

Evaluation of Catfish (*Pangasius hypophthalmus*) By-Products as Protein Sources for Pigs in the Mekong Delta of Viet Nam

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2010

Acta Universitatis agriculturae Sueciae
2010: 69

ISSN 1652-6880
ISBN 978-91-576-7514-9
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Print: SLU Service, Uppsala 2010

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Abstract

The aim of this study was to evaluate preservation methods of catfish by-products (CBP), and the effects of replacing marine fish meal (FM) with these processing by-products on diet digestibility, performance, feed efficiency and carcass quality in growing-finishing pigs.

Paper I presents data on catfish production, processing and the nutritive value of catfish by-product meals (CBM) in the Mekong Delta (MD) of Vietnam. In Paper II, CBP silage made with 20-40% addition of sugar cane molasses (M) was of a better quality than when made with rice bran (RB) at the same levels. The optimum proportion for successful silages with M was 8:2, and with RB 7:3 (CBP and M or RB, fresh weight basis). The total tract apparent digestibility (CTTAD) of CP in CBP ensiled with RB or M was comparable to that of FM, but ether extract (EE) digestibility in ensiled CBP was higher. However, N utilization was not significantly different among FM and silage diets. In Paper III, the coefficient of ileal apparent digestibility (CIAD) of CP in CBP ensiled with M (ECM) tended to be higher than that in CBM, processing waste water (WWB) and FM. However, the CIAD and CTTAD of EE in FM were lower than in CBP. Amino acid digestibility and nitrogen retention were not affected by replacement of FM. Results from Paper IV showed that, the higher the inclusion level of WWB as replacement for FM in the diet, the lower the average daily feed intake (ADFI), amino acid intake and average daily gain (ADG). However, feed conversion ratio (FCR) was improved, and the feed cost was reduced to 68% of that of the FM diet. In Paper V, a higher inclusion level of ECM as replacement for FM in the diet resulted in slightly lower ADFI, but increased ADG. As a result, FCR was improved and feed cost of the diet with 100% CP coming from ECM reduced to 72% of the cost of the diet with 100% of the CP from FM. Carcass yield and dressing percentage were not affected by ECM replacement, but leaf fat and back fat thickness were higher with higher ECM replacement of FM. There was no effect on DM and CP contents in *Longissimus dorsi* muscle, but EE and polyunsaturated fatty acid contents increased especially eicosenoic, linolenic, linoleic, DHA and DPA. Meat colour values also increased with increasing ECM replacement level.

Keywords: Catfish by-products, carcass traits, growing pigs, ileal and total tract digestibility, Mekong Delta, Vietnam, silage, waste water by-products.

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Dedication

*To the memory of my father.
To my mother, my sisters and brother
My husband Nguyen Hong Tien
My daughter Hong Nhung
My son Tien Dat*

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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 Thuy, N. T., Lindberg, J. E. and Ogle, B. 2007. Survey of the production, processing and nutritive values of catfish by-product meals in the Mekong Delta of Vietnam. *Livestock Research for Rural Development*, 19 (9).
- II Thuy, N. T., Lindberg, J. E. and Ogle, B. 2010. Effect of additive on the chemical composition of Tra catfish (*Pangasius hypophthalmus*) by-product silages and their nutritive value for pigs. *Asian-Australasian Journal of Animal Science*, 23 (6), 761-771.
- III Thuy, N. T., Lindberg, J. E. and Ogle, B. 2010. Digestibility and nitrogen balance of diets that include marine fish meal, catfish (*Pangasius hypophthalmus*) by-product meal and silage, and processing waste water in growing pigs. *Asian-Australasian Journal of Animal Science*, 23(7), 924-930.
- IV Thuy, N. T., Lindberg, J. E. and Ogle, B. 2010. Effects of replacing fish meal with catfish (*Pangasius hypophthalmus*) processing waste water on the performance of growing pigs. *Tropical Animal Health and Production* (In press).
- V Thuy, N. T., Lindberg, J. E. and Ogle, B. 2010. Effects of replacing fish meal with ensiled catfish (*Pangasius hypophthalmus*) by-products on the performance and carcass quality of finishing pigs (Submitted).

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Abbreviations

AA	Amino acids
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
CBM	Catfish by-product meal
CBP	Catfish by-products
CF	Crude fibre
CIAD	Coefficient of ileal apparent digestibility
CP	Crude protein
CRB	Catfish by-products ensiled with rice bran
CTTAD	Coefficient of total tract apparent digestibility
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentaenoic acid
EAA	Essential amino acids
ECM	Catfish by-products ensiled with sugar cane molasses
EE	Ether extract
EPA	Eicosapentaenoic acid
FA	Fatty acids
FCR	Feed conversion ratio
FM	Fish meal (marine)
LA	Lactic acid
LAB	Lactic acid bacteria
LD	<i>Longissimus dorsi</i> muscle
M	Sugar cane molasses
MD	Mekong Delta
MUFA	Mono-unsaturated fatty acids
NDF	Neutral detergent fiber

OM	Organic matter
PUFA	Polyunsaturated fatty acids
RB	Rice bran
SFA	Saturated fatty acids
WWB	Processing waste water

1 Introduction

Vietnam is an agricultural country located in South East Asia, with a total area of 331,051 km² and a population of 86 million. Of these the proportion residing in urban areas is around 27.9%, and in the rural areas 72.1%. The Mekong Delta (MD) is situated in the far South of Vietnam, and is the major agricultural production region of the country as a result of its fertile soils and abundant water resources. According to Dai *et al.* (2008), the growth rate of the agriculture sector in the MD is higher than the national average, and the main agriculture products exported from Vietnam are produced in the MD, which has a population of 17.21 million and an area of 40,518 km². The ambient temperature fluctuates between 22–27°C in the coolest months and 32–36°C in the warmest months, and the average humidity varies from 76–80%. The MD is four million hectares of flat lowland plain with alluvial, acid and saline soils watered by the Mekong River and its canal networks. Around 78.6% of the population works in the agricultural sector, mainly small-scale farmers who are dependent on rice, aquaculture and livestock production (GSO, 2010). There are considerable quantities of by-products from these sectors available throughout the year that are potentially valuable as feed for livestock.

Pig production is a very important part of the livestock sector of Vietnam generally, and in the MD in particular, and plays a major role at both family and national level. The pig population was estimated at 27.6 million animals in 2009 (GSO, 2010), and pork is the major source of animal protein for the people of Vietnam, accounting for 81% of the total meat consumption, with chicken meat and beef accounting for only 11.5% and 7.5% of the total, respectively (Kinh & Hai, 2008). In the MD, around 71% of farm households own pigs and around 80% of pig production is small-scale (Lapar *et al.*, 2003), while the rest comes from commercial farms, which are defined as having more than 20 sows, and using mainly commercial feed.

Under village conditions in the MD, the majority of the pigs raised by smallholders are crossbreds between exotic and local breeds, because the local breeds, which have high carcass fat and low lean meat percentage, have decreased in numbers recently, partly as a result of changing consumer preferences in the urban areas for leaner meat. According to Kinh and Hai (2008), mainly crossbreds and exotic breeds are raised in the South of the country, while local breeds still dominate in the rural areas of the North. In this system, the crossbreds are mainly fed using cheap local materials and unconventional feed resources instead of high quality imported feed resources, which are expensive and likely to rise in price. Feed represents approximately 65% of the production costs of market pigs, so decreasing the cost of feed is important to the producers. In recent years, the imbalance between feed and animal product prices has resulted in very low economic returns for pig producers. Because domestic production of protein feeds in Vietnam is limited, around 60-70% of the total consumption is imported, including around 90% of the fish meal used in the animal feed industry (Edwards *et al.*, 2004).

The MD has a total freshwater area of 641,350 ha, which comprises 67.2% of the total water surface (Phuong & Oanh, 2010). So, the region is rich in aquatic resources and has a great potential for aquaculture development. Recently, catfish has begun to play a very important and significant role in the aquaculture sector of Vietnam. Among the catfish species, *Pangasius hypophthalmus*, so-called Tra catfish, is the main farmed fish in the MD. Their cultivation involves the use of floating cages, fence/pens enclosures placed in rivers or pond culture, and they are cultured both extensively and intensively, depending on feed availability and rearing system. According to Da *et al.* (2010) the Tra catfish production in the MD was over 1 million tonnes in 2009, and is expected to increase to up to 1.5 million tonnes this year. As a result, there are several factories in the Delta that produce aquaculture products for export, and Tra catfish fillets are exported to around 80 countries in the world (Lam *et al.*, 2009). In fillet processing, considerable amounts of processing by-products are available, accounting for 60-65% of the weight of the whole fish, and these are potentially valuable protein resources for monogastric livestock. The Tra catfish by-products, which include head, bone, skin and abdominal organs that remain after the two side fillets have been removed, are abundant and transported fresh to catfish by-product factories nearby, where they are ground fresh, boiled, and the oil removed, then dried to produce catfish by-product meal. This is a high value product that is mainly exported to China, at a price similar to that of marine fish meal. Recent research from Tuan

(2010) on the use of residue meals from catfish by-products as feed for growing pigs reported results comparable with marine fish meal. In small scale factories, the by-products are processed to dried fishmeal for livestock, or wet fish meal as feed for other fish species. Waste water, which is a by-product of the process, is also a potentially valuable protein resource. However, it is not common that pig farmers use processing waste water and wet fish meal as feed, because of the constraints with respect to transport and preservation, as quality deteriorates very rapidly.

The present study was conducted to identify and evaluate ways of using catfish by-products with the expectation that they can be preserved by ensiling and acidification, and can replace commercial marine fish meal in diets for pigs without affecting performance, carcass and meat quality, and will reduce feed costs for the pig producers of the Mekong Delta.

Objectives of the study

The overall aim of the studies in thesis was to identify and evaluate ways of using catfish by-product to improve pig performance, and reduce feed costs for pig production. The specific objectives were to:

- Determine the most appropriate preservation method of catfish by-product by ensiling with different ratios of by-product to rice bran or sugar cane molasses.
- Determine the nutritive value of the most suitable catfish by-product silages and processing waste water in growing pigs
- Determine the optimum proportion of waste water from catfish by-product meal processing as replacement for marine fish meal in growing pig diets in terms of feed efficiency and cost.
- Evaluate the effect of using ensiled catfish by-product as replacement for marine fish meal on the performance and carcass quality of fattening pigs.

Hypotheses of the study

- Catfish by-product can be preserved by ensiling without negative effects on the nutritive value.
- The digestibility of ensiled catfish by-product will not be different to that of dried catfish by-product meal and marine fish meal.
- Catfish by-product waste water from by-product meal processing can be included in diets for growing pigs as replacement for marine fish meal without affecting performance.
- The ensiled by-products can replace marine fish meal in diets for fattening pigs without affecting performance and carcass quality.

2 Background

2.1 Catfish production systems in the Mekong Delta

2.1.1 Catfish production and by-product availability in the Mekong Delta

Pangasius hypophthalmus, so-called Tra catfish, is one of the major fish species in the MD fishery, and is the key fish species in Vietnamese aquaculture. Tra catfish is considered the major aquaculture success story of the country and is a significant source of socio-economic development, which is essential for earning foreign currency through the export of valuable fish, providing employment opportunities and encouraging local and foreign investors (Phuong & Oanh, 2010). Catfish farming started in the 1960s mainly for the domestic market, and the catfish were usually cultured in small household ponds and ditches. In 1986, through the support of Australian experts and the establishment of the Agifish Company in An Giang Province, Tra catfish fillets were produced and exported to Australia. From around 1990 onwards the fillets were exported to other countries including the USA, the EU countries and many other countries throughout the world (Lensink & Nam, 2008). Since catfish products became primarily for export, most households that were involved in pangasius farming were changing from traditional culture into intensive culture. Before 2000, cage culture with home-made feed was common, but after 2000, most producers changed from cage farming to intensive, high density farming in riverside ponds and fences (Trong, 2010).

According to Khanh (2004) Tra catfish is the second biggest aquaculture export after shrimps in the MD. Due to high export value, farmers have been encouraged to utilize poor quality agricultural land for aquaculture, and especially to adopt systems based on intensive ponds and river islands for

raising Tra catfish for export. The production of Tra catfish cultured in intensive farming systems in the MD in 2005 was around 375,000 tonnes, which increased to 1,115,000 tonnes in 2007 and 1,128,000 tonnes in 2008, and with a further expansion of around 1 million tonnes in 2009 (Trong, 2010). As the development of catfish farming in recent years exceeded the planned sector output, currently there are several constraints for catfish culture, such as poor water quality and disease problems, as well as market problems such as the stricter requirements for the quality of processed products of importers (Da *et al.*, 2010). The variation in quality of fillets, which are classified by colour (white or yellow; the whiter the colour the better the quality) is due to the different farming conditions, such as feed and water quality (Sorensen, 2005).

Because of the continuing high value of Tra catfish fillets internationally, the production of Tra catfish in the MD is developing very rapidly, especially in An Giang, Dong Thap and Can Tho provinces. Currently, there are more than 19 pangasius processing and exporting companies, and more than 4 million people are involved in fishery activities in the MD (Dzung, 2008). As a result, catfish by-products (CBP) from filleting are abundant. These include head, bone, skin, scrap meat and viscera, and account for 60–65% of the total raw whole catfish. The CBP contain relatively large concentrations of protein, fat and moisture, which means that CBP deteriorate quickly and make it difficult for them to be used directly to feed animals, unless processed quickly into a meal. Several commercial CBP processing companies produce catfish by-product meal (CBM) using advanced equipment in closed procedures. This CBM is then sold to animal feed factories for producing commercial animal feed. Some small-scale animal feed factories collect the CBP directly from the fillet export factories and produce dried and wet meals as feed for livestock and aquaculture. In the processing, considerable quantities of processing waste water (WWB) are produced, which is released after the boiling process, and which is mixed with cassava root meal and sold as wet meal. This is used immediately as feed for aquaculture, especially as feed for other species of fresh water fish. A small amount of CBP is transferred to small producers for processing into feed for animals. However, the processing results in considerable volumes of WWB, which give off unpleasant odours.

2.1.2 By-product processing methods and problems of catfish by-product meal producers

Catfish by-product is a high fat product, and many ways for processing it have been developed, depending on the purpose, equipment and local feed

requirement. Most CBP is used for producing CBM, which is considered to be a more suitable processing method for large amounts of by-products. There are several different methods for producing CBM, which include the following steps: firstly, the by-products are finely ground, cooked and then separated into three fractions: oil floats on the surface and is removed by bucket to storage tanks. The middle level is liquor (waste water) which is high in protein, and also is a cause of environmental pollution. The lowest fraction is waste material, which is used to produce CBM, after being pressed and dried by machine. The final step is dry grinding with an antioxidant substance added. The products are either mixed or separated into skin and scrap meat and head and bone by-products, as they differ in chemical composition and price. In general, there are three kinds of CBM produced, the best quality being catfish scrap meat meal, which is only a small proportion of the total amount (Tuan, 2010). The most common meal product is head and bone meal, which has a medium CP content, while the lowest protein content meals come from WWB.

However, the basic process in most of the methods involves separating the fat out of the product and then the drying process, which requires considerable investments in equipment. Many of the small by-product meal companies in the MD are changing to producing wet meal for the aquaculture industry, because of the higher benefits compared to producing dry CBM. The main constraint is that the price of the meal and oil is fluctuating and sometimes is lower than the costs of production. In addition, CBM has a high fat content, which means that the quality quickly deteriorates on storage. Environmental pollution is also an important problem for catfish by-product producers. It would be preferable if the producers of the CBM could improve or change the processing techniques to reduce the release of waste to the environment, or ensile the by-products with cereal waste for use as animal feed, and feed the WWB directly to pigs. In theory, ensiling may be a potentially feasible option for producers in some parts of the region where other options are limited. In practice, ensiled seafood by-products are popular among livestock and fish producers, and ensiling can be carried out either on a small scale, at household level, up to large scale plants taking all the input material from a region (Seafish, 2008). However, large-scale ensiling of CBP has not yet developed.

2.1.3 Preservation of fishery by-products

Drying

The most common method of preservation for high protein fish by-products is through production of meal by drying, the methods that are applied depending on the kind of fish by-products. Catfish by-product contains a high amount of fat and moisture, which make drying of the waste and extraction of the fat difficult, and oxidation occurs with storage. So the procedure for producing fish meal from CBP is always drying after the cooking and oil extracting processes, which is completely different and costly compared with marine fish meals, which are produced from both whole small fish, and the bone and offal from processed fish.

Ensiling

An alternative to drying is ensiling, and given the right conditions, good weather, sufficient substrate and good management it is possible to make well fermented silage (Henderson, 1993). Ensiling of fish by-products is a technique that has been developed over a long period of time, and using these ensiled by-products for animal feed would improve the economic efficiency as well as reducing the environmental pollution caused by disposal of these materials (Kajikawa, 1996). Ensiling is a process that converts filleting waste or fish by-products into fish silage. The aim of ensiling is to reduce the pH of the material to the critical level, at which the material cannot be damaged by microbial action and will keep for several months. The rapid drop in pH and the increasing concentrations of non-dissociated organic acids will inhibit the growth of microorganisms that cause spoilage (Pahlow *et al.*, 2003). In fact, during the fermentation process, many types of enzymes, such as glucoamylase, α -glucosidase, α -amylase and acidic protease are produced, and these enzymes may play a role in the digestion process of animals when used as a feed ingredient (Yamamoto *et al.*, 2005). Fish silage is usually manufactured and stored in liquid form after processing (Pahlow *et al.*, 2003). Several studies on ensiling protein-rich by-products have been carried out, and the resulting silages were found to be of good quality, for example, shrimp by-product silage (Ngoan & Lindberg, 2000), Golden apple snails ensiled with molasses and rice bran (Kaensombath & Ogle, 2005) and freshwater fish ensiled with rice bran and sugar palm syrup (Phiny & Rodríguez, 2001).

Silage additives

Silage additives may be chemical or biological and can be categorized as stimulants, inhibitors, nutrients or absorbents (McDonald *et al.*, 1991). Biological additives provide additional substrate for the indigenous population of micro-organisms or increase the population of lactic acid bacteria (LAB). In some products, the LAB is added with a substrate or with enzymes to provide an additional substrate (Henderson, 1993). According to McDonald *et al.* (1991), there are four groups of silage additive, classified according to their activities. The first group supports the fermentation of the LAB, and includes molasses, cereals, cassava and potatoes as carbohydrate sources. The second group acts as a fermentative control by inhibiting microbial growth, such as formic acid, acetic acid, benzoic acid and formaldehyde, and antibiotics. The third group includes propionic and sorbic acids and ammonia, which can control the deterioration of silage from air contamination. The last group improves the nutritional value of the silage, and includes minerals and urea. Based on the type of silage additive, silages can be classified into two types, acid or fermented silage.

Acid silage

Addition of acids to the silage, such as phosphoric, sulphuric, hydrochloric, propionic, formic and lactic acids or a combination of them results in so-called acid silage (Henderson, 1993). Acid additives act as stimulants or inhibitors of LAB depending upon the concentration of the ingredients in the product, and on the ratio at which the product is applied to the silage material. The silage is defined as a liquid product made from fish wastes that are liquefied by the action of enzymes in the fish in the presence of an added acid. The enzymes break down fish proteins into smaller soluble units, and the acid helps to speed up their activity while preventing bacterial spoilage. Research from Vidotti *et al.* (2003) demonstrated that the acid silage produced from three raw materials, commercial marine fish waste, freshwater fish waste, and tilapia filleting residue, ensiled with acid formic and sulphuric acid gave a pH that stabilized at 2.5 to 2.8, and the products were satisfactory with respect to essential amino acids (EAAs). The results from a study by Levin *et al.* (1989) also showed that, the addition of sulphuric or phosphoric acid to reduce pH to 4.0 achieved long-term preservation when propionic acid was added as a microbial inhibitor at a level of 0.1-0.2%. Recent research from Ramirez *et al.* (2008) showed that the fisheries by-catch and processing waste for lactic acid fermented silage was more successful when lactobacillus was used as starter, and a highly stable product was obtained.

Fermented silage

Fermented silage is produced by an alternative method that can be applied for high protein source materials. In the biological silage, the fermentation process requires fermentable carbohydrate for microorganisms to convert sugar or starch from the carbohydrate source to lactic acid (LA), which reduces the pH to about 4.5. At this level spoilage organisms, especially clostridia, are inhibited. Anaerobic condition is established in the fermentation environment, which suppresses the majority of the flora by facultative and obligate anaerobes, because the initial majority of the flora are aerobes. These undesired flora, such as clostridia and enterobacteria, will be suppressed by homofermentative and heterofermentative LAB, which will multiply quickly by using soluble carbohydrate as substrate and produce organic acids, mainly LA (Pahlow, 1991). A source of fermentable carbohydrate must be added, such as molasses (Zahar *et al.*, 2002; Ngoan & Lindberg, 2000) or rice bran (Phiny & Rodríguez, 2001), which are convenient and readily available sources of fermentable carbohydrate for the preservation of fish by-products in Vietnam. The advantage of stabilizing fish by-products by fermentation rather than acidification is that the fermentation process reduces odor, improves the nutritive value, and enhances probiotic activity. However, lipid and protein quality can change depending upon the conditions of acidification. Vidotti *et al.* (2003) found that the fermented silage of marine fish waste, freshwater fish waste and tilapia filleting residue made with 15% molasses and 5% *Lactobacillus plantarum* culture reduced the pH to 4.06, 4.26 and 4.48, respectively, and the products investigated were found to be appropriate for use in balanced fish diets.

2.1.4 Characteristics and chemical composition of catfish processing by-products

Catfish by-product meal (CBM)

The chemical composition of Tra CBM will depend on the proportion between head and bone and scrap meat or skin. According to Honoroad (2006), when CBM was produced on a commercial-scale, through a series of technical treatments, such as steam desiccation, the product had a CP content of around 56.2% and EE of 9.5%, with high Ca and P contents. Catfish by-product meal produced from scrap meat contains an excellent balance of EAAs, especially with respect to lysine and methionine, and is considered to have a high nutritive value for livestock. However, head and bone by-product meal is less well balanced in amino acid (AA) composition.

Similar results were reported by Tuan (2010), who included three kinds of catfish residue meals in diets for growing pigs, with the best result from scrap meat meal (71.5% CP) followed by oil extracted meal (45.5% CP), and bone and head meal (33% CP).

The oil content in CBM is generally high, as this is a specific characteristic of catfish as compared with marine fish meal, which means that it is quickly oxidized and destroyed during storage. Almost all the products from small-scale systems have higher EE contents (>10% of EE) than the products from commercial companies, probably because of less efficient equipment and procedures for oil extraction. However, CBM is high in minerals such as calcium and phosphorus, which are required by breeding animals, and calcium and phosphorus were found to range from 7-13% and 2-3%, respectively (Honoroad, 2006).

Catfish by-product waste water (WWB)

In the small-scale processing of CBM, large amounts of WWB are discarded and this is a wasted resource that is not normally used in animal feed. In some small factories, cassava root waste is added to produce wet fish meal for aquaculture. The chemical composition of WWB varies, the DM and CP contents in WWB are very variable among factories, depending on the processing procedure and the composition of the input material. Dry matter content varied between 24 and 30% and CP content between 29 and 38% of DM. Ether extract contents were also high, around 18.2%. The AA in WWB are considered to be less well balanced than in CBM.

Ensiled catfish by-products

Many studies have established that fish by-products can become a valuable protein in animal feed if converted into fish silage, and the ensiling of the by-products in combination with other fermentable carbohydrate sources, such as molasses (M) or rice bran (RB), is a simple and appropriate method of conservation (Zahar *et al.*, 2002). The boiled ground catfish by-product was mixed with 20-40% M or RB, and the mixture placed in plastic bags and sealed to prevent air contamination and then ensiled for 3 to 4 weeks. The DM and CP contents of the silage with M at a ratio of 8:2 were 45-55% and 26-30%, respectively, and the change with storage time was only around one percentage unit. The most important improvement in silage by-products is that they contain high concentrations of organic acids, especially LA, which increased from 121-172 g/kg DM in silage fermented with RB (6:4) and with M (8:2). These organic acids reduce the activity of harmful bacteria species and especially coliforms (Mikkelsen & Jensen, 1997).

2.2 Pig production in Vietnam

2.2.1 Number and distribution

Livestock production is the second most important source of income after crop cultivation in Vietnam, and pig production plays an important role as it provides jobs for a large number of small-scale farmers, as well as providing meat at household level. Pig production has dominated the livestock sector (Figure 1), and pig numbers have increased rapidly from 20.2 million head in 2000 to 27.4 million head in 2005, and since then have remained stable, at 26.8 million head in 2008 (GSO, 2010). The reason for the peak in the number of pigs in 2005 is the outbreak of avian influenza in 2003-2004, which resulted in a rapid reduction in the number of poultry, with pig production increasing as replacement. Pig meat accounts for more than 70% of total meat production in Vietnam (Fisher & Gordon, 2008), as pork is the most popular type of meat in the diets of most Vietnamese (Minh *et al.*, 2006). The demand for pork continues to increase because the standard living and per capita incomes of the Vietnamese is increasing, as well as the population. So, almost all pork production is for local consumption, with only 1-2% of total domestic pig meat being exported, due to strong and growing domestic demand (Que, 2006).

However, small-scale pig farmers are faced with fluctuating pig meat and animal feed prices, and also with the problem of disease outbreaks. Recently, some epidemic diseases such as Porcine Reproductive and Respiratory Syndrome (PRRS) and foot and mouth diseases have occurred more often than usual, when the price of pig products quickly declines, resulting in loss of income. At the same time the price of animal feed has continued to increase. This accounts for the fact that the number of small-scale pig farmers is decreasing and that of large-scale commercial farms increasing.

The distribution of pig production varies between the different ecological regions of Vietnam, depending on the socio-economical and meat consumption habits in the different regions (Len, 2008). Pigs are reared mainly in the Red River Delta, Mekong Delta and North Central Coast. In general, exotic and crossbred pigs dominate, accounting for around 74% of the total national pig herd in Vietnam, while indigenous local breeds are raised more in the North than in the South of Vietnam, and mostly in uplands, and rural and remote areas (Huyen *et al.*, 2005). In the MD, crossbreeds between local and exotic breeds are popular. However, in the urban areas of the South of Vietnam, commercial farms have an

increasing tendency towards rearing exotic pigs to meet the demands of consumers for both high quality and increased quantity of meat.

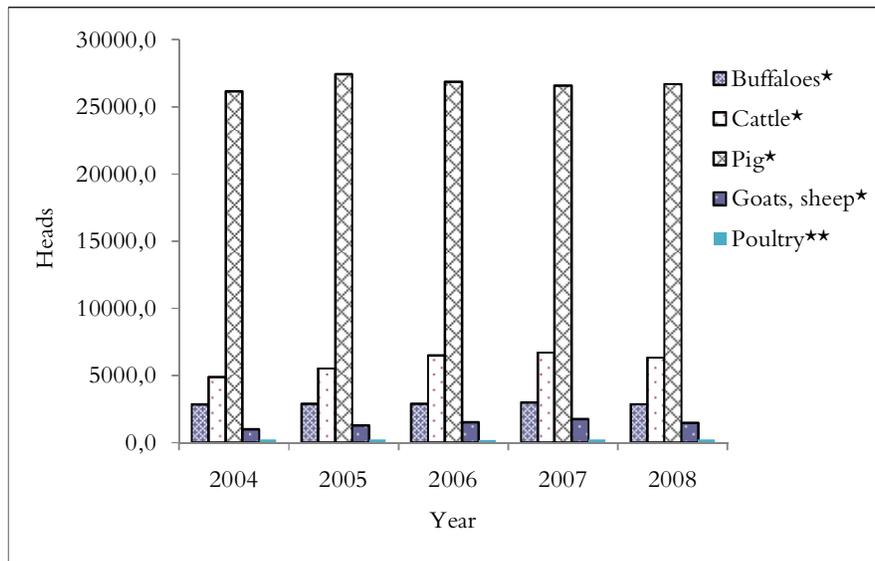


Figure 1: Livestock production
 Source: General Statistical Office of Vietnam, 2010
 * Thousand head
 **Million head

2.2.2 Production systems

According to Gautier and Phuong (2009), the main pig farming systems in Vietnam can be classified as large-scale (>100 sows), medium-scale (<100 sows) commercial farms, that account for around 20% of the total, while small-medium scale (5-20 sows), smallholder or backyard, and scavenging systems (1-10 pigs) account for the rest. The large-scale farms are still mainly owned by the state, and have around a 6% share of the national herd, and provide grand-parent breeding pigs. The medium-scale producers that have more than 20 sows and fatteners, have a 10% share of national production, and mostly rear exotic breeds on the same commercial feeds as the large scale producers. The small-medium scale producers who raise from 5-20 sows are increasing in number, and account for around 20% of the national herd. The most popular systems are small-holder and backyard, which are still dominant and account for around 64% of the national pig population (Kinh & Hai, 2008). The backyard and scavenging systems are more common in the rural mountainous areas, where there are many ethnic minorities and poor farmers, who construct simple pig houses in their back

gardens and allow their pigs to scavenge, and where the local and wild breed pigs are dominant. Fisher and Gordon (2008) reported that the proportion of the pig population held by smallholders has declined over recent years. Growth has mainly been concentrated in small-medium and medium-scale systems, especially in the MD. In the North of Vietnam, the proportion of small-scale farmers and of local breeds is higher than that in the South of Vietnam.

2.2.3 Feeding systems

In Vietnam, many foreign animal feed companies have been established and produce commercial feeds for livestock. Using commercial feeds improves pig performance and reduces labor requirements, and also reduces the time to slaughter, which results in a lower risk of outbreaks of epidemic diseases, and a quicker return on investment. However, the disadvantage of using commercial feed is always the much higher price than local feed. In order to reduce feed costs, producers tend to use whatever feedstuffs are available and reasonably palatable, which are very variable geographically (Len, 2008). It is also common that commercial concentrate feed is used together with locally available energy feed sources. These sources vary between regions, and include crop products and by-products, aquaculture by-products, agro-industrial by-products, and fresh forages. These local feed ingredients can be classified into energy feed resources, such as maize, cassava root waste, rice bran, broken rice and sweet potato root, and protein feeds, such as soya bean meal and cake, groundnut cake meal, fish meal and cottonseed cake. In addition, some agro-industrial by-products, such as Tra catfish by-products, shrimp by-products, tofu residue, brewer's grain and distiller's grain are also used to replace commercial feed. Several types of forage are also used as feeds for pigs, such as sweet potato vine, water spinach, cassava leaves, water hyacinth, and duckweed. However, the main constraint in using locally available feeds is lower performance compared with commercial feed.

2.3 Effect of dietary protein on pig performance

2.3.1 Protein requirement of pigs

The protein requirement of farm animals is influenced by many different factors, including animal characteristics, such as weight, daily gain, sex, genotype, environment and health status (Fuller & D'Mello, 1994). The protein requirement as a percentage of diet steadily declines to maturity, and especially smaller pigs require a higher level of protein in the diet. However, the amount of protein in the diet is less important than the protein quality,

or balance of the AA. This thus depends on its AA composition, and good quality protein should contain all the EAAs in correct proportions and amounts. The optimal dietary pattern is called Ideal Protein, which is defined as the perfect ratio among the EAAs required for maintenance and production (Boisen *et al.*, 2000). Buttery and D'Mello (1994) showed that a deficiency of an AA caused a reduction of performance, and excesses of AA can also be deleterious. Thus, the most important single factor affecting the efficiency of protein utilization for meat production is the dietary balance of AA (Cole *et al.*, 1994). The concept of AA balance is the relationship between the AA composition of a protein and its biological value, and a protein that provides AA in roughly the proportions in which they are required by the body is termed a balanced protein and has a high biological value. An AA imbalance results in a fall in the efficiency of nitrogen utilization. De Lange *et al.* (1999) showed that a diet with a good balance of AA but with a lower protein level can result in better growth performance in pigs compared with a diet with a high level of protein but unbalanced amino acids. In general, animal proteins such as fish meal and meat meal are rich in lysine and methionine and have higher biological values than plant proteins, which often lack lysine, methionine and tryptophan (McDonald *et al.*, 1995). Many researchers have suggested that both animal and plant proteins should be included in the diets for pigs and poultry.

2.3.2 Effect of fish meal on the feed intake and growth performance of pigs

Fish meal (FM) is recognized as a safe and natural balanced feed ingredient that is high in protein, energy and minerals (calcium and phosphorus) and is a natural source of vitamins (B₁₂, A, D and E) and a range of micronutrients, including selenium and iodine. Fish meal of high quality is one of the best sources of protein for pigs due to its favorable AA composition and digestibility for monogastric animals (Kjos *et al.*, 1999). Many studies have shown that growth, feed intake and feed conversion efficiency in pigs are improved when FM is included in the diet, especially in early weaned piglets. According to Stoner *et al.* (1990) FM generally improves growth and performance of starter and grower pigs.

2.4 Effect of dietary fat on pig performance and meat quality

There are many advantages of adding fat in pig diets. Firstly, increasing the caloric content of the diet, as fat has more than twice as much energy as an equivalent amount of carbohydrate or protein. Secondly, reducing feed intake, because pigs tend to eat a given amount of energy according to their

body weight and the temperature at which they are kept, so when the energy concentration of the diets increases by fat addition, the pigs will eat less feed in order to consume a given amount of energy. According to White and Latour (2007), pigs fed added fat gain weight more rapidly as well as having improved feed efficiency, reproductive efficiency and reduced heat increment, which is important in tropical climates.

However, depending on the fat type used in the diet, the impact on carcass quality can be very different, for example it will influence the fatty acid (FA) composition of the back fat and the FA content of the meat. A diet with high polyunsaturated fatty acids (PUFA) resulted in higher levels of PUFA in back fat (Bryhni *et al.*, 2002). In theory, fat type in the diet can affect the physical, sensory and chemical characteristics of associated meat products. Physical assessment of fat quality is through fat firmness and color, while sensory assessment measures palatability and chemical factors that are evaluated include the FA composition of meat fat. Many studies have shown that the FA composition of the carcass fat is influenced by the dietary fat (Ahn *et al.*, 1996; Warnants *et al.*, 1996), because the transfer coefficient of dietary fat to carcass lipid is high, 31–40%, depending on the specific FA (Kloareg *et al.*, 2007). The FA composition of pig muscle can be altered rather quickly, depending upon the polyunsaturation of the fat source incorporated into diets of growing-finishing pigs.

2.5 Evaluation of nutritive value in pigs

The digestion processes of animals are grouped into mechanical, chemical and microbial activities. In pigs, both mechanical and chemical (enzymes) processes occur in the front parts of the digestive tract, while the microbial processes occur mainly in the large intestine by the action of microorganisms (An, 2004). The digestibility of feed can be determined with direct or indirect methods. Direct methods are commonly used, in which all feed consumed and faeces and urine excreted are quantitatively collected during a certain number of days (Guevara *et al.*, 2008). Normally male animals are used in digestibility trials, and the loss of nutrients between feed offered and feed refused is the portion digested and absorbed. Indirect methods base on spot sampling of faeces or digesta (cannulation), and the use of an indigestible marker in the feed are also used for calculations of the digestibility (Hong, 2008). The marker can be added to the feed (external) or could be a natural component of the feed (internal) and should not be absorbed or be toxic to the animals. There are many kind of markers, and commonly the internal marker is acid-insoluble ash (Lyberg *et al.*, 2006),

while the most common external markers are chromic oxide (Cr_2O_3) (Van Leeuwen *et al.*, 1996), and titanium dioxide (TiO_2) (Weatherup & McCracken, 1998).

2.6 Determination of ileal digestibility in pigs by the cannulation technique

Sales and Janssens (2003) reported that using an internal marker to estimate the coefficient of apparent digestibility is more advantages than in comparison with the total collection method. The main site of absorption of nutrients (sugars, amino acids and fatty acids) in the pig is in the small intestine, while the enzymatically non-digested feed mainly is microbially fermented in the hindgut. Therefore, in order to have a more precise estimate of the nutrients absorbed by the pig it is preferable to determine the ileal digestibility instead of the faecal digestibility (Hong, 2008). This is especially valid for the determination of amino acid digestibility in different feedstuffs. In order to collect the digesta at the end of ileum, the cannula technique has been used (Van Leeuwen *et al.*, 1991). In the PVTC-technique the caecum is removed and a T-cannula is joined with the remnants of the caecum directly opposite the ileo-caecal valve. When the cannula is open the ileo-caecal valve protrudes into the cannula and thus digesta can be collected. In order to calculate the digestibility, without having to do total collections, an indigestible marker has to be used (Marais, 2000). The markers are indigestible substances not absorbed or affected by the digestive tract. They should also not affect or be affected by the microbial population in the digestive tract. In the current thesis chromium oxide was used as digesta marker.

3 Summary of materials and methods

3.1 Experimental site

The survey (Paper I) was conducted among catfish farmers, export processing factories and catfish by-product meal processing companies in 5 provinces (Can Tho, An Giang, Dong Thap, Vinh Long and Tien Giang) in the Mekong Delta of Vietnam. The experiments reported in Paper II and Paper III were conducted at the old Experimental Farm of Can Tho University in Can Tho City. The research reported in Paper IV and Paper V was conducted at the new Hoa An Experimental Farm of Can Tho University. The silage feed samples were analyzed at the Advanced Laboratory of Can Tho University. Amino acids and fatty acids were analyzed at the Service and Analysis Center in Ho Chi Minh City and the National Institute of Animal Husbandry (NIAH) in Ha Noi. The chemical composition of feed, faeces and ileal digesta was analyzed at the Animal Science Department Laboratory of Can Tho University.

3.2 Experimental design

Information collected in the survey included catfish cultivation systems, catfish processing techniques, different kinds and quality of catfish by-product meals available, and the main problems that by-product processing factories are experiencing. Around ten official companies and many smallholders who process catfish by-products were interviewed, and meals were sampled and analyzed for chemical composition, including amino acids and fatty acids.

Paper II includes ensiling and digestibility experiments. The aim of the silage trial was the evaluation of the chemical composition of the different silages in order to select the optimum additive and proportion of silage for

the digestibility experiment. The ensiling trial had a factorial arrangement with two silage additives, three silage ratios and seven ensiling times. The trial was conducted with boiled, ground catfish by-product (CBP), which included head, bone, skin and abdominal organs that remain after the two side fillets have been removed. After boiling the oil is removed. The CBP was mixed with RB or M of 71 degree Brix at a ratio of 8:2, 7:3 and 6:4 (CBP and RB or M, fresh weight basis). The mixtures were placed in plastic bags, and then stored at room temperature (29-31°C) for two months. There were 126 bags of 2 kg with 6 treatments, 7 sampling times and 3 replications per treatment. Samples were taken at 0, 7, 14, 21, 28, 42 and 56 days of ensiling for measurement of pH, N-NH₃, chemical composition and organic acids.

The digestibility trial (Paper II) and ileal digestibility experiment (Paper III) were designed as 4 x 4 Latin squares, with four treatments and four periods of data collection. In Paper II, the silages were prepared under conditions selected from the results of the ensiling trial. In Paper IV and V, the experiments were arranged as a randomized complete block design, with five experimental diets and six replications. The study reported in Paper IV included 30 growing pigs, and in Paper V 30 fattening pigs, kept in individual pens with each pig as the experimental unit and blocked according to initial body weight.

3.3 Experimental diets

Dietary treatments consisted of basal ingredients as the energy source, and including broken rice, rice bran and maize meal (Paper II, III, IV and V). In Paper II, for the determination of total tract digestibility, the basal diet was mixed with different protein sources, with FM as control and with the experimental diets including catfish by-product ensiled with either RB (7:3) or M (8:2). The experimental diets were made each week for the six weeks of the experiment, so that the time of preservation of the silage was the same. In Paper III, there were four different test protein sources: fishmeal (FM) as control, and diets including catfish by-product meal (CBM), ensiled catfish by-product (ECM) ensiled with M at a ratio 8:2 and processing waste water (WWB). All diets were supplemented with chromium oxide as an indigestible marker at 5 g/kg DM of the diet.

Five diets in Paper IV were formulated to contain 14% CP and 13.1 MJ/kg of ME. The protein source was FM alone in the basal diet and in the other diets 25%, 50%, 75% and 100% of the CP from FM was replaced by WWB. The diets were fed *ad-libitum*. Similarly, in Paper V, five

experimental diets were fed, and were formulated to contain 13.3 to 13.4% CP, and 13.0 to 13.1 MJ/kg of ME. The basal ingredients were the same, with FM as the sole protein supplement, and the four experimental diets were formulated so that 100%, 75%, 50% and 25%, respectively, of the CP from FM was replaced by the CP from ECM. All diets were supplemented with a standard mixture of vitamins and minerals.

In Paper IV, the WWB was collected each week, preserved with acetic acid and then stored before being mixed daily with the basal ingredients. The FM and CBM were mixed with the basal ingredients initially and stored for the whole period of data collection and fed *ad libitum*. In the study in Paper V, FM was bought from factories that specifically process small marine fish. The boiled, ground CBP was collected once per week at a catfish by-product meal factory near to the experimental farm, ensiled with M each week, and then mixed daily with the basal ingredients.

3.4 Animal management and measurements

3.4.1 Animals

In Paper II, III, IV and V, castrated male pigs (crossbreeds between Yorkshire and Landrace) were used. Animals in Paper II and III were from the same litter, with an initial body weight of 35.1 ± 0.5 (Paper II), 35.5 ± 0.4 kg (Paper III) at the start and 55.3 ± 1.3 (Paper II) and 60.1 ± 1.3 kg at the termination of the digestibility experiments. All pigs were treated against intestinal parasites and vaccinated against hog cholera and foot and mouth disease before the experiments started. The pigs in Paper III were surgically fitted with post-valve T-caecum cannulas to allow collection of ileal digesta. After surgery, the pigs were kept in individual pens for two weeks to recover before the start of data collection. The pigs in Paper II and III were housed in individual metabolism cages in an environmentally controlled house with an average temperature of 30–33°C.

The feeding trial was over a seventy-day period (Paper IV), with a total of 30 pigs at an average live weight of 23.6 ± 1.6 kg at the start and 58 to 64 kg at the termination of the trial, allocated into six groups of five, balanced for initial body weight. The pigs were housed in individual cages measuring 60 x 150 cm. Similarly in Paper V, 30 pigs with an average initial weight of 45.0 ± 3.1 kg were used and were slaughtered after 70 days on experiment. Before starting the experiment, the pigs were given the same commercial feed until 35 kg live weight, and then were given the experimental feed for 2 weeks of adaptation.

3.4.2 Measurements

In Paper II, there were four periods of data collection. In each period, pigs were fed during four periods of 12 days, consisting of seven days of adaptation to each diet followed by five days of quantitative collection of faeces and urine. During the collection period, feed offered and refusals were recorded. Faeces and urine were quantitatively collected two times per day for five days, and at the end of each period, samples were thawed, mixed within animal and diet, and sub-samples were taken for chemical analysis. Urine was collected in 50 ml of 10% H₂SO₄. However, in the ileal digestibility experiment (Paper III), each period was 12-days, consisting of five days of adaptation to each diet followed by four days of collection of feces and urine, one day of ileal digesta collection, one day of rest and the last day was a second day of ileal digesta collection. Ileal digesta samples were collected at ileum using the PVTC cannulation technique, and then all samples were immediately frozen at -20°C pending analysis. The rations were given in three meals per day (Paper IV) or twice per day (Paper V) and were offered *ad-libitum*. Refusals were collected the following morning before the first meal, and feed offer and refusal samples were stored in a freezer for DM analysis. The experiments lasted ten weeks and the pigs were weighed every two weeks. Feed intake was recorded, and then feed conversion ratio was calculated as feed intake divided by weight gain.

3.5 Slaughter

In Paper V, after the ten weeks of the feeding trial, all pigs were slaughtered after 12 h of starvation. The hot carcass weight was the weight after slaughter, but excluding blood, hair, visceral organs and gastrointestinal tract. Dressed weight was the hot carcass weight minus head, lower legs, tail and leaf fat. All the parameters were measured on the left side of each carcass, including back fat thickness at the 10th rib, three-quarters of the lateral length of the *Longissimus dorsi* (LD) muscle perpendicular to the outer skin surface, and at three other points at the 1st rib, last rib and last lumbar vertebra, using a ruler. The loin eye area was measured by tracing the LD muscle surface area at the 10th rib on acetate paper by using a compensating planimeter at slaughter. Finally, fresh loin muscle samples were taken at the 10th rib for chemical analysis.

3.6 Chemical analysis

Analyses of dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash of feed, faeces, digesta and meat (Paper II, III, IV and V) were carried out using standard Association of Official Analytical Chemists methods (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Paper II and III) were determined by the methods of Van Soest *et al.* (1991). Ammonia nitrogen (Paper II) was determined by distilling with water and MgO, collecting the distillate in 0.3% H₂BO₃, and then titrating with standard 0.1 N H₂SO₄. Organic acids (Paper II) were measured by reverse-phase High Performance Liquid Chromatography (HPLC). Amino acids (Paper I, III and IV) were analyzed on an ion-exchange column using an HPLC (Spackman *et al.*, 1958). Chromium (Paper III) was measured by atomic absorption spectrophotometer after ashing and digesting the sample in a mixture containing perchloric and nitric acid (Fenton & Fenton, 1979).

In Paper V, the pH of the meat was measured at 24 h post mortem. Approximately 10 g samples were ground into small pieces and 90 ml of distilled water was added. Slurry was then made using a homogenizer and the pH was recorded. Fatty acid composition of loin muscle samples from the 10th rib was analyzed by gas chromatography (GC), and total lipid content of muscle was determined following the chloroform-methanol extraction procedure of Folch *et al.* (1957). The objective meat color of the LD was evaluated on the 10th-11th rib surface using a colorimeter (Chromameter Minolta, CR- 200, Japan), and expressed as Hunter L* reading, which is a measure of the lightness or darkness of a meat sample. The a* reading is a measure of the redness or greenness of the sample, and the b* reading is a measure of yellowness and blueness.

3.7 Statistical analysis

Analysis of variance was performed using the General Linear Model of Minitab Statistical Software Version 15. Tukey's pair-wise comparison procedures test was used to determine differences between treatment means at P<0.05 in Paper III, IV and V, and the Bonferroni procedure was used for pair-wise comparisons in Paper II.

4 Summary of results

4.1 Catfish production and characteristics of catfish by-product meal, silage and waste water (Paper I and II)

The results from Paper I show that recently Tra catfish production in the MD has developed rapidly. There are three main catfish raising systems in the MD: floating cage, pond and Dang Quang (fence/pen). Most catfish farmers are changing to culturing catfish in ponds and in the Dang Quang system, while raising Tra catfish in floating cages is becoming less popular. The highest volume of production in the MD is in An Giang Province, followed by Can Tho Province. Around 60-65% of the whole catfish by weight are by-products, which are mainly used to produce CBM for animal and aquaculture feed. The amount of catfish by-products produced in the MD is around 710 tonnes/day, of which around 53% is CBM for animal feed, 42% is wet fish meal for aquaculture, and around 5% is for human consumption. At present, the majority of these by-products are sent to catfish by-product meal processing factories, where CBM and fish oil are produced. The processing of catfish by-products is only a little different between provinces. In general, the first stages include mincing, cooking, oil removal, and pressing of solid fish by-products, from which processing waste water and solid cake are generated. Then the waste water is centrifuged to separate the oil fraction, and this crude fish oil is then further processed and transferred to tanks or sold as such. The waste water can be fed back to the solid cake and then dried and ground to CBM. In small-scale factories cassava root waste is added to the waste water and either dried to produce meal or fed directly as a wet meal for fish.

Catfish by-product meals which are produced from scrap meat and skin are always higher in CP and lower in mineral contents than in head and bone or waste water by-product meals. Scrap meat and skin by-product

meals are considered to be higher quality meals compared with small marine fish meal. Also amino acids in scrap meat by-products meal contain an excellent balance of EAAs, especially with respect to lysine and methionine. The EE content of CBM is different between factories as a result of different procedures and input material. In particular, the CBM were found to be high in fat (10-33% EE) and calcium (4-13%), while the CP content in head and bone by-product meals was rather low (35-42%). The scrap meat and skin by-product meals had relatively high CP content (45-62%), a good balance of EAAs, and were high in lysine (5.45-9.33% of DM).

There were overall differences among CBP silages made with the two additives, M or RB, and among the three 3 ratios (8:2, 7:3 and 6:4 [CBP: M or RB]) in pH, CP, DM, N-NH₃, LA and total organic acids. The lowest pH values were after 14-28 days, and silages made with M had lower pH values than silages made with RB. There were no significant differences in DM and CP contents after different ensiling times, and these were stable until 28 days and then decreased slowly up to 56 days, but there was a trend of lower DM and CP and higher N-NH₃ during ensiling.

Lactic acid and total organic acids increased from day 0, with the highest value at 28 days in silage with M at ratio 8:2, and remained stable until 56 days. Overall, the concentrations of LA and total organic acids were significantly lower in silage made with RB at the ratio 8:2 than in all other levels of additives. Acetic and butyric acid proportions decreased slowly, but the proportion of propionic acid increased during ensiling. There was a decrease in N-NH₃ with increasing proportion of RB, whereas it increased with increasing proportion of M in the silage. The WWB is the product that is released in catfish by-product meal processing, The DM, CP and EE contents of WWB products varied according to differences in the procedures and equipment used in each of the factories, and in input materials. The chemical composition thus varied between experiments, with DM, CP and EE contents of 29.2, 38.0 and 19.8%, respectively, in Paper IV, and 24.2%, 32.5% and 19.8%, respectively, in Paper III. Acetic acid was added to prevent deterioration of the product for 1 week, without having any effect on the palatability.

4.2 Total tract apparent digestibility of diets with catfish by-product meal or catfish by-product ensiled with rice bran or sugar cane molasses (Paper II)

The DM and CP contents of CBP were lower than in FM, in contrast with the EE content. The average daily feed intake (ADFI) and CP intakes were

higher in the diet with catfish by-product ensiled with molasses (ECM) or rice bran (CRB) in comparison with the FM diet and the basal diet (BD). Silages were prepared once per month before being used in the experiment. There were no differences in the coefficient of total tract apparent digestibility (CTTAD) of DM and organic matter (OM) among diets. The CP and EE digestibility did not differ between the FM, CRB and ECM diets, but was significantly lower in BD ($P<0.01$) than in the other diets. Nitrogen retention in BD was lower ($P<0.01$) than in the other diets. However, there was no significant difference in nitrogen utilization among diets. As a result, there was no difference in the calculated digestibility of DM, OM, CP and EE among the ECM, CRB and FM.

4.3 Ileal digestibility of catfish by-product meal, silage and processing waste water (Paper III)

The DM content was lowest in the diet with WWB in comparison with the other three diets, FM, CBM and ECM. However, there were no significant differences in OM, CF, ADF, NDF and NFE content among the four treatment diets. Overall, the ADFI, N intake and N-retention and utilization were not different among diets. However, the coefficient of apparent ileal digestibility (CIAD) of OM in the WWB diet was higher than in the other three diets, while the CIAD of EE was lower ($P<0.05$) in the FM diet compared with ECM diet and WWB diet. The CTTAD of EE was lower in the FM diet compared with the WWB diet. There was a trend towards higher CP digestibility at both ileal and total tract level in the ECM diet than in the other diets. The CIAD and CTTAD of AA were not different among diets ($P>0.05$). In all diets, the highest CIAD of EAAs such as leucine, lysine and methionine was in the ECM diet, and similarly the highest CTTAD of EAAs was for arginine, lysine, methionine and valine, also in the ECM diet.

4.4 Effects of processing waste water on the performance of growing pigs (Paper IV)

The WWB used in Paper IV was the product that is released in the catfish by-product meal processing, and was low in DM and CP but high in EE content. So the DM of diets decreased with increasing inclusion of WWB, in contrast with the EE content, that increased with increasing inclusion of WWB. The AA composition did not differ significantly among diets.

For the feeding experiment with growing pigs, when diets were equal in CP and ME content, it was found that, replacing 75% and 100% of the CP from FM in the diet by WWB caused a significant depression in DM intake, average CP and ME intakes and average daily gain (ADG), but improved feed conversion ratio (FCR). The ADFI was highest in the diet with 100% of the CP from FM (1409 g DM/day), and lowest (1084 g DM/day) in the diet with 100% of the CP from WWB. As a result the essential amino acid intakes were highest in WW0 and lowest in WW100. The ADG was highest in WW0 (582 g/day) and lowest in WW100 (501 g/day). The FCR, PCR and ECR were lowest in WW100 and highest in WW0. The cost of feed per kg gain decreased with increasing level of replacement of the FM by WWB, as the cost of the WW100 diet was 68% of the cost of the WW0 diet.

4.5 Effects of catfish by-product silage on performance, carcass characteristics and meat quality of finishing pigs (Paper V)

4.5.1 Effect of ensiled catfish by-product on pig performance

The catfish by-product used for the silage was made from head and bone by-products. The DM of the diets decreased with increasing inclusion of ECM in the diet, while EE and ash contents increased. Results indicate that, the higher the ECM inclusion in the diet, the higher the ADG in finishing pigs, which thus was highest in ECM100 (767 g/day) and lowest in ECM0 (691 g/day). Decreasing ADFI ($P < 0.05$) was found with increasing inclusion of ECM. The highest ADFI was in ECM0 (2.47 kg DM /day) and lowest in ECM75 (2.35 kg DM/day). However, FCR was improved when 100% of FM was replaced by ECM. As a result, the cost of feed per kg gain decreased with increasing inclusion of ECM in the diet, and was 72% in ECM100 compared with ECM0.

4.5.2 Carcass characteristics and meat quality

There was no significant difference in carcass yield and dressing percentage among pigs in diets with different proportions of ECM replacement. However, leaf fat and back fat thickness increased ($P < 0.05$) when pigs were fed higher levels of ECM. The leaf fat, back fat thickness at the 10th rib and average back fat thickness were highest in ECM100 and lowest in ECM0. In contrast, loin eye area was lowest in ECM100 and highest in ECM0. There was no significant difference in DM, CP and ash contents of the LD muscle, but the EE content of pigs fed ECM100 was higher than in ECM0. The meat color values of the LD muscle increased with increasing inclusion

of ECM. In particular, the L* and a* values increased from 40.8 and 7.26 in ECM0 to 42.2 and 9.09 in ECM100, and the b* values were highest in pigs fed ECM100 (6.07) and lowest in pigs fed ECM25 (5.04).

4.5.3 Fatty acid composition of *Longissimus dorsi* (LD) muscle

There was no significant difference among diets ($P > 0.05$) in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in the LD muscle. However, polyunsaturated fatty acids (PUFA) increased with higher inclusion of ECM in the diet, with the highest total PUFA in ECM100 (19.3%) and the lowest value ($P < 0.05$) in ECM0 (18.2%).

5 General discussion

5.1 Preservation methods of catfish by-product by ensiling (Paper I and II)

Silage technology is recognized as being most useful for solving the waste problem in the fish processing industry. Fish silage can be produced from all kinds of low-value fish by-products, and is almost entirely used for animal feed as a source of highly digestible protein. The advantages of producing fish silage are the low capital investment and simple processing equipment, but the disadvantage is the high transport cost due to the high water content (Rustad, 2003). Dapkevicius *et al.* (2000) reported that fish waste can be upgraded into feed by fermentation with LAB, and the procedure is economical and environmentally safe. Fish silage can be prepared either by acid treatment or by fermentation with bacterial culture and sugar (Rai *et al.*, 2010), because carbohydrate-rich materials as additives in the silages are necessary to facilitate the fermentation of LAB. Carbohydrates provides a source of potentially available energy for growth of the LAB (McDonald *et al.*, 1991), and carbohydrates may be added in order to improve the fermentation process when low quality fish wastes are used (Ramirez *et al.*, 2008).

In the present study, fermentation of catfish by-products was based on sugar and starch from molasses (M) and rice bran (RB) as silage additives for fermentation, without any bacterial culture added. The results from the study showed that M was more effective than RB as a silage additive. The main purpose of using M or RB as additives when making catfish by-product silage is to obtain a LA fermentation, and as a result the pH fell rapidly, and the silage was well preserved. The preservation occurs due to the anaerobic environment and bacterial fermentation of carbohydrates, the lower pH being primarily through the production of lactic and acetic acids.

The pH value of the silage decreases below 4.5 during ensiling and this pH decrease is partially responsible for the preservation (Soltan *et al.*, 2008). In fact, the lower the pH in the silage, the higher the LA concentration, especially in the M silages.

In this study, at 4 weeks of ensiling, all silages with M at all the proportions studied stored well, with low pH values (4.2–4.5) and remained stable until 56 days of ensiling. However, silage with RB at an 8:2 ratio showed signs of spoilage towards the end, with the pH value exceeding recommendations (5.4 – 6.1) (Espe & Lied, 1999), and the concentration of N-NH₃ was significantly higher at all times than in the other treatments. In addition, the LA content in treatment CRB8:2 did not increase as much as in the other treatments, resulting in a poor fermentation. This was most likely due to the low level of carbohydrate added, resulting in a too slow drop in pH and the development of a bacterial population with a high content of clostridia and enterobacteria (McDonald *et al.*, 1991). Ramirez *et al.* (2008) reported that the amount of carbohydrate in the source materials and addition of starters are the most important factors for controlling the LA fermentation. So the CBP ensiled with RB can only achieve a good fermentation with more than 30% RB in the material. The explanation for this is that, M has a high water-soluble carbohydrate content (McDonald *et al.*, 2002), and the sugar in M is more rapidly fermented than the starch in RB (Ngoan & Lindberg, 2000). Consequently, increasing the amount of CBP in the silage with RB adversely affected the fermentation characteristic of the silage, whereas the addition of M improved it, which is in agreement with Rasool and Gilani (1996), who ensiled poultry litter with molasses. (Ramirez *et al.*, 2008) carried out a study on using molasses as additive to silage made from three sources of fish waste, and the fermented products were well preserved due to the acid produced and the reduction of water activity that inhibited spoilage microorganisms and putrefaction.

The DM and CP contents of silage decreased slightly with ensiling time, in contrast with N-NH₃, probably because the low pH suppressed DM loss of the silage. The reduction in DM content can be explained by the conversion of starch in the RB and sugar in the M to volatile fatty acids (Vidotti *et al.*, 2003). Also the reduction of CP levels and increasing N-NH₃ in silage are a consequence of protein hydrolysis, which may transform CP into N-NH₃, which volatilizes during the process of storage (Geron *et al.*, 2007). In addition, there is the action of clostridia, which in wetter silage also showed a higher potential for fermentation to produce N-NH₃. According to McDonald *et al.* (2002) clostridia are able to grow at high pH levels and ferment LA to butyric acid, and can also break down amino acids

to amines and N-NH₃. According to Kung and Shaver (2001) a high concentration of butyric acid and N-NH₃ in silage indicates that the silage has undergone clostridia fermentation, and the actions of bacteria from the propionibacteria family generate a high proportion of propionic acid in silages with high protein and high moisture materials.

5.2 Digestibility of catfish by-product meal, silage and waste water in growing pigs

5.2.1 Digestibility of ensiled catfish by-product with molasses or rice bran compared with marine fish meal in diets for growing pigs (Paper II)

The results from the ensiling trial showed that the best proportion of catfish by-product with RB was 7:3, and 8:2 in the silage made with M. With these proportions, the silages were optimal with respect to pH, chemical composition and organic acids. The DM of CBP ensiled with M (47.5%) was lower than that in silage with RB (52%), and much lower than the DM of FM. The CBP used for ensiling was in fresh form and consisted of head and bone by-products, which explains the low DM and CP contents, and caused the lower DM in the diet supplemented with catfish by-product silages. The EE contents in the silages were variable and much higher than the EE in FM, as a result of an inefficient oil extraction process. It has been demonstrated that both the CTTAD (Paper II) and the CIAD (Paper III) of CP and EE tended to increase when the silages were supplemented into the diets for growing pigs, although there was no significant difference in CP digestibility in the FM diet compared with the two silage diets. Some possible explanations are that the fermented feed contained high concentrations of organic acids, especially LA, and the low pH can reduce the microbial activity of harmful bacteria species and change the bacterial ecology of the gastrointestinal tract, resulting in improved nutrient digestibility (Hong *et al.*, 2009; Moon *et al.*, 2004). Another explanation was put forward by Hong (2008), who showed that the microflora has an important role in the digestive tract of the animal, and fermented liquid feed has been shown to alter the population of the microbiota and influence volatile fatty acid levels in the intestine, which plays a very important role in the elimination of many pathogens. Similarly, Plumed-Ferrer and von Wright (2009) reported that fermenting a liquid diet with LAB improved the quality of the feed and was beneficial to the health of the animals. Also Rai *et al.* (2010) reported that the protein in fish silage is highly digested as well as being a good protein source. An additional explanation, according to Scholten *et al.* (1999), is that fermented feed may stimulate pancreatic

secretion and positively influence villus architecture, and these factors may also have contributed to the improved digestion and absorption of nutrients such as CP and EE. On the other hand, the higher EE in the silage diets (Paper II and III) improved the apparent EE digestibility. This is in agreement with a previous study on growing pigs by Noblet and Perez (1993), who showed a lower EE digestibility when dietary fat content was low, and concluded that the amount of digestible EE was positively related to the dietary content.

The N-retention and N-utilization in the FM diet were not significantly different from the two silage diets (Paper II), probably because the balance of EAAs did not differ markedly among diets (Otto *et al.*, 2003), so it is not surprising that there was no difference in the digestibility of CP among the protein sources FM and CBP silages made with RB or M. It can be concluded that silage made from fish and fish by-products has a nutritional value comparable with that of marine fish meal.

5.2.2 Ileal and total tract digestibility of diets that include marine fish meal, catfish by-product meal and silage, and processing waste water in growing pigs (Paper III)

In the ileal digestibility trial, ECM was selected, because M was shown to be very effective in reducing pH, and the silage rapidly attained a pH of 4.5, with significant increases in LA content. Similarly, there was a trend towards increasing ileal and total tract digestibility of CP in the silage diet, due to the impact of microbial activity in the diet prior to feeding, which has been suggested to increase feed utilization and digestibility, as well as significantly improve the growth performance of pigs (Kim & Easter, 2001).

In general, the digestibility of EE and AA (Paper II and III) was lower than in previous studies by Ngoan and Lindberg (2000), Jorgensen *et al.* (1984) and Knabe *et al.* (1989) on FM. There could be several explanations for this, such as a high ash content in the products, heating during processing and diet CP content. Diets with high ash content were shown to have lower faecal apparent digestibility of amino acids (Noblet & Perez, 1993). In the present study, FM and catfish by-products silage materials, CBM and WWB, were all processed at temperatures higher than 80°C, which could possibly have reduced amino acid digestibility. Similarly results from Wang and Parsons (1998) showed that, high processing temperatures of meat and bone meals generally resulted in lower amino acid digestibility than did low processing temperatures. The FM produced at processing temperatures below 70-80°C had higher CP digestibility than when the meal was processed at temperatures above 100°C. Ohh *et al.* (2002) also

concluded that the quality of FM may show variation depending on the quality of the raw material and the processing method. However, all the diets (Paper II and Paper III) were formulated to have dietary CP contents that were lower than the pig's requirement (NRC, 1998). This may have caused the lower CP digestibility than was found in other studies with growing pigs, as Fan *et al.* (1994) reported that a reduction of dietary CP content decreased the ileal apparent digestibility of most amino acids in weaning pigs.

The ileal and total tract apparent digestibility of AA was not significantly different among the four protein source diets, and N-retention and N-utilization were also similar. This indicates a similarity in AA utilization among diets, as well as a similar balance of AA in FM, CBM, ECM and WWB. However, the ileal digestibility of EE in the ECM and WWB diets (Paper III) was significantly higher than in the FM and CBM diets. This was probably because of higher EE dietary content, which is positively related to higher digestibility. Also individual fatty acid digestibility increases with increasing unsaturation, and digestibility of fat sources is a function of fatty acid content (Duran-Montgé *et al.*, 2007). In fact, catfish oil is very high in PUFA, with concentrations of between 68% (Sathivel *et al.*, 2003) to 75% (Men *et al.*, 2007) of PUFA, which are more efficiently digested and absorbed than saturated fatty acids.

5.3 Effect of replacing fish meal with catfish processing waste water (WWB) or ensiled catfish by-products on the performance of growing-finishing pigs (Paper IV and V)

In small-scale CBM processing, considerable quantities of WWB are generated in the form of edible and non-edible by-products. The characteristics of WWB are low DM and high EE contents, because of the water added before cooking and an inefficient oil extraction process in the factory. The WWB was preserved by the addition of acetic acid at 3 g/kg (Bórquez & Gonzalez, 1994) and kept for around 1 week without affecting the acceptability by the pigs. Both ECM and WWB were very low in DM and high in EE contents, and as a result the diets into which they were included had lower DM and higher EE contents. In general, the feed intakes of the pigs in the present experiments were quite low compared with that in pigs of the same breed, age and live weight in the study of Le Bellego *et al.* (2002) with growing pigs, and Len *et al.* (2008) with finishing pigs. The explanation is probably that the mean ambient daytime temperature was 30–34°C and would most likely have reduced feed intake of pigs in all

treatments. Lebret (2008) stated that the ambient temperature influences the energy requirements and the growth performance of pigs, and the maintenance energy requirement decreases as temperature increases over the higher critical temperature and leads to reduced feed intake in pigs.

However, the feed intake decreased significantly with increased inclusion of the WWB (Paper IV), and was slightly reduced with inclusion of ECM (Paper V), probably because of the high fat content of these diets and differences in palatability. Michael (2001) showed that daily feed intake was reduced in diets with high levels of fat. In Paper IV, even though the WWB was acidified by addition of acetic acid, it is still possible that there was some bacterial deterioration, which would have negatively affected palatability. In Paper V, the ECM had a high concentration of acetic acid of around 21% of total organic acids, which possibly affected the palatability of the ensiled by-products. Brooks *et al.* (2001) also showed that a high level of acetic acid in fermented liquid feed is undesirable because it can reduce the palatability.

Decreasing daily feed intake resulted 12–20% lower AA, metabolisable energy and nutrient intakes in the diets with more than 50% replacement of the FM, and consequently reduced ADG (Paper IV). This may be because the FM used was a higher quality product and therefore gave a better response than the alternative, poorer quality WWB, and also probably because of lower nutrient intakes compared with the pig's requirement. Rosenvold and Andersen (2003), Terlouw (2005) and Lebret (2008) concluded that the growth performance of pigs, carcass composition and quality of pork products depend on multiple interactive effects of genotype, feeding level, housing, environmental conditions and production systems. In fact, pigs fed a high lysine level had a higher ADG than pigs fed lower lysine levels (Coma *et al.*, 1995), and Lebret *et al.* (2001) reported that restricting feed intake by around 25% reduced growth rate by around 27%. In contrast, the ADG of pigs fed the diet with 100% of ECM replacement with FM were 11% higher than in the FM diet (Paper V). This can be explained by the fact that fish silage is considered to be an excellent protein source, with a high biological value, because during silage processing, proteins are hydrolyzed to free amino acids, thus increasing their availability for protein biosynthesis (Vidotti *et al.*, 2003). In addition, silage feed also improved growth performance and reduced mortality and morbidity in growing-finishing pigs, because of enhanced nutrient availability and reduced growth and shedding of pathogenic bacteria due to the low pH, and improved animal health (Van Winsen *et al.*, 2001). Moreover, the higher fat content in high silage diets, has been shown to improve the efficiency of energy

utilization in pigs maintained at high ambient temperatures, and these grew significantly faster than those given no fish silage (Stahly, 1984).

Although ADFI and ADG were reduced when increasing the inclusion of WWB in the diets, FCR for DM, CP and ME were improved (Paper IV and V). This can be explained by the low CP intake contributing to a marked reduction of nitrogen excretion, and generating less heat due to the reduced energy expenditure for urea synthesis and turnover of body protein (Kerr *et al.*, 2003). Moreover, Noblet *et al.* (2001) also showed that heat production was reduced when dietary CP was reduced or when fat was added. An additional explanation for the lower FCR of the diets with high inclusion rates of WWB could have been a higher nutrient digestibility, especially of EE (Paper III) in WWB compared to FM. Supplementation of ECM in the diets for growing-finishing pigs improved the performance (Paper V), but not inclusion of WWB (Paper IV). The main benefit of using these by-products would be to reduce feed cost. For example, 100% replacement of FM by WWB reduced feed cost per kg gain to around 68% (Paper IV) and around 72% (Paper V). This is in agreement with Arvanitoyannis and Kassaveti (2008), who reported that using fish by-product as animal feed not only resulted in environmental and public benefits but also reduced the cost of animal production. However, in practice, using WWB and ECM in diets for growing pigs is not common in the MD due to the high cost of transport and difficulties in preservation.

5.4 Effect of replacing marine fish meal with ensiled catfish by-products on carcass and meat quality of finishing pigs (Paper V)

In general, the supplementation of ECM improved the performance of finishing pigs, especially with respect to ADG. However, back fat thickness and leaf fat increased with higher ECM inclusion in the diet, even though there was no significant difference in the hot carcass and dressing percentage. These results are in agreement with Kjos *et al.* (1999), who reported that the inclusion of defatted fish silage at the level of 50 g/kg diet had no negative effects on the dressing percentage or sensory quality of pig meat. According to Stahly and Cromwell (1979), increasing dietary fat supplementation increased carcass back fat thickness in pigs raised at high ambient temperatures, which is in agreement with the results of the present study, where the higher inclusion of ECM, the higher fat consumed and the higher the back fat thickness. Urlings *et al.* (1993) also showed that feeding

pigs with fermented poultry by-product which was heated before fermentation increased back fat thickness.

There were no differences in moisture, CP and ash of *Longissimus dorsi* (LD) in all pigs in the experiment. However, the EE content of LD was higher in the high ECM diets. Kim *et al.* (2006) reported that addition to the diet of fermented persimmon shell, which has a high content of EE, increased the EE content of the LD muscle. However, the meat from pigs fed higher inclusion of ECM had higher a* and b* values than those given lower levels of ECM, which had lower pH values. The lower pH in the FM diet resulted in a lower colour value in LD, because the lower the pH the paler the meat colour. According to Teye *et al.* (2006), the higher a* and b* colour values on these diets were probably because of the higher concentration of C16:0 and C18:0 in the LD fat, which makes the lipid less translucent and affects the colour of the pork. Moreover, Kouba (2006) reported that a high degree of tissue unsaturation can result in darker meat color, because tissue unsaturation can cause a more rapid conversion of the red muscle pigment myoglobin to brown metmyoglobin.

The fatty acid composition of pork can be easily manipulated through the feeding regime, as a consequence of the well known influence of the dietary fatty acids on fatty acid deposition in both subcutaneous and intramuscular lipids in pigs (Lebret, 2008). In fact, fat and fatty acids are important because of their effects on human health, and fatty acid composition is a major factor in the nutritional value of meat. Studies in pig nutrition have shown that the fatty acid profile of pork fat can be altered by feeding diets containing different fatty acid concentration, as the fatty acid composition of the diet has significant effects on tissue fatty acid composition (Mas *et al.*, 2010). The pig is unable to synthesize essential fatty acids such as linoleic and linolenic acid, and they must be supplied in the diet, and the fatty acid composition of pig meat is mostly controlled by diet (Kristinsson *et al.*, 2001). The fat in the ECM diet was mainly from catfish oil which is a rich source of essential PUFA in both the omega-3 and omega-6 families. Ho and Paul (2009) showed that the high absolute contents of DHA (C22:6, n-3) and EPA (C20:5, n-3) in Tra catfish makes it a potentially valuable source of omega-3 fatty acids. Linoleic acid (8.4%) is the major PUFA in Tra catfish, followed by DHA (4.7%). Thus, the higher the inclusion rates of ECM in the diet, the higher the DHA, EPA, DPA and other PUFA in LD. This is in agreement with Lauridsen *et al.* (1999), Hallenstvedt *et al.* (2010) and Bryhni *et al.* (2002), who showed that feeding fish oil to pigs did not affect the sensory quality of loin, but increased the level of DHA, EPA and DPA in muscle. Although FM normally contains

high levels of PUFA (Jónsdóttir *et al.*, 2003), the fish meal used in the present experiment was made from small marine fish which probably had lower PUFA contents than catfish by-product silage.

6 General conclusions and implications

6.1 Conclusions

- Tra catfish farming plays a vital role in the aquaculture sector in Vietnam, and is in a fast growth phase in terms of culture and production, in which the pond and fence systems are developing rapidly in the Mekong Delta.
- Catfish by-products are abundant and account for an estimated 65% of the whole fish, and most is used to produce dried and wet catfish by-product meals, which are mainly used locally for livestock and aquaculture.
- Catfish by-products ensiled with sugar cane molasses gives a better quality product than when ensiled with rice bran. The present data suggest that catfish by-products can be successfully ensiled with 20-40% of sugar cane molasses or 30-40% of rice bran (fresh and air-dry weights, respectively).
- The total tract apparent digestibility of crude protein in catfish by-products ensiled with rice bran or sugar cane molasses was comparable to that of small marine fish meal. However, the ether extract digestibility of ensiled catfish by-products was higher than that in fish meal.
- The ileal digestibility of crude protein and ether extract in ensiled catfish by-product tended to be higher than that in catfish by-product meal, processing waste water and marine fish meal, but amino acid digestibility was similar. As result, it can completely replace this type of fish meal in diets for growing pigs without affecting digestibility and nitrogen retention.
- Replacement of fish meal by catfish by-product processing waste water in the diet for growing pigs reduced the performance, but improved the feed efficiency and reduced feed cost.

- Replacing fish meal by ensiled catfish by-product improved the performance and meat quality of finishing pigs, but resulted in an increase in back fat thickness. However, because of the reduced feed costs, complete replacement of fish meal would still be profitable for pig producers in the Mekong Delta.
- Due to the high level of catfish oil remaining in catfish by-products, feeding ensiled catfish by-products to finishing pigs increased the level of DHA, EPA, DPA and other polyunsaturated fatty acids in LD muscle, but did not affect the sensory quality of loin.

6.2 Implications

- Catfish by-products can be preserved by ensiling and can be included in the diets of growing-finishing pigs without affecting acceptability. Dried meal, wet meal, silage and waste water can be valuable protein sources and completely replace marine fish meal in the diets for pigs without affecting the digestibility and nitrogen retention.
- Silage made from catfish by-products can not only improve performance but also reduce the cost of pig production in the Mekong Delta, where there are large quantities of catfish by-products available and an increasing number of processing companies.

6.3 Further research

- Studies are needed to test the preservation process and quality of silage made with raw catfish by-product material by acid ensiling in terms of pH and organic acids values.
- Research on feeding catfish by-product silage should be carried out on a large scale to show that there are cheaper alternatives to commercial feed in order to reduce feed costs for pig production.

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Acknowledgements

I am very grateful to the Swedish International Development Authority/Department for Research Cooperation (Sida/SAREC), through the regional MEKARN program for financial support.

My thanks also to the Animal Science Department and Experimental Farm of CanTho University, for allowing me to study and conduct the experiments, and to the Department of Animal Nutrition and Management of the Swedish University of Agricultural Sciences (SLU) for providing the facilities for my work in Sweden.

I am deeply grateful to my main supervisor, Professor Brian Ogle, who was my first supervisor in the MSc program, and afterwards enabled me to continue with PhD studies. During the course of my studies, I have received a lot of strong support, useful guidance, and valuable advice from him, particularly patient correction of my English.

I am also greatly indebted to my co-supervisor, Professor Jan Erik Lindberg, who introduced me to new ideas concerning experiments, and gave me useful guidance and correction of my articles.

My special thanks to Professor Inger Ledin, who first interviewed and accepted me for the MEKARN MSc program, and provided me with such comfortable living conditions during my stays in Sweden.

I would like to express my sincere gratitude to:

Professors, lecturers and assistant lecturers in courses which I have attended during my studies for sharing their valuable knowledge.

I am very grateful to Associate Professor Vo Van Son, Vice Dean of the Faculty of Agriculture and Applied Biology of Can Tho University, who encouraged me to study English when I was working at the Experimental Farm of Can Tho University, and gave me the opportunity to be interviewed for the MSc program and to become a lecturer.

Associate Professor Nguyen Anh Tuan, Rector of Can Tho University and Associate Professor Nguyen Van Thu, Head of the Department of Animal Science, for allowing me to study in the PhD program.

Thanks to Associate Professor Do Van Xe, Vice Rector of Can Tho University and Associate Professor Lennart Norell for their useful statistics lectures and valuable assistance concerning the statistics in my articles.

Dr. Ngo Van Man, Mr. Nguyen Van Cong and the members of SAREC/MEKARN project committee for their help, support and for transferring money on time for my studying and traveling. Especially, Associate Professor Le Van Tho, of Nong Lam University, Thu Duc, Ho Chi Minh City, for the surgery on the cannulated pigs and his guidance.

Mr. Trieu Cong Tam, the director of the Experimental Farm for supplying pigs and equipment for the experiments, and the staff of the experimental farm for their help.

Associate Professor Nguyen Nhut Xuan Dung, and Ms. Tran Thi Diep for their guidance of my students for analyses at their laboratory.

My students at Can Tho University and the College of Economics and Technology in Can Tho City: Tan Loc, Hoang Ky, Le, Anh Tuan, Dang Thang, Tan Tai, Trong Dai, Minh Phuc, Thanh Dat, Thai and Thong for their valuable cooperation and collection the data for the experiments.

My friends at SLU from Vietnam: Hong, Len, Giang, Tram, Thang, Thieu, , Khai, Quang, Xuan, Trien, Lam, Tong Anh, Tham, Thi Da, Sen, Ngoc, Hue, Thao and Uyen, and friends from various other countries: Oudom, Seuth, Daovi, Malavanh and Lampheuy (from Laos), Malin and Karin (from Sweden), Kerya and Sath (from Cambodia), Salimata and Alice (from Burkina Faso), Liu-Haoyu (from China) and Bryan from Nicaragua, for their friendship, help and encouragement during my stays in Sweden.

My mother, sisters and brother for their help and for encouraging me. Especially to the memory of my father, who was always encouraging my study. I am very sad at his passing away and cannot wait until my studies are finished.

Lastly, but not least, my husband Nguyen Hong Tien, who always accompanies me in my life; thanks for your love, support and taking care our family during my times in Sweden, and to my daughter, Hong Nhung, and my son, Tien Dat, as you are my present and future life.