MINISTRY OF EDUCATION AND TRAINING
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CASSAVA LEAVES (*Manihot esculenta* Crantz) AND WATER SPINACH *(Ipomoea aquatica)* AS PROTEIN SOURCES FOR GROWING PIGS IN CAMBODIA

DOCTORAL THESIS IN AGRICULTURAL SCIENCE

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PhD THESIS IN AGRICULTURAL SCIENCE

Specialization: Animal Sciences

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Supervisors:

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Associate Professor Le Dinh Phung, PhD

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GUARANTEE

I hereby guarantee that scientific work in this thesis is mine. All results described in this thesis are righteous and objective. They have been published in Journal of Livestock Research for Rural Development and Hue University Journal of Sciences (in press).

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Chhay Ty, PhD student

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Abstract

The study was aimed at evaluating and improving cassava leaves *(Manihot esculenta* Crantz*)* and water spinach *(Ipomoea aquatica)* as protein sources in diets for pig production in small scale production systems in Cambodia. Four experiments were carried out with pigs using cassava leaves and water spinach as forage protein sources. In the first experiment (paper 1), eighteen crossbred castrated male pigs of 12.9 (±1.04) kg live weight were allocated to a 2*3 factorial arrangement of 6 treatments and 3 replications. The first factor was with or without DL-Methionine (Met) supplementation; the second factor was source of high protein forage including fresh water spinach (WS); fresh cassava leaves (FCL); and a mixture of the two proteins forages (WSFCL) [40:60 DM basis]. Pigs fed FCL as a main protein source had lower feed intake, live weight gain (LWG) and feed conversion ratio (FCR) than pigs fed WS or WSFCL (P<0.05). Supplementing Met to the diet did not affect LWG and FCR (P>0.05). In the second experiment (paper 2), twenty four crossbred castrated male pigs of 17.5 (±0.76) kg live weight were allocated to a 2*3 factorial arrangement of 6 treatments and 4 replications. The first factor was energy source from broken rice (BR) and a combination of rice bran (RB) with cassava root meal (CRM); the second factor was the combination between fresh water spinach (WS) and fresh cassava leaves (FCL) in proportions of 10:40, 20:30 and 30:20 in the diets, respectively. Pigs fed WSFCL (30:20) had higher feed intake, LWG and better FCR than WSFCL (20:30) and WSFCL (10:40) (P<0.05). Pigs fed RB and CRM as energy source had higher LWG and better FCR than BR (P<0.05). In the third experiment (paper 3), eighteen crossbred castrated male pigs of 24.5 (±0.84) kg live weight were allocated to a 2*3 factorial arrangement of 6 treatments and 3 replications. The first factor was with or without supplementation of DL-methionine and the second factor was methods of processing cassava leaves: ensiled (ECL), dried (DCL) and fresh (FLC). Feed intake was significantly higher for ECL than for DCL and FLC treatments (P<0.001). DL-methionine supplementation resulted in higher feed intake and LWG (P<0.05). However, there were no significant differences among cassava leaves processing methods in terms of LWG (P>0.05). The fourth experiment (paper 4) was arranged as a Latin-square design and included 4 crossbred castrate male pigs of 50.3

(±1.44) kg live weight. The pigs were fed randomly one of four diets based on rice bran, broken rice and fishmeal as the control diet (CTRL), and fresh water spinach (WS) or with fresh cassava leaves (FCL) or mixture with ratio 1:1 (WSFCL) as the main protein source. The DM intake was significantly higher for the CTRL diet than the others (P<0.05). Apparent organic matter, DM, CP, CF and NDF digestibility coefficients were significantly (P<0.05) higher for the WS than FCL and WSFCL treatments. The essential amino acid digestibility was higher for WS and CTRL than for WSFCL and FCL treatments. Nitrogen retention was higher for the CTRL than the WS diet, which were superior to diets WSFCL and FCL (P<0.05). In all experiments, there were no ill-health symptoms in any of the pigs that could have been ascribed to HCN toxicity arising from fresh cassava leaves. The average intake of HCN was 152 mg/day or 7.35 mg/kg live weight. There appeared to be an interaction between Met supplementation and protein sources from the forages for total DM and CP intake but no interaction for LWG and FCR. It is conclude that cassava leaves and water spinach could be used as protein sources for pig production in small scale production system in Cambodia.

Key words: *Protein, Amino acid, Cassava leaves, Water spinach, Fish meal, HCN Digestibility, Growing pigs*

Dedication

To my parents, brothers and sisters

My wife Sok Chenda

My son Chhay Kolsambath

My daughter Chhay Danapich

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LIST OF ABBRIVIATIONS

AA	Amino Acid
ADF	Acid Detergent Fiber
Arg	Arginine
BR	Broken rice
CF	Crude Fiber
CL	Cassava leaves
CLS	Cassava leaves silage
CLM	Cassava leaves meal
CP	Crude Protein
CRM	Cassava root meal
DCL	Dry cassava leaves
DF	Dry fish
DM	Dry Matter
DWG	Daily weight gain
EAA	Essential Amino Acid
EE	Ether Extract
ECL	Ensiled cassava leaves
FCL	Fresh cassava leaves
FCR	Feed conversion ratio
FM	Fish meal
FMD	Food and mouth disease
GDP	Gross domestic product
HCN	Hydrogen Cyanide
His	Histidine
Iso	Isoleucine
Leu	Leucine
LW	Live weight
LWG	Live weight gain
Lys	Lysine
Met	Methionine
ML	Mulberry leaves
MAFF	Ministry of Agriculture, Forestry and Fishery
N	Nitrogen
NRC	National Research Council
NGO	Non Government Organization
NDF	Neutral Detergent Fiber
NEAA	Non essential amino acid
OM	Organic Matter
Phe	Phenylalanine
P.D.R	People's Democratic Republic
RB	Rice bran
SPS	Sugar palm syrup
Thr	Threonine
TLS	Taro leaf silage
Tyr	Tyrosine
Val	Valine
WS	Water spinach

CHAPTER I: INTRODUCTION

I. 1. Rationale

Cambodia has a total land area of 181,035 km^2 (land: 176,515 km^2, water: 4,520 km^2), consisting of 24 provinces/cities comprising 185 districts, 1621 communes and 14073 villages. Cambodia is located on the mainland of Southeast Asia between Thailand to the west and north and Vietnam to the east and southeast. It shares a land border with Laos in the northeast. Cambodia has a sea coast on the Gulf of Thailand. Cambodia is a tropical country, with rainy, monsoon season starting from May to November and dry season from December to April. The total population was estimated to be about 14,453,680 persons in 2010 with population growth 1.705% and with 22.8% of the population in urban areas (Cambodia General Statistics Office, 2010).

Agriculture is one of the most important economic sectors in Cambodia. The sector contributed a share of 33.5% to the country's gross domestic product (GDP) in 2009 (US Department of Statistic, 2010) compared with 30.5% in 2008. Agriculture is the primary source of employment and income for the vast majority of the rural population that makes up about 80% of the country's population. The livestock sector also has a key role to play in a strategy for rural development in Cambodia. Livestock sector development appears to be one important pillar of any development strategy for agriculture in Cambodia. Such a strategy aims at achieving higher and more stable rural incomes, reducing the incentives for a flow of migrants from rural to urban areas, making farming system more sustainable in the long run, and alleviating rural poverty. Among many animal species, pigs play an important role for small holder farmers because they are an extra source of family income, are used for festivity events, for paying a debt or acting as a savings bank. Pigs can be confined in a small area, and can covert to meat a variety of crop and kitchen wastes and give a rapid return on investment (Steinfeld, 1998). Pig production in rural areas is dependent on agriculture by-products (rice bran and broken rice). Agriculture by-products are cooked with other water plants that can be collected near the households or from lakes and ponds. The quality and quantity of local feeds for pigs are usually not sufficient for them to reach their productive potential. Preston, (2006) reported that the leaves from shrubs such as cassava, mulberry, and sweet potato, and water plants such as duckweed and water spinach are locally available and can be used successfully in diets for pigs to replace at least half the protein usually supplied as soybean and fish meals which are expensive and can not be afforded by small scale farmers. Based on the recommendation, it is considered that high protein forages such as cassava leaves and water spinach have potential for pig production in Cambodia.

Cassava leaves (CL) are a good source of crude protein (CP) and minerals particularly Ca, Mg, Fe, Mn and Zn. The chemical composition of cassava leaves is variable depending on variety, growing conditions, fertilizer application, processing and age of the plant. Animal Feed Resources Information Systems, (2004) reported a range in CP content of the leaves from 22.8 to 24.1% and from 25.4 to 29.0% (DM basis) in the rainy and dry seasons, respectively. Cassava leaves have a good amino acid profile similar to alfalfa and soybean meal although the concentration of methionine is considered to be limiting (Bui Huy Nhu Phuc et al., 2001). Several studies have been done on the use of cassava foliage as animal feed, using different forms such as meal, silage or hay. Cassava foliage hay has been used for cattle (Chet Sophal et al., 2010; Keo Sath et al., 2007), cassava foliage silage and hay for goats (Iv Sophea et al., 2010; Seng Sokerya, 2009), cassava leaf silage or meal for pigs (Khieu Borin, 2005; Nguyen Hoa Ly, 2006; Du Thanh Hang, 2007; Bui Huy Nhu Phuc et al., 2000b) and cassava leaf meal for poultry (Khieu Borin, 2005). However, few researches have been done on using fresh cassava leaves for pigs, probably due to the content of anti-nutritional factors (HCN and tannins).

Water spinach *(Ipomoea aquatica)* is a plant that grows equally well in water or in soil. It responds dramatically in biomass yield and protein content when fertilized with biodigester effluent (Kean Sophea and Preston, 2001), earthworm compost (Tran Hoang Chat et al., 2005) or urea (Ly Thi Luyen and Preston, 2004; Tran Hoang Chat et al., 2005). The yield of water spinach can be as high 15 tonnes ha^{-1} $month^{-1}$ of fresh biomass with CP content ranging from 20 to 35% in DM (Ho Bunyet, 2003). The amino acid profile in water spinach is good but there is a deficiency of methionine. In Cambodia, water spinach (WS) is cultivated for human food and also is fed to pigs, chicken and rabbits; it does not appear to contain anti-nutritional compounds and has been used successfully for rabbits as the only source of supplementary protein in a diet (Pok Samkol et al., 2006abc). However, no comparisons have been made between fresh cassava leaves, fresh water spinach and combinations of fresh cassava leaves and water spinach as sources of protein in diets with or without supplementation of methionine. The combination of water spinach and cassava leaves as a source of protein for pigs may improve nutrient digestibility and animal performance.

I. 2. Objectives

The goal of the present research was to study and improve the use of water spinach and cassava leaves as a source of protein in diets for pig production in small scale in Cambodia. The specific objectives were to study:

- Effect of water spinach and fresh cassava leaves on growth performance of pigs fed a basal diet of broken rice
- Effect of different ratios of water spinach and fresh cassava leaves on growth of pigs fed basal diets of broken rice or mixture of rice bran and cassava root meal
- Effect of processing cassava leaves and DL-methionine supplementation on growth performance of crossbred pigs fed basal diets of rice bran and cassava root silage.
- Effect of fresh cassava leaves and water spinach as protein sources on N balance and amino acid digestibility in growing crossbred pigs.

I. 3. Innovation of thesis

This thesis is the output from four experiments; of which three experiments concentrate on pig growth performance and one on apparent digestibility. The results from experiments can help researchers, students and extension workers from government and non-government organizations to understand more about high protein forages from cassava leaves and water spinach and the degree to which they can be used as protein sources in diets to improve small scale pig production in Cambodia.

Reference

Animal Feed Resources Information Systems (2004). Updated from B. Göhl, (1981) Tropical feeds. FAO. http://www.fao.org./ag/AGa/agap/FRG/AFRIS/DATA/535.htm

Bui Huy Nhu Phuc, Ogle B, J E Lindberg (2000b) Effect of replacing soybean protein with cassava leaf protein in cassava root meal based diets for growing pigs on digestibility and N retention. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala

Bui Huy Nhu Phuc, Ogle B & Lindberg J K (2001) Nutritive value of cassava leaves for monogastric animals; Proceedings of workshop on "Use of cassava as animal feed" Khon Kaen University, Thailand http://www.mekarn.org/prockk/phuc.htm

Cambodia General Statistics Office, (2010). http://www.nis.gov.kh/index.php/pop-demog-stat/censuses

Cheat Sophal, Khieu Borin & Preston T R (2010) Effects of supplements of water hyacinth and cassava hay on the performance of local "Yellow" cattle fed a basal diet of rice straw. MSc Thesis, MEKARN-SLU http://www.mekarn.org/MSC200810/theses/sophal.htm

Du Thanh Hang (2007) Cassava leaves as protein source for pigs in Central Vietnam. Chapter 1: Cassava (Manihot esculenta Crantz) production: nutritive value and toxic factors. Doctoral thesis, Nederland University 2007

Ho Bunyeth (2003) Biodigester effluent as fertilizer for water spinach established from seed or from cuttings; Retrieved, from MEKARN Mini-projects. http://www.mekarn.org/msc2003-05/miniprojects/webpage/buny.htm

Iv Sophea, Khieu Borin, Leng R A & PrestonT R (2010) Effect of different levels of supplementary potassium nitrate replacing urea on growth rates and methane production in goats fed sugar palm-soaked rice straw and mimosa foliage MSc Thesis, MEKARN SLU http://www.mekarn.org/MSC2008-10/theses/sophea.htm

Kean Sophea & Preston T R (2001) Comparison of biodigester effluent and urea as fertilizer for water spinach vegetable. Livestock Research for Rural Development 13 (6) http://www.cipav.org.co/lrrd/lrrd13/6/kean136.htm

Keo Sath, Khieu Borin & Preston T R (2007) Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. MSc Thesis, MEKARN-SLU http://www.mekarn.org/MSC2005-07/thesis07/sath2.htm

Khieu Borin (2005) Cassava Foliage for Monogastric Animals. Forage Yield, Digestion, Influence on Gut Development and Nutritive Value. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2005

Ly Thi Luyen & Preston T R (2004) Effect of level of urea fertilizer on biomass production of water spinach (*Ipomoea aquatica*) grown in soil and in water. *Livestock Research for Rural Development. Vol. 16, Art. #81*. Retrieved, from http://www.cipav.org.co/lrrd/lrrd16/10/luye16081.htm

Nguyen Thi Hoa Ly (2006) The use of ensiled cassava leaves for feeding pig's on-farm in Central Vietnam. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN-CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006. Article # 13. Retrieved March 16, 107, from http://www.mekarn.org/proprf/hoaly.htm

Pok Samkol, Preston T R & Leng R A (2006a) Effect of offering leaves or stems of water spinach on patterns of eating, consumption of soft faeces and excretion of hard faeces by growing rabbits. Livestock Research for Rural Development. Volume 18, Article # 46. Retrieved, from http://www.lrrd.org/lrrd18/3/samk18046.htm

Pok Samkol, Preston T R & Ly J (2006b) Digestibility indices and N balance in growing rabbits fed a basal diet of water spinach (*Ipomoea aquatica*) supplemented with broken rice. Livestock Research for Rural Development. Volume 18, Article # 19. Retrieved, from http://www.lrrd.org/lrrd18/2/samk18019.htm

Pok Samkol, Preston T R & Ly J (2006c) Effect of increasing offer level of water spinach (*Ipomoea aquatica*) on intake, growth and digestibility coefficients of rabbits. Livestock Research for Rural Development. Volume 18, Article # 22. Retrieved, from http://www.lrrd.org/lrrd18/2/samk18022.htm

Preston T R (2006) Forages as protein sources for pigs in the tropics. Workshop-seminar on forages for pigs and rabbits MEKARN–CelAgrid, Phnom Penh, Cambodia, 22–24 August, 2006.

Seng Sokerya (2009) The effect of cassava foliage (Manihot esculenta) on gastrointestinal parasites in small ruminant in Cambodia. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2009.

Sorn Suheang and Preston T R (2005) Utilization of sugar cane stalks and water spinach for fattening pigs and their residues as substrate for the earth worm. http://www.mekarn.org/msc2003-05/theses05/content/suheanglr.pdf

Sovann S & San S (2002) Pig production in Cambodia In: Priorities for pig research in Southeast Asia and the Pacific to 2010. pp. 22–28. http://www.aciar.gov.au/sites/aciar/files/node/472/wp53.pdf.

Steinfeld H (1998) Livestock production in the Asia and Pacific region, current status issues and trends. In: hursey BS (ed), World Animal Review 90–1998/81. Animal Production and health Division, FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.

Tran Hoang Chat, Ngo Tien Dung, Dinh Van Binh & Preston T R (2005) Water spinach *(Ipomoea aquatica)* as replacement for guinea grass for growing and lactating rabbits; Workshop-seminar "Making better use of local feed resources" (Editors: Reg Preston and Brian Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005. Article #48. Retrieved, from http://www.mekarn.org/proctu/chat48.htm

US Department of Statistic (2010) http://www.state.gov/r/pa/ei/bgn/2732.htm#profile

CHAPTER II: LITERATURE REVIEW

II. 1. Pig production in Cambodia

II. 1.1. Role of pig production in livelihoods of Cambodian

Pig production is important for poor farmers because it provides extra source of family income, festivals, paying a debt or as a savings bank, providing employment (CelAgrid, 2006). Pigs are easy to raise, easy to sell, can be confined in a small area, can covert a variety of crop and kitchen wastes and can bring about higher incomes compared to ducks or chickens etc (Steinfeld, 1998; Ngo Thuy Bao Tran and Brian, 2005ab). Pigs manure can be used as fertilizer for cropping and animal feed which can be a source of protein for growing pigs (Lemke et al., 2002). Riethmuller et al, (2002) reported that farmers in Vietnam sell pig manure to plantations for fertilizing and improving the soil quality. A survey in Laos showed that 60 pigs can produce enough manure for one hectare of fish pond in which fish stock is around 40,000 heads and can produce up to 4,000 kg of fish year^{-1}(Hoffmann, 1999).

Increased human population leads to an increased demand of meat in Cambodia. However, farmers in rural area are keeping their pigs in traditional scavenging or semi confinement systems as a means of risk management rather than in systems more oriented toward increased production and income. The majority of farmers raise local pig breeds while small and medium scale close to the cities and towns keep crossbred (local*improved breed) or (improved breed*exotic breed) and exotic pigs in order to meet the demand of pork for local consumers.

II.1.2. Pig population

Pig population has been increasing year by year since 2000 to 2006. However, the numbers of pigs declined remarkably from 2007 to 2009 due to smuggling from neighboring countries where live pigs are cheap (Dam Am Pil Newspaper 2010).

Photo 1: Illegal pig importation in Cambodia

Animal Statistics from Ministry of Agriculture, Forestry and Fishery, (2009) reported that total pig population in the country is about 2,126,304 heads (figure 1). However, the compositions between local, crossbred or exotic breeds are difficult to be addressed. According to the record of pig farms in 8 provinces in 2007, small scale farms keep local breeds and crossbreds which occupy from 92 to 96% of total pig population; medium and large pig farms keep exotic breeds and crossbreds which occupy from 4 to 8% of total pig population (Table 1).

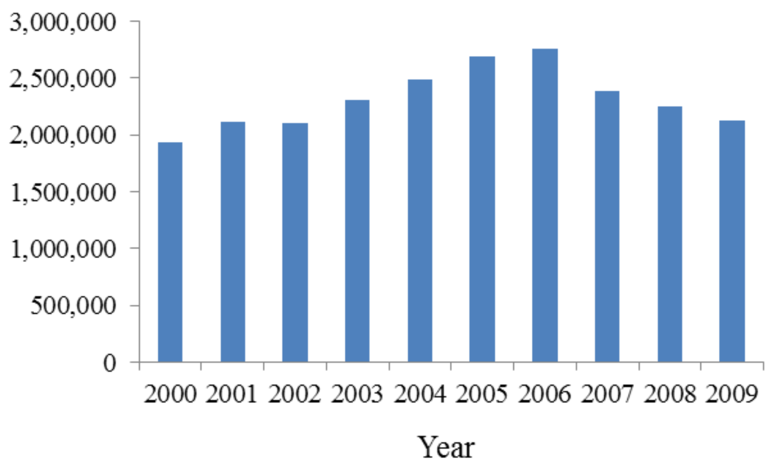

Figure 1: Number of pigs in Cambodia from 2000 to 2009
Sources: MAFF, (2007, 2009)

Table 1: Classification of pig production systems in Cambodia

Scale	Herd, head	Breed	Proportion of breed as total of pig population, %*
	Sow: 1	Local: rural area	
Small	Fattener: 1-4	Crossbred (local*improved): town	92-96
	Sow: 10-50	Crossbreed (improved*exotic)	
Medium	Fattener: <100	Exotic	3-5
	Sow: > 200	Exotic	
Large	Fattener: >400	Exotic	1-3

* Estimate based on agriculture statistics in 2007

Animal statistics from Ministry of Agriculture, Forestry and Fishery, (2009) showed that a large numbers of pigs are located near the borders with Vietnam and Thailand because the farmers easily imported good breeds and commercial feeds from these countries. The average percentages of pigs in the whole country in 2009 indicated that 51% are fatteners, 37% are piglets and the rest is between sows and boars. Sows are preferred by medium and large scale farms in order to distribute piglets to other pig farms.

Table 2: Characteristic of pig in the whole country in 2009

No	Province/city	Boar	Sow	Fattener	Piglet	Total	% Boar	% Sow	% Fattener	% Piglet
1	Bantaymeanchhey	818	20,878	51,743	54,424	127,863	0.6	16.3	40.5	42.6
2	Battambong	537	7,400	48,399	34,670	91,006	0.6	8.1	53.2	38.1
3	Kampong Cham	883	11,458	112,996	57,579	182,916	0.5	6.3	61.8	31.5
4	Kampong Chhang	314	10,149	72,653	63,381	146,497	0.2	6.9	49.6	43.3
5	Kampong Speu	413	8,295	59,623	21,204	89,535	0.5	9.3	66.6	23.7
6	Kampong Thom	594	10,235	54,697	37,322	102,848	0.6	10	53.2	36.3
7	Kampot	519	6,412	74,662	22,631	104,224	0.5	6.2	71.6	21.7
8	Kandal	420	7,120	70,866	6,007	84,413	0.5	8.4	84	7.1
9	Kep	25	1,240	6,395	3,120	10,780	0.2	11.5	59.3	28.9
10	Koh Kong	123	788	4,535	4,980	10,426	1.2	7.6	43.5	47.8
11	Kratie	279	3,849	41,969	16,971	63,068	0.4	6.1	66.5	26.9
12	Mundul Kiri	143	1,895	3,102	2,569	7,709	1.9	24.6	40.2	33.3
13	Oudor MeanChhey	437	3,945	38,200	39,349	81,931	0.5	4.8	46.6	48
14	Pailin	58	663	2,680	3,040	6,441	0.9	10.3	41.6	47.2
15	Phnom Penh	79	635	11,696	0	12,410	0.6	5.1	94.2	0
16	Prasihanouk	57	864	6,052	4,988	11,961	0.5	7.2	50.6	41.7

17	Pravihear	1,870	9,563	11,362	22,043	44,838	4.2	21.3	25.3	49.2
18	Prey Veng	1,780	47,608	201,427	124,246	375,061	0.5	12.7	53.7	33.1
19	Pursat	448	8,961	34,953	20,716	65,078	0.7	13.8	53.7	31.8
20	Ratanakiri	1,736	6,898	7,731	15,417	31,782	5.5	21.7	24.3	48.5
21	Siem Reap	716	14,571	72,340	47,373	135,000	0.5	10.8	53.6	35.1
22	Steung Treng	226	639	7,883	21,281	30,029	0.8	2.1	26.3	70.9
23	Svay Reing	602	19,409	0	95,991	116,002	0.5	16.7	0	82.7
24	Takeo	700	25,296	94,050	74,440	194,486	0.4	13	48.4	38.3
	Total	13,777	228,771	1,090,014	793,742	2,126,304	0.6	10.8	51.3	37.3

Source: Ministry of Agriculture, Forestry and Fishery, (2009)

II.1.3. Typical pig production systems

In Cambodia, typical pig production systems can be divided in small scale, medium scale and large scale pig production.

II.1.3.1. Small scale pig production

The small scale pig farms can supply pigs to the local markets or towns and to Phnom Penh. Small scale pig production is run by small farm holders in rural areas. The number of pigs/farm varies among countries (Jones, 2002; Lapar et al., 2003; FAO, 2005). For instance, in the Philippines and Vietnam, small scale pig farms have less than 20 pigs while small scale farms in Cambodia and Laos have less than 5 pigs. A study by CelAgrid, (2006) showed that number of pigs per household ranges from 1.25 to 3.78 heads and most farmers prefer keeping 2 heads. Small scale pig farms keep local and crossbred breeds. Local breeds are *Kandol (mouse)*, *Domrei (elephant)*, *Hainam*, and *Kampot.* The numbers of local breeds have been decreasing. They are mainly kept by ethnic minorities in scavenging systems. Increased human population leads to higher pork demand. Small scale farmers start keeping crossbred pigs and they can access pig stock from medium farms and middlemen. However, it is difficult to access pig stock from medium farms because it is far from villages. In addition, the price is normally high compared with that of middlemen who travel around villages every day.

Photo 2: Local sow

Photo 3: Local piglets

Photo 4: Crossbred piglets

Rice bran is the most common feed ingredient for pigs. Rice bran from village rice mills is generally poor quality (8-9% CP) due to the high content of husk while rice bran from larger rice mills is better quality (12–13% CP). Rice bran is cooked with other ingredients which farmers can collect near their households, or with water plants from lakes and ponds. The type and quantity of feed vary on a seasonal or area basis and family situation (Kunavongkrit and Heard, 2000). The number of pigs per family increases when they make rice wine. Sometimes the production of rice wine is not profitable but is justified in order to get the residue for pig production. Feeding systems are different among farmers. Mixing ingredients and feeding directly to pigs is preferred by many farmers because it is easy and no need to use firewood (extra cost) or spend time cooking. There are still some farmers who mix ingredients and cook before feeding the animal. The amount of feed offered per day is 9 to 14 kg for sows and 7 to 15 kg for fattening pigs but these amounts mainly come from water rather than from feed ingredients. So through this system, pigs can not get enough nutrients to grow healthy and protect themselves from diseases, and subsequently farmers are not able to generate enough profit from this practice. Small scale pig production in Cambodia can be categorized into three types i) scavenging system, ii) scavenging plus confinement system and iii) confinement system while in Laos P.D.R, small scale pig production systems can be categorized into four types, namely, free range scavenging, semi-intensive (confine within a large area), intensive (confine to a pig pen), and integrated pig and fish farming (Hoffmann, 1999). Pig housing is built with local materials which find can be found in the villages such as bamboo, grass leaves and wood. In terms of disease prevention, farmers have poor understanding, and poor access to drugs and other veterinary services. However, the government agencies and non-government organizations (NGOs) try to implement simple interventions to improve farmer’s knowledge on pig production.

Farmers sell their pigs through middlemen and piglets can be sold to other farmers in or outside the villages. About 70 to 90% of farm households check live pig price before they intend to sell their pigs (CelAgrid, 2006). To get information about the price, pig producers often ask farmers in the village and middlemen. There is information about price of agricultural products which the government launches on radio and TV but farmers rarely pay attention. The price of live pigs can be set by either producers (10-45%) or traders/middlemen (35-70%) (CelAgrid, 2006). However, farmers generally never get the desired price when they sell pigs because prices are not fixed by the government. The traders and slaughterhouses display little or no loyalty to pork produced from locally raised pigs. Local breeds generally yield lower percentages of lean meat and therefore, are less attractive to traders/slaughterhouses especially those that are involved in product distribution to urban markets. At the "traders/ slaughterhouse" level, Cambodian pork costs more than imported pork due to difference in total meat output. To be able to sell pigs to traders/slaughterhouses, local pig raisers have to decrease prices.

Photo 5: Pig market for middlemen

Photo 6: Middlemen carry pigs

Photo 7: Pork in wet market

Constraints in small scale pig production

Several factors have been reported to limit pig production in small scale pig production. These constraints can be found in the areas of nutrition, animal health, animal productivity/genetic make-up, extension services, provision of loan to small-scale producers, high cost of feed and marketing. CelAgrid, (2006) found that there are four important constraints on pig production such as, unstable price of live pigs, lack of capital, diseases, lack of feed and low quality of feed. All these constraints are variable among provinces in Cambodia. Some provinces realize that unstable price is one of the main problems because most pigs are sold through middlemen and many pigs are imported from the neighboring counties. Other provinces find that animal health is a critical problem. Overall, nutrition is the main constraint on small scale pig production because farmers provide only rice bran from village rice mill which is of poor quality so that pigs can not get enough nutrients to grow well and protect themselves from diseases, and subsequently farmers are not able to generate enough profit from this practice. Recently, there are many animal feed companies operating in Cambodia in order to help animal raisers to meet the demand of local consumers, however, small scale pig producers don not have enough resources to buy those feeds and other feed ingredients such as fish meal and soybean meal while small scale farmers still continue practices of scavenging and use of local breeds. In addition, fish meal and soybean are expensive and are challenging with human food. Therefore, it is necessary to explore high protein forages in the region especially cassava leaves and water spinach in order to meet the needs of the animal for healthy growth and economic return to the farmer. The government of Cambodia also sees improved pig production as a means to reduce poverty in rural and remote areas.

Some solutions for improving small scale pig production

Even though there are many constraints on small scale pig production, there are also many ways to solve those constraints. Government policies on animal health, extension service, marketing and credit play an important role as a means to improve the economics of small scale pig production. The two main priorities for which urgent solutions are needed are nutrition and breed improvement. More than 90% of small scale producers keep local breeds and crossbreeds in rural areas and towns (Cambodian agriculture statistics in 2007) which make productivities low and not attractive to farmers to invest in pig rising. In terms of nutrition, the small scale producers still practice with scavenging traditional systems or semi confinement and provide animals with low nutrient diets which normally come from agriculture by-products especially poor quality rice bran from village rice mills. To improve pig production performance, improving nutrition situation for pigs based on locally available resources should be given priority. High protein forages such as cassava leaves, water spinach, mulberry leaves, sweet potato vine, taro and duckweed could be added to, or replaces other expensive sources of protein such as fish meal.

II.1.3.2. Medium scale pig production

Numbers of pigs per medium scale pig farm are variable and dependent on resources. Around Phnom Penh and other cities, a medium pig farm keeps about 10-50 sows, more than 100 fatteners and they can be a mix of production categories (sows, piglets, and fatteners) (Khieu Borin, 2006). In Vietnam, a medium pig farm keeps 50-500 sows and 400-4000 fatteners (Lapar et al., 2003) and some farms produce only weaning piglets or only fatteners or both (Villar et al., 2002; Steinfeld et al., 2006). In Cambodia, there are 8 provinces/cities with 287 pig farms with a total of 60,147 pigs (Ministry of Agriculture, Agriculture and Forestry, 2007); however, pig production does not meet the meat demand of local consumers. Medium scale pig production is facing many obstacles such as limited funds; limited technology and disease control (Sovann and San, 2002). In additional, most of producers rely on imported feed or feed ingredients, this subsequently increases production cost. Medium scale pig producers prefer keeping exotic breeds (Yorkshire, Landrace and Duroc) and sometimes with crossbreds between improved local and exotic breeds. Most of these breeds are selected from large pig farms. Exotic breeds are available around cities and their outskirts; however the prices of exotic breeds are very high. The proportion of crossbred and exotic breeds is about 3-5% in the total pig population in the country (Ministry of Agriculture, Forestry and Fishery, 2007).

Photo 8: Crossbred pigs

Photo 9: Exotic pigs

Rice bran, broken rice and maize are the main feed ingredients for pig production. Some producers mix by products with commercial feed, some farmers feed directly commercial feed and some use “homemade” concentrate feeds. In terms of housing, some producers build pig pens with simple materials; others use bricks, concrete and steel. Most producers make vaccinations based on their experience; the vaccination can be against Salmonellosis, Swine Pest, Pasteurellosis and some farms have access to other vaccines such Aujeszky and Food and Mouth Disease. Pigs can be sold through wholesale buyers/slaughterhouses that manage and distribute pork meat in the market. However, some farms sell pigs to small illegal slaughterhouses.

II.1.3.3. Large scale pig production

Typical big commercial pig farms in Cambodia are those belong to the "CP" Company. They supply almost all the grandparent stock, breeding sows, and piglets to medium scale producers particularly around Phnom Penh and other large cities. These farms are very well-equipped, well managed and have a high productivity (Khieu Borin, 2006). Besides supplying breed stocks to medium scale producers, the CP Company has signed contracts with farmers to grow fattening pigs (Lapar et al., 2003). The company provides all inputs and other technical support, while farmers supply laborers and housing facilities. The increase in the number of this type of contracts will most likely result in the domination of the meat market by giant companies. Under the current scenario, millions of smallholder households will stand to lose their market share and subsequently become employees of these large companies (Cameron, 2000; Lapar et al., 2003). In year 2009, local big commercial pig farm named Mong Reththy group was created and imported 600 genetically advanced breeding pigs from Britain. The breeds are exotic breeds such as Yorkshire, Landrace and Duroc and crossbreeds between those exotic breeds. These breeds are famous for growth rate, feed conversion efficiency and carcass conformation. According to Marian and Boquiren, (2009) exotic breeds are more attractive to traders/slaughterhouses especially those that are involved in pork distribution to urban markets especially in Phnom Penh.

Photo 10: Large pig farms in the outskirt of the city

Large pig farms use concentrates feed imported from outside or they can produce by themselves. The main protein ingredients in the industrial feed come from fish meal and soybean meal. These farms are very well equipped, well managed and have a high productivity, high quality of building with concrete floors providing greater protection and hygiene. Pigs are vaccinated against important diseases such as Salmonellosis, Swine Pest, Pasteurellosis, Augestky and FMD. Beside vaccination, visitors are banned to enter pig farms**.** Pigs are sold to market by their own companies and to whole buyers/slaughterhouses who they manage and distribute pork meat in market in the country.

II.2. Protein and amino acids in pig nutrition

II.2.1. Role of protein and amino acids for pigs

Amino acids are classified into two general types, essential amino acids (EAA) and non essential amino acids (NEAA). Essential amino acids are those that can not be synthesized by the animal body and need to be acquired through diets. Non-essential amino acids are those that the animal body can produce, specifically by the liver without any help from outside. Amino acids are normally supplied by dietary protein and required for maintenance, muscle growth, development of fetuses and supporting tissues in gestating and milk production in lactating sows. Thus, pigs require amino acids, not crude protein. Diets must be balanced with respect to a desirable level and ratio of the essential 10 AAs. Among 20 AAs, only 10 AAs are synthesized by the animal, other 10 AA must be provided in the diet for normal growth and two other amino acids (cysteine and tyrosine) are semi-essential since both can be synthesized if adequate amounts of Met and Phe are present. The 10 EAAs for growing pigs are Arg, His, Iso, Leu, Lys, Met, Phe, Thr, Try, and Val. Most cereal grains are limiting in Lys, Try, Thr, and Met. Therefore, when evaluating feed ingredients, the concentration of these AAs, especially Lys is most important in determining protein quality.

Table 3: List of essential and non essential amino acids

Essential Amino Acids	**Non-Essential Amino Acids**
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic Acid
Leucine	Glutamic Acid
Lysine	Glutamine
Methionine	Glycine
Phenylalanine	Proline
Threonine	Serine
Tryptophane	Hydroxyproline
Valine	

Amino acids are the building blocks of protein as they are essential for the synthesis of structural proteins, enzymes, some hormones and neurotransmitters. When animals are fed protein, the animal body digests the protein into individual AA and short-linked AA that are small enough to be absorbed into the bloodstream. The primary functions of AAs are to build and repair muscle tissue. The AA composition of animal body protein is quite independent of live weight, genotype and sex (Bikker, 1994; Hess, 1999). Essential amino acid requirement is generally expressed relatively to the most limiting Lys. The ideal protein is a dietary AA profile in which all EAAs are balance against Lys in order to maximize N retention and performance. Limiting feed protein content by reducing the amount of protein rich feedstuffs in the diet can reduces the amount of both EAAs and NEAAs fed to the animal. Without any compensatory supplement of EAA such as Lys, Thr, Met, Try, levels may fall below a critical level, which is the animal requirement for each individual AA.

II.2.2. Protein and amino acid requirement for growing pigs

The AA requirements of growing pigs include two components; the first component is requirement for maintenance and the second is a requirement for tissue protein accretion. The patterns of AA requirement are quite different, and the animal requirement must depend on the relative contribution of maintenance and tissue protein accretion to total needs. Below in the table is the recommendation of protein and amino acid for growing pigs at different live weights.

Table 4: Nutrient requirement of growing pigs allowing *ad libitum* feed (90% DM basis)

	Body weight, kg					
	3-5	**5-10**	**10-20**	**20-50**	**50-80**	**80-120**
DE content of diet (kcal/kg)	3400	3400	3400	3400	3400	3400
ME content of diet (kcal/kg)	3265	3265	3265	3265	3265	3265
Estimated DE intake (kcal/day)	855	1690	3400	6305	8760	10450
Estimated ME intake (kcal/day)	820	1620	3265	6050	8410	10030
Estimated feed intake (g/day)	250	500	1000	1855	2575	3075
Crude protein (%)	26.0	23.7	20.9	18.0	15.5	13.2
True ileal digestible basis, % DM basis						
Arginine	0.54	0.49	0.42	0.33	0.24	0.16
Histidine	0.43	0.38	0.32	0.26	0.21	0.16
Isoleucine	0.73	0.65	0.55	0.45	0.37	0.29

Leucine	1.35	1.20	1.02	0.83	0.67	0.51
Lysine	1.34	1.19	1.01	0.83	0.66	0.52
Methionine	0.36	0.32	0.27	0.22	0.18	0.14
Methionine + cosine	0.76	0.68	0.58	0.47	0.39	0.31
Phenylalanine	0.80	0.71	0.61	0.49	0.40	0.31
Phenylalanine + tyrosine	1.26	1.12	0.95	0.78	0.63	0.49
Threonine	0.84	0.74	0.63	0.52	0.43	0.34
Tryptophan	0.24	0.22	0.18	0.15	0.12	0.10
Valine	0.91	0.81	0.69	0.56	0.45	0.35
Apparent ileal digestible basis, % DM basis						
Arginine	0.51	0.46	0.39	0.31	0.22	0.14
Histidine	0.40	0.36	0.31	0.25	0.20	0.16
Isoleucine	0.69	0.61	0.52	0.42	0.34	0.26
Leucine	1.29	1.15	0.98	0.80	0.64	0.50
Lysine	1.26	1.11	0.94	0.77	0.61	0.47
Methionine	0.34	0.30	0.26	0.21	0.17	0.13
Methionine + cystine	0.71	0.63	0.53	0.44	0.36	0.29
Phenylalanine	0.75	0.66	0.56	0.46	0.37	0.28
Phenylalanine + tyrosine	1.18	1.05	0.89	0.72	0.58	0.45
Threonine	0.75	0.66	0.56	0.46	0.37	0.30
Tryptophan	0.22	0.19	0.16	0.13	0.10	0.08
Valine	0.84	0.74	0.63	0.51	0.41	0.32
Total basis, % DM basis						
Arginine	0.59	0.54	0.46	0.37	0.27	0.19
Histidine	0.48	0.43	0.36	0.30	0.24	0.19
Isoleucine	0.83	0.73	0.63	0.51	0.42	0.33
Leucine	1.50	1.32	1.12	0.90	0.71	0.54
Lysine	1.50	1.35	1.15	0.95	0.75	0.60
Methionine	0.40	0.35	0.30	0.25	0.20	0.16
Methionine + cystine	0.86	0.76	0.65	0.54	0.44	0.35
Phenylalanine	0.90	0.80	0.68	0.55	0.44	0.34
Phenylalanine + tyrosine	1.41	1.25	1.06	0.87	0.70	0.55
Threonine	0.98	0.86	0.74	0.61	0.51	0.41
Tryptophan	0.27	0.24	0.21	0.17	0.14	0.11
Valine	1.04	0.92	0.79	0.64	0.52	0.40

Source: National Research Council, (1998).

The National Research Council (NRC, 1998) recommends that pigs require AAs and CP based on their body weight. To support the recommendation from NRC, series of studies have been done by Taylor et al, (2010) on diets containing CP from 10 to 17.6% which lysine is maintained at 0.95% in the diets of pigs ranging from 25-55kg. The results showed that growth performance was unaffected by a reduction in CP level from 17.6 to 14.5% but below 14.5% CP, daily live weight gain and feed conversion ratio were deteriorated linearly.

A study by Anugwa and Okwori, (2008) on two genetic groups of pigs {local and crossbred (local*large white)} at body weight of 7.3 and 10.2kg, respectively and two protein levels (12 and 16% of CP) showed that local pigs performed better than crossbred on the lower CP (12%) in the diet while crossbreds performed better than local pigs on the 16% CP in diet. González et al, (2003) studied effects of graded levels of CP, 25.1, 23.7, 17.0 or 13.2% for pigs of 30-60kg and 23.0, 20.6, 14.5 and 9.9% for pigs of 60-90kg fed fresh sweet potato foliage and showed that in both periods feed intake, live weight gain and feed conversion ratio were decreased when CP was declined in the diet but intake of sweet potato foliage showed a non - linear response with lower levels of CP in the diets.

It is possible to have a high level of performance (growth and feed conversion) in pigs fed *ad libitum* sweet potato foliage and provided feed supplement containing high protein. A study was made by Nguyen Thi Hoa Ly et al, (2003) on evaluating the effect of increasing the protein level from 12 to 14, 16 and 18% for pigs from 20-50kg (phase I) and 10, 12, 14 and 16% CP for pig from 50-90kg (phase II) with protein supplement consisted of mixture of 30% fishmeal and 70% sweet potato leaf meal (as DM), replacing a mixture of rice bran and ensiled cassava roots. The results showed that there were no treatment effects on final live weight, daily live weight gain and feed conversion ratio, but the cost of feed per kg live weight gain was significantly higher for the very high protein level, because of the high cost of fish meal. Dietary protein levels of 14% CP for pigs from 20-50kg and 12% CP for pigs from 50-90kg (as DM) with the protein supplied by fishmeal and sweet potato leaf meal can be recommended as they resulted in reasonably good growth performance and gave the best economic efficiency. The early study by Devendra and Clyde Parris, (1970) on the optimum protein levels for growing and finishing pigs in a tropical environment showed that there were statistically significant differences in daily live weight gain and feed conversion efficiency between pigs fed a diet containing 16% CP and those receiving 18% CP but no differences in response were found to diets containing 12 and 13% CP. Feed conversion efficiency improved with increased dietary levels of CP.

Briefly, local pigs performed better than crossbred on the lower CP (12%) in the diet while crossbred performed better than local pigs on the 16% CP in diet. High level of performance (growth and feed conversion) in pigs fed *ad libitum* high protein forages with supplementation from fish meal but the cost of feed per kg live weight gain was significantly higher for the very high level of fish meal, because of the high cost of fish meal. However, NRC recommends that pigs require protein levels in the range from 26 to 13% for exotic breeds of 3-5kg to 80-120kg of body weight, respectively.

II.3. Locally available feeds

Water spinach and cassava leaves are productive tropical/subtropical plants that are traditionally cultivated for both human consumption and animal feeds. Water spinach and cassava leaves have been used successfully for animal feed in different forms such as fresh, dry and ensiled cassava leaves and fresh water spinach in Cambodia. For a deeper understanding of both forages for animal feeds, yield and nutritive value are reviewed from other authors.

II.3.1. Water spinach *(Ipomoea aquatica)*

II.3.1.1. Yield and availability

Water spinach (WS) *(Ipomoea aquatica)* is a plant growing equally well in water or in soil. It responds dramatically in biomass yield and CP content when being fertilized with biodigester effluents (Kean Sophea and Preston, 2001), earthworm compost (Tran Hoang Chat et al., 2005) or urea (Ly Thi Luyen and Preston, 2004; Tran Hoang Chat et al., 2005). Ly Thi Luyen and Preston, (2004) reported that the biomass yield was higher when WS was grown in soil rather than in water. Fresh biomass yields were higher when WS was established from seed than from stem cuttings (15 compared with 9.18 tonnes ha^{-1} $month^{-1}$) (Ho Bunyeth, 2003). The N content of the WS leaves increased from 3.08 to 5.56% in DM (from 19.3 to 34.8% crude protein) by application of 200 kg N^{-1} ha^{-1} as biodigester effluents. Stems are much lower in N (1.2 to 2.0% in DM) and this index tends to be decreased with increasing application of N from effluent but the trend was not uniform. Le Thi Men and Preston, (2005) suggested that small-holder farmers should cultivate vegetables as supplements for pigs, using effectively animal excreta. San Thy and Preston, (2001) also reported that the effluent from a biodigester loaded with pig manure was a good fertilizer for WS production, and improve soil productivity.

Photo 11: Water spinach grown in the soil

Photo 12: Water spinach grown in water

II.3.1.2. Nutritive value

Water spinach is a good source of protein and vitamins for human and animal feed because it contains no anti-nutritional compounds. Chemical compositions of water spinach (WS) are different depending on the quality of WS, maturity, growing condition, fertilizer application and management. The dry matter content of WS is from 8.09 to11.2%, while protein content is from 25.6 to 31.6%, Ether extract is from 2.6 to 9.49, ash is from 11.2 to 14.1%, crude fibre is from 15.5 to 16.2 and neutral detergent fibre is from 22.9 to 34.9% in DM basis (Table 5).

Table 5: Chemical composition in water spinach (% in DM basis)

DM	CP	EE	Ash	CF	ADF	NDF	Ca	P
10.6[a]	26.4[a]	2.6[a]	11.2[a]	-	-	22.9[a]	-	-
8.12-11.2[b]	25.6-31.6[b]	-	-	15.8[b]	-	-	-	-
8.30[c]	26.7[c]	6.7[c]	14.1[c]	15.5[c]	-	-	0.90[c]	0.50[c]

[a] Bui Huy Nhu Phuc, (2000), [b] Chhay Ty and Preston, (2005), [c] Le Thi Men and Preston (2005)

A study of Le Thi Men and Preston, (2005) on the evaluation of the effect of different fertilizers on WS yields and of including WS and catfish oil in diets for fattening pigs in the Mekong Delta of Vietnam, showed that AA content in WS varies with type of fertilizers (compost or effluent). Lys content in WS was higher when it was fertilized by compost compared with biodigester effluents but Met was higher for biodigester effluents rather than compost. In order to minimize Met deficiency, WS should be incorporated with other rich protein sources or supplemented with crystalline AA, when they are used as a protein source for pig production.

Table 6: Amino acid in water spinach plant

	g/16g N	% in DM	% in DM
Essential AA	1	2. Compost	2.Biodigester
Arginine	6.0	1.25	1.12
Histidine	1.6	0.55	0.60
Isoleucine	3.8	1.51	1.37
Leucine	7.2	1.66	1.70
Lysine	4.2	0.97	0.89
Methionine	1.7	0.45	0.57
Phenylalanine	5.2	1.10	1.11
Threonine	4.1	1.09	0.96
Tyrosine	3.7	-	-
Valine	5.2	1.49	1.45
Non-essential AA			
Alanine	5.2	-	-
Aspartic acid	16.2	-	-
Glutamic acid	4.5	2.04	1.79
Glycine	4.3	1.02	0.98
Proline	3.3	-	-
Serine	4.1	-	-

1. Bui Huy Nhu Phuc, (2000); 2. Le Thi Men and Preston, (2005)

II.3.2. Cassava *(Manihot esculenta* Crantz*)*

II.3.2.1. Yield and availability

Cassava plantation is mainly for root production. The yields of root are variable depending on soil fertility, management and irrigation system. Cassava root yields can be from 10 to15 tonne ha^{-1} without inputs on eroded soils (Howeler, 1991) but soil fertility eventually decreases after the cultivation of cassava for root production. The yield of root can be decreased when leaves are harvested during the maturity period. If cassava is managed for foliage, the annual yields of fresh foliage of about 80-120 tonnes ha^{-1} were recorded during two year periods in Vietnam and Cambodia with continuous harvesting of the foliage every 2 months, accompanied by heavy fertilization with goat manure or biodigester effluent (Preston, 2001).

Ravindran (1993) reported that the annual cassava leaves (CL) DM yield can be over 21 tonnes ha^{-1} when harvested from 4 months of age in a cycle of 60-75days with adequate irrigation and fertilization. The yield of CL is possible to obtain more than 6 tonnes of CP ha^{-1} year^{-1} with proper agronomic practices directed toward foliage harvesting (Animal Feed Resources Information Systems, 2004). In Thailand application of approximately 10kg N ha^{-1} from cow manure resulted in DM yields and CP contents of cassava foliage from 3.6 to 4.4 tonnes ha^{-1} and 20.6 to 22.0% in DM, respectively (Poungchompu et al., 2001).

Photo 13: Sweet cassava variety

Photo 14: Bitter cassava variety

II.3.2.2. Nutritive value

Cassava leaves (CL) are a good source of CP and minerals particularly Ca, Mg, Fe, Mn and Zn and are also rich in ascorbic acid and vitamin A. Cassava leaves contain significant amounts of riboflavin but considerable losses of vitamins, particularly of ascorbic acid, may occur during processing (Ravindran, 1993). The CL chemical composition is variable depending on varieties, growing conditions, fertilizer application and processing. The DM content of FCL is from 23.9 to 32.1%, CP content is from 23.9 to 34.7%, EE is from 6.4 to 15.6%, Ash is from 3.48 to 10.5%, CF is from 9.28 to 16.7%, ADF is from 20.2 to 22.1%, NDF is from 24.8 to 37.6% in DM basis and HCN in fresh cassava leaves is from 475 to 507mg/kg DM (Table 7).

Table 7: Chemical composition of cassava leaves in DM basis

	DM, %	**CP, %**	**EE, %**	**Ash, %**	**CF, %**	**ADF, %**	**NDF, %**	**HCN, mg/kg**	**Sources**
Fresh									
Leaves	26	23.9-34.7	11.3-15.6	5.0-8.1	9.7-16.5	-	32-33.5	-	Bui Huy Nhu Phuc, (2000)
Leaves	23.9-32.1	23.5-27.5	-	3.48-6.48	9.28-10.8	-	-	475-507	Chhay Ty et al, (2007, 2009) and Chhay Ty and Preston, (2005)
Ensiled									

Leaves	35	27.0	-	-	15.3	-	-	70.7	Du Thanh Hang et al, (2007)
Leaves	-	31.7	8.1	7.2	-	-	24.8	-	
Leaves	-	24.5	11.5	10.5	16.7	20.2	32.6		Bui Huy Nhu Phuc, (2000)
Sun dried									
Leaves	-	32.4	6.4	7.2	-	-	27.5	59	Bui Huy Nhu Phuc, (2000)
Leaves	-	26.4	11.4	8.7	16.4	22.1	32.1	-	
Oven dried									
60 ^{0}C	-	32.7	7.5	7.6	-	-	25.3	86	Bui Huy Nhu Phuc, (2000)
105 ^{0}C	-	32.2	8.2	7.2	-	-	37.6	28	

Bui Huy Nhu Phuc et al, (2001) declared that the CL has acceptable AA profile in comparison with alfalfa and soybean meal. The content of Thr in CL is quite acceptable compared with the ideal AA pattern for pigs, while the concentration of Met would be limiting (NRC, 1998). The AA composition of CL is of great importance when they are used as a protein source for mono-gastric animal species. In order to minimize Met deficiency, CL should be incorporated with other rich protein sources or be supplemented with crystalline AA, when they are used as a protein source for pig production. Table 8 shows that most AA is slightly decreased when cassava leaves are processed.

Table 8: Essential amino acid and non-essential amino acid (g/16g N) in two batches of cassava leaves in DM basis

	Batch A			**Batch B**	
	Sun dried	**Dried 60 ^{o}C**	**Dried 105 ^{o}C**	**Sun dried**	**Ensiled**
Essential amino acids					
Arginine	6.3	6.4	5.8	6.5	5.6
Histidine	2.2	2.0	2.0	1.8	1.7
Isoleucine	4.1	4.4	4.5	4.2	4.2
Leucine	8.7	8.9	9.1	8.3	8.3
Lysine	5.1	5.1	4.2	5.5	5.4
Methionine	1.6	1.4	1.5	1.6	1.4
Phenylalanine	6.3	6.2	6.2	6.2	5.6
Threonine	4.4	4.2	4.4	4.1	3.9
Tyrosine	4.3	4.6	4.6	4.4	4.4
Valine	5.9	5.6	5.7	5.6	5.3
Σ EAA	48.9	48.8	48.0	48.2	45.8
Non-essential amino acids					
Alanine	6.3	6.5	6.4	6.0	6.4

Aspartic acid	9.7	9.4	9.6	10.3	9.3
Glutamic	11.0	10.4	10.7	9.3	9.6
Glycine	4.5	4.4	4.8	4.3	4.1
Proline	3.8	3.7	3.6	4.1	4.3
Serine	3.4	4.1	4.2	3.3	3.8
Σ NEAA	38.7	38.5	39.3	37.2	37.5
Σ AA	87.6	87.3	87.3	85.5	83.3

Bui Huy Nhu Phuc, (2000)

Table 9: Amino acids in cassava leaves, g/16g N in DM basis

Essential amino acids	**a.**	**b.**	**Non-essential AA**	**a.**	**b.**
Arginine	-	5.9	Alanine	-	5.7
Histidine	2.2	1.9	Aspartic	-	9.7
Isoleucine	4.9	4.4	Glutamic	-	11.2
Leucine	8.6	8.0	Glycine	-	4.1
Lysine	6.2	5.6	Proline	-	3.6
Methionine + Cystine	2.8	1.5	Serine	-	4.7
Phenylalanine + Tyrosine	9.4	5.7			
Threonine	4.7	4.0			
Tryptophan	1.5	-			
Valine	5.7	5.3			
Σ EAA	46.0	42.3	Σ NEAA		39.0

a. Woolfe, (1992), b. Bui Huy Nhu Phuc et al, (2001)

II.3.2.3. Constraints of using cassava

Cyanogenic glycosides

The cyanogenic glycosides present in CL can cause toxicity to animals when hydrocyanic acid is generated (Van Soest, 1994). The glycosides are decomposed by beta glycosidase and hydroxynitrile xylases to form hydrocyanic acid (HCN). Although these enzymes are not present in mammalian tissues, the micro flora in the human intestine is able to produce them (California Air Resource Board, 1997). HCN is colorless, volatile and extremely poisonous. HCN is rapidly converted in the body to thiocyanate, which is no longer toxic (Hartung, 1983). Due to this rapid detoxification, animals are able to ingest small amounts of cyanide safely (Humphreys, 1988). Stosic and Kaykay, (1981) noted that small quantities of HCN ingested on a regular basis, though not large enough to cause mortality, might be sufficient to affect the general health and productivity of the animal. According to Humphreys, (1988), intake of feeds containing over 20mg HCN/100g is potentially dangerous to livestock. The presence of HCN could lead to a deficiency of the EAA (Met); if dietary supply of this AA is marginal (Oke, 1978). Simple sun drying or oven drying has been reported to eliminate almost 90% (Oke, 1994), and is more effective than ensiling because of the stability of the linamarase at low pH values (Oke, 1994). Despite the high cyanide levels in CL, documented cases of poisoning due to the ingestion of CL are rare (Ravindran, 1993). The study on feeding fresh CL to pigs showed that palatability was depressed and growth performance was low. However, there were no symptoms of ill-health and no apparent relationship between intake of HCN and production response as measured by DM intake and LWG (Du Thanh Hang and Preston, 2005; Chhay Ty et al., 2009). According to Chhay Ty et al, (2007) 24h wilting of CL reduced slightly the HCN content of CL but had no effect on apparent DM digestibility and N retention. It is suggested that AA imbalances are probably of greater importance than the presence of HCN as factors limiting the utilization of this feed resource. It is logical to expect that provision of supplementary Met would be beneficial in diets containing CL, as it is well established that the process of detoxification of HCN causes an increased demand for sulfur-containing AAs (Maner and Gomez, 1973) or elemental sulphur (Oke, 1978).

Tannin

Tannins can be divided into two classes: hydrolysable and condensed compounds. Hydrolysable tannins such as tannic acid are present only in low concentrations in commonly consumable foods and therefore most of the focus has been on the condensed tannins. Animals respond differently to dietary tannins, because there is variation in the biological activity of the tannins themselves (Hagerman et al., 1992; Reed, 1995). Some researchers have shown that tannins and other polyphenolic compounds cause increased endogenous losses and altered digestibility at the site of digestion of nutrients, including minerals, protein and AAs in monogastric animals and ruminants (Wang et al., 1994; Tanner et al., 1994; Reed, 1995; Yu et al., 1996). Tannins have been considered as anti-nutrients or ANF due to a range of adverse effects including reduced feed intake and digestibility, poor FCR and reduced LWG (Chung et al., 1998; Onwuka, 1992). Tannin content in CL is reported to increase with maturity and varied between cultivars, from 30 to 50g/kg DM (Ravindran, 1993). Harvesting of CL at an early growth stage (3 months) reduces condensed tannin content and increased the protein content, resulting in higher nutritive value (Wanapat, 1995). However, the anti-nutrient factors coming from hydrocyanic acid (HCN) and tannin can be reduced and safety used for animal feed by sun drying or ensiling.

II.3.3. Constraint of fiber in available feed resources for pig production

Fiber is an important factor because it influences the composition and nutritive value of plant products. It plays a major role in development of structural elements in the plant by formation of fibrous tissues and the content of lignin-rich structural carbohydrates (Bui Huy Nhu Phuc, 2000). The CP content of plants decreases with the plant age while the fibrous constituents increase (Bui Huy Nhu Phuc, 2000). High CF content reduces the nutrient digestibility, especially of CP (Ogle, 2006). Fibrous feeds in the diet also lead to an increased rate of passage of digesta through the gut and reduced ileal and total tract digestibility (An et al., 2004). High CF in the diets affects gut size and development, particularly the large intestine (Jorgensen et al., 1996). Nearly all CF digestion takes place in the caecum and colon, where bacteria break down fermentable carbohydrates that have escaped digestion in the stomach and small intestine. Volatile fatty acids from fiber fermentation can provide from 5%-28% of the energy requirements of the growing pigs (Kass et al., 1980). However, levels of more than 7-10% of CF in the diet will generally result in decreased growth rates (Kass et al., 1980). However, pigs are able to digest a substantial part of plant fiber pre-caecally (Lindberg and Anderson, 1998). The authors reported that increasing the level of leaf meal from white clover, lucern, red clover or perennial rye grass in basal diets from 10 to 20% reduced the digestibility of OM, but increased the digestibility of CF. Ravindra et al, (1987) observed pigs a depression in digestibility of DM, EE, cell wall components and hemicelluloses in diets in which CLM is replaced by coconut oil meal. Sarwat et al, (1988) also observe lower digestibility of DM and OM when CLM was included in a sorghum based diets of growing pigs. Bui Huy Nhu Phuc et al, (1996) report a significant reduction in apparent digestibility of DM, OM, CF and EE as the level of inclusion of CLM increased from zero to 216g/kg diet. For this reason, feeds of zero fiber diets such as sugar cane juice (Rodriguez et al., 2006) and sugar palm syrup (Pheng Buntha et al., 2008) or with very low fiber such as cassava root meal/cassava root silage (Chittavong Malavanh and Preston, 2008), have been investigated as the energy source to accompany leaves from high fibrous feed.

Briefly, water spinach is a good source of amino acids, protein and vitamins for pigs. In addition it contains no anti nutritional compounds. Protein in water spinach can reach to 25.6-31.6%. However water spinach is lacking in Met. Annual yield of fresh foliage is increased when cassava is managed for foliage crop and harvesting of the foliage every 2 months with an accompanying heavy fertilization. Cassava leaves are a good source of CP, minerals and vitamin they contain anti-nutritional compounds such as HCN and tannin which affect feed intake and growth performance. The CP content ranges from 23.9 to 34.7% and AA profile is acceptable but the Met is deficient. Overall, amino acids in fresh water spinach and fresh cassava leaves are of great importance when used as a protein sources for mono-gastric animal species. In order to minimize Met deficiency in fresh water spinach or fresh cassava leaves, both forages should be incorporated with other rich Met sources or supplemented with crystalline AAs.

II.4. Preserving and processing local feeds

Improving the quality and preserving foliages rich in protein is important because it allows animal feeds supply sufficient around the year when the original material is not available. Other advantages are improvement of the palatability of the original material, reducing toxicity and destroying harmful bacteria. Two common techniques, drying and ensiling, have been used with the aim of improving the quality and preserving forages.

II.4.1. Ensiling

Ensiling is the preservation of forages (or crop residues or by-products) of high moisture content based on a lactic acid (ideally) fermentation under anaerobic condition (Moran, 2005; McDonald et al., 2002). Ensiling can also render some previously unpalatable products useful to livestock by changing the chemical nature of the feed (Chedly and Lee, 1998). Ensilage is a simple and low cost option, which can preserve feeds that are seasonally abundant for later feeding during periods of feed shortage (Kaensombath, 2005). Ensiling material with less than 30% DM may create an environment that is totally anaerobic (suited to clostridia bacteria) rather than micro-aerophilic (suited to lactic acid bacteria). In addition, it may result in the loss of valuable nutrients because water and soluble nutrients accumulate at the bottom of the silo as silage effluent (Titterton and Bareeba, 1999). If crops are cut and ensiled on the same day, nutrient losses are negligible and even over a 24 hour wilting period, losses of dry matter may not be more than 1 or 2% (McDonald et al., 2002). With wilting longer than 48 hours, considerable losses of nutrients can occur depending upon weather conditions. Dry matter losses can be as high as 6% after 5 days and 10% after 8 days of wilting in the field. Silage additives can be classified into two main types: fermentation stimulants, such as sugar-rich materials, inoculants and enzymes, which encourage the development of lactic acid bacteria, and fermentation inhibitors, such as acids and formalin, which partially inhibit microbial growth (McDonald et al., 1995). The main function of a silage additive is to increase the nutritional value or improve fermentation (Ohio State University Extension, 2001). Molasses is most frequently used, and is of particular benefit when applied to crops with low soluble carbohydrates. Good silages have reported when molasses is applied at 3-5% (Chhay Ty et al., 2001). However, if the treated silage has a very low DM content, most of the carbohydrate source may be lost in the effluent during the first few days of ensilage in pits or bunkers.

II.4.2. Drying

The aim of this preservation process is to reduce the moisture content of the green materials to a minimal level, resulting in the inhibition of plant cell enzymes and microbial activities (McDonald et al., 1995). Studies have reported that for good quality hay, the moisture content should be between 13-14% on dry matter basis. In moisture hays (>200g water kg^{-1}), microbial respiration, primarily via oxidation of non-structural carbohydrates, can cause negative changes via spontaneous heating within the hay mass, increased concentration of field and storage fungi (Roberts, 1995) and potentially also toxic metabolites (Oke, 1994). Sun drying is probably the cheapest method in the tropics for preserving feeding materials for animal use. Sun-drying is a common practice in the dry season, which is the usual harvest time of the tubers and leaves, due to strong sunlight, high temperatures, low humidity and availability of space. However, when cassava leaves are harvested in the rainy season sun-drying is difficult, due to the lack of sun and high frequency of rain. Prolong drying due to bad weather creates favorable conditions for bacteria and fungi, producing moldy hay and mycotoxins (Cole and Cox, 1981). Another problem is that in windy conditions major losses of the dry material will occur if not properly handled. However, sun drying for 2-3 days is sufficient enough for leaves to be milled into meal before storage. To speed up the drying process, the forage material is first chopped into 3-5 cm lengths, allowing quicker evaporation of moisture and subsequently the release of volatile toxic substances such as HCN and anti trypsin.

Ensiling material with less than 30% DM can be loss of valuable nutrients because water and soluble nutrients accumulate at the bottom of the silo as silage effluent. Silage can be development of lactic acid bacteria and fermentation inhibitors. Molasses is most frequently used, and is of particular benefit when apply to crops low in soluble carbohydrates. Sun drying is cheap method for preserving feeding materials. However, it is difficult in the rainy season due to the lack of sun and high frequency of rain which creates favorable conditions for bacteria and fungi, producing moldy hay and mycotoxins. However, both methods can reduce anti nutrient compounds in forages and be safe for animals, improve the quality and palatability and allow forages as feeds for livestock around the year.

II.5. Studies on using high protein forages as a main protein sources for pigs

II.5.1. Effect of using high protein forages on growth performance

Cassava leaves have been used as pig feeds in different forms such as fresh, dry and ensiled. However, there were no ill-health symptoms in any of the pigs that could have been ascribed to HCN toxicity. According to Du Thanh Hang and Preston, (2005) when pigs are given free access to FCL, which have been washed, chopped and washed or wilted for 24h, DM feed intake of pigs are similar between treatments ranging from 27 to 32 g DM/kg live weight. Other study of Chhay Ty et al, (2009) on fresh and wilted CL for 24h shows that, the live weight gain (LWG) is better for 24h wilted (334g) compared with FCL (317g), event there is a bit difference on LWG but HCN is not toxic under condition of experiment, intake of HCN ranges from 3.08-3.19 mg/kg LW for fresh and 1.85-2.16mg/kg LW 24h for wilted CL.

A study by Phiny et al, (2008b) on growth performance of pigs fed water spinach (WS) or WS mixed with mulberry leaves (ML), as protein sources in basal diets of cassava root meal (CRM) plus rice bran (RB) or sugar palm syrup (SPS) plus broken rice (BR) showed that the feed intake and LWG were tended to be higher when WS mixed with ML. Other study by Sorn Suheang and Preston, (2005) reported a growth rate of 320g/day when WS replaced FM in diets for growing crossbred pigs fed whole sugar cane stalks or sugar cane juice. However, Prak Kea and Preston, (2003a) reported that the LWG (436g/day) is improved when FM is fed at a level of 6% in the diet of BR and WS.

II.5.2. Effect of using high protein forages on digestibility

Chhay Ty et al, (2007) did not show any differences in the nutritive value for pigs fed bitter and sweet CL varieties, nor are there benefits from wilting for 24h, as measured by apparent digestibility of DM, CP, CF, and N retention. Bui Huy Nhu Phuc et al, (2000b, 2000c) and Bui Huy Nhu Phuc and Lindberg, (2000a) report that increasing the level of ECL to replace the CP from conventional sources led to a reduction of feed intake, apparently due to the associate increased in the fibre content of the diet. Du Thanh Hang, (2007) reported that increased levels of the ECL resulted in a reduction in N retention. However, Ly and Pok Samkol, (2001) showed that DM feed intake and N retention was normal, this can be due to in the nature of the CL that used. In the Cambodian research, the CL is obtained from foliage harvest at 2-2.5months intervals (Preston, 2001). According to Chhay Ty et al, (2003) silage made from young CL (from 2-2.5 months re-growth) are more digestible and higher N retention in growing pigs than silage made from the old CL (harvested 4.5-5 months after planting). In general, there appears no relationship between intakes of cyanogenic glycosides and performance traits. This confirms several earlier findings that FCL can be fed safely to growing pigs (Chhay Ty and Preston, 2005; Chhay Ty et al., 2009; Du Thanh Hang and Preston, 2005; Nguyen Thi Hoa Ly, 2005; Nguyen Thi Loc and Le Khac Huy, 2003).

Phiny et al, (2008a) found that digestibility of DM, OM, CF and CP are not different when pigs were fed WS alone or the mixture of WS and ML. However, N retention is lower when pigs were fed WS compared to the mixture of WS and ML. It is contrast with the study of Chhay Ty and Preston, (2005) who found that total DM intake, digestibility of DM, OM, N and CF, and N retention were higher when pigs were fed WS alone, the mixture between WS and FCL compared with FCL alone. Mixing two sources of leaves may be beneficial in view of possible complementary effects of the two arrays of AAs. Prak Kea et al, (2003b) did a study on feed intake, digestibility and N retention of a diet of WS supplemented with palm oil and or BR and FM for growing pigs and found that the apparent digestibility of DM and OM decreased, as the proportion of palm oil increased but it had no effect on digestibility of CF and N, nor on N retention with level of palm oil add to WS.

II.5.3. Effect of amino acid supplementation to diets containing high protein forages on pig performance

A study of Chhay Ty et al, (2009) showed that total DM feed intake and intake g/kg LW are the highest for pigs supplementing Met at level of 0.3% in diet with fresh or 24h wiled CL as a source of protein compared with diet without Met supplementation. DM feed intake and LWG are 894 and 312g, and 794 and 248g, respectively for with and without Met supplementation. This confirms the findings of Du Thanh Hang et al, (2007), who showed that LWG and FCR are improved when supplementing Met from 0.1-0.2% in the diets with ECL or FCL as a protein source. The LWG and FCR are 712g and 2.79 compared with 540g and 3.37 for diet without Met supplement. Nguyen Thi Hoa Ly, (2005) confirmed that increased level of inclusion of AAs (Lys and Met) in 15% ECL improve feed intake, LWG and FCR compared with low level of AAs in diets. Diets with high level of AAs give highest economic efficiency of pig production. Portela and Maner, (1972) reported that pigs fed a diet with 55% CRM supplemented with 0.1% Met have higher LWG and better FCR compared with non supplemented diets or with 0.2% Met. This finding was confirmed by Nguyen Thi Hoa Ly, (2006) who reported a 23% increase in LWG of pigs when Met is added at 0.15% of the DM in diets with 17-25% CRS and 15% ECL (DM basis). Job, (1975) reported that when diets are supplemented Met, LWG and FCR are improved in cassava root based diet because the AAs is needed for detoxification of the HCN in the cassava. In the animal body, the detoxification of HCN needs Met and sulfur as donors of AAs, this converts HCN to non-toxic thiocyanate (Oke, 1973).

A study of Ly et al, (2002) on the effect of Met supplementation on digestibility and performance traits of growing pigs fed BR and WS showed that there is no treatment effect on feed intake (in DM) when supplemented with Met from 0, 0.25, 0.50 and 0.75% in the diet (DM basis) but find significant improvements in LWG (442g/day) when 0.5% of synthetic Met is added to a basal diet of BR and WS. This was confirmed by Pech Sovanno et al, (2002), who reported an improvement in LWG (417g/day) when Met was supplemented at 0.5% on WS with a basal diet of full fat rubber seed and CRM. These results imply that Met may be a limiting AA in WS as claimed by Le Thi Men, (1999) and Bui Huy Nhu Phuc, (2000).

II.5.4. Effect of amino acid supplementation to diets containing high protein forages on nutrient digestibility

Bui Huy Nhu Phuc and Lindberg, (2001) mentioned that most of the AAs are digested to a greater extent than the CP. As reported by Reverter et al, (1999) the reduction in apparent ileal digestibility of EAAs when CL is included in the diets, due to an increase in the ileal flow of AAs with increasing fiber content in the diets. Furthermore, an increase in endogenous AAs secretions could also be expected (Sauer and Ozimek, 1986; Boisen and Moughan, 1996; Jondreville et al., 2000). The magnitude of this effect depends on the digestibility of the basal diet, the level and type of the fiber and the contribution of the fiber sources to the dietary AAs supply (Lenis et al., 1996). In addition to fiber level and quality, the presence of tannins and enzyme inhibitors may also influence the digestibility of CP and AAs (Kidder and Manner, 1978). The estimated apparent ileal digestibility of His, Lys and Tyr are finding to be highest, while that of Thr is the lowest. Also the low Arg digestibility of CL basal diet could be due to the binding of these AAs to lignin (Nongyao et al., 1991). The average apparent ileal AAs digestibility is 0.56, close to the value reported for perennial ryegrass meal (0.61) (Reverter et al., 1999). A study of Ly, (2002) on the effect of Met supplementation on digestion indices and N balance of young Mong Cai pigs fed high levels of CLS show that the high level of Met result in an increase in the digestibility of DM and OM. As there are also trends for N digestibility and N retention to be improved with Met supplementation, the implication is that the effect is mediated though a better balance of AAs at the sites of metabolism, and the Met assist in the detoxification of the HCN. A similar trend for a positive effect of Met addition on N digestibility is observed in pigs fed high levels of full-fat rubber seeds (Pok Samkol et al., 2002) and water spinach (Ly et al., 2002). NDF digestibility is high when diets are supplemented with Met suggesting that the physio-chemical characteristic of CL favors cell wall degradation in the alimentary canal. In this connection, Le Goff et al, (2002) suggested that the botanical origin of fiber can affect its digestibility in pigs, and this may be more decisive than other factors (Varel and Yen, 1997). It is suggested that N balance in young pigs fed very high levels of CLS can be improved by supplementation with Met. Le Duc Ngoan and Nguyen Thi Hoa Ly, (2007) studied the use of CRS and CLS for feeding pigs in Vietnam, results show that digestibility of OM and CP are high when including level of 0.1, 0.2 and 0.3% (as DM) Met in CRS based diet for pigs. Inclusion of Met in diets significantly reduced the amount of N secretion in urine but increase N retention and growth performance. Nguyen Thi Loc and Le Khac Huy (2003) show that N retention values in growing pigs fed a cassava root based diet are 19.3, 20.1, 20.8 and 21.9g for supplementation with 0, 0.1, 0.2 and 0.3% Met, respectively.

Ly et al, (2002) studied the effect of Met supplementation on digestibility and performance traits of growing pigs fed BR and WS. Results showed that DM and N digestibility are not varied according to the level of Met (0, 0.25, 0.50 and 0.75%) in WS based diets, however, a trend is observed for OM to decrease with increased level of Met added to the diet. This probably reflects the higher intake of WS in the diet when Met is added. However, N digestibility is improved when Met is supplemented. Through the results of this study, very simple diets, formulate to contain BR as energy source and WS as source of protein for growing pigs, can greatly be improved with the addition of no more than 0.5% Met to the total daily feed intake. Even in this study a very high AA level is used in the diet the useful information that Met could not used more than 0.5% in the diet containing WS.

Briefly there were no ill-health symptoms in any of the pigs that could have been ascribed to HCN toxicity in fresh, dry and ensiled cassava leaves. However, feed intake, growth rate, nutrient digestibility and N retention were affected. Growth performance, feed conversion ratio, apparent digestibility and N retention are improved when cassava leaves or water spinach based diets are supplemented with amino acids, especially methionine.

Reference

Animal Feed Resources Information Systems (2004). Updated from B. Göhl, (1981) Tropical feeds. FAO. http://www.fao.org./ag/AGa/agap/FRG/AFRIS/DATA/535.htm

An L V, Hong T T T & Lindberg J E (2004) Ileal and total tract digestibility in growing pigs fed cassava root meal diets with inclusion of fresh, dry and ensiled sweet potato leaves. Animal Feed Science and Technology 114, 127-139.

Anugwa and Okwori (2008) Performance of growing pigs of different genetic groups fed varying dietary protein levels African Journal of Biotechnology Vol. 7 (15), pp. 2665–2670, 4 August 2008 ISSN 1684-5315 © 2008 Academic Journals

Bikker P (1994) Protein and lipid accretion in body components of growing pigs. Effects of body weight and nutrient intake. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, the Netherlands.

Boisen S & Moughan P J (1996) Dietary influences on endogenous ileal protein and amino acid loss in the pig-A review. Acta Agric. Scand. 46:154–164.

Bui Huy Nhu Phuc & Jan Erik Lindberg (2000a) Ileal digestibility of amino acid in growing pigs fed a cassava root meal diets with inclusion of cassava leaves, leucaena leaves and groundnut foliages. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Bui Huy Nhu Phuc (2000) Tropical Forages for growing pings. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Bui Huy Nhu Phuc, Jan Erik Lindberg, Brian Ogle & Sigvard Thomke (2000c) Nutritive evaluation with rats of tropical biomass products for monogastrics: 2. Effects of drying temperature, ensiling and level of inclusion of cassava leaves and sweet potato vine. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Bui Huy Nhu Phuc, Ogle B, J E Lindberg (2000b) Effect of replacing soybean protein with cassava leaf protein in cassava root meal based diets for growing pigs on digestibility and N retention. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Bui Huy Nhu Phuc, Ogle B & Lindberg J K (2001) Nutritive value of cassava leaves for monogastric animals; Proceedings of workshop on "Use of cassava as animal feed" Khon Kaen University, Thailand http://www.mekarn.org/prockk/phuc.htm

Bui Huy Nhu Phuc, Preston T R, Ogle B & Lindberg J E (1996) the nutritive value of sun-dried and ensiled cassava leaves for growing pigs. Livestock Research for Rural Development 8: (3) http://cipav.org.co/lrrd/lrrd8/3/bui.htm

California Air Resource Board (CARB) (1997) Cyanide compounds (online). California Air Resource Board. Available from: http://www.arb.ca.gov/toxics/tac/factshts/cyanidec.pdf (23 Feb 2001)

Cameron R D A (2000) "A Review of the Industrialization of Pig Production Worldwide with Particular Reference to the Asian Region". Animal Health and Area-wide Integration. FAO, Brisbane, Australia.

CelAgrid (2006) Assessment of impacts of action research on pig production systems, management, feeding and economic returns in Sras Takoun, Louk and Krom village, Sanlong Communes, Trang district, Takeo province. Cambodia

Chedly K & Lee S (1998) Silage from by-products for smallholders. Institute National Agronomique de Tunisie and Brooklyn Valley, New Zealand

Chhay Ty & Preston T R (2005) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. Livestock Research for Rural Development. Vol.17, Art. #23. Retrieved June 30, 2005, from http://www.cipav.org.co/lrrd/lrrd17/2/chha17023.htm

Chhay Ty, Borin K & Preston T R (2007) Effect of mixtures of water spinach and fresh water hyacinth leaves on growth performance of pigs fed a basal diet of rice bran and cassava root meal. *Livestock Research for Rural Development. Volume 19, Article #194.* Retrieved September 10, 2008, from http://www.cipav.org.co/lrrd/lrrd19/12/chha19194.htm

Chhay Ty, Khieu Borin & Preston T R (2009) Effect of wilting cassava leaves and supplementing them with DL-methionine, on intake, growth and feed conversion in growing pigs. *Volume 21, Article # 12.* Retrieved December 12, 2008, from http://www.lrrd.org/lrrd21/01/chha21012.htm

Chhay Ty, Ly J & Rodriguez Lylian (2001) An approach to ensiling conditions for preservation of cassava foliage in Cambodia. Livestock Research for Rural Development 13 (2): http://www.cipav.org.co/lrrd/lrrd13/2/chha132.htm

Chhay Ty, Preston T R & Ly J (2003) The use of ensiled cassava leaves in diets for growing pigs. 2. The influence of type of palm oil and cassava leaf maturity on digestibility and N balance for growing pigs. Livestock Research for Rural Development (15)8 Retrieved, from http://www.cipav.org.co/lrrd/lrrd15/8/chha158.htm

Chittavong Malavanh & Preston T R (2006) Intake and digestibility by pigs fed different levels of sweet potato leaves and water spinach as supplements to a mixture of rice bran and cassava root meal. *Livestock Research for Rural Development.* http://www.cipav.org.co/lrrd/lrrd18/6/mala18086.htm

Chung K, Wong T Y, Wei C, Huang Y & Lin Y (1998) Tannin and human health. Critical Reviews in Food Science and Nutrition, 38:421-464.

Cole R J & Cox R H (1981) Handbook of Toxic Fungal Metabolites. Academic Press, New York.

Dam Am Pil newspaper (2010) Pig import from Vietnam. Thursday, 23 December 2010 17:23. http://www.dap-news.com/index.php?option=com_content&view=section&layout= blog&id=1&Itemid =465

Devendra C & Clyde Parris E C (1970) Optimum crude protein levels for growing finishing pigs in a tropical environment. Trop. Anim. Hlth. Prod., 2, 162– 165.

Du Thanh Hang & Preston T R (2005) The effects of simple processing methods of cassava leave on HCN content and intake by growing pigs. Livestock Research for Rural Development. Volume 17, Article #99. Retrieved September 7, 2005, from http://www.cipav.org.co/lrrd/lrrd17/9/hang17099.htm

Du Thanh Hang (2007) Cassava leaves as protein source for pigs in Central Vietnam. Chapter 1: Cassava (Manihot esculenta Crantz) production: nutritive value and toxic factors. Doctoral thesis, Nederland University 2007

Du Thanh Hang, Nguyen Quang Linh, Preston T R, Everts H & Beynen A C (2007) Cassava leaves as protein source for pigs in Central Vietnam. Chapter 6 Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source. (Doctorial Thesis 2007)

FAO (2005) Livestock sector brief. Livestock information, sector analysis and policy branch, AGAL. http://www.fao.org/ag/againfo/resources/en/publications/sector_briefs/lsKhM .pdf. FAO, Rome, Italy. pp. 1–21.

González AM, Brehm A, Perez JA, Maca-Meyer N, Flores C & Cabrera VM (2003) Mitochondrial DNA affinities at the Atlantic Fringe of Europe. Am J Phys Anthropol 120: 391–404.

Hagerman A E, Robbins C T, Weerasuriya Y, Wilson T C & McArthur C (1992) Tannin chemistry in relation to digestion. Journal of Range Management, 45: 57-62

Hartung R (1983) Cyanides and nitriles. In: Clayton, G.D; Clayton, F.E. (Eds), 3rd ed. Patty's industrial hygiene and toxicology. John Eiley and Sons, Inc., New York.

Hess V (1999) Disponibilité vraie des acides aminés et impact des pertes azotées endogène spécifiques selon la source de protéines alimentaires chez le porc. Ph.D. Diss. Univ. Rennes, Rennes, France

Ho Bunyeth (2003) Biodigester effluent as fertilizer for water spinach established from seed or from cuttings; Retrieved, from MEKARN Mini-projects. http://www.mekarn.org/msc2003-05/miniprojects/webpage/buny.htm

Hoffmann D (1999) Manual on Improved Rural Pig Production. RAP Publication 1999/25. http://www.aphca.org/publications/files/laosEu/Pig%20Eng%20-%20ebook.pdf.

Howeler R H (1991) Long-term effects of cassava cultivation on soil productivity. Field Crop Research 26, 1-18.

Humphreys D J (1988) Veterinary Toxicology, 3rd ed. Bailliere Tindall, London

Job T A (1975) Utilization and protein supplementation of cassava for animal feeding and the effect of sulfured source on cyanide detoxification. PhD Thesis. University of Ibadan, Nigeria. (Astr. On cassava Vol III, pp 97-98

Jondreville C, Van Der Broecke J, Grosjean F, Van Cauwenberghe S & Gatel F (2000) Ileal true digestibility of amino acids in wheat milling by-products for pigs. Annales de Zootechnie 49, 55-65.

Jones R (2002) Priorities for pig research in Southeast Asia and the Pacific to 2010. http://www.aciar.gov.au/sites/aciar/files/node/472/wp53.pdf. ACIAR (Australian Centre for International Agricultural Research), Canberra, Australia. pp 5–13.

Jorgensen H, Zhao X, Eggum B O & Zhao X Q (1996) The influence of dietary fiber and environmental temperature on development of the gastrointestinal tract, digestibility, degree of fermentation in the hind gut and energy metabolism in pigs. British Journal of Nutrition 75, 365-378.

Kaensombath L (2005) Evaluation of the Nutritive Value of Ensiled and Fresh Golden Apple Snails (GAS) *(Pomacea* spp) for Growing Pigs. Master of Science thesis, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. http://www.mekarn.org/msc200305/theses05/lamp1.pdf

Kass M L, Van Soest P J, Pond W G, Lewis B & McDowell R E (1980) Utilization of dietary Fiber from alfalfa by growing swine. I. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. Journal of Animal Science 50. 175-181

Kean Sophea & Preston T R (2001) Comparison of biodigester effluent and urea as fertilizer for water spinach vegetable. Livestock Research for Rural Development 13 (6) http://www.cipav.org.co/lrrd/lrrd13/6/kean136.htm

Khieu Borin (2005) Cassava Foliage for Monogastric Animals. Forage Yield, Digestion, Influence on Gut Development and Nutritive Value. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2005

Khieu Borin (2006) The current situation of pig production in Cambodia. Personal communication.

Kidder D E & Manners M J (1978) Digestion in the Pig. Scientechnica, Bristol, UK.

Kunavongkrit A & TW Heard (2000) “Pig Reproduction in South East Asia”. Animal Reproduction Science, 60–61: 527–533.

Lapar M L, Vu Trong B & Ehui S (2003) Identifying barriers to entry to livestock input and output markets in Southeast Asia: The case of Vietnam. Socio-economic and Policy Research Working Paper 56. ILRI (International Livestock Research Institute), Nairobi, Kenya. 58 pp.

Le Duc Ngaon & Nguyen Thi Hoa Ly (2007) Use of cassava root and leaves for feeding pigs in Vietnam. In Proc of 7th Regional workshop held in Bangkok Thailand from 28th Oct-1st Nov 2002 pp 518-523 http://webapp.ciat.cgiar.org/asia_cassava/pdf/proceedings_workshop_02/518.pdf

Le Goff G, Van Milgen J & Noblet J (2002) Influence of dietary fiber on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows. Animal Science 74:503-515

Le Thi Men (1999) Evaluation of water spinach (Ipomoea aquatica) for Baxuyen and Large White sows and fattening crossbred pigs. Master in Sciences Thesis. Swedish University of Agricultural Sciences. Uppsala pp 71

Le Thi Men & Preston T R (2005) On-farm evaluation of effect of different fertilizers on water spinach (Ipomoea aquatica) yields, and of including water spinach and catfish oil in diets for fastening pigs in the Mekong Delta of Vietnam, from: http://www.mekarn.org/proctu/lemen38.htm

Lemke U, Thuy L T, Zárate A V, Kaufmann B & Vang N D (2002) "Characterization of Smallholder Pig Production Systems in Mountainous Areas of North Vietnam". Paper presented at the Deutscher Tropentag 2002 Conference on International Agricultural Research for Development, held 9-11 October 2002 in Witzenhausen.

Lenis N P, Van der Meulen J, Van Diepen J T M, Bakker J G M & Jongbloed R (1996) Utilization of nitrogen and amino acids in growing pigs as affected by the ratio between essential and nonessential amino acids in the diet. J. Anim. Sci. 74 (Suppl.1):172.

Lindberg J E & Andersson C (1998) The nutritive value of barley-based diets with forage meal inclusion for growing pig base on total tract digestibility and nitrogen utilization. Livestock Production Science 56, 43-52

Ly J & Pok Samkol (2001) The nutritive value of ensiled cassava leaves for young Mong Cai pigs fed high levels of protein. Livestock Research for Rural Development.13 (4): http://www.cipav.org.co/lrrd/lrrd13/4/ly134b.htm

Ly J (2002) The effect of methionine on digestion indices and N balance of young Mong Cai pigs fed high levels of ensiled cassava leaves; *Livestock Research for Rural Development (14) 6.* Retrieved October 18, 2010, from http://www.lrrd.org/lrrd14/6/ly146.htm

Ly J, Hean Pheap, Keo Saeth & Pok Samkol (2002) The effect of DL-methionine supplementation on digestibility and performance traits of growing pigs fed broken rice and water spinach (*Ipomoea aquatica).* Livestock Research for Rural Development (14)5 http://www.cipav.org.co/lrrd/lrrd14/5/ly145b.htm

Ly Thi Luyen & Preston T R (2004) Effect of level of urea fertilizer on biomass production of water spinach (*Ipomoea aquatica*) grown in soil and in water. *Livestock Research for Rural Development. Vol. 16, Art. #81.* Retrieved, from http://www.cipav.org.co/lrrd/lrrd16/10/luye16081.htm

Maner J H & Gomez G (1973) Implications of cyanide toxicity in animal feeding studies using high cassava rations in chronic cassava toxicity: Proceedings of an interdisciplinary workshop, London, England, 29 – 30 January 1973. IDRC 010e p113 –120.

Marian E & Boquiren (2009) Strengthening Micro, Small and Medium Enterprises in Cambodia, Sponsoring USAID.

McDonald P, Edwards R A, Greenhalgh J F D & Morgan C A (2002) Animal Nutrition. Sixth Edition. Longman Scientific and Technical, Harlow, Essex, England.

McDonald P, Edwards R, Greenhalgh J F D & Morgan C (1995) Additive-treated silage. Animal nutrition. 5th Edition. Longman Scientific & Technical, Essex CM20 2JE, England, pp. 456.

Ministry of Agriculture, Forestry and Fishery (2007) Statistics of livestock.

http://www.maff.gov.kh/en/statistics/livestock.html

Ministry of Agriculture, Forestry and Fishery (2009) Statistics of livestock. http://www.maff.gov.kh/en/statistics/livestock.html

Moran J (2005) Tropical dairy farming: Feeding management for small holder dairy farmers in the humid tropics. Department of Primary Industries. 312 pp.

National Research Council (NRC) (1998) Nutrient Requirements of Swine, 10th Revised Edition. National Academy Press, Washington D.C., p. 189

Ngo Thuy Bao Tran & Brian O (2005a) Surveys on small-scale urban and peri-urban livestock production in Chau Doc Town. Master thesis. http://www.mekarn.org/msc200305/theses05/Trancont.htm

Ngo Thuy Bao Tran & Brian O (2005b) Surveys on small-scale urban and peri-urban livestock production in Long Xuyen City. Master thesis. http://www.mekarn.org/msc2003-05/theses05/Trancont.htm

Nguyen Thi Hoa Ly, Nguyen Thi Loc, Du Thanh Hang & Truong Thi Thuan (2003) Effect of dietary protein level on the performance of growing pigs under village conditions in Central Vietnam. 94-98 pp.

Nguyen Thi Hoa Ly (2005) Effect of supplementation of l lysine and dl methionine on utilization of diets containing ensiled cassava leaves as protein source for pig in central Vietnam " (Editors: Reg Preston and Brian Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005. http://www.mekarn.org/proctu/hoal36.htm

Nguyen Thi Hoa Ly (2006) The use of ensiled cassava leaves for feeding pig's on-farm in central Vietnam. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN- CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006. Article#13. Retrieved March 16, 107, from http://www.mekarn.org/proprf/hoaly.htm

Nguyen Thi Loc & Le Khac Huy (2003) Effect of DL-methionine supplementation levels in ensiled cassava root-based diets on the performance and economic efficiency of F1 (Large White x Mong Cai) fattening pigs. In: *Proceedings of Final National Seminar Workshop on Sustainable Livestock Production on Local Feed Resources (Editors: Reg Preston and Brian Ogle)*. HUAF-SAREC, Hue City, 25 – 28 March, 2003. Retrieved July14, 107, from http://www.mekarn.org/sarec03/loc1.htm.

Nongyao A, Han I K & Choi Y J (1991) Amino acid digestibility to pigs in various fibers sources 2. True digestibility of amino acids in ileal digesta and feces. *Asi. Austr. J. Anim. Sci.* 4 (3): 211-218.

Ogle B (2006) Forages for pigs: nutritional, physiological and practical implications. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN-CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006 http://www.mekarn.org/proprf/ogle.htm

Ohio State University Extension (2001) Silage Additives, Department of Horticulture and Crop Science, Columbus, Ohio, USA. http://ohioline.osu.edu/agf-fact/0018.htm

Oke O L (1973) The mode of cyanide detoxification. In McIntyre E, Nestle C Eds: chronic cassava toxicity

Oke O L (1978) Problems in the use of cassava as animal feed. Anim. Feed Science and Technology. 3:345-380.

Oke O L (1994) Eliminating cyanogens from cassava through processing: Technology and tradition. In: Acta Horticulturea Cassava Safety 375. Proc.Int. Workshop in Nigeria. pp. 163-174

Onwuka C F I (1992) Tannin and saponin contents of some Tropical browse species fed to goats. Tro. Agrc. (Trinidad) 69:1442-1440

Pech Sovannoh, Pok Samkol, J Ly & T R Preston (2002) Performance traits of growing pigs fed diets based on Cambodia rubber seeds and soybeans supplemented with water spinach. M.Sc. Thesis. University of Tropical Agricultural Foundation

Pheng Buntha, Khieu Borin, Preston T R & Ogle B (2008) Digestibility and nitrogen balance studies in pigs fed diets with ensiled taro (Colocasia esculenta) leaves as replacement for fish meal. Livestock Research for Rural Development. Volume 20, supplement. http://www.cipav.org.co/lrrd/lrrd20/supplement/bunt2.htm

Phiny C, Ogle B, Preston T R & Borin K (2008b) Growth performance of pigs fed water spinach or water spinach mixed with mulberry leaves, as protein sources in basal diets of cassava root meal plus rice bran or sugar palm syrup plus broken rice. *Livestock Research for Rural Development. Volume 20, supplement.* Retrieved September 28, 2009, from http://www.lrrd.org/lrrd20/supplement/phin2.htm

Phiny C, Ogle B, Preston T R & Khieu Borin (2008a) Digestibility and N-retention in crossbred pigs of diets with water spinach or water spinach mixed with mulberry leaves as protein sources in basal diets of cassava root meal plus rice bran, or sugar palm syrup plus broken rice. MSc Thesis, MEKARN-SLU

Pok Samkol, Pech Sovanno, Preston T R and Ly J 2002 Digestibility study in growing pigs fed diets based on full-fat rubber seeds or soy beans supplemented with water spinach ad libitum, Livestock Research for Rural Development 14(5): http://www.lrrd.org./lrrd14/5/ly145.htm

Portela J & Maner J H (1972) Chronic cassava toxicity. International Development Research Center, London, Monograph No. 010e, pp: 113-120.

Poungchompu O, Wanapat S, Polthanee A, Wachirapakorn C & Wanapat M (2001) Effects of planting method and fertilization on cassava hay yield and chemical composition. In: Proceeding of the International Workshop "Current Research and Development on Use of Cassava as Animal Feed. Khon Kaen University, Thailand, July 23-24, 2001. http://www.mekarn.org/procKK/poun.htm

Prak Kea, Preston T R & Ly J (2003a) Response of pigs fed a basal diet of water spinach (Ipomoea aquatica) to supplementation with oil or carbohydrate. MSc. thesis. Swedish University of Agricultural Sciences, Uppsala http://www.mekarn.org/msc2001-03/theses03/contkea.htm

Prak Kea, Preston T R & Ly J (2003b) Feed intake, digestibility and N retention of a diet of water spinach supplemented with palm oil and / or broken rice and dried fish for growing pigs. *Livestock Research for Rural Development (15) 8* Retrieved, from http://www.cipav.org.co/lrrd/lrrd15/8/kea158.htm

Preston T R (2001) Potential of cassava in integrated farming systems. International Workshop Current Research and Development on Use of Cassava as Animal Feed, Khon Kaen, Thailand July 23-24, 2001. http://www.mekarn.org/procKK/pres.htm

Ravindran V (1993) Cassava leaves as animal feed: Potential and limitation. Journal of the Science of Food and Agriculture 61, 141-150.

Ravindran V, Kornegay E T & Rajaguru A S B (1987) Influence of processing methods and storage time on the cyanide potential of cassava leaf meal. Animal Feed Science and Technology 17: 227-234.

Reed J D (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science, 73: 1516-1528

Reverter M, Lundh T & Lindberg J E (1999) Ileal amino acid digestibility in pigs of barley based diets with inclusion of lucerne, white clover, red clover or perennial ryegrass. *Br. J. Nutr*. 82: 139-147.

Riethmuller P, Hoffmann D & Chalermpao N (2002) "Some Issues Associated with the Livestock Industries of the Asia-Pacific Region". Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific (APHCA)

Roberts C A (1995) Microbiology of stored forage. Page 21 in Post-Harvest Physiology and Preservation of forages. CSSA Special Publication No 22 Moore, K.J., Peterson, M.A., (Eds.). Am. Soc. Agron., and Crop Sci. Soc. Am. Madison, WI.

Rodríguez L, Lopez D, Preston T R & Peters K (2006) New Cocoyam (*Xanthosoma sagittifolium*) leaves as partial replacement for soya bean meal in sugar cane juice diets for growing pigs. Workshop on Forages for Pigs and Rabbits, Phnom Phen, 22-24 August 2006.

http://www.mekarn.org/proprf/rodr2.htm.htm
San Thy & Preston T R (2001) Evaluation of the effluent from different retention times as fertilizer for growing water spinach (*Ipomoea aquatica*), from: http://www.mekarn.org/msc200103/theses03/santexp2.htm
Sarwat S R, Katak S N & Kategile J A (1988) Performance of growing-finishing pigs when given diets containing fresh cassava leaves and roots. East African Agriculture and Forestry Journal. 53:111-115.
Sauer W C & Ozimek L (1986) Digestibility of amino acids in swine: results and their practical application a review. Livestock Production Science 15:367.
Sorn Suheang and Preston T R (2005) Utilization of sugar cane stalks and water spinach for fattening pigs and their residues as substrate for the earth worm. http://www.mekarn.org/msc2003-05/theses05/content/suheanglr.pdf
Sovann S & San S (2002) Pig production in Cambodia In: Priorities for pig research in Southeast Asia and the Pacific to 2010. pp. 22–28. http://www.aciar.gov.au/sites/aciar/files/node/472/wp53.pdf.
Steinfeld H (1998) Livestock production in the Asia and Pacific region, current status issues and trends. In: hursey BS (ed), World Animal Review 90–1998/81. Animal Production and health Division, FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.
Steinfeld H, Costales A, Rushton J, Scherf B, Bernett T, & Hall D (2006) The Livestock Report 2006, edited by A. McLeod, 85pp. Rome: FAO, Animal Production and Health Division
Stosic D D & Kaykay J M (1981) Rubber seed as animal feed in Liberia. World Animal Review 39, 29 39.
Taylor A E, Jagger S, Toplis P, Wellock I & Miller H M (2010) Can Hampshire tolerant low lysine diets post weaning? Proceeding of the British Society of Animal Science and the Agriculture Research Forum 2010. Advances in Animal Biosciences.
Tanner G J, Moore A E & Larkin P J (1994) Proanthocyanidins inhibit hydrolysis of leaf protein by rumen micro flora in vitro. British Journal of Nutrition, 71:947-958.
Titterton M & Bareeba F B (1999) Grass and legume silages in the tropics. Proceedings of the FAO Electronic Conference on Tropical Silage. 1 September to 15 December 1999. http://www.fao.org/docrep/005/x8486e/x8486e0c.htm#bm12
Tran Hoang Chat, Ngo Tien Dung, Dinh Van Binh & Preston T R (2005) Water spinach *(Ipomoea aquatica)* as replacement for guinea grass for growing and lactating rabbits; Workshop-seminar "Making better use of local feed resources" (Editors: Reg Preston and Brian Ogle)

MEKARN-CTU, Cantho, 23-25 May, 2005. Article #48. Retrieved, from http://www.mekarn.org/proctu/chat48.htm

Van Soest P J (1994) Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY.

Varel V H & Yen J T (1997) Microbial perspective on fiber digestibility by swine. Journal of Animal Science 75:2715-2722

Villar E C, Maleon M O & Facundo D T (2002) "The Swine Industry in the with Particular Reference to the Asian Region". Animal Health and Area-wide

Wanapat (1995) The use of local feed resources for livestock production in Thailand. Proceedings of the International Conference on Increasing Animal production with local Resources. (Editor: Guo Tingshuang). China Forestry Publishing House, Ministry of Agriculture, China

Wang Y, Waghorn G C, Barry T N & Shelton I D (1994) The effect of condensed tannins in Lotus Corniculatus on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. British Journal of Nutrition, 72: 923-935.

Woolfe J A (1992) Sweet potato: an untapped food source. Cambridge University Press, Cambridge, 643 pp

Yu F, Moughan P J & Barry (1996) The effect of cottonseed condensed tannins on ileal digestibility of amino acid in casein and cottonseed kernel. British Journal of nutrition, 75: 683-698.

CHAPTER III

Effect of water spinach and fresh cassava leaves on growth performance of pigs fed a basal diet of broken rice

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Abstract

Eighteen crossbred (local*Landrace) castrated male pigs weighing from 11 to 14 kg were used in a 2*3 factorial arrangement to study the effect of source of supplementary protein (fresh water spinach, fresh cassava leaves or a mixture [40:60 DM basis] of the two) and DL-methionine on growth performance traits with a basal diet of broken rice. The pigs were housed in individual pens and allotted at random to the three experimental diets within three blocks based on live weight. The feeding trial lasted for 100 days from 16 November to 24 February 2005. The pigs are up to 38% of their DM intake with no symptoms of HCN toxicity. Growth rates on all treatments increased as the pigs became heavier, but were always lower on the cassava leaf supplement compared with water spinach or the mixture of cassava leaves and water spinach. There were no differences between water spinach as the only protein supplement and the mixture of water spinach and cassava leaves. There was no effect on performance traits of the DL-methionine. It is concluded that there appear to be synergistic positive effects from combining two sources of protein-rich leaves as supplements to a low protein basal diet for growing pigs.

Key words: *cassava, growth, leaves, methionine, pigs, protein, water spinach*

Introduction

Monogastric animals play an important role in agricultural activities in rural areas in Cambodia. On average, a farmer raises 2 to 5 pigs (local or crossbred). The major problem in pig production is the lack of protein because protein-rich feed resources are scarce and, if available, the prices are often prohibitive. There is therefore a need to identify feeds, which can compensate for these deficiencies.

Water spinach *(Ipomoea aquatica)* is a plant that grows equally well in water or in soil. It responds dramatically in biomass yield and protein content when fertilized with biodigester effluent (Kean Sophea and Preston 2003), earthworm compost (Tran Hoang Chat et al 2005) or urea (Li Thi Luyen et al 2004; Tran Hoang Chat et al 2005). In Cambodia, it is cultivated for human food and also is fed to pigs and other animals. It does not appear to contain anti-nutritional compounds and has been used successfully for growing pigs as the only source of supplementary protein in a diet based on broken rice (Ly et al 2002). In that research there was a significant response in growth rate and feed conversion to supplementary DL-methionine. Prak Kea et al (2003) reported a linear increase in growth rates in pigs fed water spinach, palm oil and broken rice when up to 6% fish meal (in diet DM) replaced equivalent amounts of water spinach, which they attributed to an improved amino acid balance, especially in terms of the sulphur-rich amino acids.

Cassava **(***Manihot esculenta* Crantz*)* is a widely grown crop in many tropical counties (Calpe 1992). Traditionally it is cultivated for root production, but recently attention has focused on managing it as semi-perennial forage with repeated harvesting at 2-month intervals (Preston 2001). In this system, when the cassava is fertilized with high levels of organic manure, annual protein yields can reach 3 to 4 tonnes/ha.

The protein in cassava leaves has been reported to be rich in lysine but limiting in methionine (Eggum 1970). Water spinach also appears to be limiting in methionine in view of the growth response when synthetic DL-methionine was added to a diet in which water spinach provided most of the protein (Ly et al 2002). Mixing two sources of leaves as sources of protein may be beneficial in view of possible complementary effects of the two arrays of amino acids.

The aim of the present experiment was to study the effect of water spinach and fresh cassava leaves, fed alone or mixed together, on growth performance of pigs fed a basal diet of broken rice, with or without a supplement of DL-methionine.

Material and methods

Location

The experiment was carried out from 16 November 2004 to 24 February 2005 in the Center for Livestock and Agriculture Development (CelAgrid-UTA Cambodia), located in Kandal village, Rolous Commune, Kandal steung district, Kandal province about 25km from Phnom Penh City, Cambodia .

Experimental animals, treatments and design

Eighteen crossbred (Local*Landrace or Local*Duroc) castrated male pigs with initial body weight from 11 to 14 kg were allocated to 6 treatments in a 2*3 factorial arrangement. The factors were:

Methionine:

- With (M)
- Without (NM)

Protein supplement:

- Fresh cassava leaves (FCL)
- Fresh water spinach (WS)
- Mixture of water spinach and cassava leaves (40:60 DM basis) (WSFCL)

The pigs were allocated into 3 blocks according to live weight and the nutritional treatments were applied at random within each block. The pigs were housed in individual pens with concrete floor and provided with feeders and drinking nipples. The pigs were vaccinated against Salmonellosis and were adapted to the feeds and the housing for a 10 day period before starting the experiment.

Feeding and management

Broken rice is a by-product of Cambodian rice mills and was available in the local market. Premix and methionine were purchased from shops in the city. Fresh cassava leaves were harvested every day from plots in CelAgrid (Photo 1). The water spinach was purchased from traders who harvested it from lagoons receiving waste water from Phnom Penh city.

Photo 1: Cassava plantation in CelAgrid

Photo 2: Water spinach being harvested in the lagoon

The leaves plus stems of the water spinach and the leaves of cassava (after removing stems and petioles) were chopped into small pieces, mixed with the other ingredients of the diet (Tables 1 and 2) and fed immediately. The amounts of feeds offered were based on the broken rice being fed at the rate of 2 kg DM per 100 kg live weight (equivalent to 4 kg of DM per 100 kg live weight of the total feed), and were given in 3 meals daily (8.00, 12.00 and 17.00h).

Table 1: Chemical characteristics of the ingredients of the diets

		As % of DM			
	% DM	**N*6.25**	**Ash**	**OM**	**HCN, mg/kgDM**
Fresh cassava leaves	32.1	23.5	3.48	96.5	475
Water spinach	11.2	25.6	13.3	86.7	-
Broken rice	89.7	7.5	0.53	99.5	-
DL-Methionine	100	100	-	-	-

Table 2: Composition (DM basis) and analysis of diets (M = DL-Methionine)

	WS-M	**FCL-M**	**WSFCL-M**	**WS**	**FCL**	**WSFCL**
Ingredients, % DM basis						
Fresh cassava leaves	0	48.7	29.2	0	49.0	29.5
Fresh water spinach	47.7	0	19.5	48.0	0	19.5
Broken rice	50.0	50.0	50.0	50.0	50.0	50.0
DL-Methionine	0.3	0.3	0.3	0	0.0	0
Vitamins and minerals	2.0	1.0	1.0	2.0	1.0	1.0
Total	100	100	100	100	100	100
Analysis (% in DM except for DM which is on fresh basis)						
Dry matter	50.3	60.5	56.4	50.4	60.6	56.5
Ash	6.61	1.96	3.87	6.52	1.97	3.89
Organic matter	93.4	98.0	96.1	93.5	98.0	96.1
Crude protein (Nx6.25)	15.9	15.2	15.6	15.8	15.3	15.7
HCN, mg/kg DM	-	231	139	-	233	140

Data collection and analyses

The pigs were weighed every 10 days until 100 days. Individual daily weight gains were calculated by the regression of live weight on time in days. Individual feed intake and crude protein intake were recorded daily from weight of fresh material offered minus the residue collected the next morning. Feed conversion ratio was calculated from individual daily DM intake and live weight gain. Feed samples were taken every 5 days to determine DM and every 10 days for N and HCN. The DM content was determined using the microwave method of Undersander et al. (1993). Ash, N and HCN were analyzed following procedures of AOAC (1990).

Statistical analysis

Data for weight gain, feed DM intake, crude protein intake and feed conversion were analyzed using the general linear model (GLM) option of the ANOVA software of Minitab (2000). The sources of variation were blocks, diets, methionine, interaction diets*methionine and error.

Results and discussion

Feed intake

The intake of the water spinach (as DM) was almost twice that of the cassava leaves (84% higher) when each was given separately and resulted in an associated (since all ingredients were mixed together) greater intake of the broken rice, and thus of total DM (Table 3). Intakes of total foliage on the mixed supplement (WSFCL) were slightly above the mean value for foliage given alone. As the content of crude protein was similar in the cassava leaves and the water spinach, intakes of crude protein followed those of the DM. The foliages supplied from 38% (cassava alone) to 49% (water spinach alone) of the total DM intake and from 65 to 75%, respectively, of the crude protein intake.

Addition of methionine to the diets led to a lower intake of broken rice, a higher intake of cassava leaves and no change in water spinach intake. The overall effect was a slightly higher (6%) intake of DM as a function of live weight. Total crude protein intake was not affected by methionine supplementation but the proportion of the total diet DM as forage and of total diet protein from foliage protein was higher with methionine supplementation. There appeared to be an interaction between methionine supplementation and the source of forage protein (Figure 1), with no differences in the proportions of protein derived from the foliage on the diets of cassava leaves and water spinach as separate supplements but a 25% increase on the mixed foliage diet.

Table 3: Mean values (main effects) for feed intake of pigs fed broken rice supplemented with fresh cassava leaves (FCL), fresh water spinach (WS) or mixture (WSFCL), with (M) or without (NM) DL-methionine (from 0-100 days)

	M	**NM**	**SEM**	**Prob.**	**FCL**	**WS**	**WSFCL**	**SEM**	**Prob.**
Intake of feed ingredients, g/d									
BR	462a	516b	6.49	0.001	436a	527c	504b	7.95	0.001
CL	191b	162a	3.51	0.001	270	0	258	-	-
WS	211	213	5.09	0.90	0	498	139	-	-
Total DM intake									
g/d	863	891	11.8	0.07	706a	1025c	901b	14.5	0.001
g/kg LW	38.5	36.1	0.19	0.001	33.2a	39.8b	38.9b	0.24	0.001
Total crude protein									
g/d	137	136	1.65	0.54	94a	167c	149b	2.03	0.001
% in DM	15.9	15.3	-	-	13.3	16.3	16.5	-	-
Foliage as proportion of total intake									
DM #	0.44	0.40	0.0040	0.001	0.38	0.49	0.39	0.0050	0.001
Crude protein ##	0.724a	0.660b	0.014	0.002	0.65a	0.77b	0.65a	0.017	0.001
HCN									
mg/d	89.9b	75.8a	2.16	0.001	130	0	123.3		
mg/kg LW	4.02b	3.32a	0.07	0.001	6.28	0	4.86		

Foliage as proportion of total DM intake

Crude protein in foliage as proportion of total crude protein intake

ab Means within main effects within rows without common letter are different at P<0.05

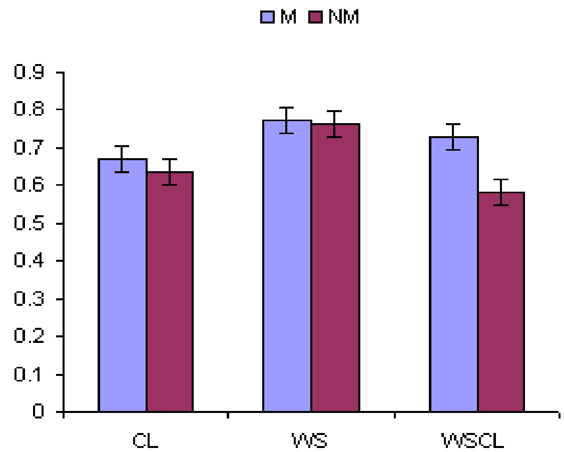

Figure 1: Proportion of diet protein supplied by each ingredient on the diets
with fresh cassava leaves (CL), water spinach (WS) and the mixture (WSCL),
with (M) and without (NM) supplementation with DL-methionine

Growth and feed conversion

There were no ill-health symptoms in any of the pigs that could have been ascribed to HCN toxicity. On a live weight basis the HCN levels consumed on the FCL and WSFCL diets (6.28 and 4.86 mg/kg LW) were considerably higher than the maximum levels recommended by other authors, which were 1.4 (Getter and Baine 1938), 2.1 to 2.3 (Johnson and Ramond 1965), 3.5 (Tewe1992) and 4.4mg/kg LW (Butler 1973).

There were marked differences in growth rate due to the source of supplementary protein (Table 4; Figure 2). Supplements containing water spinach supported higher growth rates compared with cassava leaves alone. Growth rates on the mixture of water spinach and cassava leaves were similar to those on water spinach alone and considerable higher than the average of cassava alone and water spinach alone. Data for feed conversion rate (Table 6 and Figure 3) showed a similar trend with the differences in favor of the mixed foliages being more marked than in the case of live weight gain. The implication from these findings is that there was a synergistic effect from combining the two sources of protein, presumably because of a superior array of essential amino acids in the mixture compared with either supplement fed alone. For all supplements, growth rates increased and feed conversion improved as the trial progressed, with consistent (over 50%) differences in favor of the diets containing water spinach. Trends for live weight at the end of each period were similar to those for growth rate (Table 5).

Supplementation with DL-methionine had no effect on growth rate or feed conversion in any of the periods of the trial (Table 4). This result was unexpected as in a similar trial with broken rice and water spinach there was a significant improvement in growth rate due to methionine supplementation (Ly et al 2002). Significant improvements in growth and feed conversion when DL-methionine was added to diets containing ensiled cassava leaves were also reported by Nguyen Thi Hoa Ly (2005).

Table 4: Mean values for growth rate (g/day) of pigs fed broken rice supplemented with fresh cassava leaves (FCL), fresh water spinach (WS) or a mixture (WSFCL), with (M) or without (NM) DL-methionine, during successive periods of the trial and overall

Days	M	NM	SEM	Prob.	FCL	WS	WSFCL	SEM	Prob.
0-40	214	236	19.7	0.46	151a	248b	276b	24.1	0.01
40-70	341	351	21.5	0.46	242a	377b	420b	26.3	0.002
70-100	448	402	35.4	0.37	287a	478b	511b	43.3	0.009
0-100	321	321	19.0	0.99	225a	356b	383b	23.3	0.002

ab Means within main effects within rows without common letter are different at P<0.05

Table 5: Mean values for live weight (kg) of pigs fed broken rice supplemented with fresh cassava leaves (FCL), fresh water spinach (WS) or a mixture (WSFCL), during successive periods of the trial and overall

Days	FCL	WS	WSFCL	SEM	Prob.
0-40	13.8	13.4	11.4	1.76	0.33
40-70	19.9	23.4	22.4	1.74	0.37
70-100	27.2a	34.7b	34.8b	2.39	0.07
0-100	35.8a	49.2b	50.2b	29.2	0.008

ab Means within rows without common letter are different at P<0.05

Table 6: Mean values for DM feed conversion of pigs fed broken rice supplemented with fresh cassava leaves (FCL), fresh water spinach (WS) or a mixture (WSFCL), with (M) or without (NM) DL-methionine

Days	M	NM	SEM	Prob.	FCL	WS	WSFCL	SEM	Prob.
0-40	2.37	2.43	0.14	0.77	3.23a	2.25b	1.72c	0.17	0.001
40-70	2.34	2.34	0.11	0.97	2.62a	2.37a	2.02b	0.14	0.03
70-100	2.97	3.14	0.17	0.48	3.31	3.0	2.79	0.2	0.24
0-100	2.83	2.87	0.08	0.74	3.15a	2.85b	2.56b	0.09	0.05

ab Means within main effects within rows without common letter are different at P<0.05

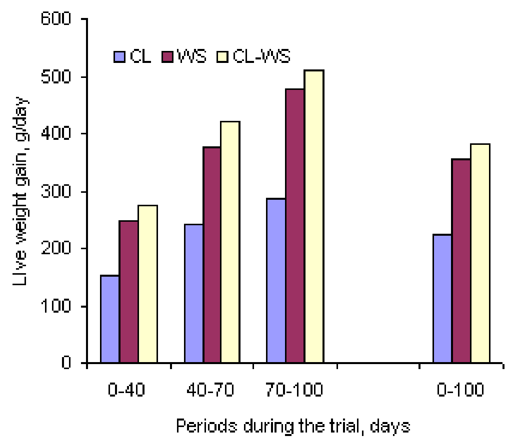

Figure 2: Growth rates of the pigs during successive periods according to supplementary protein source

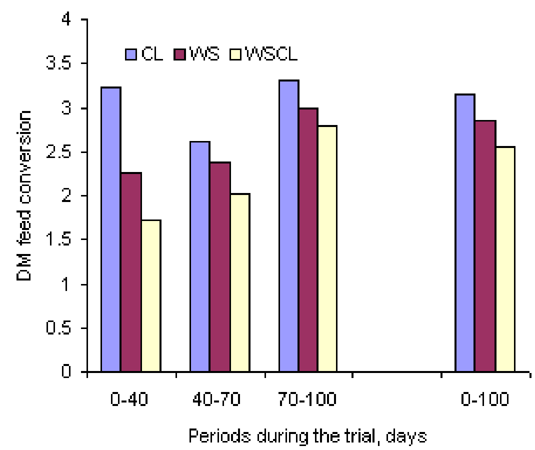

Figure 3: Feed conversion rates of the pigs during successive periods according to supplementary protein source

A lower consumption of broken rice, of total DM and of crude protein (Table 6) would appear to be the reason for the lower growth performance of pigs fed only the cassava leaves as protein supplement. The intake of crude protein appeared to be the critical factor, and accounted for 92% of the variation in growth rate (Figure 4).

According to Ravindran et al (1987), the bitter taste of the cassava leaves could negatively influence their intake by pigs. The presence of tannins in the may also have reduced intake. However, such potential effects of the non-nutritional compounds does not explain why DM intake on the mixture of water spinach and cassava leaves was the same (in fact slightly though not significantly higher) as on water spinach alone, when the expectation was that it should be midway between the values for each foliage given separately.

DM intake as a percentage of live weight over the overall period of 0-100 days for the CL diet (3.31%) was the same as was reported by Chhay Ty et al (2005) (3.30%) for the same feeds in a digestibility trial and similar to that reported by Du Thanh Hang (2005) (range of 2.7 to 3.3%) for crossbred pigs fed a 2:1 mixture of ensiled cassava root and rice bran and fresh cassava leaves that had been washed prior to feeding. It was higher than the values reported by Nguyen Duy Quynh Tram (2003) and Bounhong et al (2004) (2.6 and 3.11%, respectively), who used the same diet as in the present study. However, a much higher intake (4.4% of live weight) was recorded for pigs fed broken rice and cassava leaves that had been ensiled before feeding (Chhay Ty et al 2003a). It would appear that ensiling the leaves increases their palatability; however, a comparison of fresh versus ensiled leaves in the same experiment does not appear to have been made. Also, in the experiment of Chhay Ty et al (2003), 3% of dried fish was in the diet and the pigs weighed only 9 kg. Both these differences could have accounted for the higher DM intakes as a percentage of live weight.

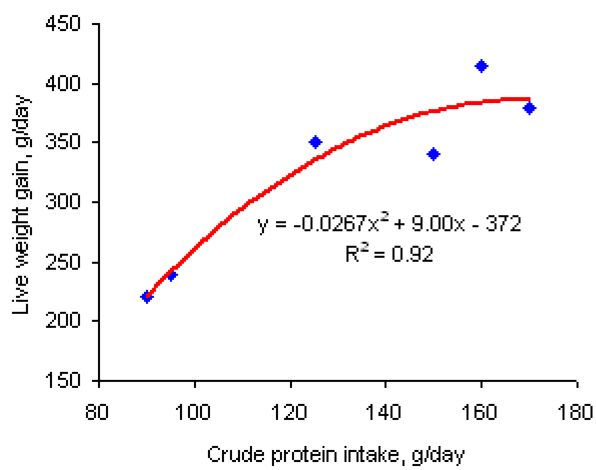

Figure 4: Relationship between crude protein intake and growth rate

Conclusion

In a feeding system for growing pigs (LW range 11 to 50 kg), with broken rice as the only other ingredient restricted to 2% of LW, and with supplements of water spinach, fresh cassava leaves or a mixture (400:60 DM basis) of the two:

- Fresh cassava leaves supplied up to 40% of the DM without apparent signs of toxicity of HCN.
- Fresh water spinach was eaten in greater quantities than fresh cassava leaves, when compared on a DM basis.
- The mixture of leaves was eaten in similar amounts (of DM) as for water spinach as the only supplement.
- The growth and conversion rates were 50% better for the diets with water spinach alone, or as a mixture with fresh cassava leaves, compared with fresh cassava leaves alone.
- There appear to be synergistic positive effects from combining two sources of protein-rich leaves as supplements to a low protein basal diet.
- Performance was not affected by supplementation with DL-methionine.

Acknowledgments

The authors would like to express their gratitude to the MEKARN project financed by the SIDA-SAREC Agency and to the Center for Livestock and Agriculture Development (CelAgrid UTA-Cambodia), for providing resources for conducting this experiment

References

AOAC (1990) Official Methods of Analysis. Association of Official Analytical Chemists.15th Edition (K Helrick editor). Arlington pp 1230

Bounhong Norachack, Soukanh Keonouchanh, Chhay Ty, Bounthong Bouahom & Preston T R (2004) Stylosanthes and cassava leaves as protein supplements to a basal diet of broken rice for local pigs. *Livestock Research for Rural Development.Vol. 16, Art. # 74.* Retrieved, from http://www.lrrd.org/lrrd16/10/boun16074.htm

Butler G W (1973) Physiological and genetic aspects of cyanogenesis in cassava and other plants, chronic cassava toxicity. Proceedings of the Interdisciplinary Workshop, London England, 29-30 Jan., 1973. IDRC -010e, pp. 65-71

Calpe C (1992) Root, tubers and plantains: recent trends in production trade and use. In: Machin F and Nyvild S (Editors) Root, tuber, Plantains and Bananas in Animal Feeding. FAO Animal Production and Health Paper 95, pp. 11-25

Chhay Ty, Preston T R & Ly J (2003) The use of ensiled cassava leaves in diets for growing pigs. 2. The influence of type of palm oil and cassava leaf maturity on digestibility and N balance for growing pigs. *Livestock Research for Rural Development 15 (8).* Retrieved June 30, 2005, from http://www.lrrd.org/lrrd15/8/chha158.htm

Chhay Ty & Preston T R (2005) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. *Livestock Research for Rural Development .Vol. 17, Art. #23.* Retrieved June 30, 2005, from http://www.lrrd.org/lrrd17/2/chha17023.htm

Du Thanh Hang & Preston T R (2005) The effects of simple processing methods of cassava leave on HCN content and intake by growing pigs. Proceedings of Workshop-seminar "Making better use of local feed resources" MEKARN-CTU, Cantho, 23-25 May, 2005. http://www.mekarn.org/procctu/hang.htm

Eggum B O (1970) The protein quality of cassava leaves. British Journal of Nutrition. 24: 761-768

Getter A O & Baine J (1938) Research on cyanide detoxification. American Journal of Medical Science. pp. 185-189

Johnson R M & Ramond W D (1965) The chemical composition of some Tropical food plants: Manioc. Tropical Science 7, pp. 109-115.

Kean Sophea & Preston T R (2001) Comparison of biodigester effluent and urea as fertilizer for water spinach vegetable. Livestock *Research for Rural Development* http://www.lrrd.org/lrrd13/6/kean136.htm

Ly Thi Luyen & Preston T R (2004) Effect of level of urea fertilizer on biomass production of water spinach (*Ipomoea aquatica*) grown in soil and in water. *Livestock Research for Rural Development. Vol.16, Art. #81*.Retrieved, from http://www.lrrd.org/lrrd16/10/luye16081.htm

Ly J, Hean Pheap, Keo Saeth & Pok Samkol (2002) The effect of DL-methionine supplementation on digestibility and performance traits of growing pigs fed broken rice and water spinach (*Ipomoea aquatica).* Livestock Research for Rural Development (14) 5: http://www.lrrd.org/lrrd14/5/ly145b.htm

Ly J (2002) The effect of methionine on digestion indices and N balance of young Mong Cai pigs fed high levels of ensiled cassava leaves. Livestock Research for Rural Development. (14) 2: http://www.lrrd.org/lrrd14/6/Ly146.htm

MINITAB (2000) Minitab Reference Manual release 13.31.

Nguyen Duy Quynh Tram & Preston T R (2004) Effect of method of processing cassava leaves on intake, digestibility and N retention by Ba Xuyen piglets. *Livestock Research for Rural Development.Vol.16, Art. # 80.*Retrieved, from http://www.lrrd.org/lrrd16/10/tram16080.htm

Prak Kea, Preston T R & Ly J (2003) Feed intake, digestibility and N retention of a diet of water spinach supplemented with palm oil and / or broken rice and dried fish for growing pigs. Livestock Research for Rural Development (15) 8 Retrieved, from http://www.lrrd.org/lrrd15/8/kea158.htm

Preston T R (2001) Potential of cassava in integrated farming systems. International Workshop. Current Research and Development on Use of Cassava as Animal Feed, Khon Kaen, Thailand July 23-24, 2001.
http://www.mekarn.org/procKK/pres.htm

Ravindran V, Kornegay E & Rajaguru E S (1987) Influence of processing methods and storage time on the cyanide potential of cassava leaf meal. Animal Feed Science and Technology 17:227-23

Tewe O O (1992) Detoxification of cassava products and effects of residual toxins on consuming animals. In: Roots, tubers, plantains and bananas in animal feeding. (D. Machin and S. Nyvold, editors) FAO Animal Production and Health Paper No 95. Rome p 81-98

Tran Hoang Chat, Ngo Tien Dung, Dinh Van Binh & Preston T R (2005) Water spinach *(Ipomoea aquatica)* as replacement for guinea grass for growing and lactating rabbits**;** Workshop-seminar "Making better use of local feed resources" (Editors: Reg Preston and Brian Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005. Article #48.Retrieved, from http://www.mekarn.org/proctu/chat48.htm

Undersander D, Mertens D R & Theix N (1993) Forage analysis procedures. National Forage Testing Association. Omaha pp 154

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CHAPTER IV

Effect of different ratios of water spinach and fresh cassava leaves on growth of pigs fed basal diets of broken rice or mixture of rice bran and cassava root meal

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Abstract

Twenty four crossbred (Local*Landrace or Local*Duroc) castrated male pigs weighing from 16.0 to 18.4 kg were used in a 2*3 factorial arrangement to study the effect of (i) different levels of substitution of wilted cassava leaves by water spinach; and (ii) a basal diet of broken rice or a mixture (50:50) of rice bran and cassava root meal. The feeding trial lasted for 120 days from 8 August to 6 December 2005. Higher intakes of water spinach and cassava leaves, and of total DM, were observed when the basal diet was broken rice rather than rice bran mixed with cassava root meal. Increasing the replacement of cassava leaves by water spinach from 10 to 30% resulted in increases in intake of the energy component, in the total quantity of foliage, and of total DM. Growth rates tended to be higher on the broken rice diet than on rice bran and cassava root meal; while the 30% level of water spinach was superior to the 10% level. The overall trend of live weight gain as a function of level of water spinach was curvilinear and positive, indicating a synergistic effect on performance from mixing increasing amounts of water spinach with fresh cassava leaves.

Key words: *cassava leaves, growth, broken rice, cassava root meal, rice bran, pigs, protein, water spinach*

Introduction

In a previous experiment (Chhay Ty and Preston 2005) with growing pigs fed a basal diet of broken rice, we showed that a supplement of a 40:60 mixture (DM basis) of water spinach (*Ipomoea aquatica*) and fresh cassava leaves (*Manihot esculenta*) supported growth rates that were 32% higher than the average of the growth rates on the two sources of foliages fed separately. In the present experiment the hypothesis to be tested was that lower proportions of water spinach would also have a synergistic effect on growth of pigs fed low protein basal diets.

Materials and methods

Location

The experiment was carried out from 8 August to 6 December 2005 in the Center for Livestock and Agriculture Development (CelAgrid-UTA Cambodia), located in Kandal village, Rolous Commune, Kandal Steung district, Kandal province, about 25km from Phnom Penh City, Cambodia .

Experimental animals, treatments and design

Twenty four crossbred (Local*Landrace or Local*Duroc) castrated male pigs with initial body weight from 16 to 18.4 kg were allocated to six treatments with 4 replicates per treatment in a 2*3 factorial arrangement. The factors were:

Energy

- Broken rice
- Cassava root meal mixed with rice bran

Protein supplement (Proportions of water spinach (WS) and fresh cassava leaves (FCL) in ratios (DM basis) of:

- WS 10% and FCL 40%
- WS 20% and FCL 30%

- WS 30% and FCL 20%

The pigs were allocated into 2 blocks according to live weight (means for each block were 14.6 and 20.3 kg). The nutritional treatments were applied at random within each block. The pigs were housed in individual pens with concrete floors and provided with feeders and drinking nipples. The pigs were vaccinated against Salmonella disease and were adapted to the feeds and the housing for 7 days before starting the experiment.

Feeding and management

Broken rice and rice bran are by-products of Cambodian rice mills and were available in the local area. Cassava root meal was purchased from a store in the city. Fresh cassava leaves were harvested every day from plots in CelAgrid (Photo 1) or they were purchased from a farmer near the center. The water spinach was purchased from traders who harvested it from lagoons receiving waste water from Phnom Penh city. The leaves plus stems of the water spinach and the leaves of cassava (after removing stems and petioles) were chopped into small pieces and wilted over-night and then mixed with the other ingredients of the diet (Tables 1 and 2) before being offered in 3 meals at 8.00, 12.00 and 17.00h. The amounts of feeds offered were based on the allowance of 40 g DM per 1 kg live weight of the pigs, with the broken rice (or mixture of rice bran and cassava root meal) supplying 50% and the mixtures of leaves providing the remainder.

Table 1: Chemical characteristics of the ingredients of the diets

	DM, %	N*6.25 , % in DM	HCN, mg/kg DM
Cassava leaves	29.8	27.1	351
Water spinach	8.47	31.1	-
Broken rice	86.5	7.90	-
Rice bran	89.4	10.8	-
Cassava root meal	97.2	3.12	-

Table 2: Composition (DM basis) and analysis of diets

	WS10BR	WS20BR	WS30BR	WS10RBCRM	WS20RBCRM	WS30RBCRM
Ingredients, % DM basis						
Water spinach	10.0	20.0	30.0	10.0	20.0	30.0
Cassava leaves	40.0	30.0	20.0	41.0	30.0	20.0
Broken rice	49.5	49.5	49.5			
Cassava root meal	-	-	-	24.0	25.0	25.0
Rice bran	-	-	-	24.5	24.5	24.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Analysis						
Dry matter, %	55.6	53.5	51.3	58.3	55.9	54..7
N*6.25, % in DM	17.8	18.3	18.6	17.6	17.8	18.2
HCN, mg/kg DM	14.0	10.5	3.51	14.4	10.5	3.51

Data collection and analyses

The pigs were weighed every 10 days during the trial which lasted 120 days. Individual feeds offered and residues were recorded daily. Samples of feeds and residues were analyzed for DM, N and HCN. The DM content was determined using the micro-wave method of Undersander et al (1993). N and HCN were determined following procedures of AOAC (1990).

Statistical analysis

Data for weight gain, DM feed intake, crude protein intake, HCN intake and feed conversion rate were analyzed using the general linear model (GLM) option of the ANOVA software of Minitab (2000). The sources of variation were blocks, level of water spinach, energy, interaction energy*level of water spinach and error.

Results and discussion

Feed intake

Higher intakes of water spinach and cassava leaves were observed when the basal diet was broken rice rather than rice bran mixed with cassava root meal (Table 3), with total DM intakes being higher for the latter when expressed on a live weight basis. Replacing cassava leaves with water spinach resulted in increases in intake of the energy component, and in the total quantity of foliage, but a decrease in the intake of cassava leaves.

Table 3: Mean values (main effects) for feed intake of pigs fed broken rice or rice bran + cassava root meal supplemented with mixtures of fresh cassava leaves and fresh water spinach

	Energy				Leve of water spinach					Interaction
	BR	**RBCRM**	**SEM**	**Prob.**	**WS10**	**WS20**	**WS30**	**SEM**	**Prob.**	**Prob.**
Intake of feed ingredients, g DM/day										
RBCRM	0	611	-	-	279	284	352	6.28	-	-
BR	586	0	-	-	275	277	327	4.37	-	-
CL	310a	288b	4.60	0.010	354^{a}	279b	264c	5.64	-	-
WS	264a	242b	3.69	0.001	127	248	384	4.53	-	-
Foliage DM	575	530	-	-	481	528	647	-	-	
Total DM	1160	1140	11	0.200	1036^{a}	1089b	1326c	13.9	0.001	0.07
g/kg LW	35.2a	37.3b	0.190	0.001	34.2^{a}	37.0b	37.6b	0.23	0.001	0.033
N*6.25, g/d	34.1a	31.3b	0.34	0.001	28.0a	30.8b	39.3c	0.42	0.001	NS
HCN										
mg/d	124a	115b	1.45	0.001	147^{a}	116b	96.6c	1.78	0.001	0.001
mg/kg LW	3.97	3.99	0.04	0.72	4.99^{a}	4.10b	2.86c	0.05	0.001	0.02

abc Means within main effects within rows without common letter are different at P<0.05

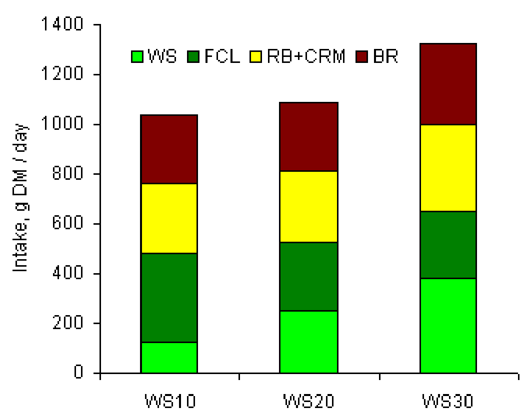

Figure 1: Relative intakes of diet ingredients according to degree of substitution of cassava leaves by water spinach

On a live weight basis the HCN levels consumed were 4.99, 4.1 and 2.86 mg/kg LW, for the water spinach 10, 20 and 30% levels, respectively. These were lower than in the earlier study (Chhay Ty et al., 2005) and in the range reported as potentially toxic: 1.4 by Getter and Baine (1938), 2.1 to 2.3 by Johnson and Ramond (1965), 3.5 by Tewe (1992) and 4.4% of LW by Butler (1973).

Growth and feed conversion

Growth rates tended to be higher on the broken rice diet than on rice bran and cassava root meal during the initial, final and overall trial period. For the periods 40 to 80, 80 to 120 and overall, the 30% level of water spinach was superior to the 10% level.

Table 4: Mean values for initial and final live weights, and growth rates, of pigs fed broken rice or a mixture of rice bran and cassava root meal supplemented with different levels of water spinach replacing fresh cassava leaves

	Energy				Water spinach, % replacement of cassava leaves				
Days	**BR**	**RBCRM**	**SEM**	**Prob.**	**WS10**	**WS20**	**WS30**	**SEM**	**Prob.**
Growth rate, g/day									
0-40	273	225	16.9	0.07	234	229	284	20.8	0.15
40-80	327	293	19.3	0.24	249[a]	321[ab]	360[b]	23.8	0.013
80-120	427	341	18.5	0.06	3394[a]	355[a]	456[b]	22.6	0.004
0-120	337	290	17.5	0.079	267[a]	306[ab]	367[b]	21.4	0.015
Live weight, kg									
Initial	17.7	17.3	0.69	0.67	18.0	16.0	18.4	0.84	0.13
Fina1#	58.2	50.2	2.3	0.025	49.7a	52.6a	60.4b	2.9	0.044

ab Means within rows within main effects, without common letter are different at P<0.05
 # Adjusted for differences in initial weight

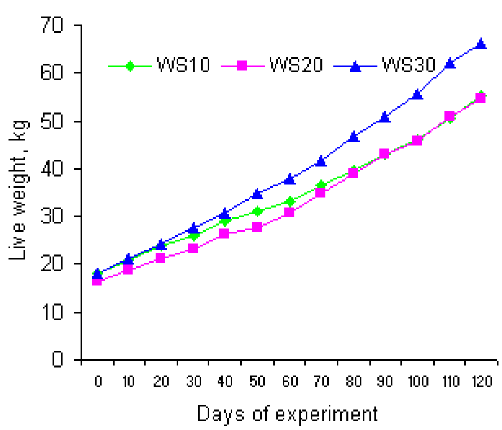

Figure 2: Growth curves of pigs fed basal diet of broken rice supplemented with different levels of water spinach (WS) replacing cassava leaves

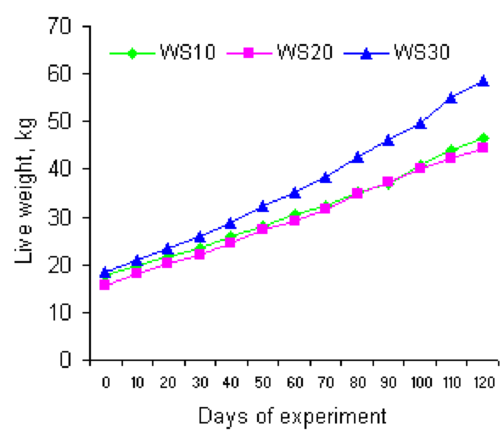

Figure 3: Growth curves of pigs fed basal diet of rice bran and cassava root meal supplemented with different levels of water spinach (WS) replacing cassava leaves

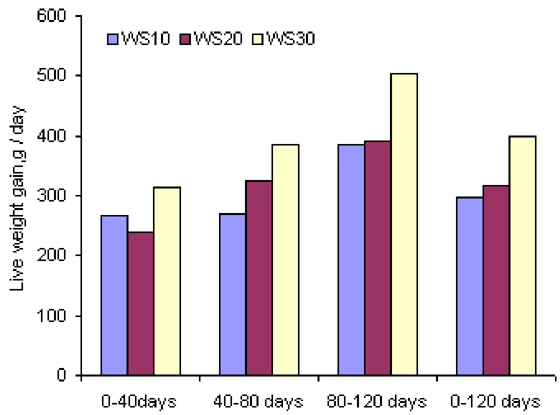

Figure 4: Growth rates of the pigs during successive 40 day periods and overall, according to supplementary level of water spinach with basal diet of broken rice

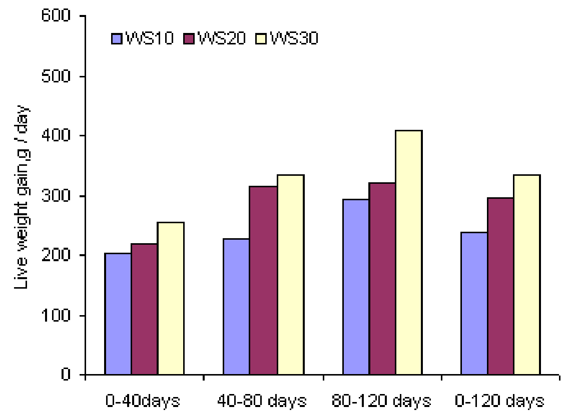

Figure 5: Growth rates of the pigs during successive 40 day periods and overall, according to supplementary level of water spinach with basal diet of rice bran and cassava root meal

The overall trend of live weight gain as a function of level of water spinach was curvilinear and positive (Figures 6 and 7), indicating that the rate of increase in live weight gain by replacing cassava leaves with water spinach was more marked the higher the level of water spinach in the diet. The growth curves in Figures 2 and 3 indicate a similar trend.

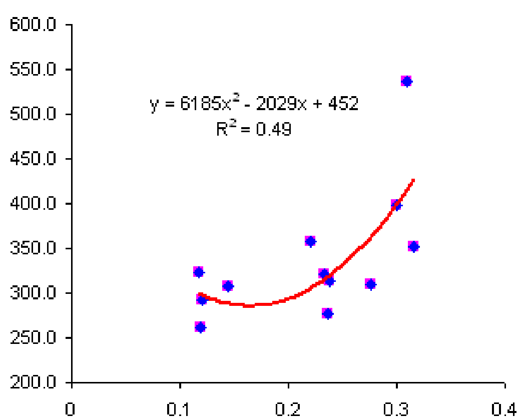

Figure 6: Relationship between growth rate and replacement of cassava leaves by water spinach on broken rice diet

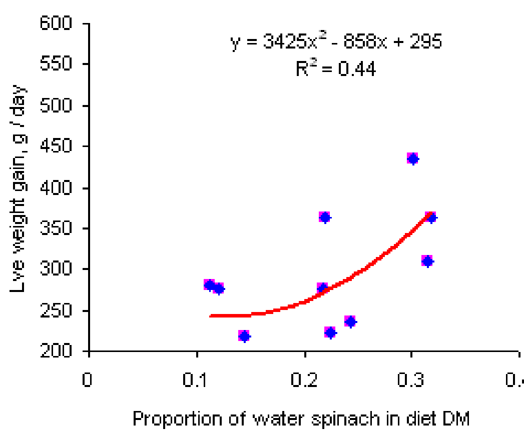

Figure 7: Relationship between growth rate and replacement of cassava leaves by water spinach on diet of rice bran and cassava root meal

Feed conversion was better on the basal diet of broken rice than on the mixture of rice bran and cassava root meal and tended to improve (P=0.12 for the overall trial period) with increasing levels of water spinach (Table 5).

Table 5: Mean values for DM feed conversion of pigs fed broken rice or a mixture of rice bran and cassava root meal supplemented with different levels of water spinach replacing fresh cassava leaves

Energy	Level of water spinach

Days	BR	RBCRM	SEM	Prob.	WS10	WS20	WS30	SEM	Prob.
0-120	3.44	4.06	0.07	0.001	3.91	3.70	3.65	0.08	0.12

Conclusions

- The synergistic effect on growth rate of pigs of replacing cassava leaves with water spinach, as the main protein source, was confirmed in that the relative response to substituting cassava leaves with water spinach increased as the degree of replacement was increased.
- There were no indications of HCN toxicity on any of the diets

Acknowledgments

The authors would like to express their gratitude to the MEKARN project financed by the SIDA-SAREC Agency and to the Center for Livestock and Agriculture Development (CelAgrid UTA-Cambodia), for providing resources for conducting this experiment

References

AOAC (1990) Official Methods of Analysis. Association of Official Analytical Chemists15th Edition (K Helrick editor). Arlington pp 1230

Butler G W (1973) Physiological and genetic aspects of cyanogenesis in cassava and other plants, Chronic cassava toxicity. Proceedings of the Interdisciplinary Workshop, London England, 29-30 Jan., 1973. IDRC -010e, pp. 65-71

Chhay Ty & Preston T R (2005) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. Livestock Research for Rural Development. Vol. 17, Art. #23.Retrieved June 30, 2005, from http://www.lrrd.org/lrrd17/2/chha17023.htm

Getter A O & Baine J (1938) Research on cyanide detoxification. American Journal of Medical Science. pp. 185-189

Johnson R M & Ramond W D (1965) The chemical composition of some Tropical food plants: Manioc. Tropical Science 7, pp. 109-115.

MINITAB (2000) Minitab Reference Manual release 13.31.

Tewe O O (1992) Detoxification of cassava products and effects of residual toxins on consuming animals. In: Roots, tubers, plantains and bananas in animal feeding. (D. Machin and S. Nyvold, editors) FAO Animal Production and Health Paper No 95. Rome p 81-98

Undersander D, Mertens D R & Theix N (1993) Forage analysis procedures. National Forage Testing Association. Omaha pp154

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CHAPTER V

Effect of processing cassava leaves and supplementing them with DL-methionine on intake, growth and feed conversion in crossbred growing pigs

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Abstract

Eighteen crossbred (local x landrace) castrated male pigs with initial mean body weights at 24.5±0.84 kg (range of 19 to 30 kg) were allocated to individual pens in 3 blocks according to body weight, and within blocks to a 2*3 factorial arrangement of 6 treatments. The first factor was with or without supplementary DL-methionine; the second factor was processing cassava leaves (dried, ensiled or fresh). The other dietary ingredients were rice bran, cassava root silage and dried fish. The feeding trial lasted for 90 days from 03rd November 2008 to February 2009. There was no health problem in any of the pigs during the whole of experiment. Total DM intake was increased by supplementation with DL-methionine and was highest when cassava leaves were ensiled. Cassava leaves provided close to 50% of the diet DM and over 60% of the dietary crude protein. HCN levels were highest on the treatment with fresh cassava leaves and lowest when the leaves were sun-dried. Growth rates were increased by methionine supplementation and tended to be lower when the cassava leaves were processed by sun-drying. There were no interactions between methionine supplementation and cassava leaf processing. Feed conversion ratio was improved when the cassava leaves were fed fresh compared with sun-drying, with intermediate values when the leaves were ensiled. There was no relationship between intake of cyanogenic glucosides (measured in terms of potential release of HCN) and performance traits.

***Key words**: Drying, ensiling, fresh leaves, HCN, toxicity*

Introduction

Early maturing cassava varieties (sweet) are cultivated by most rural families in Cambodia for root production. The leaves are seldom used for other purposes especially for animal feed. Cassava leaves are reported to be rich in lysine but limiting in DL-methionine (Eggum 1970). The cyanogenic glycosides in cassava leaves have been considered to be a limitation to their use for mono-gastric animals (Tewe 1992). Levels of hydrocyanic acid (HCN) produced by the action of hydrolytic enzymes on the cyanogenic glycocides that are present in the plant are influenced by the nutritional status and age of the plant (Ravidran and Ravindran 1988). However, several studies (Chhay Ty and Preston 2005a; Du Thanh Hang and Preston 2005; Du Thanh Hang et al 2009a, b) have shown that cassava leaves can be fed in the fresh state with no apparent toxicity. It has been suggested that the acid condition in the pig stomach may de-activate the enzymes that cause the release of the HCN (R ALeng, unpublished observations). Elemental suphur and the sulphur amino acids, cysteine and methionine, may also play a role in facilitating the detoxification of HCN into the non-toxic thiocyanate (Oke 1978).

The hypothesis that was tested in the following experiment was that in the case of feeding pigs with the fresh cassava leaves, there would be improvements in performance by also providing supplementary DL-methionine.

The objective of the study was to determine the effect of different ways of processing cassava leaves and supplementing them with DL-methionine, on intake, growth and feed conversion in crossbred growing pigs in Cambodia

Materials and methods

Location

The experiment was carried out from 03rd November 2008 and finished in 01st February 2009 at the Center for Livestock and Agriculture Development (CelAgrid), located in Phras Teat village, Rolous Commune, Kandal stung district, Kandal province about 25km from Phnom Penh City, Cambodia.

Experimental animals, treatments and design

Eighteen crossbred (local x landrace) castrated male pigs with mean body weight of 24.5±0.84 kg (range from 19 to 30 kg) were allocated to individual pens in 3 blocks according to body weight, and within blocks to a 2*3 factorial arrangement of 6 treatments. The first factor was supplementary DL-methionine (with or without) and the second factor was processing cassava leaves (dried, ensiled or fresh). The factors were

Methionine

- With
- Without

Cassava leaves processing

- Fresh
- Dried
- Ensiled

Individual treatments were

- DCL: Dried cassava leaves without supplementary DL-methionine
- ECL: Ensiled cassava leaves without supplementary DL-methionine
- FCL: Fresh cassava leaves without supplementary DL-methionine
- DCL-M: Dried cassava leaves plus 0.3% DL-methionine
- ECL-M: Ensiled cassava leaves plus 0.3% DL-methionine
- FCL-M: Fresh cassava leaves plus 0.3% DL-methionine

The other dietary ingredients were rice bran, cassava root silage and dried fish (Tables 1 and 2).

Table 1: Chemical composition of diet ingredients (based on samples taken prior to starting the experiment)

	% DM	DM basis	
		Crude protein, %	HCN, mg/kg DM
Rice bran	90.8	10.1	-
Cassava root silage	39.2	2.6	61.7
Dry cassava leaf	90.5	25.3	60
Ensiled cassava leaf	31.4	23.7	131
Fresh cassava leaf	25.4	25.7	378
Dried fish	91.5	47.3	-
DL-Methionine	100	55.7	-

Table 2: Composition of the diets (% DM basis; based on samples taken prior to starting the experiment)

	DCL	ECL	FCL	DCL-M	ECL-M	FCL-M
Rice bran	55	55	55	55	54	55
Cassava root silage	10	9	10.3	10	10	10.2
Dry cassava leaf	31	0	0	31	0	0
Ensiled cassava leaf	0	31	0	0	31	0
Fresh cassava leaf	0	0	31	0	0	31

Dry Fish	3	4	2.7	2.7	3.8	2.5
DL-Methionine	0	0	0	0.3	0.3	0.3
Salt (NaCl)	1	1	1	1	0.9	1
Total	100	100	100	100	100	100
% DM	84.7	66.9	64.3	84.7	66.5	64.4
% Crude protein in DM	15.1	15.0	15.1	15.1	15.0	15.1
HCN, mg/kg DM	2.48	4.63	12.4	2.48	4.69	12.4

The pigs were housed in individual pens (1.5m and 2m) with concrete floor and provided with feeders and drinking nipples. They were vaccinated against salmonella and swine fever and were de-wormed with ivomectin prior to being adapted to the feeds and the housing for 10 days before starting the experiment.

Feeds, feeding and management

Rice bran was purchased from the rice mill near CelAgrid center and cassava leaves and roots from farmers who plant cassava along the banks of the Mekong River. Cassava leaves were harvested when the plant was 3-4 months of age; roots were harvested after 6 months. The cassava variety, known locally as "DamlongKor" was a "sweet" variety. Dried fish and DL-methionine were purchased from animal feed shops in Phnom Penh city.

Stems and petioles were removed from the cassava foliage prior to processing by sun-drying (3-4 days exposure to sunlight) or ensiling. The sun-dried leaves were ground in a hammer mill. Ensiling of the leaves was done with 10 kg of rice bran plus 0.5g of salt for 100kg of fresh leaves and stored for one month before feeding. Cassava roots were cleaned with water and chopped and ensiled with 0.5 kg of salt for 100 kg of fresh roots. On the FCL treatments, the fresh cassava leaves, after removal of the stems and petioles, were chopped into small pieces and offered immediately to the pigs. The daily feed allowance was given in three meals at 7:30, 12.00 and 16.00h. The rice bran, cassava root silage, dried fish and DL-methionine were mixed together and given first followed by the cassava leaves which were placed in separate troughs. The total quantities offered were based on an expected total daily intake of 40 g DM per 1 kg LW. The basal diet (excluding the cassava leaves) was offered at approximately 28 g/kg LW and was fed first. When the pigs had eaten all the basal diet the cassava leaf component was fed at about 12 g DM/kg live weight. These offered levels were varied according to recorded intakes with the objective to minimize refusals and maintain the balance between basal diet and the cassava leaf fraction.

Data collection and analyses

The pigs were weighed every 10 days during the 90 days of the experiment. Feeds offered and residues were recorded daily. Representative samples of feeds offered and residues were taken one time per 10 days to estimate content of DM, N and HCN. DM was determined using the micro-wave procedure of Undersander et al (1993). N and HCN were analyzed following procedures of AOAC (1990).

Statistical analysis

Data of DM feed intake, weight gain and feed conversion rate were analyzed using the general linear model (GLM) option of the ANOVA software of Minitab 2000 version 13.31 (Minitab 2000). The sources of variation were blocks, processing, methionine, interaction processing * methionine and error. When the "F" test was significant (P<0.05) the means were separated by the Tukey test in the Minitab software. The model used was:

$Y_{ijk} = \mu + B_k + E_i + L_j + E_i*L_j + e_{ijk}$

μ = Overall mean

B_k= Block effect

E_i = Cassava leaves processing effect

L_j = With or without methionine supplementation effect

E_i*L_j = Interaction between cassava leaves processing * methionine supplementation

e_{ijk} = random error

Results and discussion

Feed intake

Total DM intake was increased by supplementation with DL-methionine and was highest when cassava leaves were ensiled. Cassava leaves provided close to 50% of the diet DM (Figure 1) and over 60% of the dietary crude protein (Figure 2). Earlier experiments with cassava leaves in pig diets were focused on reducing the risk of toxicity from hydrocyanic acid, arising from the cyanogenic glucosides present in high concentrations in the fresh leaves of most cassava varieties (Bui Huy Nhu Phuc et al 2001). In this experiment, HCN levels were highest on the treatment with fresh cassava leaves and lowest when the leaves were sun-dried. Expressed as daily intakes per kg LW, the HCN levels on the treatment with fresh cassava leaves were higher than the levels of 1.4 (Getter and Baine 1938) and 2.1 - 2.3 (Johnson and Ramond 1965) but slightly lower than the level recommended by Butler (1973) (4.4) although similar to that recommended by Tewe (1992) (3.5 mg/kg LW). However, in contrast to the reports by these authors, in this experiment fresh cassava leaves were fed with no signs of toxicity (Table 3) despite the high HCN levels.

Table 3: Mean values for feed intake of pigs fed dried, ensiled or fresh cassava leaves with or without DL-methionine (main effects)

	DL-methionine				Processing				
	With	**Without**	**SEM**	**P**	**DCL**	**ECL**	**FCL**	**SEM**	**P**
DM intake, g/day									
Rice bran	254	229	10.2	0.081	263a	250a	212b	12.5	0.013
Cassava root silage	166	150	1.96	0.001	161a	169a	145b	2.40	0.001
Cassava leaves	421	370	5.84	0.001	410b	482a	295c	7.15	0.001
Dried fish	50.0	44	0.55	0.001	47.9b	50.1a	42.9c	0.68	0.001
DL-Methionine	3.05	0.00	0.02	0.001	1.48c	1.51b	1.58a	0.02	0.003
Total	894	794	10.7	0.001	883b	951a	697c	13.0	0.001
DM intake, g/kg LW/day	27.4	25.8	0.43	0.01	28.2a	29.6a	21.9c	0.53	0.001
As % of diet DM									
Cassava leaves	48.7	48.0	0.62	0.440	49.0	51.7	44.5	0.76	0.001
Crude protein	18.1	17.9	0.10	0.060	18.3	17.7	17.9	0.13	0.002
As % of diet crude protein									
Cassava leaves	64.3	63.6	0.63	0.40	65.2	66.0	60.7	0.77	0.001
HCN, mg/day									
Cassava leaves	69.6	60.6	1.00	0.001	24.6c	59.2b	111.5a	1.23	0.001
Cassava roots	9.76	8.84	0.09	0.001	9.57b	9.82a	8.51c	0.11	0.001
Total	79.4	69.4	1.06	0.001	34.2c	69.0b	120.0a	1.30	0.001
HCN, mg/kg LW/day	2.31	2.12	0.02	0.001	1.04c	2.08b	3.52a	0.03	0.001

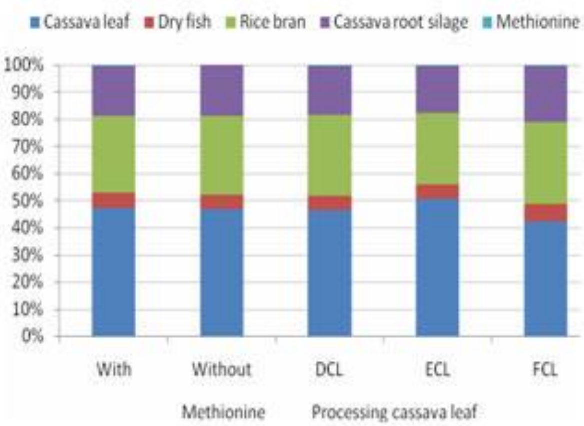

Figure 1: Relative DM intake from dietary ingredients

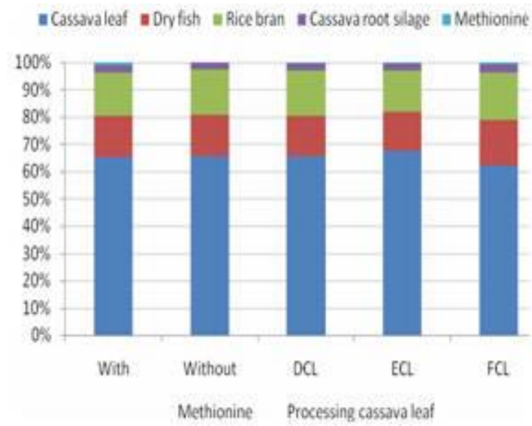

Figure 2: Relative crude protein intake from dietary ingredients

Live weight gain and feed conversion

Growth rates were increased by methionine supplementation and tended (P=0.10) to be lower when the cassava leaves were processed by sun-drying (Table 4). There were no interactions between methionine supplementation and cassava leaf processing. The growth curves (Figures 3 and 4) for the pigs on the different treatments suggest that the beneficial effects of DL-methionine and of fresh compared with dried cassava leaves were enhanced the longer the pigs were fed the diets.

Feed conversion ratio was improved when the cassava leaves were fed fresh compared with sun-drying, with intermediate values when the leaves were ensiled. The improved growth rate that resulted from DL-methionine supplementation supports the findings of Du Thanh Hang et al (2009a) and Chhay Ty et al (2009). The lack of interaction between DL-methionine supplementation and processing was in contrast to a previous study, when DL-methionine supplementation was only effective with fresh cassava leaves and not with leaves wilted for 24h (Chhay Ty et al 2009). The lack of relationship between intakes of cyanogenic glucosides and performance traits confirmed several findings that fresh cassava leaves can be fed safely to growing pigs (Chhay Ty and Preston 2005a; Chhay Ty et al 2009; Du Thanh Hang and Preston 2005; Nguyen Thi Hoa Ly 2005; Nguyen Thi Loc and Le Khac Huy 2003). The fact that growth rate and DM conversion rates were best for the treatment with fresh leaves and poorest for sun-dried leaves, is in marked contrast with estimated HCN intakes which were highest on fresh leaves (3.52 mg/kg live weight) and lowest on sun-dried leaves (1.04 mg/kg live weight). These findings indicate that sun-drying or ensiling of cassava leaves may have negative effects on the biological value of the leaves. The fermentation taking place in ensiled forages converts soluble carbohydrates to organic acids and also promotes proteolysis (McDonald 1981). Oshima and McDonald (1978) considered that, after ensiling, 10 to 25% of the total nitrogen in fresh forages would be non-protein nitrogen. There appear to be no comparable data on the ensiling of cassava leaves but it is probable that some loss in protein quality occurs during the ensiling process.

The detoxification of HCN in the animal body needs a sulphur-donor to facilitate the conversion of hydrogen cyanide to non-toxic thiocyanate (Oke 1973). DL-methionine would fulfill this purpose and this could be the explanation for the better growth rate when this synthetic amino acid was added to the diets. However, the lack of an interaction between methionine supplementation and cassava processing, does not supportive such as hypothesis, especially when HCN intakes were almost 3 times higher when the cassava leaves were fed fresh compared with the sun-dried form. The improvement in the amino acid balance resulting from methionine supplementation would seem to be a more probable explanation for the beneficial effects of methionine supplementation on growth rates for all the diets (Figure 5).

Table 4: Mean values for live weight gain of pigs fed dried or ensiled or fresh cassava leaf with or without methionine

	DL-methionine				Processing					Interaction
	With	**Without**	**SEM**	**P**	**DCL**	**ECL**	**FCL**	**SEM**	**P**	**P**
Initial, kg	24.8	24.2	1.40	0.784	24.7	24.2	24.7	1.71	0.972	0.908
Final, kg	52.8	48.8	2.51	0.183	46.0	51.2	52.2	3.08	0.349	0.806
Daily gain, g	312	248	18.8	0.033	236	298	306	23.1	0.107	0.696
DM conversion	2.93	3.35	0.23	0.23	3.92^{a}	3.20^{ab}	2.32^{b}	0.29	0.007	0.25

^{ab} *Means in same row without common superscript are different at P<0.05*

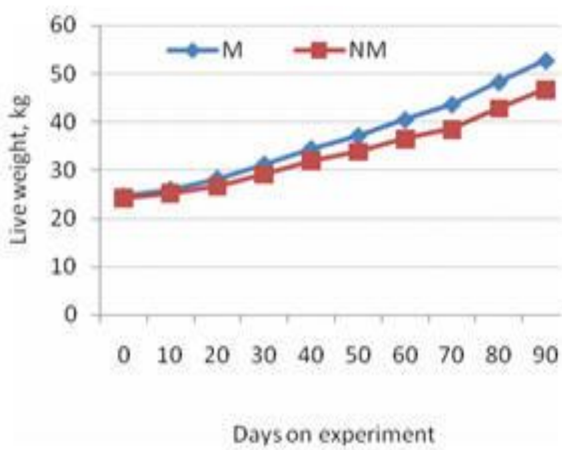

Figure 3: Growth curves of pigs according to supplementation with methionine (M) or none NM)

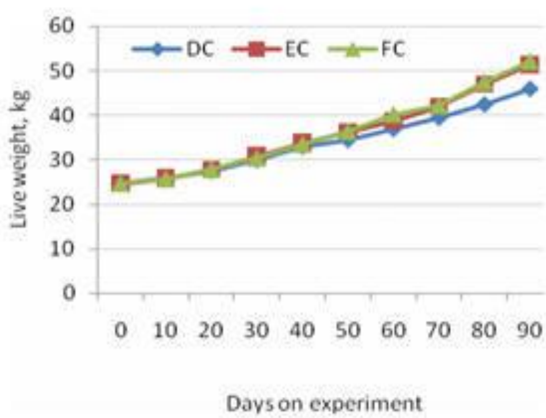

Figure 4: Growth curves of pigs according to processing of the cassava leaves

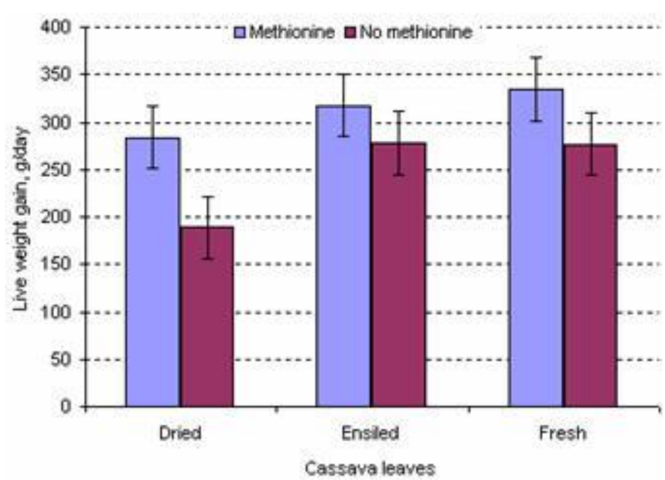

Figure 5: Effect of supplementary methionine on growth rate of pigs fed fresh, dried or ensiled cassava leaves

Conclusions

- Growth rates were higher for DL-methionine supplementation and were better when the cassava leaves were fed fresh compared with being sun-dried.
- DM feed conversion was not influenced by DL-methionine supplementation but was best when cassava leaves were fed fresh with poorest conversion registered for the treatment with sun-dried leaves.
- There was no relationship between intake of cyanogenic glycosides (measured in terms of potential release of HCN) and performance traits.

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References

AOAC (1990) Official Methods of Analysis. Association of Official Analytical Chemists.15th Edition (K Helrick editor). Arlington pp 1230

Bui Huy Nhu Phuc, Ogle B & Lindberg J E (2001) Nutritive value of cassava leaves for monogastric animals. International Workshop on Current Research and Development on Use of Cassava as Animal Feed. KhonKaen University, Thailand, July 23-24, 2001 http://www.mekarn.org/procKK/phuc.htm

Butler G W (1973) Physiological and genetic aspects of cyanogenesis in cassava and other plants, chronic cassava toxicity. Proceedings of the Interdisciplinary Workshop, London England, 29-30 Jan., 1973. IDRC -010e, pp. 65-71

Chhay Ty & Preston T R (2005a) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. Livestock Research for Rural Development. Vol. 17, Art. No. 23.http://www.lrrd.org/lrrd17/2/chha17023.htm

Chhay Ty, Borin Khieu & Preston T R (2009) Effect of wilting cassava leaves and supplementing them with DL-methionine, on intake, growth and feed conversion in growing pigs. Livestock Research for Rural Development. Volume 21, Article #8. http://www.lrrd.org/lrrd21/1/chha21008.htm

Du Thanh Hang & Preston T R (2005) The effects of simple processing methods of cassava leave on HCN content and intake by growing pigs. Livestock Research for Rural Development. Volume 17, Article No. 99. http://www.lrrd.org/lrrd17/9/hang17099.htm

Du Thanh Hang, Linh Nguyen Quang, Everts H & Beynen A C (2009a) Ileal and total tract digestibility in growing pigs fed cassava root meal and rice bran with inclusion of cassava leaves, sweat potato vine, duckweed and stylosanthes foliage. Livestock Research for Rural Development. Volume 21, Article #12. http://www.lrrd.org/lrrd21/1/hang21012.htm

Du Thanh Hang, Linh Nguyen Quang, Preston T R, Everts H & Beynen A C (2009b) Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source. Livestock Research for Rural Development. Volume 21, Article #15. http://www.lrrd.org/lrrd21/1/hang21015.htm

Eggum B O (1970) The protein quality of cassava leaves. British Journal of Nutrition. 24: 761-768
Getter A O & Baine J (1938) Research on cyanide detoxification. American Journal of Medical Science. pp. 185-189

Johnson R M & Ramond W D (1965) The chemical composition of some Tropical food plants: Manioc. Tropical Science 7, pp. 109-115.

McDonald P (1981) Biochemistry of Silage. John Wiley and Sons Ltd 226 pages

MINITAB (2000) Minitab reference Manual release 13.31. User's guide to statistics. Minitab Inc., USA

Nguyen Thi Hoa Ly (2005) Effect of supplementation of l-lysine and dl-methionine on the utilization of diets containing ensiled cassava leaves as a protein source for pigs in central Vietnam. Workshop-seminar "Making better use of local feed resources" (Editors: Reg Preston and Brian Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005. Article #24.Retrieved July 17, 109, from http://www.mekarn.org/proctu/hoal36.htm

Nguyen Thi Loc & Le Khac Huy (2003) Effects of DL-methionine supplementation levels in ensiled cassava root-based diets on the performance and economic efficiency of F1 (Large White x MongCai) fattening pigs. In: Proceedings of Final National Seminar-Workshop on Sustainable Livestock Production on Local Feed Resources (Editors: Reg Preston and Brian Ogle). HUAF-SAREC, Hue City, 25 – 28 March, 2003. Retrieved July 17, 109, from http://www.mekarn.org/sarec03/loc1.htm

Oke O L (1973) The mode of cyanide detoxification. In McIntyre E, Nestle C Editors: Proceedings of an interdisciplinary workshop on *chronic cassava toxicity*, London, England, 29–30 January

Oke O L (1978) Problems in the use of cassava as animal feed. Animal. Feed Science and Technology. 3:345-380.

Oshima M & McDonald P (1978) A review of the changes in nitrogenous compounds of herbage during ensilage. Journal Science Food Agriculture; 29:497-505

Ravindran V & Ravindran G (1988) Changes in the nutritional composition of cassava (*Manihotes Culenta* Crantz) leaves during maturity. Food Chemistry 27:299-239

Tewe O O (1992) Detoxification of cassava products and effects of residual toxins on consuming animals. In: Roots, tubers, plantains and bananas in animal feeding. FAO Animal Production and Health Paper 95. http://www.fao.org/Docrep/003/T0554E/T0554E06.htm

Undersander D, Mertens D R & Theix N (1993) Forage analysis procedures. National Forage Testing Association. Omaha pp 154

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CHAPTER VI

Effect of fresh cassava leaves and water spinach as protein sources in diets on N and amino acid digestibility in growing crossbred pigs

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Hue University Journal of Sciences (in press)

Abstract

The objective of this experiment was to study effects of fresh cassava leaves and water spinach as protein sources in diets on N and amino acid digestibility in growing crossbred pigs. A balance experiment was arranged as a Latin-square design and included 4 male castrates of 50.3 (±1.44) kg live weight. Four treatments included diets based on rice bran, broken rice and fishmeal (as the control diet – CTRL), and with fresh cassava leaves (FCL) or fresh water spinach (WS) or mixture of WS and FCL with ratio 1:1 (WSFCL) as the main protein source. Feed intake and nutrient digestibility were measured. Results showed that control diet had higher feed intake than diets with fresh cassava leave, water spinach and mixture of fresh cassava leave and water spinach as the main protein source. Fresh water spinach was more palatable than the cassava leaves as reflected in higher total dry matter intake but not differences in proportion of the diet (26.7% for fresh cassava leaves and 26% for fresh water spinach and 26.3% for fresh cassava leave mix with water spinach). Digestibility of DM, OM, N, CF, NDF and essential amino acid in WS diet was higher than those in other treatments. There appeared to be some synergic effects between fresh cassava leaves and the fresh water spinach as the N retention of pigs fed the mixture fresh water spinach and fresh cassava leave diet (21.0g/day) was almost as high as on the fresh water spinach diet (24.6g/day) and higher than cassava leave diet (16.7 g/day). In conclusion, fresh water spinach and mixing water spinach with cassava leaves could be a good protein sources for pigs.

Key words: *Digestibility, growing pigs, fresh cassava leaves, water spinach*

Introduction

In Cambodia, pig production is important for poor farmers in terms of main family income sources, money savings and festivals. Normally, farmers keep around 1-3 heads/ household. Most still practice the traditional scavenging system or a form of semi confinement using poor quality rice by-products without protein/amino acid supplementation and local and/or crossbred between local and exotic breeds. For these reasons pigs grow slowly and take a long time to reach slaughter weight (CelAgrid, 2006a).

Recently, many researchers have developed systems to use protein-rich forages for pigs to replace expensive and imported fish meal and soybean meal. Several studies have been done on amino acid profiles of forages rich in protein and found that Lysine, Isoleucine and Leucine are limiting amino acids for pigs (Le Duc Ngoan and Lindberg, 2001; Le Van An et al., 2003). Apparent ileal digestibility of some local protein sources has been studied in growing pigs (Bui Huy Nhu Phu, 2000; Le Duc Ngoan and Lindberg, 2001; Le Van An et al., 2003). Positive effects of supplementation of Methionine and Lysine in diets based on cassava foliages were reported by Nguyen Thi Loc and Le Duc Huy, (2003) and Du Thanh Hang et al, (2009b). Mixing cassava leaves with water spinach in pig diets led to improve intake and animal growth rates (paper 1 and 2). However, it is not known if this is due to improving digestibility of the protein in water spinach and cassava leaves or an improvement in the amino acid profile balance in the diets.

The aim of the present experiment was therefore, to study the total tract digestibility of DM, N, amino acids, OM, CF and NDF on crossbred pigs fed diets contained the fresh cassava leaves, fresh water spinach and a mixture of fresh water spinach and cassava leaves as protein sources with a basal diet of rice bran, broken rice and fish meal in Cambodia.

Materials and Methods

Experimental animal and treatments

Four crossbred (Hainam breed x Large White) castrate male pigs with a body weight of 50.3kg ±1.44 (Mean ±SEM) were used in this study. There were four dietary treatments, including Control diet (CTRL), Fresh cassava leaves (FCL), Fresh water spinach (WS), and Mixture of fresh water spinach and fresh cassava leaves (WSFCL) (50:50 DM basis). The control diet was composed of fish meal (FM), broken rice (BR), rice bran (RB) and premix, while other foliages treatment were composted of the same ingredients in CTRL treatment but low level of fish meal in the diets, however, all diets were balance crude protein and energy. The treatments were:

CTRL: Control diet

FCL: Fresh cassava leaves

WS: Water spinach

WSFCL: Mixture of fresh water spinach and fresh cassava leaves (50:50 DM basis)

Experimental feeds and feeding

The cassava variety used in this experiment was known locally breed as called Damlong Kor (sweet variety) and was harvested everyday for pigs after planting 3-4 months. Stems and petioles were removed from the leaves and then the leaves were chopped into small pieces (about 2-3cm) and offered to pigs immediately. Water spinach was chopped into small pieces and offered immediately at the same time as the fresh cassava leaves (FCL).

The daily feed offer in dry matter was calculated based on feed consume daily and varied from 2.5-4.5% of the body weight. Pigs were daily fed three times at 8:00, 12:00 and 17:00h and given as a wet mash. The amount of feed offer was reduced if refusals were observed. Fish meal (FM), broken rice (BR), rice bran (RB) and premix were thoroughly mixed together and then fed to pig first to prior foliage (FCL, WS and mixture WSFCL). The mixture of FCL and WS were in the ratio 50:50 in DM basis (the proportion used in this experiment was different with proportion used in experiments number 1 and 2 (40:60 and 60:40 in DM). The reason is that the results from experiment number 1 and showed both proportion (40:60 and 60:40 in DM) had no effluence on live weight gain of pigs. Water was permanently supplied through low pressure nipples. The animals were weighed at the beginning of the trial and every 12 days. Basal diet was very homogenized, through this method, intake of feed ingredients were calculated, if feed refusals were observed.

Table 1: Ingredient content and chemical composition of the diets

	Diets[1]			
	FCL	**WS**	**WSFCL**	**CTRL**
Ingredients, % DM basis				
Cassava leaves	28	0	14	0
Water spinach	0	28	14	0
Fish meal	6	3	4.5	13.5
Broken rice	43	27	35	25.5
Rice bran	21	40	30.5	59
Bio premix 17*	2	2	2	2
Total	100	100	100	100
Calculated chemical composition in % DM basis[#]				
Dry matter	72.6	67.9	70.3	90.4
Organic matter	92.3	89.4	90.8	88.5
Crude protein	17.1	17.2	17.2	17.2
Crude fiber	8.4	15.2	11.8	15.2
Neutral detergent fiber	23.7	31.7	27.7	31.7

Ca	0.750	0.616	0.683	0.962
P	0.621	0.609	0.615	0.880
ME, Kcal/kg	3070	2932	3001	3008
HCN, mg/kg	100.2	0.0	50.1	0.0
Arginine	0.809	0.797	0.803	0.831
Histidine	0.303	0.344	0.324	0.354
Isoleucine	1.133	1.023	1.078	1.092
Leucine	0.670	0.644	0.657	0.690
Lysine	0.551	0.537	0.544	0.516
Methionine	0.199	0.173	0.186	0.187
Phenylalanine	0.752	0.698	0.725	0.766
Threonine	0.398	0.331	0.364	0.309
Valine	0.630	0.635	0.632	0.709

*Amount per kg: Vitamin A: 2800000UI, Vitamin D_3: 324000UI, Vitamin E: 2100UI, Vitamin B_1: 240mg, Vitamin B_2: 440mg, Vitamin B_{12}: 3200mcg, Biotim: 4800mcg, Pantothenic Acid: 2000mg, Fe: 25200mg, Cu: 14400mg, Zn: 37800mg, Mn: 10800mg, I: 252mg, Se: 216mg

[1] CTRL= Control diet; FCL= Fresh cassava leaves; WS= Water spinach; WSFCL= Mixture of fresh water spinach and fresh cassava leaves (50:50 DM basis)

[#] Derived from chemical analysis of samples during experiment and from reference

Experimental design

The experiment was conducted in a 4*4 Latin Square (Figure 1) with the experimental period of 12 days comprising 7 days of adaptation to each diet followed by 5 days to collect feces, feed refusal and urine. All pigs were housed in metabolism cages during the whole trial (48days).

Period/Animal	**1**	**2**	**3**	**4**
1	WSFCL	WS	FCL	CTRL
2	WS	FCL	CTRL	WSFCL
3	FCL	CTRL	WSFCL	WS
4	CTRL	WSFCL	WS	FCL

Figure 1: Experimental layout

Sample collection

Samples of feeds offered, refusals (in case), urine and faeces were collected daily and weighed, and 10% of the collected amount as faecal sample were stored at -18^{0}C until the end of each collection period. A representative sample was obtained from every treatment, mixed thoroughly by hand and then homogenized in a coffee grinder and dried at 60^{0}C prior to analyses of DM, OM, HCN, N CF, NDF and amino acids. Urine was collected in a plastic bucket to which sulfuric acid was added to maintain the pH below 4.0 (20ml of concentrated H_2SO_4). The volume of urine was measured every day and 10% of the total volume was stored at -18^{0}C until the end of each period, when a sample was taken for N analysis.

Chemical analyses

The chemical composition of the diets, faeces and urine was determined using the Association of Official Analytical Chemists (AOAC) methods (1990). Dry matter (DM) was measured by drying fresh samples at 60^{o}C for 24 hours. Total nitrogen (N) was determined on fresh samples by the Kjeldahl method and CP was calculated from total nitrogen (N*6.25). Crude fibre (CF) and Neutral detergent fiber (NDF) was determined according to Van Soest et al, (1991). All samples were analyzed in duplicates. Amino acids of feed ingredient and faeces were analyzed according to Spackman et al, (1958) on an ion exchange column using HPLC. Samples were weighed as mg and then adding 100 μl HCl 6N to hydrolyze at 110°C for 22 hours, then checking pH with V ml Na2CO3 and take 25 μl of mix add 100 ml reagent l to load of sorbet tip and elute sample by adding 50μl iso-octane and 100μl chloroform to emulsify sample, transfer part of the organic layer in vial to evaporate, after that re-dissolve in 1 ml HPLC mobile to LC/MS analysis. (Standard 20 nmol/ ml)

%AAs: (S_{sample} x $C_{standard}$ x V_{first} x V_{final} x M)/ ($S_{standard}$ x V_{taked} mix x W (g)).

Statistical analyses

Analysis of variance was performed according to a 4*4 Latin-square design using the general linear models of Minitab statistic software (Minitab 2007 version 15.12). Sources of variation were: column, row, treatments and random error. When the *F*-test was significant at P<0.05, pair wise comparisons were performed using Turkey's procedure (Minitab Statistical Software). The model used was:

$Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$

where, Yijk = Dependent variables

μ = overall mean

T_i = treatment effect (i=1-4)

P_j = column effect (j=1-4)

A_k = row effect (k=1-4)

e_{ijk} = random error

The relationship between N intake and N retention was developed by regression analyses. The best model was selected based on adjusted R^2.

Results and discussion

Chemical composition of feeds ingredients

Chemical compositions of feed ingredients were determined during the trial and indicated some remarkable differences between fresh cassava leaves and fresh water spinach. The content of DM, OM and NDF in fresh cassava leaves was significantly higher than those in water spinach but it was in the other way around for CP and CF. DM contents of basal diet ingredients were almost similar, around 90%. The highest content of CP was found in fish meal with 59.9%. Crude fiber was not found in broken rice, while it was 25% in rice bran. Organic matter and CP content of water spinach in our study were in agreement with Bui Huy Nhu Phuc, (2000) with 88.8 and 26.4%, respectively, but NDF content was higher, 38.7% compared with 22.9%. HCN content in this study was 357.7 mg/kg DM that was in agreement with experiment 2 and 3 (351 and 378mg/kg DM, respectively) and higher than the study of Nguyen Duy Quynh Tram and Preston, (2004) with 269mg/kg DM.

Table 2: Chemical composition of the dietary ingredients (in DM basis)

	Ingredients[1]				
	FCL	**WS**	**FM**	**BR**	**RB**
Dry matter, %	29.4	10.6	90	88.1	91.2
Organic matter, %	93.9	87.4	76.4	99	89.7
Crude protein, %	26.1	29.8	59.9	8.78	11.7
Crude fiber, %	10.5	18.3	3.2[b]	0	25.0
NDF, %	43.9	38.7	10.3	0	51.3
HCN, mg/kg	357.7	-	-	-	-
ME Kcal/kg	2532[a]	2533[a]	2866[a]	3704[f]	2842[a]
Ca, %	1[c]	0.9[d]	5.0[e]	0.2[f]	0.4[g]
P, %	0.5[c]	0.5[d]	2.7[e]	0.4[f]	0.7[g]
Essential amino acid, %					

Arginine	0.923	0.761	0.811	0.722	0.911
Histidine	0.399	0.417	0.483	0.175	0.414
Isoleucine	1.543	1.285	2.560	0.830	0.906
Leucine	0.922	0.820	1.548	0.435	0.627
Lysine	0.759	0.723	0.921	0.424	0.480
Methionine	0.215	0.148	0.241	0.203	0.174
Phenylalanine	1.018	0.845	1.745	0.511	0.679
Threonine	0.725	0.687	1.474	0.199	0.101
Valine	0.817	0.793	1.705	0.378	0.648
Essential amino acid, in g/16g N					
Arginine	3.562	2.572	1.354	8.187	7.796
Histidine	1.526	1.408	0.807	1.985	3.545
Isoleucine	5.908	4.333	4.276	9.423	7.754
Leucine	3.544	2.765	2.586	4.934	5.367
Lysine	2.924	2.443	1.539	4.816	4.107
Methionine	0.812	0.497	0.403	2.307	1.488
Phenylalanine	3.909	2.859	2.914	5.796	5.807
Threonine	2.789	2.328	2.462	2.256	0.864
Valine	3.148	2.677	2.848	4.294	5.547
Tot EAA	28.122	21.883	19.188	43.999	42.275

[1].FCL: Fresh cassava leaves; WS: Fresh water spinach; FM: Fish meal; BR: Broken rice; RB: Rice bran; NDF= Neutral detergent fiber; HCN= Hydrogen cyanic acid, Metabolisable energy;

[a.] Nguyen T H Ly et al, (2010); [b]. Thuy et al, (2011); [c] Gohl (1994); [d.] Le Thi Men et al, (2000); [e.] David, (1991); [f.] NIAH, (1995); [g.] Chanphone and Choke, (2003)

The essential amino acids in cassava leaves have similar value with water spinach. Both forages (cassava leaves and water spinach) have closed value of amino acid with fish meal except Iso, Leu, Lys, Phe, Thr and Val, which were higher in fish meal. Amino acid profile in broken rice were similar value with rice bran but in rice bran was high on Arg, His, Leu, Phe and Val. However, the amino acid content in water spinach and cassava leaves in this study were lower than other studies of Bui Huy Nhu Phuc, (2000); Le Thi Men and Preston, (2005) on water spinach and Bui Huy Nhu Phuc, (2000), Woolfe, (1992), Bui Huy Nhu Phuc and Lindberg, (2001) on cassava leaves. The total essential amino acids of water spinach and cassava leaves in this study were 22% and 28%, respectively which were lower than above studies nearly twice times (over 45%).

Table 3: Essential amino acid in water spinach

	g/16g N	% in DM	% in DM
	a	b. Compost	b. Biodigester
Arginine	6.0	1.25	1.12
Histidine	1.6	0.55	0.60
Isoleucine	3.8	1.51	1.37
Leucine	7.2	1.66	1.70
Lysine	4.2	0.97	0.89
Methionine	1.7	0.45	0.57
Phenylalanine	5.2	1.10	1.11
Threonine	4.1	1.09	0.96
Tyrosine	3.7	-	-
Valine	5.2	1.49	1.45

[a]. Bui Huy Nhu Phuc, (2000), [b]. Le Thi Men and Preston, (2005)

Table 4: Essential amino acid in cassava leaves (g/16g N)

	Sun dried (a)	Ensiled (a)		b	C
Arginine	6.5	5.6	Arginine	-	5.9
Histidine	1.8	1.7	Histidine	2.2	1.9
Isoleucine	4.2	4.2	Isoleucine	4.9	4.4
Leucine	8.3	8.3	Leucine	8.6	8.0
Lysine	5.5	5.4	Lysine	6.2	5.6
Methionine	1.6	1.4	Methionine + Cystine	2.8	1.5
Phenylalanine	6.2	5.6	Phenylalanine + Tyrosine	9.4	5.7
Threonine	4.1	3.9	Threonine	4.7	4.0
Tyrosine	4.4	4.4	Tryptophan	1.5	-
Valine	5.6	5.3	Valine	5.7	5.3
Σ EAA	48.2	45.8	Σ EAA	46.0	42.3

[a].Bui Huy Nhu Phuc, (2000), [b]. Woolfe, (1992), [c]. Bui Huy Nhu Phuc and Lindberg (2001)

Amino acid content in fish meal in this study was lower than fish meal that has protein content 64.5, and 62.2% which studied by Famino et al, (2004), NRC, (1994), respectively. The low crude protein content of fish meal in present study might be due to fish meal sellers who put some material such as sand or soil or other heavy materials in order to get a high profit, even though, CP content is high (59.9%) but this value can be also effect by adding some materials such as urea, animal hair or skin or nail etc.

Table 5: Essential amino acid in fish meal (% as DM)

	a. % DM		b. % of CP
%CP in DM	64.5	65	62.2
Arginine	3.61	3.81	4.02
Histidine	1.55	1.59	1.34
Isoleucine	2.10	3.06	2.72
Leucine	4.14	4.98	4.36
Lysine	4.14	5.07	4.53
Methionine	1.09	1.95	1.68
Phenylalanine	2.24	2.75	2.28
Threonine	2.38	2.82	2.57
Valine	3.00	3.46	3.02

[a] Famino et al, (2004), [b]. Poultry NRC, 1994

Feed intake

Total intake of DM, CP, CF, NDF and intake (g/kg live weight) was highest in control diet (CTRL) and then water spinach (WS) and mixture of water spinach and fresh cassava leaves (WSFCL), and lowest in fresh cassava leaves (FCL) treatment. The DM intake as percentage of live weight (LW) on WS was 3.15% that is in agreement with Prak Kea et al, (2003), who used the same diet but lower than that reported by Chhay Ty and Preston, (2005a) on both WS and WSFCL. This is due to pigs used in previous studies fed broken rice at 2% of live weight (LW) and WS fed *ad libitum* while in the present study WS and other ingredients are offered based on formula which was planned during the experiment. However, feed intake as percentage of LW was improved when pigs fed mixture WSFCL as agreed with Chhay Ty and Preston, (2005a) and Bounhong et al, (2004). DM intake as percentage of LW in FCL (2.60%) in this study was ranged to that reported by Du Thanh Hang and Preston, (2005) (2.7 to 3.3%), for crossbred pigs fed a 2:1 mixture of ensiled cassava root and rice bran and fresh cassava leaves that had been washed prior to feeding and similar values reported by Nguyen Duy Quynh Tram and Preston, (2004) and Bounhong et al, (2004) (2.6 and 3.11%, respectively), who used similar diets as in the present study but no fish meal. However, a much higher intake (4.4%) was recorded for pigs fed broken rice and cassava leaves that had been ensiled before feeding (Chhay Ty et al., 2003b). It would appear that ensiling the leaves increases their palatability and the low voluntary intake could have been due to characteristics of fiber of ingredients and the presence of cyanogenic glycosides and tannins in fresh cassava leaves. According to Ravindran et al, (1987), the bitter taste of the cassava leaves could negatively influence pig intake.

The HCN intake in FCL was nearly twice higher than mixture WSFCL. This result is similar with the study of Chhay Ty and Preston, (2005a), who reported that, got up to 125mg/day on FCL alone compared with 83.9mg/day for mixture WSFCL. The intake of HCN (mg/kg live weight) (2.52) in this study was higher than that reported by Getter and Baine, (1938) with 1.4 mg/kg BW but lower than Butler, (1973) with 4.4 mg/kg LW; Tewe, (1992) with 3.5 mg/kg LW; Chhay Ty and Preston, (2005a) with 5.1 and 7.4 mg/kg LW for WSFCL and FCL, respectively but supplementing with 0.3% Met in diets. Du Thanh Hang, (2005) recorded that even higher intakes (from 6.0 to 15 mg/kg live weight) when feeding fresh cassava leaves with ensiled cassava root and rice bran. Gómez, (1991) suggested that 100 mg HCN/kg feed could be the permissible maximum level for animal. However, many studies mentioned above did not report any health problem or death of animals caused by HCN intoxication.

Table 6: Mean values of feed intake for pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two, as supplements to rice bran, broken rice and fish meal

	FCL	**WS**	**WSFCL**	**CTRL**	**SEM**	**Prob**
Mean value of BW, kg	59.6	59.9	59.8	59.4	0.228	0.459
Intake, g/day in DM basis						
Fresh cassava leaves	413.9	0	228.5	0	9.95	0.001
Fresh water spinach	0	482.2	221.8	0	5.57	0.001
Fish meal	95.3	56.9	79.1	296.2	5.69	0.001
Broken rice	683.3	511.6	615.5	559.4	19.8	0.001
Rice bran	333.7	757.9	536.4	1294.3	26.4	0.001
Premix	31.2	37.1	34.5	43.0	1.13	0.001
Total feed intake in DM basis						
g/day	1557.4^{a}	1845.6^{b}	1715.7^{ab}	2192.9^{c}	55.8	0.001
g/kg live weight#	26.0^{a}	31.5^{b}	29.6^{b}	37.1^{c}	1.05	0.001
Total crude protein intake in DM basis						
g/day	263.6^{a}	310.8^{b}	289.3^{ab}	377.9c	9.39	0.001
g/kg live weight#	4.39^{a}	5.30^{b}	4.99^{b}	6.40^{c}	0.18	0.001

Total crude fiber intake in DM basis						
g/day	132.7^{a}	279.6^{b}	200.9^{c}	333.1^{d}	7.74	0.001
g/kg live weight#	2.4^{a}	4.93^{b}	3.69^{c}	6.4^{d}	0.15	0.001
Total NDF intake in DM basis						
g/day	370.8^{a}	581.9^{b}	468.5^{c}	694.5^{d}	16.3	0.001
g/kg live weight#	6.43^{a}	10.2^{b}	8.42^{c}	12.9^{d}	0.32	0.001
HCN in DM basis						
mg/day	149.4	0	83.6	0	2.85	0.001
mg/kg live weight#	2.52	0	1.47	0	0.06	0.001

FCL= Fresh cassava leaves; WS= Fresh water spinach; WSFCL= Water spinach mix with fresh cassava leaves; CTRL= Control diet

Range of body weight (59.4 – 59.9 kg) used for calculation

abcd: Means within main effects within rows without common letter are different at P<0.05

Faecal characteristics in pigs fed fresh cassava leaves, fresh water spinach

Faeces of pigs fed CTRL diet had higher DM, CF and NDF contents than those of FCL, WS and WSFCL. However, faeces of pigs fed CTRL diet had lower contents of OM and N than other diets. A total fresh faeces excretion was not significant difference among treatments (P>0.05) but faeces excretion in dry matter was lowest in WS (P<0.001). The DM content of faeces, excretion in dry matter and water in this study were higher than previous studies reported by Chhay Ty and Preston, (2005a). This might be due to the LW of pigs used in this study (50.3±1.44kg) were heavier than study of Chhay Ty and Preston, (2005a) with 15.1kg. In addition, in that experiment pigs were fed broken rice at 2% of body weight and WS fed *ad libitum* while in the present study WS and other ingredients were offered based on formula which was planed during the experiment.

Table 7: Fecal characteristics in pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two, as supplements to rice bran, broken rice and fish meal

Express in % DM basis	FCL	WS	WSFCL	CTRL	SEM	Prob
Dry matter	33.5^{a}	24.1^{b}	27.4^{c}	34.9^{d}	0.22	0.001
Organic matter	80.8^{a}	80.5^{a}	81.4^{a}	76.0^{b}	0.45	0.001
Nitrogen	3.80^{a}	3.12^{b}	3.93^{a}	2.74^{c}	0.05	0.001
Crude fiber	24.6^{a}	30.4^{b}	24.9^{a}	35.0^{c}	0.36	0.001
Neutral detergent fiber	52.9^{a}	52.9^{a}	52.8^{a}	56.6^{b}	0.34	0.001
Faecal excretion, g/kg DM intake						

Fresh material	689.9	695.6	741.1	610.0^{6}	38.1	0.13
Dry matter	229.3^{a}	167.2^{b}	202.1^{ab}	212.8^{a}	11.9	0.001
Water	460.6^{a}	528.4^{ab}	539.0^{ab}	397.8^{ac}	27.2	0.001

FCL= Fresh cassava leaves; WS= Water spinach; WSFCL= Water spinach mix with fresh cassava leaves; CTRL: Control diet

^{abc}: Means within main effects within rows without common letter are different at P<0.05

Nutrient digestibility

Digestibility of DM, OM, N, CF and NDF in WS treatment was higher than other treatments of CTRL, WSFCL and the lowest was FCL. The low digestibility of FCL could be affected by anti nutrition compounds in this plant such as HCN and tannin because HCN is colorless, volatile and extremely poisonous when hydrocyanic acid is generated (Van Soest, 1994). However, Stosic and Kaykay, (1981) noted that small quantities of HCN ingest on a regular basis, though not large enough to cause mortality, might be sufficient to affect the general health and productivity of the animal. According to Humphreys, (1988), intake of feeds containing over 20mg HCN/100g is potentially dangerous to livestock. Tannins have been considered as anti-nutrients due to a range of adverse effects including reduced feed intake and digestibility (Chung et al., 1998 and Onwuka, 1992). Tannin content in cassava leaves is reported to increase with maturity and varied between cultivars, from 30-50g/kg DM (Ravindran, 1993).

However, the digestibility coefficient of DM for FCL in this study (77.1%) was similar to the value of 77% as reported by Chhay Ty et al, (2003b) when the cassava leaves was ensiled but lower than that reported by Nguyen Duy Quynh Tram and Preston, (2004) with 89% and Bounhong et al, (2004) with 84%. The digestibility of N in FCL was 67.3%, higher than that reported by Chhay Ty et al, (2005a) with 60% and Bounhong et al, (2004) with 65% but lower than that reported by Nguyen Duy Quynh Tram and Preston, (2004) with 74%. This might be due to low intakes of FCL and high intake of broken rice in their study, which would have the effect on increasing digestibility. In term of digestibility for DM and N in WS treatment was 83.3 and 80.6%, respectively, and ranged values with reported by Chhay Ty and Preston, (2005) with 89 and 82% for DM and N, respectively for pig fed WS 47% in the diet. Prak Kea et al, (2003) reported that DM and N digestibility of diet with 35% water spinach as the only supplement to broken rice (in DM) were 89 and 77%, respectively.

The digestibility of CF and NDF was observed high for WS treatment than other treatments especially CTRL treatment, this could be cause of high feed intake and fiber intake in CTRL diet. Ogle, (2006) mention that high CF content in the diets reduces the nutrient digestibility, especially of CP, fibrous feeds in the diet also lead to an increased rate of passage of digesta through the gut and reduced ileal and total tract digestibility and affect on gut size and development, particularly the large intestine (An et al., 2004 and Jorgensen et al., 1996). Nearly all CF digestion takes place in the caecum and colon, where bacteria break down fermentable carbohydrates that have escaped digestion in the stomach and small intestine. However, levels of more than 7-10% of CF in the diet will generally result in decreased growth rates (Kass et al., 1980). Ravindra et al, (1987) observe in pigs a depression in digestibility of DM, EE, cell wall components and hemicelluloses in diets in which CLM is replaced by coconut oil meal. Sarwat et al, (1988) also observe lower digestibility of DM and OM when CLM is included in a sorghum base diets of growing pigs. Bui Huy Nhu Phuc et al, (1996) report a significant reduction in apparent digestibility of DM, OM, CF and EE as the level of inclusion of CLM meal increase from zero to 216g/kg diet.

Table 8: Mean values of apparent digestibility in pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two as supplements to rice bran, broken rice and fish meal

	FCL	**WS**	**WSFCL**	**CTRL**	**SEM**	**Prob**
Dry matter	77.1^{a}	83.3^{b}	79.8^{ab}	78.7^{a}	1.11	0.012
Organic matter	79.8^{a}	85.0^{b}	81.9^{ab}	81.7^{ab}	1.07	0.034
Nitrogen	67.3^{a}	80.6^{b}	70.7^{a}	78.9^{b}	1.68	0.001
Crude fiber	36.2^{a}	66.2^{b}	56.9^{bc}	51.0^{cd}	2.86	0.001
Neutral detergent fiber	50.9^{a}	71.9^{b}	60.9^{c}	62.0^{cd}	2.21	0.001
Biological value	56.6	60.8	64.1	59.2	2.52	0.215

FCL= Fresh cassava leaves; WS= Water spinach; WSFCL= Water spinach mix with fresh cassava leaves; CTRL: Control diet

^{abc}: Means within main effects within rows without common letter are different at $P<0.05$

Digestibility of essential amino acids of CTRL and WS diets were higher than those of FCL diet. Combining WS and FCL increased AA digestibility compared to FCL. The digestibility of essential amino acids in FCL treatment ranged with study of Bui Huy Nhu Phuc et al., (2000), who showed digestibility of Arg 80, 82; His 77,79; Iso 70,69; Leu 74,76; Lys 79,79; Met 70, 71; Phe 76,78; Thr 66,68 and Val 67, 66% for dry and ensiled cassava leaves which used at level of 15% in the diet and basal diet from cassava root meal and soybean meal, respectively.

Table 9: Mean values of amino acid digestibility in pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two as supplements to rice bran, broken rice and fish meal

	FCL	WS	WSFCL	CTRL	SEM	Prob
Essential amino acid, % in DM basis						
Arginine	80.6^a	92.8^b	87.1^c	93.7^b	1.16	0.001
Histidine	78.6^a	91.6^b	81.9^a	89.1^b	1.15	0.001
Isoleucine	76.4^a	88.0^b	79.1^a	86.0^b	1.33	0.001
Leucine	73.6^a	86.4^b	76.8^a	85.3^b	1.47	0.001
Lysine	75.0^a	87.3^b	76.4^a	82.2^c	1.48	0.001
Methionine	77.4^a	90.9^b	81.7^a	89.3^c	1.45	0.001
Phenylalanine	74.5^a	89.4^b	77.3^a	86.9^b	1.41	0.001
Threonine	83.0^a	84.1^a	69.0^b	88.6^a	2.43	0.001
Valine	68.5^a	87.3^b	76.8^c	84.8^b	1.69	0.001

FCL: Fresh cassava leaves, WS: Fresh water spinach, WSFCL: Fresh water spinach mix with fresh cassava leaves, CTRL: control diet

^{abc}: *Means within main effects within rows without common letter are different at P<0.05*

Nitrogen balance

Daily N intake was highest for CTRL diet followed by WS and WSFCL and lowest in FCL (P<0.001). This means total N excretion was highest for CTRL but not differences in N excretion among FCL, WS and WSFCL. Daily N retention is superior for CTRL (28.1g/d) and WS (24.6g/d) but low in FCL (16.7g/d). There appeared to be some synergism and interaction between FCL and WS as the N retention on WSFCL (21.0g/day) was almost as high as on the WS (24.6g/day). In previous experiment on growth performance with the same diets, we indicated that the mixture of cassava leaves and water spinach may even be superior to the water spinach alone, thus providing support for the hypothesis that mixtures of fresh cassava leaves and water spinach may have a complementary action in providing the required nutrients for animals. The N retention in FCL (16.7g) in this study was higher than that of Chhay Ty et al., (2003b), Chhay Ty and Preston, (2005), Nguyen Duy Quynh Tram and Preston, (2004) and

Bounhong et al, (2004). In the present study, fish meal is added at level of 6% in the diet that may improve amino acid in the diets. Eggum, (1970) reported that imbalance in the diets containing cassava leaves may be a factor affecting nutrient digestibility of pigs as well as the study of Bui Huy Nhu Phuc et al, (2001), who reported that Met as well as Lys was the major limiting amino acids for N retention, as well as biological value. Chhay Ty and Preston, (2005) reported that supplementing with 0.3% of Met in fresh cassava leaves improved significantly digestibility of DM and OM. Furthermore, Adegbola, (1977) concluded that when Met was added 0.2% in diet with 20% of dry cassava leaves, it would be improved amino acid as well protein quality.

Table 10: Mean values for N retention in pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two, as supplements to rice bran, broken rice and fish meal

	FCL	**WS**	**WSFCL**	**CTRL**	**SEM**	**Prob**
N balance, g/day in DM basis						
Intake	42.2^{a}	49.7^{b}	46.3^{ab}	60.5^{c}	1.51	0.001
Excretion, g/day in DM basis						
Faecal	13.8	9.64	13.7	12.9	0.92	0.001
Urinary	11.7	15.5	11.5	19.6	0.97	0.001
Total excretion	25.5^{a}	27.2^{a}	26.7^{a}	32.4^{b}	1.25	0.001
N retention in DM basis						
g/day	16.7^{a}	24.6b^{c}	21.0^{ac}	28.1^{b}	1.45	0.001
% digested N (BV)	56.6	60.8	64.1	59.2	2.52	0.215
% N intake	39.6	49.1	45.7	46.6	2.28	0.03

FCL= Fresh cassava leaves; WS= Water spinach; WSFCL= Water spinach mix with fresh cassava leaves; CTRL: Control diet

^{abc}: Means within main effects within rows without common letter are different at $P<0.05$

The relationship between N intake and N retention was plotted. It can be seen from figure 10 that, there was linearly increased (R^2 = 0.88) nitrogen retention as nitrogen intake increased. The result in this study was similar with study of Nguyen Duy Quynh Tram and Preston, (2004). The authors reported that relationship between N intake and N retention was very high (R^2 = 0.86).

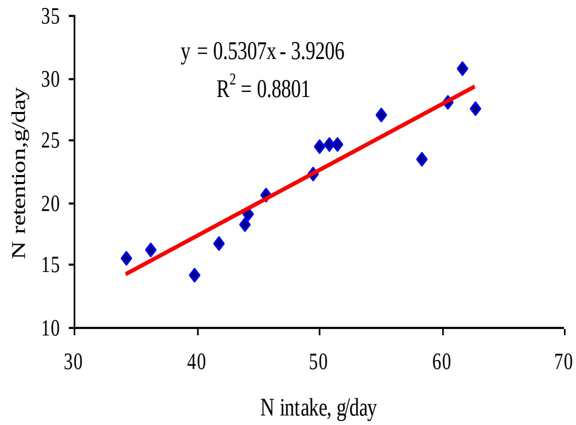

Figure 2: Effect of N intake on N retention

CONCLUSIONS

- Control diet had higher feed intake than diets with fresh cassava leave, water spinach and mixture of fresh cassava leave and water spinach as the main protein source. Fresh water spinach was more palatable than the cassava leaves as reflected in higher total dry matter intake but not differences in proportion of the diet (26.7% for fresh cassava leaves and 26% for fresh water spinach and 26.3% for fresh cassava leave mix with water spinach).
- Digestibility of DM, OM, N, CF, NDF and essential amino acid in WS diet was higher than those in other treatments.
- There appeared to be some synergism effects between fresh cassava leaves and the fresh water spinach as the N retention of pigs fed the mixture fresh water spinach and fresh cassava leave diet (21.0g/day) was almost as high as on the fresh water spinach diet (24.6g/day) and higher than cassava leave diet (16.7 g/day).

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References

Adegbola M O K (1977) Field sprays of fungicides for the control of Phytophthora pod rot. Annual Rept. Cocoa Res. Inst. Nigr. Lbadan. 1975/76:76-77opical environment

An L V, Hong T T T & Lindberg J E (2004) Ileal and total tract digestibility in growing pigs fed cassava root meal diets with inclusion of fresh, dry and ensiled sweet potato leaves. Animal Feed Science and Technology 114, 127-139.

AOAC (1990) Official methods of analysis. Association of official analytical chemists 15th Edition (K Helrick editor). Arlington pp 1230

Bounhong Norachack, Soukanh Keonouchanh, Chhay Ty, Bounthong Bouahom & Preston T R (2004) Stylosanthes and cassava leaves as protein supplements to a basal diet of broken rice for local *pigs.* Livestock Research for Rural Development Vol. 16, Art. #74. *Retrieved January 20,* 2011, from http://www.lrrd.org/lrrd16/10/boun16074.htm

Bui Huy Nhu Phuc (2000) Tropical Forages for growing pings. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Bui Huy Nhu Phuc, Ogle B & Lindberg J K (2001) Nutritive value of cassava leaves for monogastric animals; Proceedings of workshop on "Use of cassava as animal feed" Khon Kaen University, Thailand http://www.mekarn.org/prockk/phuc.htm

Bui Huy Nhu Phuc, Preston T R, Ogle B & Lindberg J E (1996) the nutritive value of sun-dried and ensiled cassava leaves for growing pigs. Livestock Research for Rural Development 8: (3) http://www.lrrd.org./lrrd8/3/bui.htm

Butler G W (1973) Physiological and genetic aspects of cyanogenic in cassava and other plants, chronic cassava toxicity. Proceedings of the Interdisciplinary Workshop, London England, 29-30 Jan., 1973. IDRC -010e, pp. 65-71

CelAgrid (2006a) Assessment of impacts of action research on pig production systems, management, feeding and economic returns in Sras Takoun, Louk and Krom village, Sanlong Communes, Trang district, Takeo province. Cambodia

Chanphone Keoboualapheth & Choke Mikled (2003) Growth Performance of Indigenous Pigs Fed with *Stylosanthes guianensis CIAT 184* as Replacement for Rice bran; *Livestock Research for Rural Development (15) 9* Retrieved, from http://www.lrrd.org/lrrd15/9/chan159.htm

Chhay Ty & Preston T R (2005) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. Livestock Research for Rural Development. Vol.17, Art.#23. Retrieved June 30, 2005, from http://www.lrrd.org/lrrd17/2/chha17023.htm

Chhay Ty, Preston T R & Ly J (2003) The use of ensiled cassava leaves in diets for growing pigs. 2. The influence of type of palm oil and cassava leaf maturity on digestibility and N balance for growing pigs. Livestock Research for Rural Development (15)8 Retrieved, from

Chung K, Wong T Y, Wei C, Huang Y & Lin Y (1998) Tannin and human health. Critical Reviews in Food Science and Nutrition, 38:421-464.

David (1991) Use of duckweed for waste treatment and Animal feed. Jaunal water pollution control federation Vol.45.pp 337-347

Du Thanh Hang & Preston T R (2005) The effects of simple processing methods of cassava leave on HCN content and intake by growing pigs. Livestock Research for Rural Development. Volume 17, Article #99. Retrieved September 7, 2005, from http://www.lrrd.org/lrrd17/9/hang17099.htm

Du Thanh Hang, Linh Nguyen Quang, Preston T R, Everts H & Beynen A C (2009b) Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source. Livestock Research for Rural Development. Volume 21, Article #15. http://www.lrrd.org/lrrd21/1/hang21015.htm

Eggum B O (1970) The protein quality of cassava leaves. British Journal of Nutrition. 24: 761-768

Famino A.O, Oduguwa B.O, Oduguwa O.O, Ajasa O.Y & Jegede O (2004) Feeding value of shrimp meal for growing pigs. Archivos de Zootecnia, ano/vol.53, numero 201 Universidad de cordoba, Espana. pp. 77-85

Getter A O & Baine J (1938) Research on cyanide detoxification. American Journal of Medical \ Science. pp. 185-189

Gohl B (1994) Tropical Feed. FAO Animal Production and Health Series

Gomez G G (1991) Use of cassava products in pigs feeding. Pigs News and Information 12, 387-390 http://www.lrrd.org/lrrd15/8/chha158.htm

Humphreys D J (1988) Veterinary Toxicology, 3rd ed. Bailliere Tindall, London

Jorgensen H, Zhao X, Eggum B O & Zhao X Q (1996) The influence of dietary fiber and environmental temperature on development of the gastrointestinal tract, digestibility, degree of fermentation in the hind gut and energy metabolism in pigs. British Journal of Nutrition 75, 365-378.

Kass M L, Van Soest P J, Pond W G, Lewis B & McDowell R E (1980) Utilization of dietary Fiber from alfalfa by growing swine. I. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. Journal of Animal Science 50. 175-181

Le Duc Ngoan & Lindberg J E (2001) Ileal and total tract digestibility in growing pigs fed cassava root and rice bran with inclusion of fishmeal and fresh or ensiled shrimp by-products. Asian-Aust. J. Anim. Sci. 14, 216-223

Le Thi Men & Preston T R (2005) On-farm evaluation of effect of different fertilizers on water spinach (Ipomoea aquatica) yields, and of including water spinach and catfish oil in diets for fastening pigs in the Mekong Delta of Vietnam, from: http://www.mekarn.org/proctu/lemen38.htm

Le Thi Men, Ogle Brian & Vovanson (2000) Comparison of biodigester effluent and urea as fertilizer for water spinach vegetative. Livestock Research of Rural Development (13)6.

Le Van An, Bodil E, Frankow Lindberg & Lindberg J E (2003) Effect of harvesting interval and defoliation on yield and chemical composition of leaves, stems and tubers of sweet potato (Ipomoea batatas L. Lam) plant parts Field Crops Research 82 (2003) 49-58. www.elsevier.com/locale/fer

MINITAB (2007) Minitab reference Manual release 15.1.2.

National Institute of Animal Husbandry, Vietnam (1995). Composition and nutritional Value of animal feed in Vietnam, Hanoi, Vietnam.

National Research Council (NRC) (1994) Nutrient Requirements of Poultry, Ninth Revised Edition, 1994. National Academy Press, Washington D.C.1994

Nguyen Duy Quynh Tram & Preston T R (2004) Effect of method of processing cassava leaves on intake, digestibility and N retention by Ba Xuyen piglets. Livestock Research for Rural Development. Vol. 16, Art. # 80. Retrieved, from http://www.lrrd.org/lrrd16/10/tram16080.htm

Nguyen Thi Hoa Ly, Le D Ngoan, Martin W A, Verstegen, Wouter H & Hendriks (2010) Ensiled and dry cassava leaves and sweet potato vines as a protein sources in diets for growing Vietnamese Large White x Mong Cai pigs. Asian-Australia Journal Animal Science. Vol. 23, No.9: 1205-1212.

Nguyen Thi Loc & Le Khac Huy (2003) Effect of DL-methionine supplementation levels in ensiled cassava root-based diets on the performance and economic efficiency of F1 (Large White x Mong Cai) fattening pigs. In: *Proceedings of Final National Seminar Workshop on Sustainable Livestock Production on Local Feed Resources (Editors: Reg Preston and Brian Ogle)*. HUAF-SAREC, Hue City, 25 – 28 March, 2003. Retrieved July14, 107, from http://www.mekarn.org/sarec03/loc1.htm.

Ogle B (2006) Forages for pigs: nutritional, physiological and practical implications. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN-CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006 http://www.mekarn.org/proprf/ogle.htm

Onwuka C F I (1992) Tannin and saponin contents of some Tropical browse species fed to goats. Tro. Agrc. (Trinidad) 69:1442-1440

Prak Kea, Preston T R & Ly J (2003) Feed intake, digestibility and N retention of a diet of water spinach supplemented with palm oil and / or broken rice and dried fish for growing pigs. *Livestock Research for Rural Development (15) 8* Retrieved, from http://www.lrrd.org/lrrd15/8/kea158.htm

Ravindran V (1993) Cassava leaves as animal feed: Potential and limitation. Journal of the Science of Food and Agriculture 61, 141-150.

Ravindran V, Kornegay E T & Rajaguru A S B (1987) Influence of processing methods and storage time on the cyanide potential of cassava leaf meal. Animal Feed Science and Technology 17: 227-234.

Sarwat S R, Katak S N & Kategile J A (1988) Performance of growing-finishing pigs when given diets containing fresh cassava leaves and roots. East African Agriculture and Forestry Journal. 53:111-115

Spackman D H, Stein W H & Moore S (1958) Automatic recording apparatus for use in chromatography of amino acids. Anal.Chem.30:1190-1206

Stosic D D & Kaykay J M (1981) Rubber seed as animal feed in Liberia. World Animal Review 39, 2939.

Tewe O O (1992) Detoxification of cassava products and effects of residual toxins on consuming animals. In: Roots, tubers, plantains and bananas in animal feeding. FAO Animal Production and Health Paper 95. http://www.fao.org/Docrep/003/T0554E/T0554E06.htm

Thuy L T, Son T H, Xa N H, Hao P X & Tuyen T Q (2011) Evaluation of performance of Pi x Du and Du x Pi crossbred pigs in Vietnam. Annually scientific conference on pig production of pig Research Center, National Institute of Animal Science, Nanoi, Vietnam, 30, Octorber, 2011

Undersander D, Mertens D R and Theix N 1993 Forage analysis procedures. National Forage Testing Association. Omaha pp 154

Van Soest P J, Robertson J B and Lewis B A 1991 Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74: 3583–3597

Woolfe J A (1992) Sweet potato: an untapped food source. Cambridge University Press, Cambridge, 643 pp

CHAPTER VII

GENERAL DISCUSSION AND CONCLUSIONS

VII.1. General discussion

Feed and HCN intake

Intake of FCL (270g) was half that of WS (527g) when a pig of 21.6-26.7kg was given separately (paper 1, chapter 3). High total DM intakes were observed when cassava leaves were given as an ensiled (482g) or sundried form (410g) and lower intake was observed in FCL (295g) (paper 3, chapter 5) in a pig of 30.8-33.3 kg, but in paper 4 (chapter 6) the intake of foliages of FCL and WS was similar (413 and 482g, respectively) in a pig of 59.4 to 59.9 kg. These differences might be due to the effect of body weight as older pigs may use foliages more efficiently than small pigs.

On percentage of live weight basis, the total DM intake of the FCL diet was high (3.32%) in paper 1 and similar to that reported by Chhay Ty and Preston, (2005a) (3.30%) for the same feeds in a digestibility trial and similar to that reported by Du Thanh Hang and Preston, (2005) (2.7 to 3.30%). However, it was higher than in paper 3 (2.19%) and paper 4 (2.60%), and higher than in the report by Nguyen Duy Quynh Tram and Preston, (2004) and Bounhong et al, (2004) (2.6 and 3.11%, respectively). Meanwhile, the value of total DM intake was higher in the WS diet (3.97%) compared with the FCL diet (3.32%) but when both foliages were combined, total DM intake as percentage of live weight was improved (papers 1, 2, 4) compared with when each forage was provided alone. The difference in total DM intake might be due to the fiber content and anti-nutritional factors (HCN and tannin) present in the fresh cassava leaves. The precursors of cyanogenic glycosides in the fresh leaves of most cassava varieties are decreased when making silage or meal. The process of ensiling is the development of lactic acid bacteria and fermentation inhibitors. Molasses is most frequently used as additive, and is of particular benefit when applied to forages is low in soluble carbohydrates. Sun drying is a cheap method for preserving feeding materials. However, it is difficult in the rainy season due to the lack of sun and high frequency of rain which creates favorable conditions for bacteria and fungi, producing moldy hay and mycotoxins. However, both methods can reduce anti nutrient compounds and make the feed safe for the animal, improve the quality and palatability and preserve the feed so it can be used to feed the livestock at all times of the year.

In all the experiments conducted in this thesis there was no sign of toxicity that could have been caused by hydrocyanic acid. On a live weight basis, the HCN intake of the FCL diet was 7.35 mg/kg LW in paper 1 which was higher than in papers 3 and 4 (3.52 and 2.52 mg/kg LW, respectively). The HCN intake in paper 1 was similar to that reported by Du Thang Hang et al, (2007) when they studied effects of simple processing methods of cassava leaves on HCN content and intake by growing pigs. The HCN intakes of pigs in papers 1, 2 and 3 were all considerably higher than the maximum levels recommended by most other authors (1.4 mg/kg LW reported by Getter and Baine, 1938; 2.1 - 2.3 mg/kg LW by Johnson and Ramond, 1965; 3.5 mg/kg LW by Tewe, 1992; and 4.4 mg/kg LW by Butler, 1973). Clarke and Clarke, (1967) reported that factors that influence HCN toxicity include the size and type of animals, the rate of ingestion, the type of feed ingested simultaneously with the cyanogenic glycoside, presence of active degradation enzymes in the animal digestive tract, and the ability to detoxify cyanide.

The presence of cyanogenic glycosides could lead to a reduced supply of DL-methionine (Met) to the animal, and hence in the presence of high levels of dietary cyanogenic glycosides, better performance can be expected with Met supplementation. This was confirmed by Nguyen Thi Hoa Ly, (2006), who observed a 23% increase in growth rate in pigs when Met was added at 0.15% of the DM of diets with 17 to 25% ensiled cassava root and 15% ensiled cassava leaves (DM basis). Nguyen Thi Loc and Le Khac Huy, (2003) also reported that the nitrogen retention values in growing pigs fed cassava root based diets were increased (19.3, 20.1, 20.8 and 21.9 g/day) for diets supplemented with 0, 0.1, 0.2 and 0.3% Met, respectively.

Growth rate and feed conversion ratio

Live weight gain (LWG) and feed conversion ratio (FCR) were best for the treatment with FCL and poorest for DCL and ECL (paper 3). The poorer results with ECL might have been due to the loss of valuable nutrients by leaching from the silage, accumulating at the bottom of the plastic bags used to ensile the cassava. Such losses have been reported for low dry matter silage made in pits and bunkers (Titterton and Bareeba, 1999). Another reason could be that fresh cassava leaves are more digestible. Also, ensiled cassava leaves were more digestible than dried leaves according to Bui Huy Nhu Phuc, (2000). However, there appear to be no reports on digestibility of fresh versus dried cassava leaves.

Pigs fed water spinach had higher LWG than those fed fresh cassava leaves as the protein source. The LWG and FCR were also improved when pigs were fed a mixture of water spinach and cassava leaves as compared with cassava leaves alone. The implication from these findings is that there was a synergistic effect from combining the two foliages as protein sources. The effect could have been due to better AA balance in the mixed foliages or that Met requirements were increased when cassava leaves were the only protein supplement, due to the need for sulfur donors to detoxify the HCN.

In paper 1, there was no effect on LWG and FCR due to Met supplementation treatments but in paper 3, pigs gained weight better with Met supplemented diets. Du Thanh Hang et al, (2007) showed that increasing levels of Met (0, 0.1 and 0.2% in diets with fresh or ensiled cassava leaves and cassava root products) resulted in increased growth rates and improved DM and crude protein conversion rates, for fresh compared with ensiled cassava leaves. For both sources of cassava leaves, LWG and FCR were improved linearly with level of supplementary Met. There were no differences in growth performance between the experimental diet (fresh cassava leaves + 0.2% Met) and the control diet containing 15% fish meal. These beneficial effects from feeding Met are similar to those reported by Job, (1975), and Portela and Maner, (1972), when cassava root meal was the basal diet. Nguyen Thi Hoa Ly, (2006) observed a 23% increase in growth rate in pigs when Met was added at 0.15% of the DM of diets with 17 to 25% ensiled cassava roots and 15% ensiled cassava leaves (DM basis). Nguyen Thi Loc and Le Khac Huy, (2003) showed that addition of 0.3% Met in a basal diet with 20-40% ensiled cassava root slightly reduced while a level of 0.2% Met increased growth rate. From a theoretical standpoint, it is to be expected that there would be nutritional benefits from Met supplementation of pig diets rich in cassava leaves because of the relative deficiency of the sulphur amino acids in cassava leaf protein and the fact that a source of sulphur is required for the detoxification of HCN. Job, (1975) also claimed that the benefit from Met supplementation to cassava root based diets, of improved LWG and FCR efficiency, was because the amino acid was needed for detoxification of the HCN in the cassava.

Feed intake and digestibility

According to Preston and Leng, (2009), feed intake mainly reflects the balance of nutrients at the sites of metabolism, and to a lesser extent, the diet digestibility in paper 1 and 2, there were close relationships between total feed intake, or total foliage intake, and live weight gain (Figures 1, 2, 3, 4). In paper 4, N retention (which is an indirect measure of growth rate) was highest on the control diet and there was a close relationship between DM intake and live weight gain on all treatments (Figure 5). In contrast, when there were simultaneous measurements of DM intake and apparent DM digestibility in paper 4, there was no relationship between these two variables (R^2 = 0.03).

The balance of nutrients would be expected to be highest for the control diet with the highest level of fish meal. However, the apparent biological value of the protein in the control diet, measured as N retained as per cent of N digested, indicated no difference between the control (59.2) and water spinach diets (60.8). The ratio of methionine to lysine in the diets was slightly in favor of the control diet (0.36 compared with 0.32) but the difference was small. It was also found that the ratio of DL-methionine to lysine in the feed ingredients showed little difference between the fish meal (0.26) and the water spinach (0.21). It would appear that some other factors were contributing to the better animal responses on the control diet.

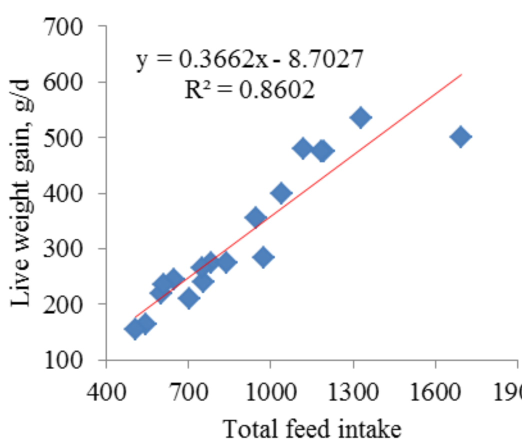


Figure 1: Relationship between total feed intake and live weight gain of pigs fed forage protein sources and Methionine supplementation with a basal diet of broken rice (Experiment 1)

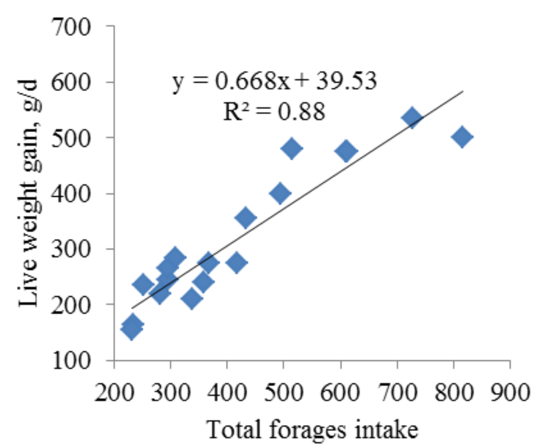
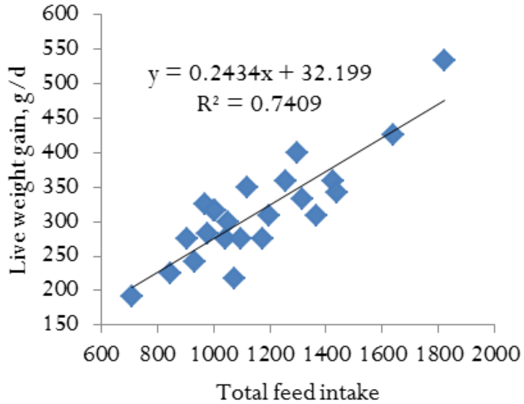


Figure 2: Relationship between total forages intake and live weight gain of pigs fed forage protein sources and Methionine supplementation with a basal diet of broken rice (Experiment 1)



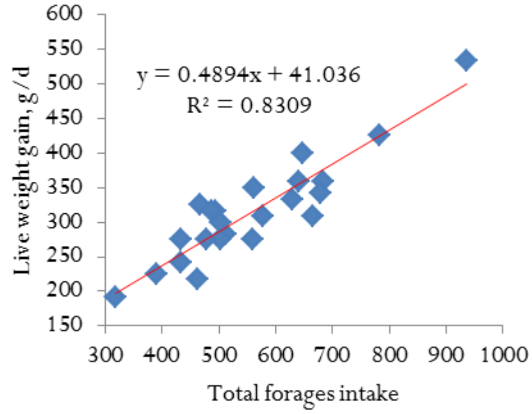


Figure 3: Relationship of feed intake, g/d and live weight gain, g/d of pigs fed BR or RB mixed CRM supplemented with different combination levels of water spinach and wiled cassava leaves (Experiment 2)

Figure 4: Relationship of total forages intake, g/d and live weight gain, g/d of pigs fed BR or mix RB with CRM supplemented with different combination levels of water spinach and wiled cassava leaves (Experiment 2)

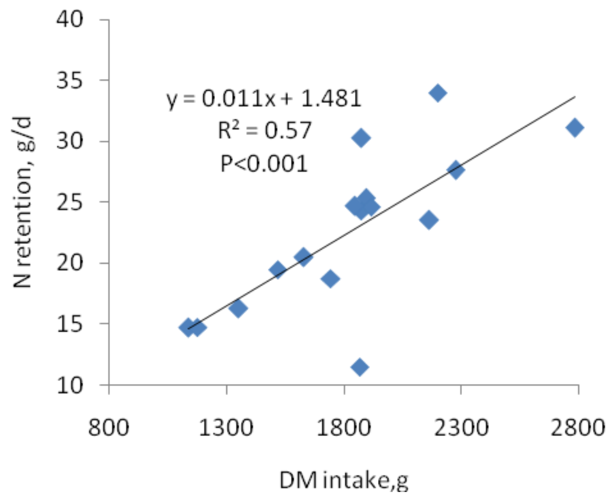


Figure 5: Relationship of feed intake, g/d and N retention in pigs fed control diet, fresh cassava leaves, water spinach or a mixture of the two, as supplements to rice bran, broken rice and fish meal

Nutrient digestibility

Apparent digestibilities of DM, OM, N, CF and NDF in the WS diet were higher than in the WSFCL diet and lowest in the FCL diet. The low digestibility of FCL could be caused by the anti-nutritional compounds in this plant such as HCN precursor and tannin. Stosic and Kaykay, (1981) noted that small quantities of HCN ingested on a regular basis, though not large enough to cause mortality, might be sufficient to affect the general health and productivity of the animal. Tannins have been considered as anti-nutrients due to a range of adverse effects including reduced feed intake and digestibility (Chung et al., 1998; Onwuka, 1992).

Ogle, (2006) reported that high CF content in the diets reduced the nutrient digestibility, especially of CP. In addition, the extent of the reduction in digestibility has been shown to vary with the level of fiber and feeding level (Oke, 1978; Ravindran and Ravindran, 1998; Dierick et al., 1989). Fibrous feed ingredients in the diet also lead to an increased rate of passage of digesta through the gut and reduced ileal and total tract digestibility and affect the gut size and development, particularly the large intestine (An et al., 2004; Jorgensen et al., 1996). Nearly all CF digestion takes place in the caecum and colon, where bacteria break down fermentable carbohydrates that have escaped digestion in the stomach and small intestine.

However, the fibre level would not appear to be the factor causing low digestibility on the FCL diet, as the level of NDF was lowest on this diet (23.7% in DM compared with 31.7% on the control diet). Thus the HCN precursors or the tannins in the cassava leaves are more likely to be the cause of the lower digestibility.

VII.2. Conclusions

- Pigs can be fed fresh cassava leaves up to a level of 38% of total DM intake and HCN intake can be up to 152 mg/pig or 7.35mg/kg live weight without toxicity.
- Feeding water spinach alone or as a mixture with fresh cassava leaves as protein source improved significantly pig performance compared with fresh cassava leaves alone.
- Supplementing methionine to diets containing cassava leaves improved pig growth rate and feed conversion ratio.
- Feeding water spinach significantly improved the apparent digestibility of DM, OM, CP, and NDF, and essential amino acids compared with the diets containing cassava leaves.
- In the specific case of lysine and methionine it appeared that the apparent digestibility of these amino acids was higher in the diet with 28% water spinach and 3% fish meal than in the control diet containing 13.5% fish meal and no water spinach.

IMPLICATION AND FURTHER RESEARCH

Implications

Cassava and water spinach grow well in different soil types and climate conditions in tropical countries. Both can be fed to livestock; however, as a supplement to rice bran in pig diets, water spinach is recommended rather than cassava leaves due to its higher digestibility and absence of anti-nutritional factors. In situations where cassava leaves are available at low cost (ie: at the time of harvesting the roots) they can be ensiled and fed safely as a protein source to pigs. However, pig performance will be improved if the ensiled cassava leaves are supplemented with fresh water spinach. As methionine appears to be the first limiting amino acid in both these forages, improvements in growth and feed conversion can be expected from the addition of 0.3% synthetic DL-methionine.

Further research

In view of the favorable growth and feed conversion response to supplementation with synthetic methionine, it would be of interest to evaluate other protein sources rich in this amino acid, for example the residues from fermentation and distillation of rice wine.

Feed intake appears to be a limiting factor in growing pigs fed high levels of protein-rich forages. It is therefore logical to evaluate their use in systems where feed intake is normally restricted, for example in the gestation phase of reproduction.

References

An L V, Hong T T T & Lindberg J E (2004) Ileal and total tract digestibility in growing pigs fed cassava root meal diets with inclusion of fresh, dry and ensiled sweet potato leaves. Animal Feed Science and Technology 114, 127-139.

Bounhong Norachack, Soukanh Keonouchanh, Chhay Ty, Bounthong Bouahom & Preston T R (2004) Stylosanthes and cassava leaves as protein supplements to a basal diet of broken rice for local *pigs.* Livestock Research for Rural Development Vol. 16, Art. #74. *Retrieved January 20,* 2011, from http://www.lrrd.org/lrrd16/10/boun16074.htm

Bui Huy Nhu Phuc (2000) Tropical Forages for growing pings. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala 2000

Butler G W (1973) Physiological and genetic aspects of cyanogenic in cassava and other plants, chronic cassava toxicity. Proceedings of the Interdisciplinary Workshop, London England, 29-30 Jan., 1973. IDRC -010e, pp. 65-71.

Chhay Ty & Preston T R (2005a) Effect of water spinach and fresh cassava leaves on intake, digestibility and N retention in growing pigs. Livestock Research for Rural Development. Vol.17, Art. #23. Retrieved June 30, 2005, from http://www.cipav.org.co/lrrd/lrrd17/2/chha17023.htm

Chung K, Wong T Y, Wei C, Huang Y & Lin Y (1998) Tannin and human health. Critical Reviews in Food Science and Nutrition, 38:421-464.

Clarke E G C & Clarke M L (1967) Cyanides; Garner's veterinary toxicology, 3 rd edition, Williams and Wilkins Co., Baltimore, pp. 75-80

Dierick N A., Vervaeke I J., Demeyer D I & Decuypere J A (1989) An approach to the energetic importance of fiber digestion in pigs. I. Importance of fermentation in the overall energy supply. Anim. Feed Sci. Technol. 23: 141-167

Du Thanh Hang & Preston T R (2005) The effects of simple processing methods of cassava leave on HCN content and intake by growing pigs. Livestock Research for Rural Development. Volume 17, Article #99. Retrieved September 7, 2005, from http://www.cipav.org.co/lrrd/lrrd17/9/hang17099.htm

Du Thanh Hang, Nguyen Quang Linh, Preston T R, Everts H & Beynen A C (2007) Cassava leaves as protein source for pigs in Central Vietnam. Chapter 6 Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source. (Doctorial Thesis 2007)

Getter A O & Baine J (1938) Research on cyanide detoxification. American Journal of Medical Science. pp. 185-189

Humphreys D J (1988) Veterinary Toxicology, 3rd ed. Bailliere Tindall, London

Job T A (1975) Utilization and protein supplementation of cassava for animal feeding and the effect of sulfured source on cyanide detoxification. PhD Thesis. University of Ibadan, Nigeria. (Astr. On cassava Vol III, pp 97-98

Johnson R M & Ramond W D (1965) The chemical composition of some Tropical food plants Manioc. Tropical Science 7, pp. 109-115.

Jorgensen H, Zhao X, Eggum B O & Zhao X Q (1996) The influence of dietary fiber and environmental temperature on development of the gastrointestinal tract, digestibility, degree of fermentation in the hind gut and energy metabolism in pigs. British Journal of Nutrition 75, 365-378.

Nguyen Duy Quynh Tram & Preston T R (2004) Effect of method of processing cassava leaves on intake, digestibility and N retention by Ba Xuyen piglets. *Livestock Research for Rural*

Development.Vol. 16, Art. # 80. Retrieved, from http://www.cipav.org.co/lrrd/lrrd16/10/tram16080.htm

Nguyen Thi Hoa Ly (2006) the use of ensiled cassava leaves for feeding pig's on-farm in central Vietnam. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN- CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006. Article # 13. Retrieved March 16, 107, from http://www.mekarn.org/proprf/hoaly.htm

Nguyen Thi Loc & Le Khac Huy (2003) Effect of DL-methionine supplementation levels in ensiled cassava root-based diets on the performance and economic efficiency of F1 (Large White x Mong Cai) fattening pigs. In: *Proceedings of Final National Seminar Workshop on Sustainable Livestock Production on Local Feed Resources (Editors: Reg Preston and Brian Ogle)*. HUAF-SAREC, Hue City, 25 – 28 March, 2003. Retrieved July14, 107, from http://www.mekarn.org/sarec03/loc1.htm.

Ogle B (2006) Forages for pigs: nutritional, physiological and practical implications. Workshop-seminar "Forages for Pigs and Rabbits" MEKARN-CelAgrid, Phnom Penh, Cambodia, 22-24 August, 2006 http://www.mekarn.org/proprf/ogle.htm

Oke O L (1978) Problems in the use of cassava as animal feed. Anim. Feed Science and Technology. 3:345-380.

Onwuka C F I (1992) Tannin and saponin contents of some Tropical browse species fed to goats. Tro. Agrc. (Trinidad) 69:1442-1440

Portela J & Maner J H (1972) Chronic cassava toxicity. International Development Research Center, London, Monograph No. 010e, pp: 113-120.

Preston T R and Leng R A 2009 Matching livestock systems with available resources in the tropics and subtropics. 2nd edition 2009. http://www.utafoundation.org/P%L/preston&leng.htm

Ravindran G and Ravindran V (1988) Changes in the nutritional composition of cassava (Manihot esculenta Crantz) leaves during maturity. Food Chem. 27: 299-309

Stosic D D & Kaykay J M (1981) Rubber seed as animal feed in Liberia. World Animal Review 39, 2939.

Tewe O O (1992) Detoxification of cassava products and effects of residual toxins on consuming animals. In: Roots, tubers, plantains and bananas in animal feeding. FAO Animal Production and Health Paper 95. http://www.fao.org/Docrep/003/T0554E/T0554E06.htm

Titterton M & Bareeba F B (1999) Grass and legume silages in the tropics. Proceedings of the FAO Electronic Conference on Tropical Silage. 1 September to 15 December 1999. http://www.fao.org/docrep/005/x8486e/x8486e0c.htm#bm12

PUBLICATIONS FROM THESIS WORK

1. **Chhay Ty and Preston T R 2005:** Effect of water spinach and fresh cassava leaves on growth performance of pigs fed a basal diet of broken rice. Livestock Research for Rural Development. Volume 17, Article No. 76. http://www.lrrd.org/lrrd17/7/chha17076.htm

2. **Chhay Ty and Preston T R 2006:** Effect of different ratios of water spinach and fresh cassava leaves on growth of pigs fed basal diets of broken rice or mixture of rice bran and cassava root meal. Livestock Research for Rural Development. Volume 18, Article No. 57.http://www.lrrd.org/lrrd18/4/chha18057.htm

3. **Chhay Ty, Borin K and Preston T R 2011:** Effect of processing cassava leaves and supplementing them with DL-methionine, on intake, growth and feed conversion in crossbred growing pigs . Livestock Research for Rural Development. Volume 23, Article #91. http://www.lrrd.org/lrrd23/4/chha23091.htm

4. **Chhay Ty, Le Duc Ngoan and Le Dinh Phung 2011:** Total tract digestibility in fattening pigs fed fresh cassava leaves and fresh water spinach as protein sources with basal diet of rice bran and broken rice. *Hue University Journal of Sciences (in press).*