate the nutritive value of the local feed ingredients available for catfish production in Laos to build a national data-base that can be used in feed formulation.
- The yield from fish ponds in the three agro-ecological areas under study was low compared with other available data, suggesting that there is potential to improve the net production of African catfish in Laos. This could be achieved by increasing the farmers' knowledge of feed formulation and by introducing better management practices.
- The protein supply appears to be the major limiting factor in currently used feeds for African catfish production in Laos. In order to reduce the use of expensive imported feed protein feedstuffs (i.e. fish meal and soybean meal), there is a need to continually search for other potentially useful protein-rich feed ingredients that are locally available.
- The Golden Apple snail (GAS) is one potentially useful nonconventional protein source for catfish production in Laos, which can be used to completely replace fish meal in the diet for African catfish.

- The GAS can be fed either raw or preserved to African catfish fingerlings. However, in order to assure a continuous supply of GAS over the whole grow-out period it could either be preserved by ensiling or sun-drying depending on the season for the collection of the snails.
- The method used for preservation of GAS will have an influence on the nutritive value when fed to African catfish. Sun-drying is suggested to be the preferred preservation method for GAS, when possible, in order to maximize the potential nutritive value.

6.2 Implications and further research

6.2.1 Implications

Most farms still depend on the natural nutrients of the ponds with the addition of low cost supplemental feeds, including non-conventional feeds and agricultural by-products. The use of feed ingredients at farm level is dictated by the availability of local feed resources and the price of external inputs. However, the inclusion of fish meal and/or soybean meal in the fish feed will not necessarily result in better feed production and feed conversion. There appears to be a great potential in using non-conventional protein-rich feed ingredients, such as the Golden Apple snail, to replace expensive fish meal and soybean meal in catfish feed.

Future African catfish culture systems under Laotian conditions should be looked at in light of the biological and ecological limits related to the production components (i.e. feed resources, fish pond) available. Sustainable production systems will require minimal external inputs, make efficient use non-renewable resources, allow maximal recirculation of nutrients within the production system and they should be possible to run with limited technical skills. It appears reasonable to assume that the introduction of semi-intensive African catfish dominated poly-culture systems, with silver carp and catla (Rahman and Varga, 1992), should be technically feasible under Laotian conditions. Such poly-culture systems will make better use of available nutrients and will allow a higher fish yield. They can therefore be made more profitable than mono-culture based systems, which will contribute to sustainability over time. Moreover, these systems will improve the income of catfish farmers and will thereby contribute to an improved livelihood.

6.2.2 Further research

Further research focusing on African catfish production systems suitable for resource-poor people in Laos and neighboring countries could include:

- A continued search for protein-rich feed resources that can be used to completely or partially replace imported fish meal and soybean meal in the diet. This could include a closer evaluation of benefits and risks of using slaughter-house wastes from livestock. Moreover, other by-products, such as dried brewers yeast, soybean residues and kitchen waste, could be potentially useful feed ingredients.
- The feeding of diets with high fiber content to catfish should be investigated to provide a better knowledge of the possible interactions between the fiber component and other nutrient components (i.e. protein, fat, minerals). These studies should include different genotypes of catfish in order to evaluate their response with respect to feeding behavior, digestive physiology and growth performance.
- Further work is required to obtain a reliable data base on digestibility of energy and nutrient components (protein, amino acids, lipids, minerals) in available feed ingredients in order to make it possible to utilize the full potential of each ingredient in practical diets. This work should include studies on catfish of various sizes in order to represent the complete production cycle.

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