

**EVALUATION OF LOCAL PROTEIN RESOURCES
FOR GROWING PIGS IN CENTRAL VIETNAM**

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EVALUATION OF LOCAL PROTEIN RESOURCES FOR GROWING PIGS IN CENTRAL VIETNAM

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Abstract

The general objectives of the work presented here were to evaluate processing methods for the preservations of cassava leaves (CL) and sweet potato vines (SPV) for later feeding during feed shortages in Vietnam. In addition, the nutritional value (including hydrogen cyanide (HCN) contents) of stored and processed CL and SPV as ingredients in diets for pigs were studied to determine their optimal use.

The impact of different levels of various carbohydrates added to CL on ensiling and chemical properties was investigated (study 1). Inclusion of rice bran or cassava root meal at 5 or 10% (fresh basis), produced good quality silage that can be stored for up to three months. Ensiling reduced the HCN content up to 80% compared to the content in fresh CL. Using ensiled or dry CL and SPV to replace 70% of the crude protein in a practical fish meal based diet commonly used in Vietnam, gave similar performance results and carcass traits of Large White×Mong Cai pigs (study 2). However, increasing ensiled CL from variety KM94 from 0 to 20% (in DM) in diets caused a significant decrease in the average daily gain of pigs but resulted in a 9-18% reduction in feed cost (study 3). Studies into the ileal and total tract apparent digestibility of amino acids and crude protein of ensiled and dried CL and SPV showed that these feed ingredients have the potential to improve the supply of amino acids and protein to growing pigs when fed practical diets (study 4). The chemical analyses indicated CL to have a higher crude protein content than SPV and that ensiling slightly decreases the crude protein as well as the amino acids content. Ensiling however, resulted in a higher digestibility of dietary nutrients compared to drying. The first and second limiting amino acids for ensiled and dried CL and SPV for growing pigs were methionine+cysteine and lysine. Mixing ensiled CL and SPV vines may provide additional benefits in terms of amino acid digestibility over feeding these ingredients alone to pigs. Supplementation of diets containing ensiled CL with methionine and lysine showed that the performance of growing pigs can be increased, as well as the economic benefits for farmers (study 5).

The work presented shows that CL and SPV are economical alternatives for more traditionally protein source (e.g. fish meal, soybean meal) for pigs in Vietnam. Ensiling appears to be a practical solution to conserve sweet potato vines and cassava leaves and provide a solution for the rainy season when preservation by sun-drying is difficult.

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Chapter 1

General introduction

Introduction

In Vietnam, livestock production is an important sector of agriculture. It provides high value protein products for human consumption and contributes to crop cultivation by supplying valuable manure. Livestock are an impetus to increase income, status and improve living standards of small-holder farmers in many countries.

Cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) are the second and third most important food crops after rice in terms of total production in Vietnam. Cassava leaves may contain 20-30% crude protein (CP) in the dry matter (DM) fraction while sweet potato vines (SPV) have a variable CP content in the DM ranging from 16 to 29%. As such, cassava leaves (CL) and SPV are good protein sources for livestock feeding although cassava leaf and SPV protein are low in especially the essential amino acids (EAA) methionine and lysine. Cassava leaves, however also contain anti-nutritional factors (ANF) such as cyanogenic glucosides and tannins. Cyanogenic glucosides can give rise to hydrogen cyanide (HCN) by the action of either enzymatic activity in damaged plant tissues or within the digestive tract when ingested by animals. When absorbed, cyanide is rapidly detoxified by conversion to thiocyanate which is subsequently excreted in the urine. Consumption of cyanide may cause depressed thyroid function, decreased utilization of oxygen and decreased weight gain in pigs (Tewe et al., 1977, 1984; Oke, 1980; Leng, 2005). The symptoms of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, stomach pains, vomiting and diarrhea (Leng, 2005). Among the main processing methods to reduce HCN concentrations in cassava (roots and leaves) are cooking, drying and ensiling. The use of SPV in animal feeding is limited by the high content in oxalic acid, phytic acid, tannic acid as well as trypsin and chymotrypsin inhibitors.

If CL and SPV could be used on a larger scale in pig production in Central Vietnam, it would result in a reduced dependency on traditional, expensive protein sources such as fish meal, soybean meal and commercial feeds. In order to increase the utilization by pigs held by small-scale farmer households in Central Vietnam, it is necessary to understand how the nutritional value of CL and SPV might be improved as well as how these feed resources can be conserved during times of oversupply for feeding during periods of low feed availability.

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Table 1. Number of animals and meat production in Vietnam between 2000 to 2009.

Species	Year					
	2000	2005	2006	2007	2008	2009
Population (million)						
Cattle	4.13	5.54	6.51	6.72	6.34	6.10
Buffalo	2.90	2.92	2.92	3.00	2.90	2.89
Pigs	20.2	27.4	26.9	26.6	26.7	27.6
Poultry	196	220	215	226	248	280
Meat production (1,000 tons live weight)						
Cattle	93.8	142.2	159.5	206.1	226.7	257.8
Buffalo	48.4	59.8	64.3	67.5	71.5	74.9
Pork	1,418.1	2,288.3	2,505.0	2,662.7	2,806.5	2,931.4
Poultry	292.9	321.9	344.4	358.8	448.2	502.8

Source: GSO (2010).

Livestock production in Vietnam

Vietnam is a country with a population of 87 million people where more than 75% of the population lives in rural areas whose livelihood depends on agricultural production. The main food crop in Vietnam is paddy rice production which is integrated with animal husbandry in the traditional agriculture production system.

In order to meet the demand of the growing population and the higher living standards of people, livestock production in Vietnam has undergone a significant development in both number of animals and in production level. During the last 10 years (2000-2009), livestock production in Vietnam has grown with 5-17% per year (Table 1). In 2000, the livestock sector accounted for approximately 20% of the total value of agriculture and increased to 27% in 2009. It is expected that production will further increase to 35% by 2015 (MARD, 2006). Currently, pork meat is by far the most produced meat and accounts for 81% of the total livestock meat production with poultry and beef meat accounting for 11 and 5%, respectively. Between 2000-2009, pork production increased by 11.9% per year to a total annual production of 2,931 thousand tonnes in 2009. Other types of meat including buffalo and goat only contribute 3% to the total meat production in Vietnam.

One of the indigenous pig breeds in Vietnam is the Mong Cai breed. Compared to exotic breeds (Large White, Landrace and Pietrain), Mong Cai pigs have

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relatively a lower carcass weight, a lower lean meat percentage and a much higher backfat thickness (Pham et al., 2010). However, Mong Cai pigs have a relatively good reproductive performance with the number of litters per sow per year between 1.5-2.0, a litter size of 10-14 piglets with a piglet birth weight of around 0.5 kg. In addition, the breed is well adapted to the hot climatic conditions in Vietnam and is tolerant to high fibre diets (Duyet et al., 2003). Due to the low daily live weight gain and high fat content in the carcass, crossbreeding programs with Large White and other exotic boars have been implemented to improve productivity and meat quality with retention of the benefits of the Mong Cai breed. Mong Cai sows are used in these breeding programmes as the dam line.

Feeding systems for pigs in many areas in Vietnam are based on locally available feed resources such as rice bran, cassava root meal, maize and SPV, and agro-industrial by-products from marine food processing and brewing. Commercial feeds are rarely used as their costs are prohibitive in rearing pigs. The proportion of commercial feeds used in the total pig diet is about 20% while 80% originates from crop residues and farm by-products. These feedstuffs are normally rich in carbohydrate, but have a low nutritional value in terms of protein quality. Protein supply in pig diets is a major challenge and contribute significantly to the costs of pig diets in Vietnam. Cassava leaves and SPV contain relatively high concentrations of protein. As such research, into the reduction of anti-nutritional factors and conservation of these protein sources is a major research focus in Vietnam.

Cassava and sweet potato: cultivation and chemical composition

Cassava cultivation

Cassava originates from Latin America, and is a drought-tolerant crop although it grows also well in a warm and humid climate. Cassava is one of the major tuber crops grown in more than 80 countries in humid and sub-humid tropical areas. Most of the world's production of cassava in tropical countries is used for human consumption, but some is used in animal feeds and by the starch industry. In Vietnam, cassava has rapidly changed its role from a food crop to an industrial crop with a large increase in the cultivated area during the first five years of the 21st Century. Recently, the yield of cassava per hectare and the area planted has further increased (Table 2). The Vietnam Cassava Program, supported by the Ministry of Agricultural and Rural

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Development (MARD) and implemented in close cooperation with the Nippon Foundation-funded Cassava Project coordinated by CIAT (International Center for Tropical Agriculture), has promoted the rapid multiplication and widespread distribution of new high-yielding and high-starch varieties. Moreover there is a development of using more sustainable cassava production practices. Many farmers are now growing the improved varieties KM94, KM140 and KM98-5, providing yields of 25-35 ton/ha in areas of 3-5 hectares (Nguyen et al., 2010). In 2009, there was a total area of 508,800 ha planted with cassava of which more than 400,000 ha consisted of new varieties (GSO, 2010). Cassava production in Vietnam was approximately 8.5 million tonnes in 2010, up by almost 4 million tonnes in 2001 as a result of both area expansion from 292,300 ha to 496,200 ha and simultaneously an increase in yield from 12.01 ton/ha in 2001 to 17.17 ton/ha in 2010 (GSO, 2011). The latter increase is made possible by a doubling in the planting of new high-yielding cassava varieties, mainly KM94 (Hoang et al., 2010).

Table 2. Cassava and sweet potato production in Vietnam between 2001 and 2010.

Year	Cassava			Sweet potato		
	Area (ha)	Yield (ton/ha)	Production (tonnes)	Area (ha)	Yield (ton/ha)	Production (tonnes)
2001	292,300	12.01	3,519,200	244,600	6.76	1,653,500
2002	337,000	13.17	4,438,000	237,700	7.17	1,703,700
2003	371,900	14.28	4,308,900	219,600	7.18	1,576,600
2004	388,600	14.98	5,820,700	201,800	7.49	1,512,300
2005	425,500	15.68	6,716,200	185,300	7.75	1,443,100
2006	475,200	16.38	7,782,500	181,700	8.06	1,460,900
2007	497,000	16.07	7,984,900	177,600	8.20	1,456,700
2008	554,000	16.80	9,309,900	162,600	8.15	1,325,600
2009	508,800	16.82	8,556,900	146,400	8.25	1,207,600
2010	496,200	17.17	8,521,600	150,800	8.73	1,317,200

Source: GSO (2011).

Normally, cassava is ready for harvest from 7 months, and harvesting can continue until 18 months after planting or later. Cassava is traditionally grown for its root which contains a high concentration of starch (~91% in the DM). Cassava leaves

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on the other hand are a good source of protein, minerals and vitamins. The typical yield of CL (as DM) as a by-product of root harvesting may amount to as much as 4.6 tons/ha (Ravindran et al., 1987). The yield of fresh foliage of an improved variety like KM94 is approximately 9 tons/ha and leaf yield approximately 5-7 tons/ha (Phuoc, 2004; Ly and Ngoan, 2007). Cassava leaves can be harvested from 3 months after planting in cycles of 60-75 days. With adequate irrigation and fertilization, annual leaf DM yield of over 21 tons/ha can be obtained (Ravindran, 1993). Planting cassava for the production of hay involves the initial cutting of leaves at three months after planting and subsequent cutting at 2-month intervals. The protein yield of cassava hay has been reported to range from 1.5-1.7 ton/ha per harvest from six consecutive leaf harvests (Wanapat, 2009).

Sweet potato cultivation

Like cassava, sweet potato (*Ipomoea batatas* L.) originates from Central America. According to Horton (1988), approximately 80% of the sweet potatoes in the world are grown in Asia, under 15% in Africa and about 6% in the rest of the world. Because of the benefits of sweet potato cultivation and its high nutritive value, sweet potatoes have been developed as a major crop to supply food for humans and feed for livestock. In Vietnam, the area where sweet potatoes are grown has decreased in recent years. In 2001, there was a total area of 244,600 ha planted with sweet potato but in 2010 the area planted was 150,800 ha (Table 2). The main reason for the decline may have been the increase in cassava cultivation area.

Sweet potato produces a high amount of biomass, which depends on the season, climate, fertilizer application, time of harvesting and defoliation. The productive potential of different varieties of sweet potato ranges from 3 to 4 ton DM/ha of root and the foliage production can be 4.3 to 6.0 ton DM/ha/crop of sweet potato (Dominguez, 1992). The biomass yield of sweet potato vines is higher than of cassava as SPV can be harvested many times throughout the year (An, 2004).

Chemical composition of cassava and sweet potato

The chemical composition of cassava and sweet potato root has been well studied (Table 3). The roots of cassava are rich in starch but very low in CP content. Cassava roots have 64-72% starch with 10-20% amylose and 80-90% amylopectin.

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Similar to cassava roots, the roots of sweet potato are rich in starch and also low in CP. The average DM content is approximately 30%, but varies depending on variety, climate and harvesting time.

Table 3. Dry matter content (%) and the chemical composition (% in DM) of cassava root and sweet potato root.

Component	Cassava root ^a	Sweet potato root ^b
Dry matter	35.0	30.0
Crude protein	3.1	5.0
Nitrogen free extract	90.5	80.0
Ether extract	1.3	1.5
Crude fibre	3.1	10
Ash	1.9	3
Vitamins and other components	-	<1

^aBuitrago (2010).

^bWoolfe (1992).

The chemical composition of CL and SPV is shown in Table 4. Eggum (1970) reported that the leaves contain an average of 21% CP (range of 16.7 to 39.0%), of which 85% was true protein. The CP content of CL ranges from 17 to 34% depending on variety (Phuc et al., 2001; Montagnac et al., 2009). Rogers and Milner (1963) reported a range of 17.8 to 34.8% in CP contents of leaves from 20 different cassava cultivars. Ravindran and Ravindran (1988) found that the CP content decreased from 38.1% in very young leaves to 19.7% in mature leaves, with a similar trend observed for most amino acids (AA). With maturation, crude fibre, hemicellulose and cellulose contents increases. In addition, CL is a rich source of most minerals. Tewe et al. (1984) reported the concentrations (% of DM) of the following macro-minerals: calcium, 1.75, potassium, 1.28, magnesium, 0.42, phosphorus, 0.45 and sodium, 0.02. Micro-element concentrations of the following minerals have been reported (mg/kg): zinc, 149, manganese, 52, iron, 259 and copper, 12.

The CP content in the DM of SPV can range from 17.6 to 20.9% (Table 4) while the sweet potato leaves (SPL) have a CP content of 25.6 to 32.4% in the DM (Woolfe, 1992; Ishida, et al., 2000). Components (% of plant DM) of SPV include the stem (26.0%), petioles (23.9%) and leaves (51.1%) (Woolfe, 1992). According to

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Ishida et al. (2000), all parts of the sweet potato plant are rich in dietary fibre. In particular, the stems have a high content of soluble and insoluble dietary fibre. The contents of minerals and vitamins such as A, B₂, C and E are relatively high in SPL in comparison to other vegetables. For this reason, both leaves and vines are, besides being used as a protein source, also used as a vitamin source for pigs, poultry, rabbits and cattle (Dominguez and Ly, 1997; Dominguez, 1992; Ali et al., 1999).

Table 4. Dry matter content (%) and chemical composition (% in DM) of cassava leaves and sweet potato vine.

Dry matter	Crude Protein	Ether Extract	Ash	Crude fibre	Source
Cassava leaves					
-	16.7-39.9	3.8-10.5	5.7-12.5	4.8-29	Ravindran, 1993
19.5-23.3	18.5-32.0	3.9-12.8	7.5-11.1	6.4-9.4	Rogers & Milner, 1963
26.0	23.9-34.7	11.3-15.6	5.0-8.1	9.7-16.5	Phuc, 2000
24.9	28.3	6.9	6.6	15.0	Kinh, 2003
Cassava foliage ^a					
28.0	24.0	6.5	6.2	20.6	Buitrago, 2010
Sweet potato vines					
14.2	18.5	-	12.5	-	Dominguez, 1992
14.2	20.6	2.5	9.1	-	Phuc, 2000
12.1	17.6	3.0	14.0	16.6	Kinh, 2003
12.5	20.9	-	13.5	14.9	Woolfe, 1992

^aLeaves and stems from young plants, with a minimum stem content.

Cassava leaves have a higher concentrations of most EAA in the DM compared to soybean meal (Table 5). It has been reported that leaves are deficient in the sulphur-containing AA (Phuc, 2000; Montagnac et al., 2009). The total content of EAA in the protein of cassava and SPL are similar to levels found in other tropical foliage (Phuc and Lindberg, 2001; An and Lindberg, 2004). However, the content of lysine is lower in SPL than in CL. The content of EAA in both ensiled cassava and SPL are lower than in the dried material. So the content of AA varies between fresh, dried and ensiled SPL (An et al., 2004; Table 5) and also varies between different conservation applications (Table 5). Rogers and Milner (1963) studied the AA

composition in a range of cassava CL varieties and concluded that leaves were deficient in methionine. Buitrago (2010) concluded that, on a total AA compositional basis, leaf-protein concentrate should be a well-balanced source of dietary protein if supplemented with synthetic methionine. Eggum (1970) confirmed that cassava leaf protein was limiting in methionine and tryptophan, but rich in lysine, with an overall biological value (BV) of 44 to 57%, depending on the methionine content. Addition of methionine can increase the BV of cassava leaf protein to 80% (Eggum, 1970).

According to Phuc et al. (2001), the sum of AA in SPV as a percentage of CP was high, but the level of lysine was low compared to the ideal protein for growing pigs. The AA profile is comparable to other tropical forages, but it is poorer compared to that of soybean meal. Reports by Woolfe (1992) and more recently by An et al. (2003) also indicated that the protein in SPL is low in lysine when compared to the ideal AA pattern required for growing pigs.

Anti-nutritional factors in cassava leaves and sweet potato vines

The most important limitation for the utilization of CL as a feed ingredient for monogastric animals is the content of plant-defence factors or ANF (Oke, 1978; Awoyinka et al., 1995; Phuc et al., 2001; Borin et al., 2005; Montagnac et al., 2009) and fibre (Diaz et al., 1997). The presence of cyanogenic glucosides in CL can give rise to HCN when the plant tissue is broken down by processing or during ingestion by animals. The cyanide levels in leaves are influenced by genetic, physiological, edaphic and climatic differences (Ravindran, 1995). Stage of maturity is one of the major causes of variation in the content of cyanogenic glucosides (Ravindran, 1995). The average HCN content found in a Sri Lankan variety (Ravindran et al., 1987) was over 4,000 mg/kg DM. It appears that both the HCN concentration and the bitterness are associated with high cyanogenetic glycoside concentrations (Sundaresan et al., 1987). With maturation of leaves, the concentration of cyanides are reduced. Ravindran et al. (1987) reported that the HCN content of cassava leaf blades during the stage of the expansion (1-4 leaves), fully expanded (5-7 leaves) and mature (8-11 leaves) were 3,161, 1,962 and 774 mg/kg DM, respectively. In a study carried out in North Vietnam, Tiep et al. (1998) reported that the HCN content of the leaves at the time of root harvest varied according to age and maturity: 442 (mg/kg fresh matter) in the green leaves at the top of the plant, 365 in green leaves below the top, 42.9 in

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mature leaves and 14.4 in the oldest leaves. Liem (1998) reported HCN contents from 305 to 425 mg/kg in fresh leaves. Hang and Preston (2005) reported that the HCN concentration in the leaves of twenty cassava varieties taken from the upper part of the plant at the time of root harvesting ranged between 610 to 1,840 mg/kg DM.

Table 5. Content of crude protein (g/kg DM) and amino acids (g/16 g N) in soybean meal, cassava leaves and sweet potato leaves.

Component	Soybean meal ^a	Cassava leaves ^a		Sweet potato leaves ^b		
		Dried	Ensiled	Fresh	Dried	Ensiled
Crude protein	482	264	245	268	269	234
Essential amino acids						
Arginine	7.5	5.9	5.6	5.2	5.2	5.0
Histidine	2.5	1.9	1.7	2.2	2.0	1.9
Isoleucine	4.2	4.4	4.2	3.7	4.2	3.6
Leucine	7.6	8.0	8.3	8.6	8.8	9.0
Lysine	5.3	5.6	5.4	4.5	4.1	3.9
Methionine	1.1	1.5	1.2	1.5	1.6	1.2
Cystine	-	-	-	3.4	3.2	2.3
Phenylalanine	5.6	5.7	5.6	7.1	6.9	7.1
Threonine	3.5	4.0	3.9	5.2	5.2	5.2
Tyrosine	3.7	4.0	4.4	4.1	4.0	3.7
Valine	4.7	5.3	5.3	5.6	5.7	5.4
Non-essential amino acids						
Alanine	4.3	5.7	6.4	5.2	5.4	5.0
Aspartic acid	11.2	9.7	9.3	10.4	11.0	11.2
Glutamic acid	17.5	11.2	9.6	11.6	9.9	10.0
Glycine	3.6	4.1	4.1	4.1	3.5	2.7
Proline	4.4	3.6	4.3	3.7	3.4	3.4
Serine	5.3	4.7	3.8	4.1	4.1	4.7
Total amino acids	91.1	85.3	83.8	90.3	88.2	85.7

^aPhuc (2000).

^bAn et al. (2004).

Tannin levels in CL vary from 30 to 50 g/kg DM (Ravindran, 1993). Harvesting of cassava at an early growth stage (3 month) to make hay will yield a

lower content of condensed tannin and an increased protein content (to 25% of DM) compared to harvest at a later maturity stage and as such results in a higher nutritive value (Wanapat, 2009). Several studies have been undertaken to determine the suitability of the use of CL as a feed ingredient for cattle (Moore and Cock, 1985; Van Man and Wiktorsson, 2001 and 2002), pigs (Ravindran et al., 1983 and 1987, Gómez, 1991; Phuc et al., 2000; Borin et al., 2005) and poultry (Ravindran et al., 1986; Chauynarong et al., 2009). In pigs, the inclusion of 15% fresh CL in the diet had no adverse effects on the performance of growing-finishing pigs (Sarwat et al., 1988). However, Ravindran (1990) reported a linear depression in weight gain and feed efficiency when cassava leaf meals were included at up to 30% in diets for growing-finishing pigs. Techniques for reducing the level of anti-nutritional factors in both CL and roots have been developed, such as ensiling, sun-drying, boiling and fermentation. These methods have been applied and shown to make the feedstuff safe for use as human food and as an animal feed ingredient (Gómez and Valdivieso, 1985 and 1988; Padmaja, 1995; Essers et al., 1995; Diaz et al., 1997).

The limitation for the utilization of sweet potato as a feed ingredient for monogastric animals are the presence of several ANF including the high content in oxalic acid (470 ± 15 mg/100 g fresh weight), phytic acid (0.46 ± 0.01 mg/100 g fresh weight), tannic acids (491 ± 7 mg/100 g fresh weight) and trypsin (52.0 ± 0.9 TIU/g) and chymotrypsin inhibitors (69.1 ± 0.6 TIU/g) (Mosha et al., 1995; Rekha et al., 1999; Hou and Lin, 1997). Mosha et al. (1999) reported that the trypsin inhibitor activity was reduced by 29.7, 34.9, 54.3, 52.3 and 65.6% in cabbage, collard, turnip, sweet potato and peanut greens, respectively, when the vegetables were blanched for 10 min. Reductions occurred for most of the treatments in either the conventional or microwave blanching method.

Processing methods to reduce hydrogen cyanide level in cassava

Cassava HCN is derived predominantly from the cyanogenic glycoside linamarin (93%) and a small amount from lotaustralin (7%) or methyl linamarin (Chauynarong et al., 2009). Catalysed by linamarinase, linamarin is rapidly hydrolysed to glucose and acetone cyanohydrin while lotaustralin is hydrolysed to botanine cyanohydrin and glucose (Leng, 2005, Buitrago, 2010). Both acetone and butanone cyanohydrin spontaneously decompose to HCN.

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Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ which is contained in the enzyme (Leng, 2005). The latter causes a decrease in the utilization of oxygen in tissues. Hydrogen cyanide will reduce the energy availability in all cells, but its effect is most direct and quick on the respiratory system and the heart. In animals, the lethal doses of HCN reported are generally between 0.66 and 15 mg/kg body weight depending on species (WHO, 1965). Buitrago (1990) classified the total cyanide value in fresh cassava products: non-toxic <50 mg/kg, mildly toxic 50-80 mg/kg, toxic 100-150 mg/kg and highly toxic >200 mg/kg. Bolhuis (1954) proposed that cassava based diets which contain less than 50 mg HCN /kg (fresh basis) can be safely used for the feeding of pigs.

Table 6. The effect of processing methods to reduce hydrogen cyanide (HCN) content in cassava leaves and foliage.

Material	Processing method	HCN (mg/kg DM)		Reduction %	References
		Before	After		
Leaves	Drying	509	86	83	Phuc, 2000
Leaves ST ^a	Drying	545	203	62.7	Borin et al., 2005
Leaves LT ^b	Drying	408	273	33	Borin et al., 2005
Leaves	Ensiling	390	147	62	Phuc, 2000
Leaves ST ^a	Ensiling	545	122	77.6	Borin et al., 2005
Leaves LT ^b	Ensiling	408	94	77	Borin et al., 2005
Foliage	Ensiling	980	236	76	Van Man and Wiktorsson, 2001
Foliage	Ensiling	978	309	68	Van Man and Wiktorsson, 2002

^aST: cassava a short term variety, harvested for roots after 6 to 8 months.

^bLT: cassava a long term variety, harvested for roots after 12 months.

Many researchers have reported that the HCN content in cassava is reduced by processing. Padmaja (1989, 1995), Phuc et al. (2001), Cardoso et al. (2005), Van Man and Wiktorsson (2001, 2002) and Borin et al. (2005) have investigated the effect of different processing methods on the chemical contents and the nutritional value of CL and roots. Suspending cassava in water is one method to reduce the level of glycosides

and HCN (Cooke and Maduagwu, 1978), because glucosides dissolve easily in water. Soaking of cassava roots normally precedes cooking or fermentation and removes approximately 20% of the free cyanide present in fresh root chips (Cooke and Maduagwu, 1978). After soaking and boiling, the free cyanide content of cassava chips is rapidly lost and 90% disappears within 15 minutes (Cooke and Maduagwu, 1978).

The HCN content in cassava can be reduced by sun-drying and ensiling. Oke (1994) reported that simple sun-drying or oven drying eliminated almost 90% of HCN present in fresh CL. Wanapat (2009) showed that sun-drying can reduce the HCN content up to 90%. In a study carried out in the South of Vietnam, Phuc (2000) found a 83% reduction in HCN content in fresh CL after sun-drying (Table 6). Van Man and Wiktorsson (2002) reported that the HCN content in the fresh cassava tops was 978 mg/kg DM and this concentration was reduced to 309 mg/kg DM, a reduction of 68%, after two months when ensiled. It was further reduced with increasing storage period and reached a concentration of 230 mg/kg DM, or a reduction of 76% after four months of ensiling. Phuc (2000) found that the HCN content in CL was reduced by around 62% after ensiling (Table 6). Similar results have been found by others (Van Man and Wiktorsson, 2001; Borin et al., 2005). Gómez and Valdivieso (1988) reported that free cyanide in cassava roots after 26 weeks of ensiling was reduced to 36% of the initial value. Drying and ensiling of CL and roots will markedly reduce their cyanide content (Table 6).

Central Vietnam has a tropical monsoon climate with two main seasons: a rainy season and dry season. The harvest of cassava roots in Central Vietnam occurs from September to November and this coincides with the rainy season. The SPV can be harvested many times throughout the entire year. But SPV are normally more abundantly available in the rainy season when sun-drying is unsuitable. So during the rainy season, it is difficult to sun-dry the cassava and SPV to a sufficiently high dry matter content for conservation.

The question remains, however how to use CL and SPV as a protein source in the diet utilising simple processing methods which are easily applied in practice by small scale farmers. Also it is not well known what the effect is of using different additives for ensiling CL on the level of HCN and on the use of the product as a protein source for pigs. In addition, there are limited data on the effect of processing

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on the AA contents of CL and SPV as well as the precise ileal digestibility of AA and CP of CL and SPV in ensiled and dried form for pigs. For practical application, the determination of optimum levels of CL as well as SPV in pig diets needs to be further investigated.

The main questions addressed in the various experiments reported in the present work are as follows:

Chapter 2: What is the impact of different levels of various carbohydrates added to cassava leaves on fermentation and on the chemical properties and especially on protein and its amino acids during ensiling?

Chapter 3: What is the effect of replacing the protein from fish meal by protein from ensiled or dry cassava leaves and sweet potato vines on the performance of growing pigs?

Chapter 4: What is the efficacy of using different levels of ensiled cassava leaves KM94 in diets on the performance and on carcass characteristics of growing pigs under practical farming conditions?

Chapter 5: What is the ileal and total tract apparent digestibility of crude protein and amino acids in ensiled and dried cassava leaves and ensiled and dried sweet potato vines alone or as a 50:50 mixture in growing pigs?

What is the influence of preservation method on the digestibility of nutrients in cassava leaves and sweet potato vines in ensiled and dried form?

Chapter 6: What is the effect of different levels of DL-methionine supplementation alone or in combination with synthetic L-lysine on growth performance of pigs given diets containing cassava leaves as the major protein source?

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Chapter 2

Ensiling techniques for preserving cassava leaves

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Abstract

The objective of the present experiment was to investigate the impact of different levels of various carbohydrates added to cassava leaves (CL) on fermentation and on the chemical properties during ensiling. A total of 8 different additions with 18 replicates each were used for ensiling during 0 to 90 days. The leaves were, mixed with 0.5% NaCl and the additives rice bran (RB), cassava root meal (CRM) at 5 or 10% addition and fresh cassava roots (FCR) at 20, 30, 40 and 50% addition based on pre-wilted weight of CL. Results showed a considerable decrease in pH after 7 days and pH remained low and stable at 3.7-3.9 thereafter in all treatments. The dry matter (DM) content of the silages increased ($P<0.05$) with increasing level of additive. There were significant differences in DM content of the silages with time of ensiling. Crude protein (CP) contents differed among leaves ensiled with RB, CRM and FCR as additions ($P<0.01$) and decreased ($P<0.05$) with increased levels of additives in all silages. Hydrogen cyanide (HCN) content of the fresh CL was 1304 mg/kg DM and was reduced to an average of 251 mg/kg DM after 90 days after ensiling. The inclusion of RB or CRM at a level of 5 or 10% and FCR at all levels (20, 30, 40 and 50% on as fresh basis) produces good quality silage that can be stored up to three months. The entire ensiling process improves the product markedly by reducing the HCN content up to 80% of the original values in the fresh cassava leaves.

Introduction

Vietnamese agriculture is mainly based on small-scale farming systems which are characterized by small farms, integrated crop-livestock systems, a low capital input and a low economic efficiency. Pig production is an important part of these farming systems, with the pig population averaging 27.6 million in 2009 (GSO, 2010). The main feed ingredients for pigs are rice bran, maize, cassava meal and vegetables which are high in energy but low in protein content and as such low in essential amino acids. The conventional protein supplements in the region are soybean meal, groundnut cake, and fish meal but these are not always available to small-scale farmers and when available, relatively expensive. Alternative protein sources, especially more locally produced feed resources, would increase the economic efficiency of small-scale pig production in Vietnam. Next to rice, cassava (*Manihot esculenta* Crantz) is the second most important food crop in Vietnam in terms of total production by volume. In 2009, root production was approximately 8.6 million tonnes (GSO, 2010) with some of the roots used as ingredients for animal feeds.

The crude protein (CP) content of cassava leaves (CL) ranges between 20-30% in the dry matter (DM) and therefore is a good potential source of amino acids for livestock animals. However, they are rarely used in animal feeds because of their high linamarin content. This cyanogenic glucoside can be hydrolysed by the enzyme linamarase present in the plant cell to a cyanohydrin which breaks down further to hydrogen cyanide (HCN). Consumption of cyanide may cause decreased weight gain, depressed thyroid function and decreased utilization of oxygen in pigs (Tewe et al., 1977, 1984; Oke, 1980; Leng, 2005). The symptoms of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, stomach pains, vomiting, diarrhea (Leng, 2005). The typical cyanide content of fresh cassava leaves (FCL) ranges from 200 to 800 mg/kg but values as low as 80 mg/kg (Wood, 1965) and as high as over 4,000 mg/kg (Ravindran and Ravindran, 1988) have been reported. Recently, Hang and Preston (2005) found that the leaves of twenty cassava varieties at the time of root harvesting, contained between 23.7 to 29.5% crude protein (CP) in the DM and a HCN concentration ranging from 610 to 1,840 mg/kg DM.

Drying or ensiling of CL and roots will markedly reduce their cyanide content (Padmaja, 1989; Phuc et al., 2001; Ravindran et al., 1987; Van Man and Wiktorsson, 2002; Borin et al., 2005). Phuc (2000) found that the HCN content in CL was reduced

by around 62% after ensiling. Sun-drying has been reported to reduce the HCN content by almost 90% in fresh foliage leaves (Wanapat, 2009) and 96 to 99% in cassava root (Montagnac et al., 2009). However, during the rainy season, ensiling is the most common method to preserve cassava roots and leaves in order to reduce HCN levels as sun-drying is not possible. The objective of the present experiment was to investigate the impact of different levels of various carbohydrates added to cassava leaves on fermentation and on the chemical properties during ensiling.

Materials and methods

Ensiling of cassava leaves

Fresh cassava leaves were collected at the time of root harvest and spread out on the floor in the open air for five hours after which the leaves were separated from the stems and petioles, chopped into small pieces (2-3 cm) using a hand knife, mixed with 0.5% NaCl and the additives. Rice bran (RB), cassava root meal (CRM) and fresh cassava root (FCR) were used as additives in making CL silage. Locally produced RB and CRM was added at 5 or 10% (RB5, RB10, CRM5, CRM10), and FCR at 20, 30, 40 and 50% (FCR20, FCR30, FCR40, FCR50) of the pre-wilted weight of the CL. One hundred and forty four laboratory silos were used according a factorial design with 8 treatments, 6 measuring times and 3 replicates per treatment. The silages were kept in the laboratory at room temperature (26-30°C) in air tight sealed plastic bags with a capacity of 2 kg each after pressing air out of the bag by hand. Silage samples were taken at 0, 7, 21, 30, 60 and 90 days of ensiling and analysed for pH, DM, CP and HCN. At each sampling time, the content of three bags of each treatment was taken for analysis and the content of each bag analysed separately.

Chemical analysis

The pH value of the silages was recorded on fresh samples by a pH electrode while the HCN content was determined on fresh ensiled samples by titration with AgNO₃ after boiling the sample and concentrating the HCN in KOH (AOAC, 1990). Samples for other chemical analyses were dried at 60°C for 24 h and ground over a 1 mm screen. The DM was determined by drying at 105°C for 24 h to constant weight (method code no. 925.09 of AOAC, 1990). The CP was calculated from total nitrogen

(N x 6.25) which was determined by the Kjeldahl (method code no. 988.05 of AOAC, 1990) in dry samples. The dried samples of FCL, RB and CRM were also analysed for DM, CP, crude fibre (CF; method 978.10 of AOAC, 1990) and HCN content (except in the RB). All samples were analysed in triplicate at the laboratories of Hue University, Hue city, Vietnam.

Statistical analysis

Data were subjected to analysis of variance using Proc GLM in the Statistical Analysis Systems statistical software package version 9.1 (SAS Institute, Cary, NC, USA). The effects of concentration of the additive and the number of days of ensiling were evaluated per type of additive (i.e. RB, CRM and FCR) using the statistical model:

$$Y_{ij} = \mu + C_i + D_j + (C_i \times D_j) + e_{ij}$$

where Y = parameter to be dependent variable, μ = mean, C_i = effect of concentration of the additive and D_j = effect of days of ensiling and $C_i \times D_j$ the interaction between concentration of the additive and time of ensiling. Differences among least square means were evaluated using the Tukey multiple range test. Differences were considered significant at $P \leq 0.05$.

Results

The chemical composition of CL and the ingredients used as silage additives are shown in Table 1. The DM of FCL was 25.5% while the DM of FCR was 35.0%. The CP content of FCL (30.5% in DM) was higher than the CP content in FCR (2.9% in DM) and CRM. The HCN content of FCL and FCR was 1304 and 378 mg/kg DM, respectively.

The effect of additives and ensiling time on pH, DM, CP and HCN content are shown in Table 2. The pH value decreased rapidly during the first week ($P < 0.001$) from starting values of 6.5-6.6 to values which ranged between 4.4 and 4.6 in all treatments (RB, CRM and FCR). These values continued to decrease in the third week to a value of 4.0 to 4.2. After this time values decreased slightly from day 21 until day 60 and remained stable until 90 days after ensiling in all treatments (Table 2). The CRM10 had an overall significant lower pH value compared to the CRM5 while no differences were observed in the inclusion level for the RB and FCR. The DM content

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of the silage decreased slightly from 0 to 90 days of ensiling. There were significant differences in DM content of the silages with time. For all three additives, there were differences in the DM content of the silages at all sampling times. Increases in the level of RB, CRM and FCR resulted in a higher DM content of all silage during all storage times ($P < 0.01$).

Table 1. Dry matter, crude protein, crude fibre and hydrogen cyanide (HCN) content of the fresh cassava leaves and the ingredients used as silage additives.

Component	Fresh cassava leaves	Fresh cassava root	Cassava root meal	Rice bran
Dry matter (% as-is)	25.5	35.0	91.0	86.3
Crude protein (% DM)	30.5	2.9	3.1	11.2
Crude fibre (% DM)	13.5	2.9	2.8	9.5
HCN (mg /kg DM)	1304	378	25	ND

ND=not determined.

For RB and CRM treatments, a significant interaction in DM content was observed (Table 2). Figure 1 and 2 show the DM content of the RB and CRM silage over time. At 21, 30 and 60 days there was a significant difference in the DM content between addition level for the RB addition to the silage. For the silage with added CRM, the highest addition level resulted in a consistent decrease over time while for the lowest level, the DM content remained constant after day 7. The CP content in the silages decreased significantly with time of ensiling from 0 to 21 days and after 21 days until 90 days only a slight decrease was observed for most treatments (see Table 2). A high inclusion of RB, CLM and FCR decreased the CP content of the silage. There were significant differences in the CP content between silages made with RB, CRM or FCR as additives ($P < 0.01$). The CP in the FCR was lowest of all treatments ($P < 0.01$, Table 2). A significant interaction was found for the CRM silages. Figure 3 provides a graphical representation of the interaction.

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Table 2. pH, dry matter (DM), crude protein (CP) and hydrogen cyanide (HCN) content of cassava leaf silages made with the addition of rice bran (RB), cassava root meal (CRM) and fresh cassava roots (FCR) over a 90 day period.

Parameter	pH			DM (% as-is)			CP (% in DM)			HCN (mg/kg DM)		
	RB	CRM	FCR	RB	CRM	FCR	RB	CRM	FCR	RB	CRM	FCR
<i>Concentration</i>												
5	4.6	4.5	-	31.5	31.6	-	28.0	28.4	-	522	540	-
10	4.5	4.5	-	33.0	33.8	-	26.2	25.5	-	466	460	-
20	-	-	4.5	-	-	26.1	-	-	26.4	-	-	654
30	-	-	4.4	-	-	27.5	-	-	23.9	-	-	573
40	-	-	4.4	-	-	28.4	-	-	21.9	-	-	528
50	-	-	4.4	-	-	29.0	-	-	20.7	-	-	455
SE	0.03	0.02	0.02	0.1	0.1	0.2	0.1	0.1	0.2	8	9	10
P-value	0.0663	0.0425	0.0944	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>Days of ensiling</i>												
0	6.5	6.6	6.6	34.3	33.7	28.8	28.8	28.0	24.0	805	834	916
7	4.6	4.5	4.5	33.3	32.7	27.9	28.2	27.4	23.7	707	756	805
21	4.2	4.2	4.1	32.0	32.6	27.3	27.1	26.7	23.5	478	412	533
30	3.9	3.9	3.8	31.8	32.6	27.1	26.5	26.6	23.2	421	412	501
60	4.0	3.8	3.8	31.7	32.5	27.7	26.2	26.6	22.7	307	34	301
90	3.9	3.7	3.8	30.4	32.3	27.7	25.8	26.3	22.3	247	247	260
SE	0.04	0.03	0.03	0.2	0.1	0.2	0.1	0.1	0.2	15	15	13
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>Concentration x days of ensiling</i>												
P-value	0.1317	0.2655	0.7783	0.0371	0.0019	0.7328	0.0884	0.0017	0.9879	0.0459	0.1897	<0.0001

^{a-c}Least square means in the same column of each main effect (concentration and days of ensiling) that do not have a common superscript differ, P<0.05.

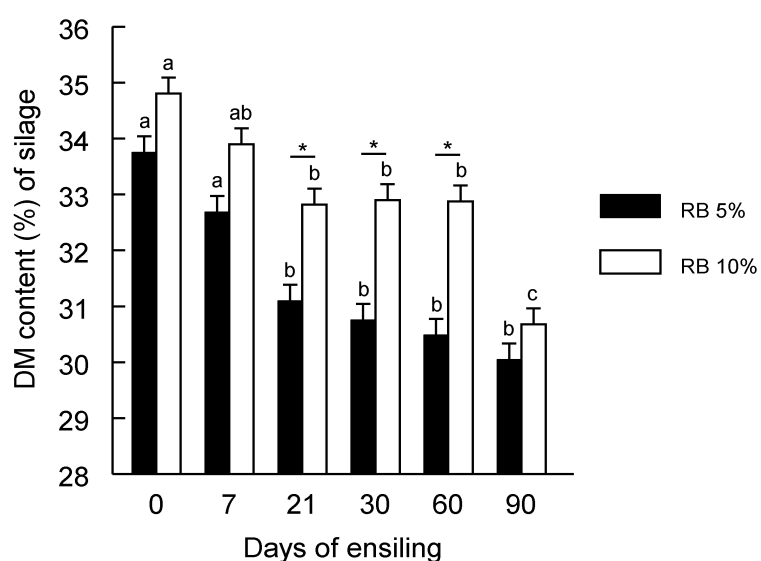


Figure 1. Effect of 5 and 10% rice bran (RB) addition and ensiling time on the DM content of cassava leave silage. Bars with different letters within addition (5 or 10%) are significantly different. * indicates difference ($P < 0.05$) between addition level.

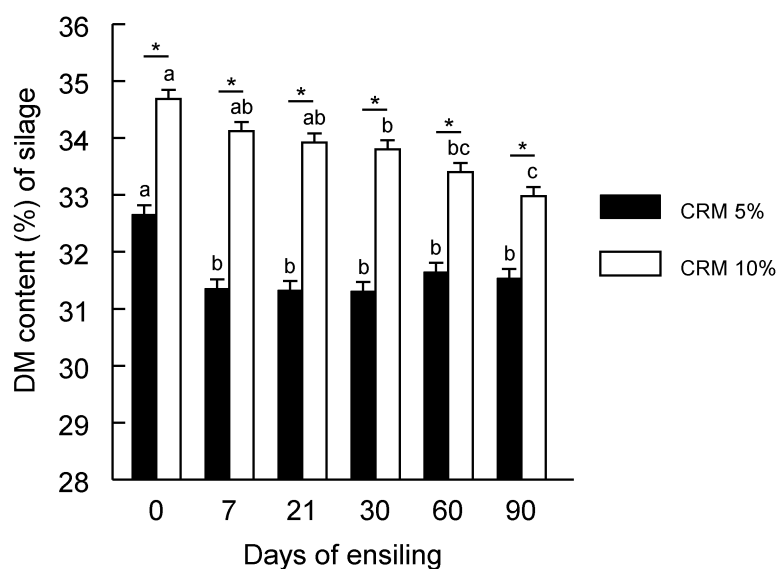


Figure 2. Effect of 5 and 10% cassava root meal (CRM) addition and ensiling time on the DM content of cassava leave silage. Bars with different letters within addition (5 or 10%) are significantly different. * indicates difference ($P < 0.05$) between addition level.

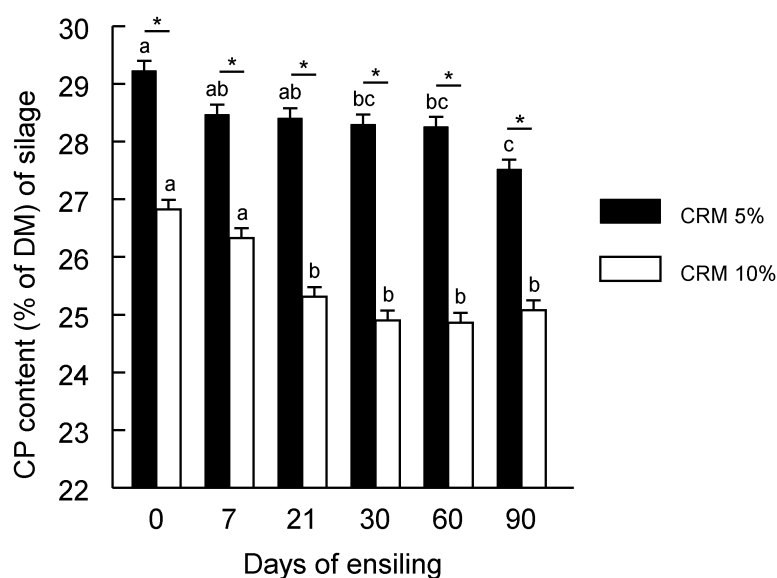


Figure 3. Effect of 5 and 10% cassava root meal (CRM) addition and ensiling time on the CP content of cassava leave silage. Bars with different letters within addition (5 or 10%) are significantly different. * indicates difference ($P < 0.05$) between addition level.

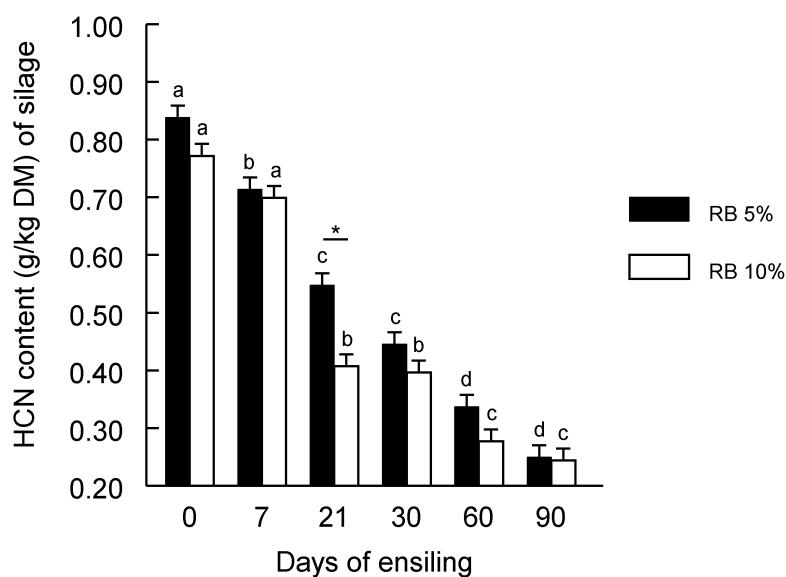


Figure 4. Effect of 5 and 10% rice bran (RB) addition and ensiling time on the hydrogen cyanide (HCN) content of cassava leave silage. Bars with different letters within addition (5 or 10%) are significantly different. * indicates difference ($P < 0.05$) between addition level.

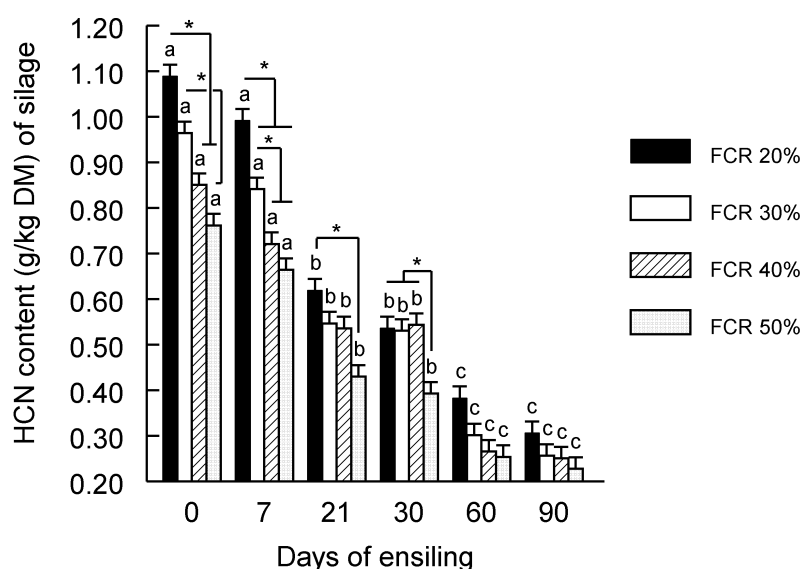


Figure 5. Effect of 20, 30, 40 and 50% fresh cassava root (FCR) addition and ensiling time on the hydrogen cyanide (HCN) content of cassava leave silage. Bars with different letters within addition (5 or 10%) are significantly different. * indicates difference ($P < 0.05$) between addition levels.

Figure 4 and 5 present the HCN concentrations over time for the RB and FCR silages for which significant interactions between concentration and ensiling time was found (Table 2). The HCN content of all treatments decreased rapidly during the first week of ensiling and then continued to decrease from day 7 to 90 days of ensiling. The initial HCN content of the FCL was 1304 mg/kg DM and was reduced to an average of 852 mg/kg DM after 5h pre-wilting. After mixing with additives and with 0.5% NaCl, a reduction to approximately 68% of the initial level due to wilting and dilution alone was observed. At 90 days after ensiling, the HCN content of the silage had fallen to an average of 251 mg/kg DM or approximately 19% of the initial concentration in the FCL.

Discussion

Ensiling is a process of fermentation of carbohydrates by means of lactic acid bacteria, clostridia and enterobacteria. As a result, fatty acids are produced, especially by the lactic acid bacteria which can be divided into two categories: the homofermentative bacteria (*Lactobacillus plantarum*, *Pediococcus pentosaceus* and

Enterococcus faecalis) and the heterofermentative bacteria (*Lactobacillus brevis* and *Leuconostoc mesenteroides*). The homofermentative lactic acid bacteria are more efficient during fermentation (McDonald et al., 2002; Filya, et al., 2007). When the crop is ensiled, the lactic acid bacteria continue to increase, fermenting the water-soluble carbohydrates into organic acids, mainly lactic acid which results in a reduction in pH value. The aim of pre-wilting is to reduce the moisture contents and to increase concentration of carbohydrates (water soluble) to be used as an energy source by microbes as well as in the case of cassava (leaves and root), decrease the HCN concentration. Pre-wilting is a very important step to reduce the moisture content of the fermenting material (An and Lindberg, 2004). In our study, we did not reach this DM level after wilting but by addition of easy fermentable carbohydrates a sufficient low pH was obtained.

The results from the present study show that after pre-wilting, CL can be successfully ensiled and preserved as silage provided that additions of RB, CRM and FCR are made to the silage. These products are locally available feedstuffs in Vietnam and are rich in easily fermentable carbohydrates in the form of sugars and starch providing a source of available energy for the growth of lactic acid bacteria (McDonald et al., 1991). The rapid drop in pH at day 7 shows that the additives provided readily available source of substrate to ensure fermentation. This finding is in accordance with a number of earlier studies where carbohydrate-rich materials have been used as additives in an attempt to improve both the fermentation process and the nutritional value of silage (McDonald et al., 1991; Ngoan et al., 2000). Ngoan et al. (2000) considered CRM to be the best additive for silage making, as cassava stimulates lactic acid fermentation to produce a very low pH. The latter study also showed that the pH of shrimp by-products ensiled with molasses at a ratio of 3 shrimps by-product units to 1 molasses (w/w), and also with shrimp by-products to CRM at a ratio of 1:1, decreased the pH during the first week to below 4.5 and remained low up to 56 days after ensiling. An and Lindberg (2004) found that CRM, sweet potato meal and molasses added at levels of 0, 30, 60 and 90 g/kg to air dried or pre-wilted sweet potato leaves gave a clearly lower pH after 7 days and this pH remained low (between 3.5-4.1) and stable until day 56 of ensiling. Increasing the level of additives to the pre-wilted CL, increased the DM content. The increase in DM content in the CL silage can be explained by the added DM from RB, CRM and FCR, and may be of significance for improving the fermentation process in forages low in

water soluble carbohydrates (WSC) (McDonald et al., 1991). However, the DM content of the silages decreased slightly from 0 to 90 days of ensiling. This is in agreement with An and Lindberg (2004). The WSC in herbage is the main substrate for microbial growth and as such concentrations of WSC in silage are reduced during fermentation. Significant losses of DM in our study are probably a consequence of losses of carbohydrates by the fermentation process and the production of volatile fatty acids during ensiling. It should be noted that most losses of nutrients occur as a result of aerobic catabolism before the material is ensiled or as a result of the import of air during storage or after opening the silo. McDonald et al. (2002) reported that losses of DM of less than 5% during ensiling are acceptable.

The CP content was highest in the treatment with 5% rice bran added (RB5) and lowest in the FCR50 treatment. The CP content was significantly decreased with increasing inclusion of the additives as CRM, and FCR50 only contained 20.7% CP per unit DM on average (Table 2). The CP content of the silages did not change during the first week of ensiling, but there was a significant decrease at day 90 in all treatments. In this study, the concentration of CP in the silage fell for all treatments by 3-5% in the DM, most likely as a result of bacterial degradation. The drop in CP content at day 90 is likely to be due to nitrogen losses by volatilization NH_3 escaping the silage in gaseous form or leakage during the time of ensiling. Ideally during the ensiling process, there is some amino acid synthesis via microbial growth in the silage. Phuc et al. (2001) and Borin et al. (2005) showed that ensiling CL resulted in higher coefficients of total tract apparent digestibility (CTTAD) or coefficients of ileal apparent digestibility (CIAD) of CP and amino acids compared to sun-dried materials. There may also be some catabolism of amino acids during fermentation which occurs if the pH is not too low (McDonald et al., 1991). Ensiling the CL was also found to result in higher CTTAD and CIAD of other dietary components compared with sun-drying (Phuc et al., 2001; Borin et al., 2005). Hong and Lindberg (2007) found that the CIAD of CP, crude fibre and NDF in pigs was higher ($P < 0.05$) on fermented diets than on raw and cooked diets.

Besides conservation of the CL, ensiling also reduces the HCN concentration. Hydrogen cyanide in cassava is derived from the cyanogenic glycoside linamarin (93%) and a small amount from lotaustralin (7%) or methyl linamarin (Chauynarong et al., 2009). Catalysed by linamarinase, linamarin is rapidly hydrolysed to glucose and acetone cyanohydrin and lotaustralin hydrolysed to a related cyanohydrin and

glucose (Leng, 2005). Under neutral conditions, acetone cyanohydrin decomposes to acetone and hydrogen cyanide. The HCN content of FCL in the present study was 1304 mg/kg DM, or 332 mg/kg fresh matter. This HCN content was less than that reported by Ravindran et al. (1987) of 4000 mg/kg DM but similar to that by Tiep and Dong (1998). The latter authors reported that the HCN content of the leaves at the time of root harvest varied according to age and maturity: 442 mg/kg fresh matter in the green leaves at the top of the plant and 365 mg/kg fresh matter in green leaves below the top. Liem (1998) also reported similar values ranging from 305 to 425 mg of HCN /kg fresh leaves. In addition Ravindran et al. (1987) reported that HCN contents of CL in the growth stage of the plant: expanding (1-4 leaves), fully expanded (5-7 leaves) and mature (8-11 leaves) were 3161, 1962 and 774 mg /kg DM, respectively. Hang and Preston (2005) found that the HCN content of leaves of twenty cassava varieties taken from the upper part of the plant at the time of root harvesting, ranged from 610 to 1840 mg/kg DM. In the present study, the HCN content of the FCL was 1304 mg/kg DM and was reduced to 251 mg/kg DM, or a reduction of 80.6%, after 90 days after ensiling. Van Man and Wiktorsson (2002) reported that the HCN content in the fresh cassava tops was 978 mg/kg and was reduced to 309 mg/kg, or a reduction of 68%, after two months of ensiling and continued to decrease with storage period reaching a concentration of 230 mg/kg, or a reduction of 76% at four months after ensiling. Phuc (2000) found that the HCN content in CL was reduced by approximately 62% after ensiling. Similar results have been found by others (Van Man and Wiktorsson, 2001; Borin et al., 2005). Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ which is contained in the enzyme (Leng, 2005) and causes a decrease in the utilization of oxygen in tissues. Hydrogen cyanide will reduce the energy availability in all cells, but its effect will be most immediate on the respiratory system and heart. In animals, the lethal doses of HCN are generally reported to be between 0.66 and 15 mg/kg body weight for various species (WHO, 1965). Buitrago (1990) classified the total HCN value in cassava fresh products: non-toxic <50 mg/kg, mildly toxic 50-80 mg/kg, toxic 100-150 mg/kg and highly toxic >200 mg/kg. Bolhuis (1954) proposed that cassava-based diets containing less than 50 mg/kg HCN (fresh basis) can be safely used for feeding to pig. In the present study, the HCN content of the CL silage was reduced with storage time reaching values at 90 day of ensiling of 247, 247 and 260 mg/kg DM for the RB, CRM and FCR treatments (Table 2) which would correspond

to 72-80 mg/kg fresh leaves. If fed as the sole source of nutrition, the present silages would probably evoke mildly toxic effects in pigs but if included in the diet at <70% would be non-toxic according to the classification on Buitrago (1990).

Conclusions

Cassava leaves can be preserved by common ensiling methods with 5-10% rice bran, 5-10% cassava root meal and 20-50% fresh cassava root. The inclusion of rice bran or cassava root meal at a level of 5 or 10% and fresh cassava roots at all levels (20, 30, 40 and 50% on as fresh basis) produces a good quality silage that can be stored for up to three months. The entire ensiling process improves the product markedly by reducing the HCN content up to 80% of the original values in the fresh cassava leaves.

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Chapter 3

Ensiled and dry cassava leaves, and sweet potato vines as a protein source in diets for growing Vietnamese Large White×Mong Cai pigs

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Abstract

The aim of the present study was to evaluate the effects of replacing 70% of the protein from fish meal by protein from ensiled or dry cassava leaves and sweet potato vines on the performance and carcass characters of growing F_1 (Large White \times Mong Cai) pigs in Central Vietnam. Twenty-five crossbred pigs (Large White \times Mong Cai) with an initial body weight (BW) of 19.7 kg ($SD = 0.84$) were allocated randomly to five treatment groups with 5 animals per group (3 males and 2 females). Pigs were kept individually in pens (2.0 x 0.8 m) and fed one of five diets during 90 days. The control diet was formulated with fish meal (FM) as the protein source while the other four diets were formulated by replacing 70% of FM protein by protein from ensiled cassava leaves (ECL), dry cassava leaves (DCL), dry sweet potato vines (DSPV) or ensiled sweet potato vines (ESPV). Animals were fed their diets at 4% of BW. Results showed that final BW, average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) among the experimental treatments were not significantly different ($p > 0.05$). Ensiled cassava leaves or DCL and ESPV reduced feed cost per unit gain by 5-16.1% compared to the FM diet. There were no significant differences in carcass characteristics among the diets ($p > 0.05$). Lean meat percentages and protein deposition ranged from 41.5-45.8% and 40.2-52.9 g/day, respectively. Using ensiled or dry cassava leaves and sweet potato vine can replace at least 70% of the protein from fish meal (or 35% of total diet CP) without significant effects on performance and carcass traits of growing (20-65 kg) pigs. Including cassava leaves and sweet potato vines could improve feed costs and therefore has economic benefits.

Introduction

In Vietnam, pig production plays an important role at both the family and national level and at present the pig population is approximately 26.6 million heads (GSO, 2008). Normally, 4-6 pigs are kept per household and pigs are fed locally available feed resources including rice bran, cassava roots or cassava meal, maize meal and vegetables. These feeds have low protein content (Pham et al. 2010), and therefore often commercial feeds are used or fish meal and soybean meal are included as a protein source. These protein supplements, however, are expensive and alternative sources are required to increase overall production efficiency of pigs raised in smallholder farms.

Cassava leaves (CL) are a rich in protein and the total essential amino acid content in the protein is higher than in soybean protein (Phuc, 2000; Montagnac et al., 2009). In addition, the CP content in the DM of sweet potato vines (SPV) ranges from 16 to 29% (Dung, 2001) and the protein has a reasonable amino acid (AA) pattern (Woolfe, 1992; Ishida et al., 2000). Being readily available throughout Vietnam, these two protein sources show great promise for livestock. In a previous study, Nguyen et al. (2009) found that AAs in a practical diet for pigs, containing approximately 90% of CP from either CL and/or SPV have an apparent ileal digestibility of 0.65 to 0.75. Cassava leaves and SPV contain a number of nutritionally active factors including linamarin, oxalic acid, phytic acid, tannic acid and trypsin and chymotrypsin inhibitors (Ngudi et al., 2003; Cardoso et al., 2005; Phuc, 2000; Man and Wiktorsson, 2001). Drying or ensiling CL and SPV reduces a number of these nutritionally active factors including linamarin (Padmaja, 1989; Cardoso et al., 2005; Nguyen et al., 2012), trypsin and chemotrypsin inhibitors (Mosha and Gaga, 1999; Peters et al., 2005). However, additional research is required on the inclusion of CL and SPV as a protein source in practical, traditional diets for growing pigs to replace part of the protein from fish meal.

The aim of the present study was to evaluate the effects of replacing 70% of the protein from fish meal by protein from ensiled or dry cassava leaves and sweet potato vines on the performance and carcass characters of growing F₁ (Large White×Mong Cai) pigs in Central Vietnam.

Materials and methods

Ensiling

Fresh cassava leaves were collected at the time of root harvest and spread out on the floor during 5 hours for wilting to increase the dry matter content from about 25 to 30%. The leaves were separated from the stems and petioles, chopped into small pieces (2-3 cm), mixed with 0.5% NaCl and then mixed with rice bran at 5% of the wilted weight of the cassava leaves. The cassava leaf silage was kept in sealed airtight plastic bags with a capacity of 30 kg, and was stored for at least 2 months prior to feeding. After opening the bag, it was fed for a maximum of 5 days.

Sweet potato vines were harvested at 60 days after planting, with subsequent harvests at 20-day intervals. At the time of harvest, 50% of the total branches were cut at 10 cm distance from the main stems. The vines were chopped into small 2-3 cm pieces and spread out on the floor overnight for wilting to reduce the moisture content (from 14 to 19%). Rice bran was used as additive at 10% of the wilted weight of the leaves and common salt was added at 0.5% of the wilted weight of the leaves. The silage was kept in sealed airtight plastic bags with a capacity of 30 kg, and was stored for at least 20 days prior to feeding. After opening a bag, it was fed for a maximum of 4 days.

The ensiled cassava leaves (ECL) or ensiled sweet potato vines (ESPV) were removed from the plastic bags daily and were mixed with the other dietary ingredients (rice bran, maize meal, ensiled cassava root (ECR), fish meal (FM), premix and soybean oil) at the time of feeding.

Drying

Fresh cassava leaves or SPV were collected and spread out on concrete outdoors in the sun for 2-3 days during which time the DM content of the material increased to 92-93%. The dried leaves were collected and milled through a 1 mm screen where after the meal was kept in plastic bags and stored in a dry place. Dry cassava leaves (DCL) and dry sweet potato vines (DSPV) were mixed with the other dietary ingredients at the time of feeding.

Animals and management

The experiment was carried out at Hue University's research farm from January 2006 to May 2006. The protocol of the experiment had been approved by the ethical committee of Hue University, Hue, Vietnam.

Twenty-five crossbred pigs (Large White×Mong Cai) with an initial weight of 19.7 kg (SD=0.84) were used. The pigs were vaccinated against hog cholera and Pasteurellosis, and de-wormed 2 weeks before starting the experiment. All pigs were allocated randomly by sex into five groups, with each group consisting of 5 pigs (3 males and 2 females). Pigs were kept individually in pens of 2.0 x 0.8 m (length x width) at the experimental farm station. Animals were fed the test diets during 90 days and were then slaughtered. The feed allowance was fixed at 4% of BW, divided equally into three meals each day (7:00 h, 11:00 h and 16:00 h). The amount was reduced if refusals were observed. Water from the tap was supplied *ad libitum* and feed refusals were collected and recorded daily. Live BWs were measured in the morning before the first meal every month.

Experimental design and diets

The experiment was designed as a completely randomized design, with 5 treatments and 5 replicates. In all the experimental diets, the basal ingredients (ECR, rice bran and maize meal) provided 50% of the protein of the total diet, the remainder being supplied from either FM or CL and SPV (ensiled or dry). The protein source in the control diet originated from FM. In the four other diets, 70% of the FM protein was replaced by ECL, DCL, DSPV or ESPV. The chemical composition of ingredients and the composition of the experimental diets are presented in Tables 1 and 2, respectively.

The pigs were fed one diet from 20 to below 50 kg (period 1) and another composition from 50 kg onwards to about 70 kg live weight (period 2). The nutrient requirement of the pigs followed the recommendations of the National Institute of Animal Husbandry, Vietnam (NIAH, 2001). The ME content was similar between diets and adjusted by supplementation of soybean oil to the diets (Table 2).

Cassava leaves and sweet potato vines as a protein source in diets for growing pigs

Table 1. Chemical composition of the dietary ingredients used to formulate the experimental diets.

	Ingredient ¹							
	ECR	Rice bran	Maize meal	FM	ECL	DCL	ESPV	DSPV
Organic matter (% DM)	97.5	91.0	98.4	70.5	92.0	92.2	85.5	86.6
Crude protein (% DM)	2.0	13.4	9.6	49.4	24.2	29.0	19.7	23.1
Crude fat (% DM)	0.5	13.1	4.6	5.3	7.0	6.7	6.4	5.3
Crude fiber (% DM)	2.9	9.4	2.6	ND	14.3	14.9	15.6	16.0
Neutral detergent fiber (% DM)	5.1	20.6	12.7	ND	36.5	36.9	41.5	41.4
Lysine (g/kg DM)	1.02	6.19	3.08	24.23	11.33	12.6	8.33	8.34
Methionine+cysteine (g/kg DM)	0.11	2.70	1.91	9.15	4.82	6.25	4.20	4.29
Metabolisable energy (MJ/kg DM)	12.6	11.9	14.9	12.0	10.8	10.6	9.5	9.5
Hydrogen cyanide (mg/kg DM)	25	ND	ND	ND	198	160	ND	ND

¹DCL: dry cassava leaves; DM: dry matter; DSPV: dry sweet potato vines; ECR: ensiled cassava root; ECL: ensiled cassava leaves; ESPV: ensiled sweet potato vines; FM: fish meal; ND, not determined.

Chapter 3

Table 2. Ingredient content (%), chemical composition (% of DM), calculated metabolisable energy content (MJ/kg DM) and hydrogen cyanide content (mg/kg DM) of the experimental diets for the pigs.

Ingredients	Diet ¹									
	Period 1 (20 to <50 kg)					Period 2 (>50 kg)				
	FM	ECL	DCL	DSPV	ESPV	FM	ECL	DCL	DSPV	ESPV
Ensiled cassava root	30.0	17.0	20.5	16.3	12.3	40.0	27.4	31.4	27.2	25.0
Rice bran	30.5	30.0	30.0	32.5	33.8	27.5	25.0	25.0	25.7	27.6
Maize meal	25.0	28.0	28.0	24.6	23.5	20.0	26.0	25.0	23.8	20.7
Fish meal	14.0	4.5	4.5	4.4	4.4	12.0	3.6	3.6	3.6	3.5
Ensiled cassava leaves	-	20.0	-	-	-	-	17.5	-	-	-
Dry cassava leaves	-	-	16.5	-	-	-	-	14.5	-	-
Dry sweet potato vines	-	-	-	20.2	-	-	-	-	18.2	-
Ensiled sweet potato vines	-	-	-	-	23.5	-	-	-	-	21.2
Premix ²	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Soybean oil	-	-	-	1.5	2.0	-	-	-	1.0	1.5
Calculated chemical composition										
Crude protein	14.0	14.0	14.0	14.0	14.0	12.0	12.0	12.0	12.0	12.0
from basal ingredients	7.0	7.0	7.0	7.0	7.0	6.0	6.0	6.0	6.0	6.0
from protein source	7.0	7.0	7.0	7.0	7.0	6.0	6.0	6.0	6.0	6.0
Organic matter	91.5	93.0	93.0	91.6	90.1	92.1	93.7	93.9	92.3	91.8
Crude fat	6.1	7.0	6.6	6.9	7.5	5.4	6.0	5.7	5.8	6.3
Crude fiber	4.4	6.9	6.5	7.5	8.0	4.3	6.3	6.0	6.8	7.3
Neutral detergent fibre	11.0	17.9	16.7	20.9	20.7	10.2	16.2	15.1	20.1	18.6
Lysine	0.64	0.63	0.6	0.58	0.6	0.57	0.55	0.53	0.5	0.53
Ileal digested lysine	0.49	0.46	0.44	0.42	0.45	0.42	0.41	0.39	0.37	0.39
Methionine	0.26	0.29	0.28	0.26	0.28	0.22	0.25	0.24	0.23	0.24
Ileal digested met+cys	0.20	0.21	0.20	0.19	0.21	0.20	0.19	0.18	0.17	0.18
Metabolisable energy	12.8	12.6	12.6	12.6	12.6	12.7	12.6	12.6	12.6	12.6
Hydrogen cyanide	7.5	43.9	31.5	4.1	3.1	10.0	41.5	31.0	6.9	6.4

¹ DCL: dry cassava leaves; DM: dry matter; DSPV: dry sweet potato vines; ECL: ensiled cassava leaves; ESPV: ensiled sweet potato vines; FM: fish meal.

² Composition per kg premix: 2400 mg retinol; 4.32 cholecalciferol, 15000 mg α -tocopherol, 5000 mg phytylmenaquinone, 2000 mg thiamin; 15000 mg riboflavin, 25000 mg calcium pantothenate, 30000 mg niacin, 30 mg cyanocobalamin, 2000 mg folic acid, 100 mg choline, 100 mg Fe, 115 mg Zn, 40 mg Cu, 0.15 mg Co, 0.6 mg I, 0.3 mg Se.

The HCN content of the ECL and DCL diets was 43.9 and 31.5 mg per kg DM for the growing period and 41.5 and 31.0 mg per kg of DM for the finishing period. The HCN of the ECL and DCL were higher than that in FM, DSPV and DSPV diets (Table 2).

Carcass measurements

For the evaluation of carcass traits, three randomly chosen pigs (2 males and 1 female) from each treatment were slaughtered at the final BW after a growing period of 160 days. The final BWs were about 60-70 kg. The pigs were starved for 24 hours, weighed and then slaughtered at the slaughter house in Hue City. Carcass weights were measured according to Kauffman and Epley (2000). Hot carcass weights were measured immediately after slaughter. Carcass weights (the body without blood, hair and internal organs) were recorded and the weight of the hot carcass without head. The carcass ratio was calculated as the ratio of carcass mass to live BW after the pigs had been starved for 24 hours. The P₂ back fat thickness was measured on the partitioned carcass 10 cm from the midline behind the tenth rib using a ruler, and loin area was measured by trace paper (70g/m²) at slaughter. Carcass length was measured from the first rib to the pubic bone. Lean percentage was calculated as the ratio of lean mass to hot carcass weight (Kauffman and Epley, 2000):

$$\text{Lean mass (Ib)} = 7.231 + (\text{hot carcass weight, Ib} \times 0.437) + (\text{loin area, Inch}^2 \times 3.877) - (\text{P}_2 \text{ backfat thickness, Inch} \times 18.746).$$

Chemical analysis

Samples of diet ingredients were dried at 60°C overnight and ground over an 1 mm screen before analysis. Dry matter, CP, crude fiber (CF), Neutral detergent fibre (NDF) and ash were determined in dry samples according to AOAC (1990). Neutral detergent fibre was analyzed according to Robertson and Van Soest (1991), with addition of sodium sulfite and alpha amylase. Amino acids were analyzed according to Spackman et al. (1958) on an ion-exchange column using an HPLC. Samples were hydrolyzed for 24 hours at 110°C with 6 mol/HCL containing 2 g/l reagent grade phenol and 5000 nmol norleucine (internal standard) in evacuated and sealed ignition tubes. Methionine+ cysteine content was determined as methionine sulphone+cysteic acid with separate samples hydrolyzed for 24 hours as described above after oxidation

with performic acid overnight at 0°C (Moore, 1963). All samples were analyzed in triplicate while AAs were determined in duplicate.

Data collection

Pigs were weighed at the start of the experiment and on the sixth day of each month during the experimental period at 06:00 h in the morning, before the first meal. Refused feed was collected and recorded daily in order to determine DMI. Feed cost/kg live weight gain, and protein and fat deposition were calculated for each treatment. Feed cost was calculated by the quantity feed eaten by each pigs and the price of 1 kg feed DM. Dry matter intake was calculate from feed refusals and the feed costs was derived by calculation using the market price for each ingredient and the amount used.

To calculate the approximate protein and fat deposition, the following assumptions were made: one gram of protein and fat contains 23.4 and 39.7 kJ of energy per g, respectively (NRC, 1998) and

$$\text{ME intake} = \text{ME}_{\text{m}} + c \times \text{protein deposition} + d \times \text{fat deposition}$$

where ME_{m} is the amount of ME required for maintenance (460 kJ of ME per kg of metabolic BW ($\text{BW}^{0.75}$)); c and d represent the amount of ME needed for the deposition of 1 g of protein and fat, respectively. The required amount of ME per g of protein and fat deposition were assumed to be 53 and 53 kJ (NRC, 1998). On the basis of the literature review of Kotarbinska and Kielanowski (1969), it can be assumed that about 10% of weight gain is gut fill and ash, thus:

$$0.9 \times \text{ADG} = \text{water} + \text{protein} + \text{fat}$$

The deposition rate of protein and fat in the empty body of the two genotypes were calculated based on the two following equations:

$$0.9 \times \text{ADG} = F + P/0.21 \text{ and } \text{MEp} = (F + P) \times 53$$

where ADG is average rate of gain (g/d), 0.21 is protein/(protein + water), F is the amount of fat deposited (g/d); P is the amount of protein deposited (g/d) and MEp is the metabolisable energy used for fat and protein deposition.

Statistical analyses

Analyses of variance was conducted using the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y is the dependent variable, μ is the overall mean, T_i is the treatment effect ($i=1, 2, 3, 4, 5$) and e_{ij} is the random error. Data collected were analyzed by ANOVA using the General Linear Model (GLM) of the Minitab Statistical Software Version 14 (2004). Tukey pair-wise comparison was used to determine differences between treatment means at $p<0.05$.

Table 3. Effect of dietary protein source on the performance of F₁ crossbred (Large White×Mong Cai) pigs.

Parameter	Diet ¹					SEM	p-value
	FM	ECL	DCL	DSPV	ESPV		
Number of pigs	5	5	5	5	5	-	-
Initial BW, kg	19.3	19.7	20	19.8	19.7	0.40	0.81
Final BW, kg	61.8	60.5	59.3	55.5	61.5	3.14	0.63
ADG, g/day	470	456	436	394	464	34.8	0.55
DMI, kg DM/day	1.46	1.41	1.42	1.34	1.42	0.08	0.88
FCR, kg DM/kg gain	3.1	3.1	3.3	3.4	3.1	0.09	0.057
Feed cost, VND/kg gain ²	11636 ^{ab}	9763 ^b	11057 ^{ab}	11158 ^a	10698 ^{ab}	318	0.002

¹FM, fish meal; ECL, ensiled cassava leaves; DCL, dry cassava leaves; DSPV, dry sweet potato vines; DMI, dry matter intake; ESPV, ensiled sweet potato vines; FCR, feed conversion ratio; SEM, standard error of the mean.

²Price of feed ingredients in Hue at the time of the study (Viet Nam Dong, VND/kg): ECR, 600; rice bran, 3100; maize, 3000; fish meal, 8500; ECL, 550; DCL, 2800; ESPV, 550; DSPV, 2500; premix, 30000; soybean oil, 12000. At the time of the study: 1 USD = 16000 VND.

^{a,b}Values within rows with differing superscript letters are significantly different ($p<0.05$).

Results

There were no significant differences in the final BW, ADG, DMI and FCR of the pigs fed the various experimental diets ($p>0.05$; Table 3). The final BW and ADG of the pigs were not different among dietary treatments ($p>0.05$). DMI of the pigs was the highest for the FM and lowest for the DSPV diet but again no significant differences were observed between treatments ($p>0.05$). Feed conversion ratio tended to be different among treatments with pigs fed the DSPV diet having higher values

than those fed the FM, ECL or ESPV diets ($p=0.057$). Calculating the feed cost per kg of live weight gain showed a significant difference among the experimental diets ($p=0.002$). The feed cost per kg of live weight gained was higher for the DSPV diet than for the ECL diet. Although not significant, compared to the control diet (FM), the DSPV was equally as expensive while the diets containing ECL, DCL and ESPV as a protein source were 16.1, 5.0 and 8.1% less expensive.

Table 4. Effect of dietary protein source on the carcass characteristics of F₁ crossbred (Large White×Mong Cai) pigs.

Parameter	Diet ¹					SEM	p-value
	FM	ECL	DCL	DSPV	ESPV		
Number of pigs	3	3	3	3	3	-	-
Live weight at slaughter, kg	63.7	62.7	61.8	57.2	61.8	3.82	0.785
Headless hot carcass weight, kg	43.7	42.3	43.2	39.5	43.2	2.95	0.857
Dressing percentage, %	68.5	67.4	69.8	69.0	69.9	0.81	0.274
Carcass length, cm	76.8	79.0	80.0	74.7	78.0	2.81	0.711
Loin muscle area, cm ²	24.4	23.7	26.2	25.6	24.8	1.19	0.633
Back fat thickness P ₂ , cm	3.1	3.2	2.9	2.9	3.1	0.13	0.388
Lean meat, %	43.1	41.5	45.8	45.2	43.0	1.49	0.301

¹DCL, dry cassava leaves; DSPV, dry sweet potato vines; ECL, ensiled cassava leaves; ESPV, ensiled sweet potato vines; FM, fish meal; SEM, standard error of the mean.

The three pigs randomly chosen for carcass evaluation (2 males and 1 female) had a somewhat higher BW than the mean values presented in Table 3. None of the carcass parameters measured were found to be significant between treatments. Loin muscle area and lean meat percentage were numerically higher for the pigs fed the DCL and DSPV diets. Carcasses of pigs fed the DCL and DSPV diets had a numerical higher lean meat percentage than pigs on the other diets.

No significant dietary treatment effects were found for any of the carcass traits measured ($p>0.05$, Table 4).

There were no significant treatment effects for the estimated fat and protein deposition of the F₁ pigs ($p>0.05$, Table 5). Protein deposition of the pigs was numerically higher when fed the FM, ECL and ESPV diets compared to the DCL and DSPV diets.

Table 5. Effect of dietary protein source on the fat and protein deposition of F₁ crossbred (Large White×Mong Cai) pigs between 20-65 kg.

Deposition	Diet ¹					SEM	p-value
	FM	ECL	DCL	DSPV	ESPV		
Fat, g/day	182.4	165.3	175.4	164.9	166.1	13.8	0.214
Protein, g/day	50.8	51.0	45.5	40.2	52.9	4.1	0.214

¹DCL, dry cassava leaves; DSPV, dry sweet potato vines; ECL, ensiled cassava leaves; ESPV, ensiled sweet potato vines; FM, fish meal; SEM, standard error of the mean.

Discussion

Cassava leaves are a rich source of protein with a total essential AA content higher than soybean protein (Eggum, 1970, Phuc, 2000, Montagnac et al., 2009). The CP content in the DM of SPV ranges from 16 to 29% (Farrell et al., 2000; Hartemink et al., 2000; Dung, 2001). Cassava leaves and sweet potato leaves have been used as a protein supplement for feeding pigs and can, at least partly, replace FM, soybean meal and groundnut cake in pig diets (Phuc, 2000; Phuc and Lindberg., 2001; Van An et al., 2005). There is limited information on the performance of pigs fed diets where traditional protein sources are replaced by ensiled or dry CL and SPV. Van An et al. (2005) reported that the daily weight gain of F₁ (Large White×Mong Cai) pig was 542 g/day when fed diets containing FM protein, while the daily BW gain of F₁ was only 482 g/day when the protein source came from sweet potato leaves. In the present study, we found that there were no significant differences in the final BW, ADG, DMI, FCR and carcass traits among the experimental diets. These results demonstrated that 70% of the CP from FM in diets for pigs can come from CL and SPV in either ensiled or dried form without reducing performance.

Fresh CL have a high HCN content and drying or ensiling of CL and roots can markedly reduce the HCN content (Phuc, 2000; Ngudi et al., 2003, Borin et al., 2005). Cassava leaves can be preserved up to 3 months by common ensiling methods which improves the nutritional quality by reducing the HCN content up to 80% of the original concentrations found in the leaves (Nguyen et al. 2012). The HCN concentration of the CL diets (ECL and DCL) was higher than in the other FM, DSPV and ESPV diets. No indication of cyanide toxicity was observed in any of the pigs fed the diets containing HCN in the present study. Growth performance of the animals on the various diets was not significantly different indicating that the pigs fed the HCN

containing diets did not have a reduction in growth. In animals, the lethal dose of HCN is generally reported to be between 0.66 and 15 mg/kg BW for various species (WHO, 1965; Leng, 2005). Tewe (1992) reported a toxicity level of HCN for pigs of 3.5 mg/kg BW. The pigs in the present study fed the ECL and DCL diets ingested approximately 1.02 and 0.75 mg HCN per kg BW or about 1/3 of the reported levels which are considered toxic.

Several studies which used forages in diets for growing pigs indicate that forages can partly replace cereals without affecting performance. Scipioni and Martelli (2001) included ensiled sugar beet pulp at 10% of DM in the diet without affecting the growth performance of fattening pigs while increasing the dietary level to 20% in the DM reduced feed intake but made no difference to the carcass quality of the pigs. Differences in lean meat content in our study were not significant different between treatments. There was however, a significant difference in feed cost among the experimental diets. The ECL, DCL and ESPV diets of growing pigs had a lower cost compared to the control diet (FM) indicating that profitability is better using these diets. Recently, Hoanh et al. (2006) found that using ESPV in diets of growing pigs improved daily BW gain by 13% and feed cost by 17.7% compared to diets using fresh sweet potato vines. In the study here, the ECL diet was the most economical to use.

Feed conversion ratio for FM, ECL and ESPV treatments, tended to be lower than for the pigs fed the DCL and DSPV diets. This finding is in accordance with a number of studies where CL or sweet potato leaves have been used as protein source in diets of pigs (Phuc et al., 2000; Nguyen et al., 2004; Van An et al., 2005). Differences in lean meat content were not significantly different between the treatments. This result is in good agreement with Van An et al. (2005) who also reported similar values for carcass traits of F₁ (Large White×Mong Cai). These authors reported that lean meat percentage of F₁ pigs were 43.6, 42.5, 42.6 and 43.4% when protein from FM, groundnut cake, ensiled sweet potato leaves or ensiled sweet potato leaves with added lysine, respectively were used. These authors also concluded that sweet potato leaves can replace FM and groundnut cake in traditional Vietnamese diets for growing pigs. Ngoan et al. (2001) found that the lean meat content was a 40.2% when using FM as a protein source in addition to cassava root meal and rice bran in the diet of F₁ (Large White×Mong Cai) pigs.

Fat and protein deposition of the F₁ pigs were not significantly affected by the dietary treatment in the present study. There were no significant differences in the intake with regard to ME, CP, lysine or methionine among diets. In a recent study into the ileal digestibility of AAs of CL and SPV both in silage and in dry form in diets for pigs, we found that AAs from CL and SPV have an ileal digestibility of 0.65 to 0.75 (Nguyen et al., 2011). The pigs in the present study received similar amounts of ileal digestible methionine+cysteine as well as similar amounts of metabolisable energy. However, the ADG of pigs fed the FM diet (470 g/day) tended to be higher than the pigs fed the CL and SPV diets. Data in Table 5 show that the estimates of fat and protein deposition in pigs fed the FM diet was slightly higher for fat and similar to the other treatments. Table 5 also shows that protein depositions of the pigs fed the FM, ECL and ESPV diets were numerically higher than the pigs fed the DCL and DSPV diets. These results can be explained by the ileal digested methionine+cysteine and lysine content of the diet which was strongly correlated with the protein deposition. Protein deposition and indirectly fat deposition are dependent on the first limiting AA. It is well known that methionine is the most limiting amino acid in CL (Eggum, 1970; Phuc, 2000; Montagnac et al., 2009) and lysine is the most limiting AA in SPV (Woolfe, 1992; An et al., 2003).

Conclusion

Using ensiled or dry cassava leaves and sweet potato vines replacing 70% of the CP from FM in diets and providing 35% of the total CP had no effect on the performance and carcass traits of the Large White×Mong Cai pigs. Cassava leaves and sweet potato vines can be used as a protein supplement for feeding pigs at a relatively high inclusion level without negative effects on performance if HCN concentrations are below levels known to be toxic to pigs. Higher returns are possible if ensiled or dry cassava leaves and sweet potato vines are used in diets for pigs.

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Chapter 4

Inclusion of ensiled cassava KM94 leaves in diets for growing pigs in Vietnam reduces growth rate but increases profitability

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Abstract

This study was conducted to determine the effect of inclusion of different levels of ensiled cassava leaves (variety KM94) in the diets on performance and carcass characteristics of growing pigs in Vietnam. A total of 40 crossbreds pigs (Large White×Mong Cai, 20 males and 20 females) with an initial live weight of 23.5 kg (SD=0.86) were randomly allocated to one of the four pens across 5 units. Four experimental diets were formulated for two growth periods, period 1 (60 days) for 20 to 50 kg and period 2 lasted 30 days, from 50 kg until slaughter. Four diets were formulated containing inclusion levels of ensiled cassava KM94 leaves diet of 0, 10, 15 and 20% in the dry matter. Diets were formulated based on previously determined ileal amino acid digestibility values of the KM94 products and were isonitrogenous and isocaloric on a metabolisable energy basis. Each pen of pigs was randomly assigned to one of the four dietary treatments. Dry matter intake and final weight tended to decrease with increasing levels of ensiled cassava KM94 leaves in the diet while there was a significant ($p=0.022$) decrease in average daily gain. Protein depositions of the F_1 pigs tended ($p=0.093$) to decrease with increasing inclusion level of ensiled cassava KM94 leaves. There was no significant difference in feed conversion ratio, carcass quality and fat gain between the groups of pigs. There were clear differences in feed costs among the experimental diets ($p=0.001$) with increasing level of ensiled cassava KM 94 leaves in the diet reducing feed costs. It was concluded that inclusion of ensiled cassava leaves reduces growth rate of growing pigs in Vietnam but increases profitability as measured by feed costs.

Introduction

In Vietnam, cassava production has rapidly changed from a food crop to an industrial crop. Each year more and more land area is used for cultivation of cassava, yielding more and more cassava roots and leaves. New high-yielding cassava varieties, such as KM94 and KM98-5 and KM140 have been distributed to various provinces in Vietnam over recent years. In 2007/2008, 560,000 ha were planted with cassava of which more than 350,000 ha with new varieties, mainly KM94 (GSO, 2008). At the time of root harvest, the yield of fresh foliage of a variety like KM94 is approximately 9 tons/ha and leaf yield approximately 5-7 tons/ha (Phuoc, 2004; Ly and Ngoan, 2007). Cassava variety KM94 has been specifically developed for its high root yield and may provide farmers an increased return. The leaves of cassava variety KM94 have a level of crude protein which ranges between 25 to 34.7% in the dry matter (DM) (Phuc et al., 2000; Kinh, 2003; Ly and Ngoan, 2007) and after suitable treatment to reduce the hydrogen cyanide (HCN) content, it be used as a protein source in animal feeds. Phuc et al. (2000) showed that cassava variety KM94 has a higher HCN content compared to other varieties. Ly and Ngoan (2007) found that the HCN content of fresh KM94 cassava leaves was 1745 mg/kg DM which was decreased by 51% after 24 h of wilting (sun drying). The initial inclusion of rice bran or cassava root meal in cassava silage at levels of 5 or 10% produces a good quality silage that can be stored for at least five months (Ly and Ngoan, 2007). The HCN content of the ensiled KM94 cassava leaves decrease very quickly during the first 30 days of ensiling, and after 90 days of ensiling it is only 10 to 13% of the initial concentration (Ly and Ngoan, 2007). There have been no studies investigating the nutritive value of ensiled KM94 cassava leaves in the diet for pigs.

The objective of the present study was to evaluate the efficacy of using different levels (from 0 to 20%) of ensiled KM94 cassava leaves in diets on the performance and on carcass characteristics of growing pigs under farming condition in Central Vietnam.

Materials and methods

Preparation and preservation of silage of cassava KM94 leaves

This experiment was carried during the wet season in Vietnam. Fresh leaves of cassava KM 94 were collected at the time of root harvest and spread out on a concrete

floor for wilting. The DM of fresh cassava (KM94) leaves was 25% and after wilting for 24 hours increased to 34%. After wilting the leaves were separated from the stems and petioles, chopped into small pieces (2-3 cm), mixed with 0.5% of NaCl and 5% of rice bran of the wilted weight of the cassava KM94 leaves. The mixture was kept in nylon bags with a capacity of 30 kg, and kept airtight and stored for 2 months before use.

Location

The experiment was carried out in five units in the Huong Van community, which is one of the main pig production areas of the Thua Thien Hue province in Vietnam. Each unit consisted of 8 pigs. The protocol of the experiment was approved by the ethical committee of Hue University, Hue, Vietnam.

Animals and experimental design and feeding

Forty (20 males and 20 females) crossbred (Large White×Mong Cai) pigs with an average initial weight of 23.5 kg (SD=0.86) and with similar age were randomly allocated within sex to five units. In each unit, 4 male and 4 female pigs were randomly allocated to four pens, with 2 pigs (1 male and 1 female) per pen. Within a unit, each pen (2 x 1 m) was randomly allocated to one of the four dietary treatments. The trials used a completely randomized block design with four levels of ensiled cassava (KM94) leaves in the diets and 5 replicates per treatment. The four experimental diets contained 0, 10, 15 and 20% of ensiled cassava KM94 on a DM basis. The pigs had been vaccinated against Hog Cholera and Pasteurellosis and were de-wormed 2 weeks before starting the experiment.

Four diets were formulated for the two growing periods of the animals: weight range 20-50 kg, duration 60 days (period 1) and weight above 50 kg, duration 30 days (period 2). In period 1, pigs received a diet with ~14% CP in which 15-22% of the total CP originated from ensiled KM94 leaves and in period 2, a diet with ~12% of CP in which 26-34% of the total CP came from the ensiled KM94 leaves mixture. The experiment lasted 90 days with feed allowance for all treatments set at 4% of body weight. The diets were distributed equally into 3 meals per day (7:00; 11:00 and 17:00 h). Refusals were collected the following morning before the first meal. Drinking water was provided *ad libitum*. The trial was designed as a complete randomized

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block with four levels of ensiled KM94 leaves of 0, 10, 15 and 20% in the DM of the diets. The treatments are called FM, 10 KM94, 15 KM94 and 20 KM94 diets.

The basal fish meal (FM) diet contained rice bran, yellow corn, ensiled cassava roots and sweet potato vines (Table 1). Metabolisable energy content in the diets was calculated according to formulas used for estimation of energy values in pig feeds in Vietnam as provided by the National Institute of Animal Husbandry (NIAH, 2001). Four diets were composed and aimed to have (in the DM) approximately 12.6 MJ of metabolisable energy (ME), 14% CP, 0.65% lysine and 0.25% methionine for period 1 and for period 2: 12.6 MJ ME, 12.1% CP, 0.55% lysine and 0.23% methionine (Table 2). The source of protein in the experimental diets was fish meal and ensiled cassava KM 94 leaves only and contained sufficient CP and apparent ileal digestible EAA to enable optimum performance.

Table 1. Ingredients and their determined chemical composition used to formulate the experimental diets.

Component	Ingredient ¹					
	Rice bran	Maize meal	ECR	Fish meal	SPV	ECL KM94
Metabolisable energy (MJ/kg DM)	12.1	14.9	12.4	13.2	9.4	10.3
Crude protein (% DM)	11.5	9.6	3.1	58.5	17.8	21.0
Crude fibre (% DM)	15.6	2.7	4.0	ND	18.0	11.7
Lysine (g/kg DM)	4.9	3.1	1.1	33.3	7.1	9.2
Methionine (g/kg DM)	2.3	1.9	0.4	10.5	2.3	3.2
Hydrogen cyanide (mg/kg DM)	ND	ND	55.0	ND	ND	195

¹ECL KM94: ensiled cassava KM94 leaves; ECR: ensiled cassava root; SPV: sweet potato vines; ECR and ensiled cassava KM94 leaves analysis at 60 days after ensiling.

DM: dry matter; ND: Not determined.

Chemical analyses

Samples of diets were dried at 60°C for 24 h and ground over a 1 mm screen before analysis. Dry matter, CP and crude fat (CF) were determined in the samples according to standard AOAC methods (AOAC, 1990). Amino acids were analysed according to Spackman et al. (1958) on an ion-exchange column using an HPLC. Samples were hydrolysed for 24 hours at 110°C with 6 M HCL containing 2 g/l

reagent grade phenol and 5,000 nmol norleucine (internal standard) in evacuated and sealed ignition tubes. Methionine was determined as methionine sulphone on separate samples hydrolyzed for 24 hours as described above following oxidation with performic acid overnight at 0°C (Moore, 1963). All samples were analyzed in triplicate except amino acids which were analysed in duplicate. All analyses except the amino acid analyses, were done at the Hue University laboratories with amino acids determined by the National Institute of Animal Husbandry (NIAH) laboratories in Hanoi.

Measurements

Feed consumption was determined by weighing the amounts of feed given and corrected for any feed remaining the following morning. This correction was done by determining the DM of feed offered and feed refusals. The pigs were individually weighed at the start and at the end of each month and also at slaughter. Daily weight gain, daily feed intake, feed conversion ratio and feed cost/kg live weight gain were estimated for each treatment. The costs were derived using the market price for each ingredient, amount used and growth of each pigs to calculate the price of 1 kg of feed DM.

For the evaluation of carcass traits, 3 representative pigs (2 males and 1 female) from each treatment were slaughtered at the final body weight (70-75 kg). The pigs were starved for 24 hours, weighed and then slaughtered at the slaughterhouse in Hue City. Carcass weights were measured according to Kauffman and Epley (2000). Hot carcass weights were measured immediately after slaughter. Carcass weights without blood, hair and internal organs were recorded and also the weight of the hot carcass without head. The carcass ratio was calculated as the ratio between carcass mass as a proportion of live body weight after the pigs had been starved for 24 h. The P₂ back fat thickness was measured on the partitioned carcass 10 cm from the midline behind the tenth rib using a ruler, and loin area was measured by trace paper (70 g/m²) at slaughter. Carcass length was measured from the first rib to the pubic bone. Lean percentage was calculated as the ratio of lean mass to hot carcass weight according to the equation published by Kauffman and Epley (2000):

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Table 2. Ingredient content and chemical composition of the experimental diets for F₁ (Large White×Mong Cai) pigs over two weight periods, 20-50 kg and 50 kg to slaughter.

Ingredients	Fish meal		10 KM94 ¹		15 KM94		20KM94	
	20-50	>50	20-50	>50	20-50	>50	20-50	>50
Rice bran (%)	31.8	37.8	31.8	35.8	29.8	34.8	29.8	27.8
Maize (%)	17.9	15.9	17.9	17.9	20.9	18.9	23.9	23.9
Ensiled cassava root (%)	28.8	29.8	29.8	29.8	25.9	26.9	19.9	24.9
Fish meal (%)	10.0	6.0	10.0	6.0	8.0	4.0	6.0	3.0
Sweet potato vines (%)	10.0	10.0	-	-	-	-	-	-
Ensiled cassava leaves (%)	-	-	10.0	10.0	14.9	14.9	19.9	19.9
Premix ³	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition (% DM)								
Metabolisable energy (MJ/kg)	12.6	12.5	12.6	12.6	12.6	12.6	12.6	12.6
from ECL (%)	0	0	8.1	8.2	12.3	12.4	16.4	16.4
Crude protein (%)	14.0	12.1	14.3	12.2	14.1	12.2	14.1	12.2
from ECL (%)	0	0	15	17	22.4	26	30	34
Crude fibre (%)	8.5	8.9	7.9	8.5	8.0	9.2	8.5	8.5
Total lysine (g/kg)	6.5	5.7	6.6	5.6	6.5	5.3	6.3	5.3
Ileal dig. Lysine ² (g/kg DM)	4.91	4.26	5.06	4.18	4.83	3.96	4.67	3.87
Total methionine (g/kg)	2.5	2.3	2.6	2.3	2.5	2.2	2.5	2.2
Ileal dig. Met ² (g/kg DM)	1.85	1.72	1.92	1.67	1.87	1.62	1.86	1.6
Hydrogen cyanide (mg/kg)	16	16	36	36	44	44	50	52

¹10KM94, 15KM94 and 20KM94 contained 10, 15 and 20% ensiled cassava KM94 leaves (ECL) in the dry matter, respectively.

²Ileal digestible lysine or methionine calculated based on the analysed crude protein and amino acid composition and apparent ileal digestibility data of Nguyen et al. (2011a) and Ngoan and Lindberg (2001).

³Contained per kg: 2,400 mg retinol, 4.32 cholecalciferol, 15,000 mg α -tocopherol, 5,000 mg phytylmenaquinone, 2,000 mg thiamin; 15,000 mg riboflavin, 25,000 mg calcium pantothenate, 30,000 mg niacin, 30 mg cyanocobalamin, 2,000 mg folic acid, 100 mg choline, 100 mg Fe, 115 mg Zn, 40 mg Cu, 0.15 mg Co, 0.6 mg I, 0.3 mg Se.

$$\text{Lean mass (Ib)} = 7.231 + [\text{hot carcass weight (Ib)} \times 0.437 + (\text{loin area (Inch}^2) \times 3.877] - [\text{P}_2 \text{ backfat thickness (Inch)} \times 18.746]$$

Protein and fat deposition calculation

To calculate the approximate protein and fat deposition, the following assumptions were made: one gram of protein and fat contains 23.4 kJ and 39.7kJ of energy, respectively (NRC, 1998) and

$$\text{ME intake} = \text{ME}_m + \text{ME}_p \text{ and}$$

$$\text{ME}_p = c \times \text{protein deposition} + d \times \text{crude fat deposition}$$

where ME_m is the amount of ME required for maintenance (460 kJ of ME per kg of metabolic BW ($\text{BW}^{0.75}$)), ME_p is the metabolisable energy used for fat and protein deposition with c and d representing the amount of energy in kJ ME needed for the deposition of 1 g of protein and fat, respectively. The required amount of ME per g of protein and fat deposition was assumed to be 53 and 53 kJ per g (NRC, 1998).

On the basis of the study by Kotarbinska and Kielanowski (1969), it can be assumed that about 10% of the average daily gain (ADG) in the type of pigs used in the present study is gut fill and ash, thus, $0.9 \times \text{ADG} = \text{water} + \text{crude protein} + \text{crude fat}$. The deposition rate of protein and fat in the empty body of the two genotypes were calculated based on the two following equations:

$$0.9 \times \text{ADG} = F + P/0.21 \text{ and } \text{ME}_p = (F + P) \times 53$$

where ADG is average rate of gain (g/d), 0.21 is protein/(protein + water), F is the amount of fat deposited (g/d); P is the amount of protein deposited (g/d) and ME_p is the metabolisable energy used for fat and protein deposition.

Statistical analysis

Analyses of variance were performed according to the following model:

$$Y_{ij} = \mu + U_j + T_i + e_{ij}$$

where Y is a dependent variable, μ is the overall mean, U_j is the unit effect ($j=1,2,\dots,5$), T_i is the treatment effect ($i=1,2,\dots,4$) and e_{ij} is the random error. Data were analyzed by ANOVA using the General Linear Model (GLM) of Minitab Statistical Software

Version 14 (2004). Tukey pair-wise comparisons were used to determine differences between treatment means at $P < 0.05$.

Table 3. Effect of using ensiled cassava KM 94 leaves in the diet on performance and economics of F_1 crossbred (Large White×Mong Cai) pigs in Vietnam.

Parameter	Diet ¹				SEM	P<
	FM	10KM94	15KM94	20KM94		
Initial BW (kg)	23.4	23.2	23.8	23.7	0.2	0.339
Final BW (kg)	73.9	71.8	70.9	69.7	1.1	0.071
ADG (g/d)	561 ^a	540 ^{ab}	523 ^{ab}	511 ^b	11	0.022
DMI (kg DM/pig/d)	1.57	1.57	1.56	1.53	0.01	0.100
FCR (kg DM/kg gain)	2.81	2.92	3.03	3.02	0.08	0.197
Feed cost ² (VND/kg gain)	8134 ^a	7386 ^{ab}	7142 ^{ab}	6638 ^b	189	0.001
Relative to control (%)	100	91	88	82	-	-

Means with different letters within row differ ($P < 0.05$). ADG, average daily gain; BW, body weight; DM, dry matter; DMI, dry matter intake; FCR, feed conversion ratio; SEM, standard error of the mean.

¹FM: Fish meal diet; 10KM94, 15KM94 and 20KM94 contain 10, 15 and 20% ensiled cassava KM94 leaves in the dry matter, respectively.

²Price (Viet Nam Dong (VND)/kg) of fresh feed in Hue at the time of the experiment: Ensiled cassava root, 500; Rice bran, 2400; Maize, 2700; Fish meal, 7000; Sweet potato vines, 500; 1US\$ = 15800 VND at the time of the experiment.

Results

Final live weight, average daily gain and feed conversion ratio

Results show that there were no significant differences in final live weight among the pigs fed the different experimental diets (Table 3). However, increased levels of ensiled cassava KM94 leaves tended ($p=0.071$) to decrease final body weight of the pigs. There was a significant ($p=0.022$) decrease in average daily gain (ADG) with an increase in inclusion levels of ensiled cassava KM94 leaves. The ADG were 561, 540, 523 and 511 g/day for pigs fed the FM, 10KM94, 15KM94 and 20KM94 diets, respectively. There was no significant ($p=0.100$) difference in the mean DM intake of the pigs on the different experimental diets. The data in Table 3 show that the feed conversion ratios (FCR) were not significantly ($p=0.197$) different between

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the pigs fed the different levels of ensiled cassava KM94 leaves although a numerical increase in FCR with KM94 inclusion level was observed (2.81, 2.92, 3.03 and 3.02 kg DM/kg gain). There were significant ($p=0.001$) differences in feed cost per kg live weight gain among treatments. The feed cost for the 20KM94 diet was lowest ($p<0.05$) among the diets. Using 10, 15 and 20% ensiled cassava KM94 leaves reduced feed costs by 9, 12 and 18%, respectively.

Table 4. Effect of inclusion level of ensiled KM94 cassava leaves in diets on the carcass characteristics of F₁ crossbred (Large White×Mong Cai) pigs in Vietnam.

Parameter	Diet ¹				SEM	P<
	FM	10KM94	15KM94	20KM94		
Hot carcass without head (kg)	49.7	48.8	48.7	47.2	0.6	0.118
Dressing percentage (%)	67.9	68.8	67.9	68.2	0.4	0.623
Carcass length (cm)	75.3	73.7	73.6	74.7	1.9	0.904
Loin muscle area (cm ²)	25.4	24.8	23.6	24.1	0.2	0.076
Back fat thickness P ₂ (cm)	3.1	2.9	2.9	3.0	0.1	0.553
Calculated lean meat (%)	43.6	44.2	43.7	43.3	0.6	0.780

¹FM: Fish meal; 10KM94, 15KM94 and 20KM94 contain 10, 15 and 20% ensiled cassava KM94 leaves in the dry matter, respectively; SEM, standard error of the mean.

No significant ($p>0.05$; Table 4) treatment effects were found for any of the carcass trait. Loin muscle area of the pigs fed the control, 10KM94, 15KM94 and 20KM94 diet were 25.4, 24.8, 23.6 and 24.1 cm², respectively. Lean meat of these pigs was 43.6, 44.2, 43.7 and 43.3%, respectively. The content of lean meat did not decrease with increased level of ensiled KM94 in the diet.

Table 5. Effect of inclusion level of ensiled KM94 cassava leaves in diets on protein and fat deposition of F₁ crossbred (Large White×Mong Cai) pigs between 70-75 kg.

Deposition	Diet ¹				SEM	P<
	FM	10KM94	15KM94	20KM94		
Protein (g/d)	71.6	66.4	62.5	62.1	3.1	0.093
Fat (g/d)	162.7	172.6	173.4	168.4	5.5	0.502

¹FM: Fish meal; 10KM94, 15KM94 and 20KM94 contain 10, 15 and 20% ensiled cassava KM94 leaves in the DM, respectively; SEM, standard error of the mean.

No significant ($p>0.05$, Table 5) treatment effects were observed for the estimated fat and protein deposition of the F_1 pigs. Protein deposition of the F_1 pigs tended ($p=0.093$) to decrease as the level of ensiled cassava KM94 leaves increased in the diet. Protein deposition of the pigs fed the FM, 10KM94, 15KM94 and 20KM94 diets were 71.7, 66.0, 62.5 and 61.2 g/day, respectively.

Discussion

New high-yielding cassava varieties, such as KM94, KM98-5 and KM140 have been distributed to various provinces in Vietnam. More than 350,000 ha of new varieties, mainly KM94 were planted in 2007 (GSO, 2008). Cassava leaves of KM94 variety have a CP content of 25-35% in the DM and are a good source of protein for animals because of its amino acid composition (Ravindran, 1993; Phuc et al., 2001; Kinh, 2003; Ly and Ngoan, 2007). The greatest limitation to the use of fresh cassava KM94 leaves as animal feed is its very high HCN content. Sun-drying is probably the cheapest method in the tropics and is also the most effective method (Cardoso et al., 2005; Chauynarong et al., 2009; Wanapat, 2009) for reduction of the HCN content in cassava. However, the harvest season of cassava root in Vietnam coincides with the rainy season making sun-drying often impossible. Ensiling is another method which is nearly as good as sun-drying for leaf preservation and reducing the HCN content (Man and Wiktorsson, 2001; Phuc et al., 2001; Borin et al., 2005). In the present study, the HCN content of fresh cassava leaves (KM94) was 1745 mg/kg DM and after 60 days of ensiling it was 11.2% of this initial level. When included in the 10 to 20% diets, the HCN contents of the diets was 16-52 mg/kg DM. The highest level is similar to the recommended safety level of 50 mg HCN/kg diet (Bolhuis, 1954) of pigs.

In this study, we did not find significant differences in final weight, DMI, FCR and for any of the carcass traits among the experimental diets. We found however, a significant decrease in ADG with increasing level of ensiled cassava leaves inclusion and indicate that ensiled cassava KM94 leaves can be used in the diets of pig although it leads to some reduction in daily gain. In addition to the depression in ADG, the calculated protein gain also tended to decrease with increasing cassava leaves intake. Fat deposition of F_1 pigs were not affected by dietary treatments. The ADG of pigs on the ensiled cassava KM94 leaves diets decreased with the level of inclusion. On the

basis of a previous experiment (Nguyen et al., 2011b), it is clear that some supplemental methionine may be required in a diet with ensiled cassava leaves to have the same performance as the low inclusion levels as the likely reason for the decrease in performance is likely a shortage of the first limiting amino acid. The ileal digestible lysine and methionine in both periods of the 15KM94 and 20KM94 diets was lower compared to the FM and 10KM94 diet. It may also be that lysine was limiting. In addition, the reduced growth rate of the pigs on the 20KM94 diet may potentially have been due to the HCN content in the diet which was 50-52 mg/kg DM. Some methionine in this diet may have been used for detoxification of the HCN. According to Tewe (1992), methionine will provide sulfhydryl groups (-SH) which are necessary for the detoxification of cyanide. In the body cyanide is detoxified by the enzyme rhodanese, forming thiocyanate, which is excreted in the urine.

In this study, we found that there were similar values for carcass traits among the experimental diets. This result is in good agreement with Van An et al. (2005) who also reported similar carcass traits of F₁ (Large White×Mong Cai) pigs which were fed diets with sweet potato leaves. These authors concluded that sweet potato leaves can replace fish meal and groundnut cake in traditional Vietnamese diets for growing pigs. This agrees with Scipioni and Martelli (2001), who concluded that increasing the level of a forage (sugar beet-pulp) from 10 to 20% in the diet does not affect carcass traits of pigs. Differences in lean meat content were not significantly different between the treatments. This result is also in good agreement with Van An et al. (2005) who reported that lean meat percentage of F₁ pigs were 43.6, 42.5, 42.6 and 43.4% when protein from fish meal, groundnut cake, ensiled sweet potato leaves or ensiled sweet potato leaves with added lysine, respectively were used. Ngoan et al. (2000) found that lean meat content was 40.2% when using fish meal as a protein source in addition to cassava root meal and rice bran in the diet of F₁ (Large White×Mong Cai) pigs.

Lindberg and Anderson (1998) reported that including 10 and 20% of the forages white clover, lucerne, red clover and perennial rye grass in barley diets for growing pigs resulted in reduced digestibility of organic matter, but increased the digestibility of crude fibre. These authors concluded that forage addition to the diet of pigs can be used to a limited extent as a protein source. Recently, Nguyen et al. (2010) also found that ensiled or dry cassava leaves and sweet potato vines can replace at

least 70% of the protein from fish meal (or 35% of total diet CP) without large effects on performance and carcass traits of growing F₁ (Large White×Mong Cai) pigs. Feed cost per kg gain were reduced by 5-16.1% compared to the fish meal diet. In the present study there were significant differences in feed cost/kg weight gain among treatments. This parameter was lowest for the KM94 diet which was not different from the other diets containing ensiled cassava KM94 leaves but significantly different from the control diet. Using 10, 15 and 20% of ensiled cassava KM94 leaves reduced the feed cost by 9, 12 and 18%, respectively. These results are in agreement with Phuc (2000) who concluded that cassava leaves can be used as a protein supplement in growing diets and that ensiled or dried cassava leaves are potentially useful feed resources in developing countries. However for optimal gain some addition of the amino acids methionine and lysine may be needed.

Conclusion

Inclusion of ensiled cassava KM94 leaves in diets for growing pigs was shown to decrease average daily gain when included up to 20% of the diet. However, feed costs per unit of live weight gain were lowest on the highest inclusion level providing a more cost effective alternative for pig farmers in Vietnam and ensiling is a practical solution to conserving cassava leaves and reducing the HCN content during root harvest.

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Chapter 5

Ileal and total tract apparent crude protein and amino acid digestibility of ensiled and dried cassava leaves and sweet potato vines in growing pigs

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Abstract

*The present study was conducted to determine the ileal and total tract apparent digestibility of crude protein (CP) and amino acids (AA) in ensiled and dried cassava (*Manihot esculenta*) leaves (CL) and sweet potato (*Ipomoea batatas*) vines (SPV) as a single ingredient or in a 50:50 mixture of CL+SPV in growing (>60 kg BW) pigs. Coefficients of ileal (CIAD) and total tract (CTTAD) apparent digestibilities of organic matter (OM), CP, AA, crude fibre (CF) and neutral detergent fibre (aNDFom) were determined in growing pigs fed practical diets. The CP in the diets originated mainly from ensiled and dried CL, SPV or CL+SPV with the main energy source originating from ensiled cassava root which provided less than 9% of dietary CP. The six diets were formulated to contain 120 g CP/kg DM, 13 MJ ME/kg DM and were fed to 60 kg growing pigs in a 6 x 6 Latin square design. Daily intake of OM, CF, aNDFom and ME differed ($P < 0.001$) among diets while for DM and CP a trend was observed. There were significant differences among diets ($P < 0.05$) for the CIAD and CTTAD of DM, OM, CP and CF and in the CTTAD of aNDFom. There were differences ($P < 0.05$) among diets for the CIAD of most AA except methionine+cysteine, glycine, glutamic acid and serine. The CIAD of AA for the ensiled CL, SPV and CL+SPV were in most cases not different from the corresponding CIAD of AA of the dried ingredients. The use of a combination of CL and SPV in diets of growing pigs resulted in higher CIAD for CF and several AA compared to expected values from the individual ingredients. The first and second limiting AA in ensiled and dried CL and SPV were found to be the methionine+cysteine and lysine. Cassava leaves and sweet potato vines have the potential to improve protein and amino acid supply in diets for pigs especially when combined with ingredients containing high concentrations of the first two limiting amino acids.*

Introduction

Cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) are important food crops in Vietnam. Cassava leaves (CL) are relatively rich in crude protein (CP, 167 to 399 g/kg DM), minerals and vitamins (Eggum, 1970; Phuc et al., 2001; Montagnac et al., 2009) but also contain high levels of cyanogenic glucosides which limit its use in animal feeding. Sweet potato vines (SPV) include in their composition the leaves (260 to 330 g CP/kg DM) and stems (100 to 140 g CP/kg DM) (Woolfe, 1992; Ishida et al., 2000; An et al., 2003). The use of SPV in animal feeding is limited by the high content in oxalic acid (470 ± 15 mg/100 g fresh weight), phytic acid (0.46 ± 0.01 mg/100 g fresh weight), tannic acids (491 ± 7 mg/100 g fresh weight) and trypsin (52.0 ± 0.9 TIU/g) and chymotrypsin (69.1 ± 0.6 TIU/g) inhibitors (Mosha et al., 1995; Rekha et al., 1999; Hou and Lin, 1997). Cassava leaves and sweet potato leaves (SPL) have been used as a protein source in diets for pigs in Vietnam and can replace partly other protein sources (Phuc et al., 2000; Phuc and Lindberg, 2001; Van An et al., 2005). Drying and ensiling are effective ways of reducing the HCN concentrations in CL (Borin et al., 2005; Cardoso et al., 2005; Wanapat, 2009) and as a result these techniques are used to increase the nutritional value.

Two studies have reported the coefficient of ileal apparent digestibility (CIAD) of amino acids (AA) for leaves in growing pigs. Phuc and Lindberg (2001) reported CIAD of AA for CL meal, ensiled CL, groundnut foliage or leucaena when included (0.15 g/g DM) in a cassava root and soybean meal basal diet in growing pigs. An et al. (2004) investigated effects of the inclusion of fresh, dry and ensiled SPL (not including stems) on the CIAD of AA in growing pigs and found for most AA no difference in CIAD. In Vietnam, smallholder farms commonly use SPV or mixtures of CL and SPV either dried or ensiled (Pham et al., 2010) as a dietary ingredient for pigs and often do not separate leaves and stems. Knowledge of the CIAD of AA in practical diets is important in order to allow improvement in dietary formulation for growing pigs and increase profitability to smallholder farmers.

The present study was conducted to determine the ileal and total tract apparent digestibility of CP and AA in ensiled and dried cassava leaves, sweet potato vines and a 50:50 mixture on a DM basis of cassava leaves and sweet potato vines in growing pigs. The effect of preservation method (ensiling vs. drying) and additiveness of the two sources used in the 50:50 mixture on the CIAD of AA were evaluated.

Materials and methods

Pigs and housing

The protocol of the experiment was approved by the ethical committee of Hue University (Hue City, Vietnam). Six castrated F₁ crossbred (Large White×Mong Cai) growing pigs of approximately 5 months of age, with an average body weight of 60.2 ± 1.0 kg were used. The pigs were surgically fitted with post-valve T-caecum cannulas for collection of ileal digesta. The cannulation followed the procedures as described by Van Leeuwen et al. (1991). Pigs were housed individually in a 2.0 x 1.0 m (length x width) pen with free access to water from nipple drinkers throughout the trial.

Ensiling and drying of cassava leaves and sweet potato vines

Fresh cassava (*Manihot esculenta*) leaves were harvested at 90 days after planting by cutting material at a height of 30 cm or above. Harvested material (leaves+stems) was thoroughly mixed and sampled before being wilted for 5 h. The wilted material was divided into two equal portions with one half used to collect the leaves which were chopped (2-3 cm) by hand. Rice bran (50 g/kg) and common salt (5 g NaCl/kg) were added to the wilted CL and thoroughly mixed by hand before storage at ambient temperature in 30 kg sealed airtight plastic bags for at least 2 months before being fed to the pigs. The ensiled CL was fed to pigs for less than 6 days after opening of a bag. A homogenous sample (500 g) was collected from each bag directly after opening, stored (-20°C) and samples pooled before a representative sample (300 g) was taken for chemical analysis. The other half of the material was left to dry in the sun for 2-3 days. Dried leaves were collected, milled over a 1 mm screen, and stored in plastic bags at ambient temperature. Dried CL samples (200 g) were collected randomly from each bag, pooled, mixed homogeneously and a sample (300 g) was analysed for DM and HCN content and stored (-20°C) in air tight containers for further analysis.

Sweet potato (*Ipomoea batatas*) vines were harvested at 60 days after planting, cut at 10 cm distance from the main stems and chopped (2-3 cm) by hand. A representative sample was stored before the material was wilted overnight and divided into two equal parts. One half was mixed with rice bran (100 g/kg) and 5 g NaCl/kg and stored in sealed airtight plastic bags (30 kg) and stored for >21 days before being

fed to pigs. The ensiled SPV was fed to pigs for a maximum of 5 days after opening a bag. The other half of the SPV was dried in the sun for 2-3 days before being milled over a 1 mm screen, and stored in plastic bags. The procedure of collecting samples for chemical analysis of ensiled or dried SPV was similar to that of ensiled and dried CL.

Table 1. Chemical composition (g/kg DM unless otherwise stated) of the ingredients.

Item ^a	Cassava	Cassava leaves		Sweet potato vines	
	root	Ensiled	Ensiled ^b	Ensiled ^c	Dried
Organic matter	975	920	922	855	867
Crude protein	17	242	299	197	229
Crude fat	6	70	67	64	53
Crude fibre	30	143	149	156	159
aNDFom ^d	49	365	369	415	412
Metabolisable energy (MJ/kg DM)	13	10.8	10.6	9.5	9.5
Hydrogen cyanide ^e (mg/kg DM)	20	152	128	ND ^f	ND

^aAll values were determined (n=3) except for metabolisable energy which was calculated from NIAH (2001) data.

^bSilage made from 945 g wilted cassava leaves, 50 g rice bran and 5 g NaCl/kg.

^cSilage made from 895 g wilted sweet potato vines, 100 rice bran and 5 g NaCl/kg.

^dNeutral detergent fibre with a heat stable amylase expressed exclusive of residual ash.

^e1221 and 342 mg/kg DM in fresh cassava leaves and roots, respectively.

^fND, not determined.

Diets and experimental design

Six experimental diets were formulated based on ensiled cassava roots as the energy source (Table 1). In four of the experimental diets, dried CL, ensiled CL, dried SPV and ensiled SPV was the main source of protein (912-929 g CP/kg dietary CP) with the remaining protein originating from the ensiled CR. In the other two diets, protein was supplied from a 50:50 mixture on a DM basis of CL and SPV in dried or ensiled form. Less than 90 g/kg of the CP originated from the ensiled cassava roots in the various diets. The six diets were formulated to contain approximately 116 g CP/kg DM with soybean oil added (30-40 g/kg) to adjust the calculated metabolisable energy

(ME) content to 12.5 MJ/kg DM. Dietary ME was calculated using the formulas and values reported by NIAH (2001) for pigs. Dietary ME was calculated using the formula:

$$\text{ME (kJ/kg)} = (5.01X_1 + 8.93X_2 + 3.44X_3 + 4.08X_4) \times 4.184$$

where: X_1 - X_4 are the digestible protein, digestible fat, digestible fiber and digestible nitrogen-free extractives content in g/kg feed. Chromium oxide was added as a digesta flow marker at 5 g/kg (Table 2).

The six experimental diets were fed to pigs according to a 6 x 6 Latin square design with each period lasting 12 days with 5 days dietary adaptation followed by 4 days of collection of faeces, one day of collection of ileal digesta, one day of rest and a second day of ileal digesta collection. The daily feeding level (3.0 kg/100 kg body weight) during the collection period was set slightly below the maximum level consumed during the adaptation period to reduce feed residues. The pigs were fed twice daily at 6:00 and 18:00 h, with the daily allowance equally divided between the two meals. Food refusals and spillage were collected, dried and used to calculate the food intake data.

Digesta, faeces collection and calculations

For the determination of the CIAD, ileal chyme was collected every 2 h during the 12 h period between the morning and afternoon feeding, giving six samples per collection day. At each sample collection, digesta were quantitatively collected for 1 h in containers through soft plastic tubing connected to the ileal cannula. The digesta were frequently removed from the tube and container and transferred to a larger container which was kept on ice during the entire sampling procedure before digesta samples were frozen at -18°C. Faeces were collected four times per day and stored at -18°C. Digestibility of a dietary nutrient/component at each sampling site was calculated using the chromium technique (Sauer et al., 2000) according to the equation:

$$\text{CAD}_D = 1 - (\text{DC}_F / \text{DC}_D \times I_D / I_F)$$

where CAD_D is the coefficient of apparent digestibility (ileal or total tract) of a dietary nutrient/component; DC_F the dietary nutrient/component concentration in ileal digesta

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or faeces (g/kg); DC_D the dietary nutrient/component concentration in the diet (g/kg); I_D the chromium concentration in the diet (g/kg); I_F the chromium concentration in ileal digesta or faeces (g/kg).

Table 2. Ingredient and analysed chemical composition, calculated metabolisable energy content and hydrogen cyanide content of the experimental diets fed to growing pigs.

Composition	Diet					
	Cassava leaves		Sweet potato vines		Mixture ^a	
	Ensiled	Dried	Ensiled	Dried	Ensiled	Dried
Ingredient (g/kg DM)						
Ensiled cassava roots	514	601	398	480	463	551
Ensiled cassava leaves ^b	446	-	-	-	246	-
Dried cassava leaves	-	359	-	-	-	202
Ensiled sweet potato vines ^c	-	-	552	-	246	-
Dried sweet potato vines	-	-	-	470	-	202
Soybean oil	30	30	40	40	35	35
Chromic oxide	5	5	5	5	5	5
Premix ^d	5	5	5	5	5	5
Chemical composition (g/kg DM)						
Organic matter	912	917	860	876	888	899
Crude protein	116.6	117.4	115.6	115.8	115.9	115.9
from ensiled cassava root	8.7	10.2	6.8	8.2	7.9	9.4
from rice bran	6.9	-	21.6	-	13.4	-
from leaves/vines	101.0	107.2	87.2	107.6	94.6	106.5
Ether extractable	61.2	57.3	77.6	57.3	69.8	62.2
Crude fibre	79.3	71.9	98.2	89.2	87.6	79
aNDFom ^e	188	162.2	248.6	217.1	214.6	184.9
Metabolisable energy (MJ/kg DM)	12.6	12.7	12.5	12.5	12.4	12.5
Hydrogen cyanide (mg/kg DM)	78.0	58.0	8.0	9.6	46.6	36.9

^a50:50 (DM basis) of ensiled or dried cassava leaves and sweet potato vines.

^bEnsiled with 50 g rice bran/kg wilted cassava leaves.

^cEnsiled with 100 g rice bran/kg wilted sweet potato vines.

^dSupplied per kg of diet: 12 mg retinol; 21.6 µg cholecalciferol, 75 mg α-tocopherol, 25 mg phytylmenaquinone, 10 mg thiamin; 75 mg riboflavin, 125 mg calcium pantothenate, 150 mg niacin, 0.15 mg cyanocobalamin, 10 mg folic acid, 0.5 mg choline, 0.5 mg Fe, 0.575 mg Zn, 0.2 mg Cu, 0.75 µg Co, 3.0 µg I, 1.5 µg Se.

^eNeutral detergent fibre with a heat stable amylase and expressed exclusive of residual ash.

Chemical analyses

Prior to chemical analyses, individual ileal digesta (2 days, 6 collections/day) and faeces (4 days, 4 collections/day) samples were thawed and pooled per pig during each period before a representative sub-sample was taken. Feed, digesta and faecal samples were dried at 45°C for 24 h and milled over a 1 mm screen before analyses. Nitrogen content of faeces and digesta was determined in fresh samples, whereas the analyses of other components in feeds, faeces and digesta were determined in dried samples. The chemical composition was determined according to standard methods (AOAC, 1990) including dry matter (method 930.15), ash (method 942.05), CP (Nx6.25; method 988.05) and crude fibre (CF; method 978.10). Ether extract was determined after extraction with petroleum ether by the Soxhlet method (method 920.39). The procedure of Van Soest et al. (1991) with addition of sodium sulfite and alpha amylase was used to determine neutral detergent fibre (aNDFom). Chromium oxide in feed, faeces and ileal digesta was determined by atomic absorption spectrometry after ashing according to Fenton and Fenton (1979). The AA were analysed according to Spackman et al. (1958) on an ion-exchange column using an HPLC. Briefly, samples were hydrolysed for 24 h at 110°C with 6 M HCl containing 2 g/l reagent grade phenol and 5 µmol norleucine (internal standard) in evacuated and sealed ignition tubes. Methionine (Met) and cysteine (Cys) were determined as Met sulphone and cysteic acid, respectively with separate samples hydrolyzed for 24 h as described above following oxidation with performic acid overnight at 0°C. The hydrogen cyanide (HCN) content was determined on fresh, ensiled or dried samples by titration with AgNO₃ after boiling the samples and concentrating the HCN in KOH (AOAC, 1990). The fresh CL and fresh SPV were analysed for DM, CP, AA and HCN content (except the SPV). Fresh cassava roots were also analysed for DM and HCN content. All samples were analysed in triplicate except amino acids which were analysed in duplicate.

Statistical analysis

Daily feed intake data were averaged per pig and per period. Data were analysed by analysis of variance according to a 6 x 6 square arrangement using the General Linear Model procedure of (SAS Inst., Inc., Cary, NC) using the following model:

$$Y_{ij(k)} = \mu + T_i + P_j + A_k + e_{ij(k)}$$

where Y is a dependent variable, μ is the overall mean, T_i the treatment effect (i =dried or ensiled CL, SPV and CL+SPV), P_j the period effect (j =1 to 6), A_k the effect of animal (k =1 to 6), $e_{ij(k)}$ is the random error. Tukey pair-wise comparisons were used to determine differences between treatment means. Differences between the actual results of mixture (dried or ensiled) and expected result based on single ingredients (i.e. $0.5 \times \text{result for cassava leaves} + 0.5 \times \text{result for sweet potato vines}$) were tested for significance using the CONTRAST statement. Probability level was set at 5%.

Results

Intake

The CP and AA content was higher in the CL ingredients compared to the corresponding SPV ingredient with the exception of Thr and valine in the fresh material (Table 3). The CP and AA content in the ensiled material was lower than in the dried or fresh material. The ensiled CL and ensiled SPV had a lower content of Lys, His, Ile, Met+Cys, Phe and Thr than fresh or dried CL and fresh SPV (Table 3). The cassava root only contained 17 g CP/kg DM.

The daily intakes of OM, CF, aNDFom and ME were different among diets ($P < 0.001$, Table 4). A trend was observed for the daily intake of DM ($P < 0.071$) and CP ($P < 0.071$) among diets. The lowest daily DM intake was recorded for the dried SPV diet, while a 12% higher intake was found for the dried CL diet. The CF and aNDFom intakes were higher ($P < 0.001$) in the dried and ensiled SPV diets compared to the corresponding CL diets.

Ileal and total tract apparent digestibility of nutrients

There were differences ($P < 0.05$) in CIAD and CTTAD between diets for DM, OM, CP and CF (Table 5). In addition, CTTAD of aNDFom was different ($P < 0.002$) among diets. The CIAD of DM, OM, CP and CF were not different ($P > 0.05$) between ensiled and dried CL. The CIAD of DM, CP and CF were higher ($P < 0.05$) in the ensiled SPV compared to the dried SPV. Only the CIAD of DM was different ($P < 0.05$) between the ensiled and dry CL+SPV. No differences were observed between the CTTAD of DM, OM, CP, CF and aNDFom between the ensiled and dry CL+SPV. The 50:50 mixture of CL+SPV gave higher ($P < 0.05$) CIAD for aNDFom

Ileal and total tract apparent crude protein and amino acid digestibility

than calculated based on individual aNDFom CIAD values of CL and SPV. Non-additivity of individual values was also found for the CTTAD of DM and OM for the dried CL+SPV as well as CF for the ensiled CL+SPV (Table 5).

Table 3. Crude protein and amino acid composition (g/kg DM) of the cassava (roots and leaves) and sweet potato vines used in the experimental diets.

Component	Cassava				Sweet potato vines		
	Roots		Leaves				
	Ensiled	Fresh	Ensiled	Dried	Fresh	Ensiled	Dried
Crude protein	17.0	299	242	299	223	197	229
Arginine	1.71	17.17	14.35	16.77	14.67	13.98	14.00
Histidine	0.54	5.45	5.31	5.33	5.37	4.42	4.78
Isoleucine	0.35	15.92	13.34	15.12	11.67	10.24	13.85
Leucine	0.80	23.82	18.46	22.70	19.48	18.21	18.83
Lysine	1.02	12.98	11.33	12.58	9.67	8.33	8.34
Methionine+Cysteine	0.11	6.24	4.82	6.25	4.50	4.20	4.29
Phenylalanine	0.47	13.39	10.12	12.72	11.76	9.41	10.24
Threonine	0.60	11.7	10.8	12.2	12.4	8.9	10.8
Tyrosine	0.46	9.59	7.97	9.07	9.94	8.90	7.73
Valine	0.43	12.66	11.71	12.30	12.81	9.47	12.01
Alanine	0.72	15.80	14.42	15.82	15.00	13.97	14.20
Aspartic acid	1.56	25.83	18.12	25.14	24.20	14.08	22.13
Glutamic acid	3.55	35.72	23.01	36.01	18.28	16.68	15.64
Glycine	0.36	16.39	13.73	15.04	9.78	13.00	15.33
Proline	0.15	11.28	12.09	13.70	8.38	8.20	8.67
Serine	0.76	13.93	7.85	12.12	9.67	5.99	7.88

There were statistically significant differences in the CIAD of CP and most AA except Met+Cys, Glu, Gly and Ser between diets (Table 6). Although for proline there was a significant diet effect ($P<0.05$), the Tukey pair-wise comparison test did not identify differences between treatments. None of the CIAD of AA were different between the ensiled and dried CL. Only the CIAD of Tyr and Asp were different between the ensiled and dried SPV. Only the lysine CIAD was different between the ensiled and dried CL+SPV. Significant ($P<0.05$) non-additive effects were observed for the CIAD of a number of AA in the ensiled and dried CL+SPV. For ensiled

CL+SPV, the CIAD of His, Lys, Thr, Asp and Pro was higher than the expected average value of a mixture of CL+SPV. For the dried CL+SPV, the CIAD of His, Leu, Thr, Tyr, Ala, Asp and Ser showed non-additivity.

Discussion

The CP contents of the CL and SPV used in the present study were in good agreement with reports in the literature (Dung et al., 2002; Montagnac et al., 2009; Woolfe, 1992). The lower CP content of the ensiled CL and SPV compared to the dried material is also in agreement with the studies of Phuc and Lindberg (2000, 2001) on processed CL. The ensiled products contained approximately 5% less CP than the dried products. This can partly be explained by the addition of the rice bran to the wilted cassava material to facilitate the ensiling process. Rice bran contains 110 g CP/kg DM compared to the 299 and 223 g CP/kg DM in the fresh CL and SPV and therefore rice bran addition dilutes the concentration of CP of the leaves/vines. The reduction in CP content in CL or SPV silage may also have been due to nitrogen losses from protein decomposition.

Both drying and ensiling reduced the concentration of anti-nutritional factors of CL and SPV. The HCN content of the fresh CL was found to be 1,221 mg/kg DM and was reduced to 152 mg/kg DM after 60 days ensiling and 128 mg/kg DM after sun-drying. The HCN content of fresh cassava roots was 342 mg/kg DM which was also reduced by ensiling to 20 mg/kg DM after 60 days. The HCN content of the ensiled CL (78 mg/kg DM) and dried CL (58 mg/kg DM) diets was higher than in the other treatments. No indications of cyanide toxicity was observed in any of the pigs fed the diets containing HCN in the present study. In animals, the lethal dose of HCN is generally reported to be between 0.66 and 15 mg/kg body weight for various species (WHO, 1965; FSANZ, 2005). Tewe (1992) reported a toxicity level of HCN for pigs of 3.5 mg/kg body weight. In the present study, pigs fed the ensiled and dried CL ingested approximately 2.0 and 1.6 mg HCN per kg body weight or about half of the reported levels which are considered toxic.

Ileal and total tract apparent crude protein and amino acid digestibility

Table 4. Average daily intake of dietary components and metabolisable energy of the experimental diets by growing pigs.

Component intake	Diet						S.E.M. ^b	P-value
	Cassava leaves		Sweet potato vines		Mixture ^a			
	Ensiled	Dried	Ensiled	Dried	Ensiled	Dried		
Dry matter (g/d)	1521	1624	1519	1451	1476	1606	44	0.071
Organic matter(g/d)	1430 ^{ab}	1536 ^a	1361 ^{ab}	1325 ^b	1359 ^{ab}	1497 ^{ab}	41	0.008
Crude protein (g/d)	183	195	182	174	177	193	5	0.071
Crude fibre (g/d)	125 ^b	120 ^b	155 ^a	135 ^b	134 ^b	132 ^b	4	<0.001
aNDFom ^c (g/d)	295 ^{bc}	272 ^c	393 ^a	328 ^b	328 ^b	308 ^{bc}	10	<0.001
Calculated metabolisable energy (MJ/d)	19.2 ^{ab}	20.6 ^a	19. 0 ^{ab}	18.1 ^b	18.3 ^{ab}	20.1 ^{ab}	0.6	0.031

Means within a row with no common superscript (a, b, and c) differ ($P < 0.05$).

^aMixture (50:50 on DM basis) of ensiled or dried cassava leaves and sweet potato vines.

^bn=6 with each sample obtained from a consecutive 4 day collection period.

^cNeutral detergent fibre with a heat stable amylase and expressed exclusive of residual ash.

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Table 5. Coefficients of ileal and total tract apparent digestibility of nutrients in the experimental diets fed to growing pigs.

Component	Diet						S.E.M. ^c	P-value		
	Cassava leaves		Sweet potato vines		Mixture ^a			Treatment	Interaction ^b	
	Ensiled	Dried	Ensiled	Dried	Ensiled	Dried			Ensiled	Dried
Ileal apparent digestibility										
Dry matter	0.697 ^{bc}	0.685 ^c	0.727 ^a	0.698 ^{bc}	0.717 ^{ab}	0.685 ^c	0.005	<0.001	0.458	0.325
Organic matter	0.737 ^{ab}	0.728 ^b	0.750 ^{ab}	0.742 ^{ab}	0.757 ^a	0.735 ^{ab}	0.005	0.012	0.052	1.000
Crude protein	0.468 ^{abc}	0.440 ^c	0.492 ^a	0.453 ^c	0.488 ^{ab}	0.457 ^{bc}	0.008	0.001	0.385	0.300
Crude fibre	0.255 ^{ab}	0.218 ^b	0.278 ^a	0.220 ^b	0.307 ^a	0.282 ^a	0.012	<0.001	0.012	<0.001
Total tract apparent digestibility										
Dry matter	0.762 ^{ab}	0.730 ^b	0.772 ^a	0.733 ^b	0.772 ^a	0.753 ^{ab}	0.008	0.003	0.619	0.041
Organic matter	0.787 ^{ab}	0.762 ^b	0.797 ^a	0.770 ^{ab}	0.798 ^a	0.800 ^a	0.006	0.002	0.435	0.001
Crude protein	0.572 ^{ab}	0.523 ^c	0.572 ^{ab}	0.542 ^{bc}	0.578 ^a	0.545 ^{abc}	0.008	<0.001	0.486	0.198
Crude fibre	0.393 ^b	0.380 ^b	0.452 ^a	0.418 ^{ab}	0.467 ^a	0.428 ^{ab}	0.013	0.001	0.012	0.082
aNDFom ^b	0.333 ^{bc}	0.307 ^c	0.400 ^a	0.365 ^{abc}	0.395 ^{ab}	0.370 ^{abc}	0.015	0.002	0.137	0.076
Difference between total tract and ileal apparent digestibility										
Dry matter	0.065 ^a	0.045 ^{ab}	0.045 ^{ab}	0.035 ^b	0.055 ^{ab}	0.068 ^a	0.006	0.008	1.000	0.001
Organic matter	0.050 ^{ab}	0.033 ^{bc}	0.047 ^{abc}	0.028 ^c	0.042 ^{bc}	0.065 ^a	0.005	<0.001	0.265	<0.001
Crude protein	0.103	0.083	0.080	0.088	0.090	0.088	0.005	0.084	0.800	0.704
Crude fibre	0.138 ^b	0.162 ^{ab}	0.173 ^{ab}	0.198 ^a	0.160 ^{ab}	0.147 ^b	0.010	0.004	0.726	0.010

Means within a row with no common superscript (a, b, and c) differ ($P < 0.05$).

^aMixture (50:50 on a DM basis) of ensiled or dried cassava leaves and sweet potato vines.

^bDifference of mixture from $0.5 \times \text{mean of cassava leaves} + 0.5 \times \text{mean of sweet potato vines}$.

^c $n=6$.

^dNeutral detergent fibre with a heat stable amylase and expressed exclusive of residual ash.

Ileal and total tract apparent crude protein and amino acid digestibility

Table 6. Coefficient of ileal apparent digestibility of amino acids of the experimental diets fed to growing pigs.

Amino acid	Diet						P-value			
	Cassava leaves		Sweet potato vines		Mixture ^a		S.E.M. ^c	Treatment	Interaction ^b	
	Ensiled	Dried	Ensiled	Dried	Ensiled	Dried			Ensiled	Dried
Arginine	0.528 ^b	0.520 ^b	0.630 ^a	0.573 ^{ab}	0.565 ^{ab}	0.565 ^{ab}	0.022	0.033	0.621	0.513
Histidine	0.694 ^{ab}	0.690 ^b	0.695 ^{ab}	0.680 ^b	0.747 ^{a*}	0.728 ^{ab*}	0.013	0.008	0.003	0.010
Isoleucine	0.648 ^b	0.653 ^{ab}	0.755 ^a	0.755 ^a	0.755 ^a	0.750 ^{ab}	0.024	0.004	0.084	0.134
Leucine	0.674 ^b	0.650 ^b	0.790 ^a	0.762 ^a	0.763 ^a	0.760 ^{a*}	0.014	<0.001	0.088	0.006
Lysine	0.697 ^{ab}	0.683 ^b	0.718 ^{ab}	0.670 ^b	0.792 ^{a*}	0.692 ^b	0.022	0.010	0.005	0.578
Methionine+Cysteine	0.706	0.712	0.737	0.695	0.715	0.715	0.010	0.170	0.614	0.369
Phenylalanine	0.584 ^c	0.613 ^{bc}	0.740 ^a	0.653 ^{abc}	0.680 ^{ab}	0.673 ^{abc}	0.022	0.001	0.372	0.154
Threonine	0.657 ^{bc}	0.630 ^c	0.748 ^a	0.712 ^{ab}	0.750 ^{a*}	0.715 ^{ab*}	0.017	<0.001	0.035	0.049
Tyrosine	0.736 ^c	0.758 ^{bc}	0.877 ^a	0.653 ^d	0.838 ^a	0.830 ^{ab*}	0.018	<0.001	0.156	<0.001
Valine	0.592	0.592	0.668	0.683	0.675	0.637	0.022	0.022	0.118	0.976
Alanine	0.721 ^{bc}	0.682 ^c	0.807 ^a	0.762 ^{ab}	0.777 ^{ab}	0.757 ^{ab*}	0.014	<0.001	0.452	0.048
Aspartic acid	0.669 ^b	0.700 ^b	0.678 ^b	0.793 ^a	0.767 ^{a*}	0.788 ^{a*}	0.014	<0.001	<0.001	0.024
Glutamic acid	0.628	0.662	0.662	0.567	0.673	0.603	0.029	0.110	0.428	0.761
Glycine	0.548	0.508	0.633	0.572	0.605	0.527	0.030	0.066	0.699	0.722
Proline	0.540	0.533	0.602	0.543	0.627	0.543	0.022	0.030	0.054	0.856
Serine	0.642	0.627	0.675	0.692	0.672	0.742 [*]	0.028	0.118	0.707	0.028

Means within a row with no common superscript (a, b, c and d) differ (P< 0.05).

^aMixture (50:50 on a DM basis) of ensiled or dried cassava leaves and sweet potato vines.

^bDifference of mixture from 0.5×mean of cassava leaves + 0.5×mean of sweet potato vines.

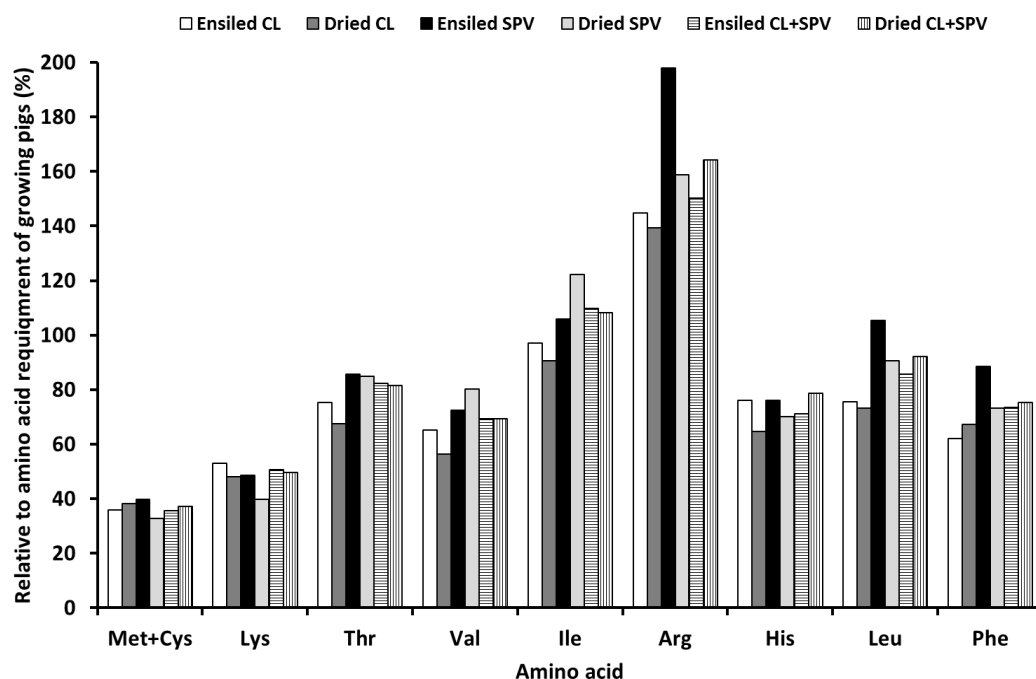


Figure. 1. Relative concentration of individual essential amino acids in the six protein sources (CL=cassava leaves, SPV=sweet potato vines) in meeting the amino acid requirements of 50-80 kg growing pigs (NRC, 1998). Values corrected to a dietary energy density of 13.6 MJ ME/kg dry matter.

As the ensiled material used in the present study contained rice bran in order to improve the ensiling process, direct comparison between the dried and ensiled products in the present study are difficult. It should be noted that the ileal digesta samples were dried at 60°C. Although unlikely, some microbial growth may have occurred when the sample was heated to this temperature and changes in the AA composition may have occurred during this time due to bacterial fermentation. The AA composition of the CL and SPV in the present study however, are in good agreement with earlier reports (Eggum, 1970; Ravindran, 1993; Phuc and Lindberg, 2001; Montagnac et al., 2009; Woolfe, 1992; Kinh, 2003). Although the protein in the diets originated from three different ingredients (CL/SPV, rice bran and ECR) with the CP of the CL/SPV making up more than 75.4 to 93.9% of the total CP, the CIAD for lysine and the sulphur amino acids reported here for the diets are in close agreement with the calculated CIAD values of the CL/SPV. Figure 1 shows the relative concentration of individual essential AA in the six diets investigated in the present study in meeting the AA requirements of growing pigs (NRC, 1998) when values are corrected for the energy density of the diet. Met+Cys were found to be the

most limiting AA in all the six diets tested, closely followed by lysine. Compared to the AA requirement estimates of 50-80 kg pig (NRC, 1998) with a high-medium lean growth rate (325 g/day of carcass fat-free lean), the ileal digestible Met+Cys content of the ensiled and dried CL, ensiled and dried SPV and ensiled and dried CL+SPV diets only met 36, 38, 45, 33, 36 and 37% of the requirements for growth, respectively. Eggum (1970) and Phuc et al. (2000) reported that the concentration of the sulphur-containing AA was rather low in CL. Recently, Chauynarong et al. (2009) and Montagnac et al. (2009) also found that the most limiting AA in CL is Met. These data are in contrast to reports of Woolfe (1992) and An et al. (2003) who found that Lys was the first limiting AA in fresh SPL. The second most limiting AA in the six diets in the present study was Lys, meeting 53, 48, 49, 40, 51 and 50% of the requirements of growing pigs (NRC 1998). Only arginine is present in excess amounts while Ile meets the requirements lean type growing pigs (Figure 1). Supplementation of diets containing dried and ensiled CL, SPV or a 50:50 mixture on a DM basis of CL+SPV as the sole proteins source with sulphur-containing AA and Lys can be expected to increase protein utilization by growing pigs.

Non-additivity was observed for a number of AA in the ensiled and dried CL+SPV. The ileal digestible Lys content of the ensiled mixture diet was 10.4% units higher than can be expected from the CIAD data of the two individual ingredients (ensiled CL and SPV) assuming additivity of digestibility values. Similarly, assuming additivity, the dried CL+SPV diet resulted in higher than expected CIAD of Leu, Thr and His than based on the CIAD of these AA from the individual dried CL and SPV. It can therefore be expected that a diet composed of a mixture of CL and SPV will result in a higher growth performance of pigs when supplemented with sulphur amino acid as the ileal digestible Lys content was the highest (0.42 g/kg DM) of all the six diets.

Our study showed that the CTTAD were higher than the CIAD for DM, OM and CF and a trend was observed from CP. In particular, the CTTAD of CF were 0.14 to 0.20 higher indicating that significant fermentation of CF occurred in the large intestine of the growing pigs. There was a net nitrogen disappearance from the large intestine resulting in a higher CTTAD for CP. The contribution of fermented carbohydrate to the energy supply of the animal can be expected to be about 70% of that of enzymatically digested starch (CVB, 1999). Our data are similar to studies by

An et al. (2004) and Phuc et al. (2000) investigating ensiled and sun dried CL, fresh, dried and ensiled sweet potato leaves, and groundnut foliage. Dung et al. (2002) reported that the capacity of the pig to digest and utilize fibre is affected by the fibre source. The fibre in dried CL and ensiled CL as well as dried SPV and ensiled SPV appears to be well fermented by the microflora in the large intestine of pigs. The 13 to 20% higher CTTAD of fibre in the large intestine of pigs found in the present study will yield additional energy in the form of volatile fatty acids and thereby meet part of the energy requirements of the pig.

Conclusion

In general, sweet potato vines have a higher nutrient digestibility compared to cassava leaves either dried or ensiled and ensiling resulted in a higher digestibility of dietary nutrients compared to drying. Hydrogen cyanide concentration of cassava leaves is reduced by both ensiling and drying with drying being slight more effective. Also, content of amino acids in cassava leaves and sweet potato vines can be affected by the preservation process, which needs to be accounted for in diet formulation. Mixing ensiled cassava leaves and ensiled sweet potato vines may yield additional benefits in terms of increased digestibility of amino acids in pigs compared to feeding these ingredients solely. The first limiting amino acid in practical diets formulated from dried or ensiled cassava leaves are methionine+cysteine and lysine. Providing dietary ingredients high in digestible methionine, cysteine and lysine or provision of these amino acids in crystalline form in diets containing cassava leaves and sweet potato vines may increase amino acid utilisation of these diets for pigs.

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Chapter 6

Pig performance increases with the addition of DL-methionine and L-lysine to ensiled cassava leaf protein diets

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Abstract

Two studies were conducted to determine the impact of supplementation of diets containing ensiled cassava leaves as the main protein source with synthetic amino acids, DL-methionine alone or with L-lysine. In study 1, a total of 40 pigs in five units, all crossbreds between Large White and Mong Cai, with an average initial body weight of 20.5 kg were randomly assigned to four treatments consisting of a basal diet 45% of dry matter (DM) from ensiled cassava leaves (ECL) and ensiled cassava root supplemented with 0%, 0.05%, 0.1% and 0.15% DL-methionine (as DM). Results showed a significantly improved performance and protein gain by extra methionine. This reduced the feed cost by 2.6, 7.2 and 7.5%, respectively. In study 2, there were three units and in each unit eight crossbred (Large White×Mong Cai) pigs with an initial body weight of 20.1 kg were randomly assigned to the four treatments. The four diets were as follows: a basal diet containing 15% ECL (as DM) supplemented with different amounts of amino acids L-lysine and DL-methionine to the control diet. The results found that diet with 15% of DM as ECL with supplementation of 0.2% lysine+0.1% DL-methionine and 0.1% lysine+0.05% DL-methionine at the 20-50 kg and above 50 kg, respectively, resulted in the best performance, protein gain and lowest costs and can be recommended for crossbred (Large White×Mong Cai) pigs. Ensiled cassava leaves can be used as a protein supplement for feeding pigs provided the diets contain additional amounts of synthetic lysine and methionine.

Introduction

Cassava (*Mani hot esculenta* Crantz) is an annually root crop grown widely in tropical and sub-tropical areas with the roots being a good source of energy while the leaves contain protein, vitamins and minerals. Cassava leaves have a high crude protein (CP) content of which almost 0.85 is true protein (Ravindran 1993). Furthermore, cassava leave protein has an essential amino acid (EAA) content which is higher than soybean protein (Eggum 1970; Phuc 2000; Montagnac et al. 2009). The high protein content and the relatively good EAA profile are reasons for the inclusion of cassava leaves as a protein source in diets for pigs in many countries. Cassava roots and leaves however contain large amounts of cyanogenic glucosides that give rise to toxic hydrocyanic acid (HCN) which limit the use of these products as an animal feed ingredient (Oke 1978; Ngudi et al. 2003; Cardoso et al. 2005). Ensiling cassava roots and leaves reduces the HCN content (Gomez and Valdivieso 1988; Phuc 2000; Loc 2004) and allows increased incorporation in animal feeds.

Several studies have determined the ileal apparent digestibility of a number of protein-rich foliages (cassava leaves, leucaena leaves, groundnut foliage, sweet potato leaves) available in tropical countries (Phuc and Lindberg 2001; An et al. 2004; Nguyen et al. 2010a). In the case of cassava leaves, the first limiting amino acid for growing pigs is methionine closely followed by lysine (Chauynarong et al. 2009; Montagnac et al. 2009; Nguyen et al. 2010a). Methionine is not only required for growth and maintenance of body protein but also for in vivo detoxification of hydrogen cyanide (Job, 1975; Tewe 1992) to non-toxic thiocyanate (Oke 1978) when pigs are fed cassava leaf or root ingredients. Although it is well-known that methionine is the first limiting amino acids in cassava protein for rats (Eggum 1970), little research has focused on the supplementation of diets containing ensiled cassava proteins with methionine or lysine on the performance of monogastric production animals. Loc (2004) reported studies in crossbred pigs (Large White×Mong Cai) fed ensiled cassava root-based diets were supplemented with methionine. Performance as measured by growth rate and feed conversion ratio were found to increase with DL-methionine supplementation. There have been no studies reported in the literature on the effects of pig performance and the economic viability of methionine and lysine addition to pig diets containing cassava protein.

The aim of the two studies reported here was to evaluate the effect of supplementation of synthetic DL-methionine alone or in combination with synthetic L-lysine to diets contain ensiled cassava leaves (ECL) and ensiled cassava roots (ECR) as the major protein source in diets for pigs.

Materials and methods

The experiments reported here were carried out in eight units in the Huong Van commune, which is one of the main pig production areas of Thua Thien Hue province in Vietnam. The protocol of the study was approved by the ethical committee of Hue University, Hue, Vietnam.

Preparation and preservation of ensiled cassava leaves

Fresh leaves of cassava were collected at the time of root harvest and spread out for 5 h on the floor for wilting during which time the DM content increased from 24% to 28-29%. After wilting the leaves were separated from the stems and petioles, chopped into small pieces (2-3 cm), mixed with 0.5% NaCl and rice bran at 5% of the wilted weight of the cassava leaves. The mixture was kept in air tight nylon bags with a capacity of 30 kg and stored during 2 months before use. This ensiling procedure resulted in a stable silage pH and a low cyanide content.

Animals, experimental design and feeding

Study 1: DL-methionine supplementation

Forty crossbred pigs (Large White×Mong Cai) with an average initial body weights of 20.5 kg (sd=0.7) and of similar ages were randomly allocated to five units. In each unit, eight pigs (four males and four females) were randomly allocated to one of four pens (2 x 1 m), with two pigs (one male and one female) per pen. Each pen was randomly allocated to one of the four dietary treatments which differed in the level of DL-methionine supplementation (0%, 0.05%, 0.10% and 0.15%) during two growing phases. Two control diets were formulated for the two growing periods; period 1 from 20 to 50 kg and period 2 above 50 kg. The control diet (Table 1) consisted of rice bran, maize, ECR, ECL and fish meal (FM). Diets for each period included 15% and 30% of ECL and ECR, respectively on a DM basis. The control diet was formulated to contain 12.6 MJ ME, 14.1% CP; 0.66% lysine and 0.28%

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Table 1. Ingredient content (%), chemical composition (% DM), calculated metabolisable energy content (MJ/kg DM) and hydrogen cyanide content (mg/kg DM) of the experimental diets for the pigs in study 1.

Ingredient/component	Diet							
	20 to <50 kg				>50 kg			
	Basal	+ DL-methionine			Basal	+ DL-methionine		
		0.05	0.10	0.15		0.05	0.10	0.15
Rice bran	29	29	29	29	30	30	30	30
Yellow maize	18	18	18	18	21	21	21	21
Ensiled casava roots	30	30	30	30	30	30	30	30
Ensiled cassava leaves	15	15	15	15	15	15	15	15
Fish meal	8	8	8	8	4	4	4	4
DL-methionine ^a	-	0.05	0.10	0.15	-	0.05	0.10	0.15
	Chemical composition							
Metabolisable energy	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Crude protein	14.1	14.1	14.1	14.1	12.2	12.2	12.2	12.2
Crude fibre	6.8	6.8	6.8	6.8	6.9	6.9	6.9	6.9
Lysine	0.66	0.66	0.66	0.66	0.55	0.55	0.55	0.55
Methionine+cysteine	0.28	0.33	0.38	0.43	0.25	0.30	0.35	0.40
Ileal digestible ^b								
Lysine	0.51	0.51	0.51	0.51	0.44	0.44	0.44	0.44
Methionine+cysteine	0.21	0.26	0.31	0.36	0.19	0.24	0.29	0.34
Hydrogen cyanide	43.5	43.5	43.5	43.5	43.5	43.5	43.5	43.5

^aNinety-eight percent DL-methionine (Sunmitomo Chemical Co., Ltd/Ajinomoto Co., Inc, Japan).

^bCalculated values based on analysed crude protein and amino acid composition and apparent ileal digestibility data from Nguyen et al. (2010) and Ngoan and Lindberg (2001).

DL-methionine and L-lysine supplementation to ensiled cassava leaf protein diets

Table 2. Dry matter and chemical composition of the dietary ingredient used to formulate the experimental diets.

Component	Rice bran		Yellow corn		ECR		ECL		Fish meal	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Dry matter (%)	88.0	86.3	85.5	84.4	40.6	41.0	28.0	32.8	90.0	90.0
Crude protein (% DM)	11.3	11.4	9.8	9.9	3.0	3.1	23.0	20.8	58.5	58.5
Crude fibre (% DM)	9.7	9.8	2.8	2.8	4.0	3.9	15.0	12.8	ND	ND
Lysine (g/kg DM)	4.8	4.9	3.2	3.3	1.2	1.1	11.5	10.8	31.6	31.6
Methionine+cysteine (g/kg DM)	2.3	2.3	2.0	2.0	0.11	0.1	4.6	3.8	10.5	10.5
Metabolisable energy (MJ/kg DM)	12.1	12.1	15.4	15.4	12.4	12.4	9.7	9.6	14.3	14.3
Hydrogen cyanide (mg/kg DM)	ND	ND	ND	ND	29	29	232	162	ND	ND

EXP, experiment; ECR, ensiled cassava root; ECL, ensiled cassava leaves analysed at 60 days after ensiling; DM, dry matter; ND, not determined.

methionine+cysteine in period 1 and 12.6 MJ ME, 12.2% CP, 0.55% lysine and 0.25% methionine+cysteine in period 2. The chemical composition of the feed ingredients used to formulate the diets in study 1 is shown in Table 2.

Study 2: DL-methionine and L-lysine supplementation

Twenty four crossbred pigs (12 males and 12 females) (Large White×Mong Cai) with an average initial weights of 20.1 kg (sd=0.2) and of similar age were randomly allocated according to gender to three units. The eight pigs (four males and four females) per unit were randomly allocated to four pens (2 x 1 m), with two pigs (one male and one female) per pen. Each pen was randomly allocated to one of the four dietary treatments with different levels of supplemented L-lysine and DL-methionine. Throughout the growing period, pigs were fed the basal diets depending on body weight (20 to 50 and above 50 kg). The control diet consisted of rice bran, maize, ECR, ECL and FM and included on a dry matter basis 15% of ECL and ECR 17% of DM for period 1 and 25% in period 2 (see Table 3). During period 1, the control diet contained 12.6 MJ ME, 14.9% CP; 0.70% lysine and 0.28% methionine while during period 2 the diet contained 12.6 MJ ME, 12.8% CP, 0.58% lysine and 0.23% methionine (Table 3). The control diet was supplemented with no, low, medium or high levels of L-lysine and DL-methionine. The low amino acid diet was supplemented with 0.10% and 0.05% L-lysine and DL-methionine, respectively during period 1 and during period 2 with 0.05% L-lysine and 0.03% DL-methionine, respectively. In the medium supplemented diet in periods 1 and 2, 0.20% and 0.10% L-lysine and 0.10 and 0.05% DL-methionine, respectively were added to the basal diet. The high supplemented diet was obtained by adding 0.30% L-lysine and 0.15% DL-methionine during period 1 and with 0.15% L-lysine and 0.08% DL-methionine during period 2. The dietary composition of the eight diets is given in Table 3.

In both studies, the pigs had been vaccinated against hog cholera and Pasteurellosis, and de-wormed 2 weeks before starting the experiment. The composition of the control diets for the two growing periods in both studies is given in Table 2. The diets were fed at a level of 4% of body weight (BW) as recommended by the National Institute of Animal Husbandry (NIAH 2001). Both experiments lasted 90 days and were conducted during the cool season in Vietnam with average daily temperatures between 22° and 26°C. The diets were distributed equally into 3 meals

DL-methionine and L-lysine supplementation to ensiled cassava leaf protein diets

Table 3. Ingredient content (%), chemical composition (% of DM), calculated metabolisable energy content (MJ/kg DM) and hydrogen cyanide content (mg/kg DM) of the experimental diets for the pigs in study 2.

Ingredient/component	Diet							
	20 to <50 kg				>50 kg			
	Basal	+ L-lysine and DL-methionine			Basal	+ L-lysine and DL-methionine		
		Low	Medium	High		Low	Medium	High
Rice bran	40	40	40	40	35	35	35	35
Yellow maize	20	20	20	20	20	20	20	20
Ensiled casava roots	17	17	17	17	25	25	25	25
Ensiled cassava leaves	15	15	15	15	15	15	15	15
Fish meal	8	8	8	8	5	5	5	5
L-lysine ^a	-	0.10	0.20	0.30	-	0.05	0.10	0.15
DL-methionine ^b	-	0.05	0.10	0.15	-	0.03	0.05	0.08
	Chemical composition							
Metabolisable energy	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Crude protein	14.9	14.9	14.9	14.9	12.8	12.8	12.8	12.8
Crude fibre	7.1	7.1	7.1	7.1	6.9	6.9	6.9	6.9
Lysine	0.70	0.80	0.90	1.00	0.58	0.63	0.68	0.73
Methionine+cysteine	0.28	0.33	0.38	0.43	0.23	0.26	0.28	0.31
Ileal digestible ^c								
Lysine	0.52	0.62	0.72	0.82	0.43	0.48	0.53	0.58
Methionine+cysteine	0.21	0.26	0.31	0.36	0.18	0.21	0.23	0.26
Hydrogen cyanide	29.3	29.3	29.3	29.3	31.7	31.7	31.7	31.7

^aNinety-nine percent L-lysine HCL (Sunmitomo Chemical Co., Ltd/Ajinomoto Co., Inc, Japan).

^bNinety-eight DL-methionine (Sunmitomo Chemical Co., Ltd/Ajinomoto Co., Inc, Japan).

^cCalculated values based on analysed crude protein and amino acid composition and apparent ileal digestibility data of Nguyen et al. (2010a) and Ngoan and Lindberg (2001).

per day (7, 11 and 17 h) with refusals collected the following morning before the first meal. Drinking water was provided *ad libitum*.

Chemical analyses

The feedstuffs in the experimental diets were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF) and HCN (AOAC 1990) and amino acids. Dry matter (DM) was measured by drying fresh samples at 105°C for 24 hours. Total nitrogen (N) was determined on fresh samples by the macro Kjeldahl method and CP was calculated from total nitrogen ($N \times 6.25$). Amino acids were analysed according to Spackman et al. (1958) on an ion-exchange column using an HPLC. Samples were hydrolysed for 24 hours at 110°C with 6 M HCL containing 2g/l reagent grade phenol and 5 µmol norleucine (internal standard) in evacuated and sealed ignition tubes. Methionine+cysteine were determined as methionine sulphone and cysteic acid with separate samples hydrolyzed for 24 hours as described above following oxidation with performic acid overnight at 0°C (Moore 1963). The HCN content was determined in the fresh ensiled samples by titration with $AgNO_3$, after boiling the samples and concentrating the HCN in KOH (AOAC 1990). All samples were analyzed in triplicate except amino acids which were analysed in duplicate. Most analyses were done in the Hue University laboratories except the amino acids (AAs) which were analyzed in the National Institute of Animal Husbandry (NIAH) laboratories (Ha Noi).

Measurements

Feed consumption was determined by weighing the amounts given and subtracting any feed remaining the following morning. Pigs were individually weighed at the start of the study, monthly and at slaughter, and average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) calculated for each treatment. Feed costs were calculated for the quantity of feed consumed by each pig, the individual feed ingredient prices and the composition of the feed.

Protein and fat deposition was calculated using the following assumptions: one gram of protein and fat contains 23.4 and 39.7 kJ of energy per gram, respectively (NRC 1998) and:

$$\text{ME intake} = \text{ME}_m + c \times \text{protein deposition} + d \times \text{fat deposition}$$

where ME_m is the amount of ME required for maintenance (460 kJ of ME per kg of metabolic BW ($\text{BW}^{0.75}$)); c and d represent the amount of ME needed for the deposition of 1 g of protein and fat, respectively. The required amounts of ME needed to deposit protein and fat deposition (ME_p) was assumed to be 53 kJ ME per g protein and 53 kJ per g of fat (NRC, 1998).

On the basis of literature review of Kotarbinska and Kielanowski (1969), it can be assumed that about 10% of weight gain is gut fill and ash, thus:

$$0.9 \times \text{ADG} = \text{water} + \text{protein} + \text{fat}$$

The deposition rate of protein and fat in the empty body of the pig was calculated based on the following two equations:

$$0.9 \times \text{ADG} = F + P/0.21 \text{ and } \text{ME}_p = (F + P) \times 53$$

where ADG is average rate of gain (g/d), 0.21 is the ratio of protein to protein + water, F is the amount of fat deposited (g/d), P the amount of protein deposited (g/d) and ME_p the metabolisable energy used for fat and protein deposition.

Statistical analysis

The experimental unit was a pen (two pigs). An analysis of variance was done according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Y is a dependent variable, μ is the overall mean, T_i is the treatment effect ($i=1, 2, 3 \dots 4$) and e_{ij} is the random error. Data were analyzed by ANOVA using the General Linear Model (GLM) of Minitab Statistical Software Version 14 (2004). Tukey pair-wise comparison was used to determine differences between treatment means at $P < 0.05$.

Results

Study 1

The data in Table 4 indicate that supplementing diets containing 45% (in DM) of ensiled cassava (30% ECR+15% ECL) with 0.05%, 0.1% and 0.15% DL-

methionine significantly increased the final BW, ADG, and decreased the FCR in the pigs. The ADG differed between treatments ($P<0.001$) and were 534, 560, 596 and 608 g/d for the control, +0.05% met, +0.10% met and +0.15% met, respectively. The FCR for these groups were 2.97, 2.86, 2.69 and 2.65 kg DM/kg gain, respectively ($P<0.001$). The data in Table 4 show that protein deposition of the F₁ pigs used increased significantly while the fat deposition was decreased as levels of supplementary DL-methionine increased in diet. Protein deposition of the pigs fed the control, control +0.05% met, +0.10% met and +0.15% met diet was 60.2, 66.3, 75.1 and 77.5 g/d, respectively ($P<0.001$).

Table 4. Performance and feed costs of growing (20-80 kg) Large White×Mong Cai pigs fed diets containing ensiled cassava leaves and supplemented with different levels of DL-methionine in study 1.

Parameter	Diet ¹				SEM	P<
	Basal	+ DL-methionine				
		0.05	0.10	0.15		
Initial body weight (kg)	20.1	20.8	20.6	20.2	0.3	0.213
Final body weight (kg)	68.2 ^a	71.2 ^{ac}	74.2 ^{bc}	74.9 ^b	1.0	0.001
Average daily gain (g/d)	534 ^a	560 ^{ac}	596 ^{bc}	608 ^b	12	0.001
Dry matter intake (kg /d)	1.59	1.60	1.61	1.62	0.02	0.475
Feed conversion ratio (kg DM/kg gain)	2.97 ^a	2.86 ^a	2.69 ^b	2.65 ^b	0.05	0.001
Calculated deposition (g/d)						
Protein	60.2 ^a	66.3 ^a	75.1 ^b	77.5 ^b	2.5	0.001
Fat	194.3 ^a	188.1 ^{ab}	179.1 ^b	178.4 ^b	3.6	0.009
Feed cost ¹ (VND/kg gain)	6289 ^a	6126 ^a	5837 ^b	5816 ^b	102	0.005
Costs compared to control (%)	100	97.4	92.8	92.5	-	-

Means with different superscripts within rows differ ($P<0.05$).

¹Price of feed ingredients at Hue during the time of the study in Viet Nam Dong (VND/kg): ensiled cassava roots, 400; ensiled cassava leaves, 500; rice bran, 2000; maize, 2000; fish meal, 6000; DL-methionine, 52000. At the time of this study: 1 USD = 15,000 VND.

Supplementing diets containing 45% (in DM) ensiled cassava (30% ECR+15% ECL) with DL-methionine at levels of 0.05%, 0.10% and 0.15% gave lower feed costs by 2.6, 7.2 and 7.5%, respectively. Differences in feed cost/kg weight gain were significant among treatments ($P<0.005$). The feed cost per kg gain for the

DL-methionine and L-lysine supplementation to ensiled cassava leaf protein diets

control+0.15% met diet was lowest although this was not significantly different from the control+0.10% met diet.

Study 2

The effects of supplementation of diets containing 15% of ECL in the DM with L-lysine and DL-methionine on the performance of pigs are shown in Table 5. The final BW and ADG were highest for pigs fed the two highest levels of supplementary L-lysine plus DL-methionine. The FCR was higher in the control diet than in the other three diets supplemented with L-lysine and DL-methionine.

Table 5. Performance and feed costs of growing (20-80 kg) Large White×Mong Cai pigs fed diets containing ensiled cassava leaves and supplemented with different levels of L-lysine and DL-methionine in study 2.

Parameter	Diet				SEM	P<
	Basal	+ L-lysine and DL-methionine				
		Low	Medium	High		
Initial body weight (kg)	20.1	20.0	20.8	19.6	0.4	0.247
Final body weight (kg)	68.8 ^a	72.6 ^a	80.3 ^b	74.2 ^a	1.5	0.001
Average daily gain (g/d)	537 ^a	584 ^a	660 ^b	604 ^b	17	0.001
Dry matter intake (kg/d)	1.60 ^a	1.59 ^a	1.71 ^b	1.64 ^{ab}	0.02	0.001
Feed conversion ratio (kg DM/kg gain)	2.99 ^a	2.73 ^b	2.59 ^b	2.72 ^b	0.06	0.001
Calculated deposition (g/d)						
Protein	59.6 ^a	71.9 ^{ab}	84.3 ^b	73.3 ^b	3.5	0.001
Fat	199.5	183.7	192.8	192.0	3.9	0.063
Feed cost ^a (VND/kg gain)	6482	6269	6193	6692	145	0.099
Costs compared to control (%)	100	96.7	95.5	103	-	-

Means with different superscripts within rows differ (P<0.05).

Supplementation with L-lysine and DL-methionine during the growing phase (20-50 and 50-80 kg) in % DM: low level, 0.10 and 0.05 and 0.05 and 0.03; medium level, 0.20 and 0.10 and 0.10 and 0.05 and high level, 0.3 and 0.15 and 0.15 and 0.08.

^aPrice of feed ingredients in Hue at the time of the study in Viet Nam Dong (VND/kg): ensiled cassava root, 400; ensiled cassava leaves, 500; rice bran, 2200; maize, 2200; fish meal, 6000; DL-methionine, 65000. L-lysine: 75000. 1 USD = 15,500 VND.

The final BW and ADG of pigs were highest, and the FCR was lowest at the medium level of L-lysine and DL-methionine supplementation in the diet. Similarly the estimated protein depositions of F₁ pigs was increased significantly ($P < 0.001$) when both L-lysine and DL-methionine were added to the diets. Protein deposition of the pigs fed the control diet+supplementary L-lysine and DL-methionine at the medium level was numerically higher than for the other three treatments. The protein deposition of the pigs fed the control diet+supplementary L-lysine and DL-methionine at the high level was lower than that of pigs fed the control diet+supplementary L-lysine and DL-methionine at the level medium. A trend ($P < 0.099$) was observed in the feed costs per kg gain with the medium supplementary L-lysine and DL-methionine.

Discussion

Cassava is a major staple root crop in many tropical and subtropical, developing countries. It is well known that cassava roots are high in starch but low in crude protein, while cassava leaves are rich in crude protein. The protein quality of a food is the product of its AA content and the nutritional availability of these AAs. Eggum (1970) and Phuc (2000) reported that the concentration of the sulphur-containing amino acids is low in cassava leaves and roots which causes a relatively low biological value of this protein ranging from 44 to 57. Recently, several researchers have reported that methionine is the most limiting AA both in cassava leaves and roots for growing pigs and poultry, followed by lysine (Loc 2004; Chauynarong et al. 2009; Montagnac et al. 2009; Nguyen et al. 2010a). In addition, the high cyanide content further limits the use of cassava leaves as a protein source in diets for pigs. Methionine not only plays a role as an EAA in protein deposition but is also important in the hydrogen cyanide detoxification process, particularly in rations with high levels of cassava roots or leaves. Therefore pig diets with a high inclusion level of cassava root and leaves could benefit from additional supplementation with synthetic methionine and lysine. In order to determine the magnitude of the effect of supplementation, we formulated diets to contain 45% of ensiled cassava root and leaves and added increasing levels of supplementary DL-methionine (from 0% to 0.15% in DM) to diets for growing pigs. The results show that there were significant effects of diet on final BW, ADG, FCR and protein

deposition. Increasing levels of supplementary DL-methionine from 0.05% up to 0.15% in the diets improved ADG and FCR. These results can be explained by the fact that the supplemental methionine was not used for protein deposition and provided sulfhydryl groups (-SH) necessary for the detoxification of cyanide as the HCN content of the diets ranged from 29.3-43.5 mg/kg DM. In the body, cyanide is detoxified by the enzyme rhodanese, forming thiocyanate, which is excreted in the urine (Oke 1978; Tewe et al. 1977).

The estimated fat and protein deposition of F₁ (Large White×Mong Cai) pigs were significantly affected by dietary treatment. The data in Table 4 show that protein deposition in the DL-methionine supplemented diets were higher than in control animals. Increased levels of supplementary methionine decreased fat deposition because at the same ME intake less of ME_p is available for lipid gain. Supplementation with 0.15% methionine gave the highest protein deposition among diets although this was not significantly different from the protein deposition of the pigs fed the control+0.10% methionine diet. These results confirm the studies by Eggum (1970) who reported that addition of synthetic methionine to diets for rats increased the biological value of cassava protein. Loc (2004) studied the addition of DL-methionine (0%, 0.1%, 0.2% and 0.3%) to diets containing 20% to 40% of the DM ensiled cassava root to F₁ (Large White×Mong Cai) pigs. This author showed that an increased performance, as measured by growth rate and feed conversion ratio could be achieved with DL-methionine supplementation. In the present study, supplementary DL-methionine at 0.05%, 0.10% and 0.15% in diets containing 45% (in DM) ensiled cassava (30% ECR+15% ECL) reduced the feed cost by 2.6%, 7.2% and 7.5%, respectively.

Study 2 was designed to test whether further increases in performance can be obtained by adding lysine to methionine supplemented ensiled cassava leave and root containing diets. Identical methionine supplementation levels were used compared to study 1. Table 5 shows that supplementary L-lysine and DL-methionine in diets of growing pigs improved ADG, FCR and protein deposition. In this study, final BW, ADG and protein deposition were highest at the two highest levels of supplementation (medium and high). The FCR was higher and protein deposition was lower in the control diet than in the other three diets with supplemented L-lysine and DL-methionine. The final BW, ADG and protein deposition of pigs were highest and the

FCR was the lowest at the medium level of supplementation with lysine and methionine. It appears that those levels met the requirements for lysine and methionine+cysteine for growing F₁ (Large White×Mong Cai) pigs. The content of lysine and methionine in the high supplemented diet was somewhat higher than the requirements for lysine and methionine as set by NRC (1998). The reason for the numerical increase in FCR from the medium to the high level of supplementation is difficult to explain. Loc (2004) also found a slightly reduced daily gain of pigs fed 0.30% supplemental methionine compared to the 0.20% when fed a ensiled cassava roots based diet. In study 1, no reduction in growth rate, FCR or protein deposition was observed indicating that this effect was likely due to the lysine addition instead of the methionine.

The most common procedures for reducing the cyanide content in cassava are sun-drying and ensiling (Phuc et al. 2001; Borin et al. 2005; Nguyen et al. 2010b). Considerable amounts of cassava leaves are readily available as a by-product at the time of harvesting the roots. However, in many tropical countries, the harvest season of cassava root coincides with the rainy season making sun-drying difficult or unfeasible. Ensiling is a suitable alternative way of preserving the roots and leaves (Van Man and Wiktorsson 2001; 2002) and recently Nguyen et al. (2012) showed that ensiling cassava leaves for 90 days reduced the HCN concentration by 70-80%. Our results in study 2 show that the diet supplemented with 0.20% L-lysine and 0.10% DL-methionine, and 0.10% L-lysine and 0.05% DL-methionine in the growing and finishing periods, respectively, resulted in the highest economic returns for farmers.

The present study further develops the practical and economical feasibility of using ensiled cassava leaves in diets for pigs. By supplementing diets containing ensiled cassava leaves with methionine and lysine the performance of Large White×Mong Cai pigs can be significantly increased as well as the economic benefits for farmers.

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Chapter 7

General discussion

Introduction

In Vietnam, pig production is an important sector in agriculture. Pigs are an impetus to increase income and to improve overall living standards of farmers. The annual growth rate of the pig population in Vietnam from 2001-2010 was 4.5%, with a total of 27.4 million pigs produced in 2010 (GSO, 2011). Farmers in the rural areas of Vietnam account for over 75% of the total pig production in the country. Typically farmers in rural areas keep around 4-6 pigs per household which are fed rice bran, maize, cassava, sweet potato and other vegetables. Cassava, maize and rice bran are rich in energy but low in protein and essential amino acids (EAA). The conventional protein supplements in the region are soybean meal, groundnut cake, and fish meal (FM). These are however, too expensive for many farmers and therefore not routinely used. It is therefore important to identify alternative, locally available high protein feed ingredients and to optimise the use of this alternative protein source by small-hold farmers in Vietnam.

Cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) are the second and third most important food crops after rice in terms of total production in Vietnam. Cassava has rapidly changed its role from a food crop to an industrial crop and in 2010, more than 496,000 ha was planted with cassava. The root production was approximately 8.52 million tonnes (GSO, 2011). At harvest time, approximately 5 tonnes/ha of fresh leaves and 7 tonnes/ha of total foliage can be harvested (Phuoc, 2004). Cassava leaves (CL) can be harvested 3-4 months after planting and subsequently in cycles of 60-75 days (Ravindran, 1993). Cassava leaves are a good source of protein for animals (Ravindran, 1993; Phuc, 2000; Wanapat, 2009) although the high levels of hydrogen cyanide (HCN) significantly hamper its potential use as an animal feed. Processes such as drying and ensiling of CL and cassava roots can markedly reduce the HCN content. A number of studies have studied the effect of different processing methods on the chemical contents and the nutritional values of CL and cassava roots (Phuc, 2000; Borin et al., 2005; Van Man and Wiktorsson, 2001).

Sweet potato is extensively grown in many countries, especially in China and other Southeast Asian countries. In Vietnam, sweet potato is the third most important crop and 150,800 ha were grown in 2010 (GSO, 2011). Sweet potato is the main food crop in areas where rice production is limited, but at present it is more commonly

used for livestock feeding. Both the tubers and vines are used as feed for pigs, chickens and cattle (Woolfe, 1992). The dry matter (DM) of the sweet potato tuber consists mainly of starch and is therefore considered an excellent energy source, while the leaves have a high protein content and can therefore, be used as a protein source for livestock feeding (Woolfe, 1992; Ishida et al., 2000, Peters et al., 2001). However, there are only few reports on the nutritive value of sweet potato vines (SPV) for pigs.

Central Vietnam has a tropical monsoon climate with two main seasons: a rainy and a dry season. The rainy season lasts from September to March and has the highest precipitation from September to November. The dry season lasts from April to August with the highest temperatures in June and July. The harvest of cassava roots in Central Vietnam occurs from September to November coinciding with highest precipitation during the rainy season. Sweet potato vines can be harvested many times throughout the year but are normally abundantly available during the rainy season. Early in the rainy season, it is difficult to sun-dry CL and SPV to a sufficiently high DM content for conservation so that it can be used when feed availability is low during the dry season as well as from November to January in the rainy season. For CL, conservation is not only important for feed shortages but also essential to reduce the concentration of HCN which is toxic to animals when consumed at high concentrations (Butler, 1973; Tewe, 1992).

The general objective of the work presented here was to evaluate processing methods for the preservations of CL and SPV for later feeding to pigs during feed shortages in Vietnam. In addition, the nutritional value (including HCN) of stored and processed CL and SPV as ingredients in diets for pigs were studied to determine their optimal use. Two processing methods were investigated namely drying and ensiling which, due to the relatively low costs and simplicity of use, could be used by many small-holder farmers in tropical countries.

Chemical composition of cassava leaves and sweet potato vines

Gross nutrient composition of cassava leaves

The chemical composition of CL reported in the present study (Chapter 2 to 6) are in general agreement with early reports (Rogers and Milner 1963; Eggum, 1970). Rogers and Milner (1963) reported that the CP content of CL in Brazil varied from 17.8 to 34.8% in the DM and from 18.5 to 32.4% in DM for Jamaican varieties.

Eggum (1970) reported that the leaves contain an average of 21% CP (range of 16.7 to 39.0%) of which 85% was true protein. In the present study, the leaves from cassava varieties, taken from the upper part of the plant at the time of root harvesting contained from 24.2 to 30.5% CP in the DM. These values varied less than those reported by Ravindran (1995) (16.7-39.9%) and by Phuc et al. (2001) (21 to 34%) for cassava varieties in the South of Vietnam. This variation in CP content can be caused by soil fertility, climatic conditions and genetics of cassava.

The crude fibre (CF) content in the DM of CL in this present study ranged from 11.7 to 15.0% and are lower than the 16.4-16.7% reported by Phuc and Lindberg (2000). However similar values were reported by Borin et al. (2005) (12.5-13.6%). The crude fat content in the DM of CL in this present study was similar to the values of 6.4-7.5% reported by Phuc et al. (2001).

Gross nutrient composition of sweet potato vines

Studies on plants that are used for forage production purposes in Vietnam show that SPV have potential as a source of protein for pigs (Phuc, 2000; Dung, 2001; Dominguez, 1992). The CP content in the DM of SPV can range from 16 to 29% (Farrell et al., 2000; Hartemink et al., 2000; Dung, 2001) which is comparable to the values of 17.8% reported in Chapter 3, 23.1% in Chapter 4 and 22.3% in Chapter 5. Similar results were reported by An et al. (2003). We also found that the content of CF in the SPV DM is rather high (~16%). This is in agreement with other authors (Woolfe, 1992; Farrell et al., 2000), while other studies have found somewhat lower CF concentrations e.g. 12.8% in the DM by An et al. (2003). According to Ishida et al. (2000), most parts of the sweet potato plant are rich in dietary fibre, particularly the stems have a high content of both soluble and insoluble dietary fibre.

Gross amino acid content of cassava leaves

The results of the chemical analyses presented in Chapter 5 show that the CP and amino acids (AA) content of the CL ingredients was higher than the corresponding SPV ingredient with the exception of threonine and valine in the fresh material. The CP content of fresh CL and dried CL was 29.9% in DM with the sum of AA as a percentage of CP in the fresh CL found to be 82.1%. The sum of EAA as a percentage of CP of fresh CL was 43%. Fresh CL contained 12.98 g/kg DM lysine

which makes up approximately 4.7% of the CP while the content of methionine+cysteine is low (2.1% of CP). The AA composition of the CL in the present study is in good agreement with reports by Eggum (1970), Ravindran (1993), Phuc and Lindberg (2001) and Montagnac et al. (2009). Eggum (1970) and Phuc et al. (2000) also reported a low concentration of the sulphur-containing AA in CL.

Gross amino acid content of sweet potato vines

Similar to CL, the results of the chemical analyses presented in Chapter 5 also show that the sum of AA as a percentage of CP in the fresh SPV, ensiled SPV and dried SPV was found to be between 81 and 88%. The sum of EAA as a percentage of CP of SPV was 46%. In general, the content of EAA in fresh SPV was similar to levels found in other tropical foliage (Phuc et al., 2001). Fresh SPV contained 9.7 g/kg DM lysine which made up approximately 4.3% of the CP while the content of methionine+cysteine was low (2.0% of CP). The content of lysine in fresh SPV was also lower than in CL (Chapter 5). The AA composition of the SPV reported in Chapter 5 is in good agreement with data in earlier reports (Woolfe, 1992; Phuc and Lindberg, 2001; An et al., 2003).

Anti-nutritional components in cassava leaves and sweet potato vines

The concentration of HCN, a highly toxic compound produced after the release of cyanogenic glucosides, varies in different cultivars from 1 up to 1,000 mg/kg fresh leaves (Bradbury, 1990). The typical cyanide contents of fresh CL range from 200 to 800 mg/kg but values as low as 80 mg/kg (Wood, 1965) and as high as over 4,000 mg/kg (Ravindran and Ravindran, 1988) have been reported. The measured HCN content of the fresh CL reported in the experiments of Chapters 2, 3, 4 and 5 ranged from 1,221 to 1,745 mg/kg DM. This HCN content was less than that reported by Ravindran et al. (1987). The latter authors reported that the HCN content of the leaves at the time of root harvest varied according to age and maturity of the plant; 442 mg/kg fresh matter in the green leaves at the top of the plant and 365 mg/kg fresh matter in green leaves below the top. Liem (1998) reported similar values ranging from 305 to 425 mg of HCN/kg fresh leaves. Cassava roots containing <50 mg HCN/kg fresh roots are considered “sweet” cultivars, while the “bitter” cultivars have >50 mg HCN/kg (Borin et al., 2005). However, the bitter cultivars also tend to

have a higher yield potential and higher starch contents (Ginting et al., 1999). New high-yielding cassava varieties, such as KM94 and KM98-5 and KM140 have been distributed to various provinces in Vietnam over recent years. In Central Vietnam, Hang and Preston (2005) found that the leaves of cassava varieties at the time of root harvesting had a HCN concentration ranging from 610 mg/kg DM in local varieties cassava such as Batrang and Nep Hong Ha, to 1,840 mg/kg DM in new varieties such as KM94, KM140-1 and KM 95. These new varieties appear to have a higher concentration of cyanogenic glucosides. As such, the importance of finding effective processing methods for CL to reduce the levels of HCN is becoming more important as high yielding varieties are being grown more widespread in many countries including Vietnam.

Although not investigated in the present work, SPV also contains a number of anti-nutritional factors. Sweet potato vines have been reported to contain high concentrations of oxalic acid (470 ± 15 mg/100 g fresh weight), phytic acid (0.46 ± 0.01 mg/100 g fresh weight), tannic acids (491 ± 7 mg/100 g fresh weight) and trypsin (52.0 ± 0.9 TIU/g) and chymotrypsin (69.1 ± 0.6 TIU/g) inhibitors have been reported (Mosha et al., 1995; Rekha et al., 1999; Hou and Lin, 1997).

Preservation methods for cassava leaves and sweet potato vines

Drying and ensiling technologies

Two of the most common techniques of processing CL and SPV in Vietnam, drying and ensiling, have been used with the aim of improving the quality and/or preservation of this material for periods of feed shortage. The aim of the drying process is to reduce the moisture content of the materials to a level so that microbial activity cannot occur (McDonald et al., 1995). Sun-drying is a common practice during the dry season and possible due to available sunlight, high temperatures, low humidity and availability of space to spread out the material. To speed up the drying process, the forage material is first chopped into 3-5 cm long parts, allowing quicker evaporation of moisture and subsequently allowing the release of volatile toxic substances such as HCN. Studies by Borin et al. (2005) and Wanapat (2009) have recommended that in order to produce a good quality hay from CL, the moisture content should be reduced from around 75% (Chapter 2) to between 13-14%. However, when CL and SPV are harvested during the rainy season, sun-drying is

difficult due to the lack of sunlight, high frequency of rain and humidity. Prolonged drying due to bad weather creates favourable conditions for bacteria and fungi, producing mouldy hay resulting in mycotoxin contamination.

Ensiling is a more practical alternative when weather conditions preclude sun-drying. This process involves the bacterial fermentation of carbohydrates under anaerobic conditions whereby fatty acids especially lactic acid are produced which causes a decrease in the pH. The low pH ensures that the feed is preserved and other microbes are unable to grow and spoil the forage material. In order for a rapid ensiling process to occur, sufficient amounts of substrates (e.g. starch) for the lactic acid bacteria to grow must be available, in addition to anaerobic conditions. The results presented in Chapter 2 show that CL can be successfully ensiled and preserved for up to 3 months when rice bran or cassava root meal at concentrations of 5 or 10% and with 20 to 50% fresh cassava roots (all on fresh basis) are used. The pH of the ensiled leaves declined from 6.6 to 4.5 within 7 days of the start of the ensiling process. After 21 days the pH was reduced to 4.1-4.2. A pH of 4.2 is sufficient to preserve a crop satisfactorily for longer periods of time (in excess of 3 months) if anaerobic conditions are maintained (McDonald et al., 2002). Rice bran and cassava root meal are locally available feedstuffs in Vietnam and from the results in Chapter 2 are suitable sources of available energy for the growth of lactic acid bacteria. Rice bran is available all year round because it is a by-product from the rice farming which is practiced by rural farmers in Vietnam.

The results presented here are in agreement with a number of earlier studies where carbohydrate-rich materials have been used as additives in an attempt to improve both the fermentation process and the nutritional value of silage (McDonald et al., 1991; Ngoan et al., 2000; Phuc, 2000; Borin et al., 2005). The latter authors reported that adding 50 g of sugar palm syrup per kg CL after sun-wilting results in a successful fermentation. Other additives, such as cassava root meal and sweet potato root meal included at a level of 60 g/kg also produced a good quality silage (An and Lindberg, 2004).

Effects of processing on nutrient composition

The results reported in Chapter 5 showed that sun-drying caused only minor changes in CP and AA content compared to the fresh of CL and SPV. This is in

agreement with early studies of Ravindran et al. (1987) and An et al. (2004). Results showed that the CP and AA content in the DM of ensiled material was somewhat lower than in the dried or in fresh material. This is in agreement with the studies of Phuc and Lindberg (2000, 2001) on processed CL. However, in a study carried out in Central Vietnam, An et al. (2004) found that sun-drying of sweet potato leaves did not affect the total content of EAA, while ensiling resulted in a reduction of the EAA and CP compared with the fresh material. Especially the sum of the AA as a percentage of CP in the ensiled CL (80.8%) was slightly lower than in fresh CL (82.1%) and in dried CL (83.3%). Similarly, the sum of AA as a percentage of CP in ensiled SPV was lower than in the fresh SPV (Chapter 3). These results can partly be explained by dilution due to the addition of the rice bran to the wilted cassava material in order to facilitate the ensiling process. The reduction in CP and AA content in CL and SPV silage may also have been due to nitrogen losses from protein decomposition and from the generation of ammonia in the process.

Effect processing on anti-nutritional components

Besides preserving feeding materials, ensiling and sun-drying are important methods to reduce the HCN content in CL to a level considered safe for animal feeding (Padmaja, 1989, 1995; Phuc et al., 2001; Cardoso et al., 2005; Van Man and Wiktorsson, 2001, 2002). It is known that HCN is released from the cyanogenetic plant after the cellular structure of the plant is disrupted (Conn, 1994). The released HCN slowly evaporates into the atmosphere during drying, thereby reducing the level of HCN in the cassava products. In the present work, ensiling and sun-drying were effective in reducing the HCN content in CL. In the experiments reported in Chapter 3 and 5, the HCN content in the fresh CL ranged from 1,221 to 1,304mg/ kg DM and this level was reduced to 128-160 mg/kg DM, or a reduction of 86.7 to 89.5% after drying in the sun for 2-3 days (Table 1). Like other studies, these results also demonstrate that sun-drying is effective in reducing the HCN content in CL. Nambisan and Sundaresan (1985) and Wanapat (2009) reported that simple sun-drying or oven-drying eliminated almost 90% of the HCN present in fresh CL. In a study carried out in South Vietnam, Phuc and Lindberg (2000) concluded that 83.1% of the HCN in fresh CL disappears by sun-drying. Ravindran et al. (1987) reported that an average of 1,436 mg HCN/kg DM of fresh CL eliminated almost 90% with

sun-drying and a combination of chopping and 3-day wilting before drying being the most effective, lowering the HCN content of the final product to 55 mg/kg DM.

Table 1. Effect of processing method on hydrogen cyanide (HCN) content in cassava leaves and foliage.

Material	Processing method	HCN (mg/kg DM)		Reduction (%)	Reference
		Before	After		
Leaves	Drying	509	86	83.0	Phuc, 2000
Leaves	Drying	545	203	62.7	Borin et al., 2005
Leaves	Drying	1,304	160	86.7	Chapter 3
Leaves	Drying	1,221	128	89.5	Chapter 5
Leaves	Ensiling	390	147	62.0	Phuc, 2000
Leaves	Ensiling	545	122	77.6	Borin et al., 2005
Leaves	Ensiling	1,304	251	80.6	Chapter 2
Leaves	Ensiling	1,304	198	84.8	Chapter 3
Leaves	Ensiling	1,745	195	88.8	Chapter 4
Leaves	Ensiling	1,221	152	87.6	Chapter 5
Foliage	Ensiling	978	309	68.4	Van Man and Wiktorsson, 2002

An overview of the reductions which can be achieved in the HCN content due to ensiling is provided in Table 1. The study reported in Chapter 2 showed that the HCN content of the fresh CL was 1,304 mg/kg DM and wilting reduced the concentration to 852 mg/kg DM. It was further decreased to an average of 251 mg/kg DM, or a reduction of 80.6% after 90 days of ensiling. Similar values were obtained in the study reported in Chapter 5 where the HCN content of the fresh CL was found to be 1,221 mg/kg DM and was reduced to 152 mg/kg DM after 60 days of ensiling or a reduction of 87.6%. Similar percentages (84.8 and 88.8) were obtained in the subsequent studies reported in Chapter 3 and 4. Part of this reduction is caused by the dilution effect of the CL due to the addition of 5% rice bran as an ensiling additive although the majority of the decrease was due to the ensiling process itself and during the pre-wilting process required for successful ensiling.

The ensiling studies conducted here appear to have been more effective in reducing the HCN content in CL compared to other studies. Van Man and Wiktorsson (2002) reported a HCN content in the fresh cassava tops of 978 mg/kg which was

reduced by 68% after two months of ensiling and continuously reduced during the additional storage period. After four months of storage, the final HCN concentration reported by these authors was 230 mg/kg, or a reduction of 76%. Phuc (2000) found that the HCN content in CL was reduced by around 62% after ensiling. Similar results have been found by others (Van Man and Wiktorsson, 2001; Borin et al., 2005). This difference between the studies reported here and those reported in the literature could be due to the preparation technique in our study, in which CL were chopped and sun-wilted before ensiling. It is known that HCN is released from a cyanogenetic plant after the cellular structure of the plant is disrupted.

The present work clearly demonstrates that sun-drying and ensiling are effective methods to reduce the HCN content in CL. The pigs fed in the diets containing ensiled CL or dried CL as protein source in Chapter 3, 4, and 6, consumed between 0.75 to 2.0 mg HCN per kg body weight (BW). No signs of cyanide toxicity were observed in any of the pigs fed the diets containing dried or ensiled CL in the present study. Reported toxic levels of HCN (in mg/kg BW) for pigs are 4.4 (Butler, 1973) and 3.5 (Tewe, 1992). Hang and Preston (2005) also found that HCN intakes from ensiled and fresh cassava leaf diets (2.8 and 4.0 mg/kg BW, respectively) did not affect the growth performance of the pigs. The results of the studies reported in this thesis indicate that farmers can significantly reduce the HCN levels in CL up to 90% after sun-dried (during the dry season) or ensiled (in rainy season) of the leaves. Ensiling CL not only reduces HCN toxicity but is also a preservation method appropriate for use during the rainy season.

There are some literature reports on the effect of drying and ensiling on the concentration of anti-nutritional factors present in SPV. Dominguez (1992) reported that trypsin inhibitors in the raw SPV decrease the protein digestibility of a mixed feed but when cooked or sun-dried these trypsin inhibitors are fully inactivated. Peters et al. (2001) reported that the ensiling process minimizes trypsin inhibitors present SPV and sweet potato roots. Mosha et al. (1999) reported that the trypsin inhibitor activity was reduced by 29.7, 34.9, 54.3, 52.3 and 65.6% in cabbage, collard, turnip, sweet potato and peanut greens, respectively, when these vegetables were blanched for 10 minutes. Reductions occurred for most of the treatments in either the conventional or microwave blanching method. To the authors knowledge, there have been no reports investigating the effect of drying or ensiling on other anti-nutritional

factors in SPV such as oxalic acid, phytic acid, and tannic acid. It can be expected however, due to their non-protein structure, that the drying and ensiling process will only have a limited effect on the reduction of these anti-nutritional factors.

Performance and carcass characteristics of growing pigs fed cassava leaves and sweet potato vines

As indicated above, CL and SPV have a relatively high CP content per unit DM and as such a high AA content. In the experiment reported in Chapter 3, the basal ingredients (ensiled cassava roots, rice bran and maize meal) provided 50% of the CP of the total diet, the remainder being supplied from either FM or CL and SPV (ensiled or dried). In the four other diets, 70% of the FM protein was replaced by ensiled CL, dried CL, dried SPV or ensiled SPV. The results from Chapter 3 showed no significant differences in the final BW, average daily gain (ADG), DM intake, feed conversion ratio (FCR) and carcass traits among the experimental diets. These results demonstrate that 70% of the CP from FM in diets for pigs can come from CL and SPV in either ensiled or dried form without a significant decrease in performance.

The pigs fed the ensiled CL and dried CL diets in Chapter 3 ingested approximately 1.02 and 0.75 mg HCN per kg BW, respectively or about 1/2 to 1/3 of the level which is considered toxic. As mentioned previously, reported toxic levels of HCN for pigs are 3.5-4.4 in mg/kg BW (Butler, 1973; Tewe, 1992). The ADG of pigs fed the FM diet (470 g/d) tended to be higher than the pigs fed the CL and SPV diets (Chapter 3, Table 3). The results can be explained by some shortage of ileal digested methionine+ cysteine and lysine in the diet. It is known that a sufficient amount of these EAA is needed for maximal protein deposition. Protein deposition and indirectly fat deposition depend on the first limiting AA.

There was a significant difference in feed cost among the experimental diets. The ensiled CL, dried CL and ensiled SPV diets for growing pigs had a lower cost (5.0-16.1%) compared to the control diet (FM diet) indicating that profitability is better when these ingredients are used in diets. The dried SPV diet had a similar cost in VND/kg gain compared to the FM control diet. This finding is in accordance with a number of studies where CL or sweet potato leaves have been used as a protein source in diet of pigs (Phuc et al., 2000; Nguyen et al., 2004; Van An et al., 2005). Hoanh et al. (2006) utilised ensiled SPV in diets of growing pigs and reported an improvement

in daily BW gain by 13% and a reduced feed cost of 17.7% compared to diets using fresh SPV. Differences in lean meat percentage were not significantly different between the treatments, a result which is in agreement with Van An et al. (2005) who reported similar values for carcass traits of Large White×Mong Cai pigs fed ensiled sweet potato leaves. Expression of the feed costs per unit lean meat shows that the ensiled and dried CL diet costs were 83.9 and 95% compared to the FM control diet while the ensiled and dried SPV diet costs were 91.9 and 95.9% relative to the FM diet. The results clearly demonstrate that CL and SPV can be economically used as a protein supplement for feeding pigs and can, at least partly, replace the traditional protein source used for pigs (such as FM) in Vietnam. As such higher returns are possible for small-holder farmers if ensiled or dried CL and SPV are more intensively used in diets for pigs.

As mentioned earlier, new high-yielding cassava varieties, such as KM94 and KM98-5 and KM140 have been distributed to various provinces in Vietnam over recent years. These new varieties appear to have a higher concentration of cyanogenic glucosides. To determine the maximal inclusion level of ensiled CL from variety KM94, a trial was conducted under practical conditions involving farmers in the Huong Van community which is located in the upland area of Thua Thien Hue Province (Chapter 4). The effect of three inclusion levels (10, 15 and 20% in the DM) of ensiled CL variety KM94 were tested against a control diet on the performance and carcass characteristics of growing Large White×Mong Cai pigs. There was a significant decrease in ADG with an increase in inclusion level of ensiled cassava KM94 leaves. Protein deposition of the F₁ pigs tended to decrease with increasing inclusion levels of ensiled cassava KM94 leaves (Table 3, Chapter 4). However, feed cost per unit BW gain decreased steadily with increasing inclusion of ensiled CL (KM94). Including 10, 15 and 20% of ensiled cassava KM94 leaves under practical rearing conditions, reduced the feed cost by 9, 12 and 18%, respectively. Similar values for carcass traits of the pigs fed the different experimental diets containing ensiled or dried leaves in Chapter 3 were found. This agrees with Scipioni and Martelli (2001), who concluded that increasing the level of a forage (sugar beet pulp) from 10 to 20% in the diet does not affect carcass traits of pigs. Lindberg and Andersson (1998) reported that including 10 and 20% of some forages such as white clover, lucerne, red clover and perennial rye grass/barley diets for growing pigs

resulted in reduced digestibility of organic matter, but increased the digestibility of CF. These authors concluded that forage addition to the diet of pigs can be used to a limited extent as a protein source. The results are also in agreement with Phuc (2000), who concluded that CL can be used as a protein supplement in growing diets and that ensiled or dried CL are potentially useful feed resources in developing countries.

Nutrient digestibility of cassava leaves and sweet potato vines

The digestibility of the dietary components depends on several factors, such as processing, fibre source and digestive capacity of the animal. The coefficient of ileal apparent digestibility (CIAD) and total tract apparent digestibility (CTTAD) in the products obtained with the different conservation methods in Chapter 5 were similar. However, the CIAD of DM, CP and CF were higher in the ensiled SPV compared to the dried SPV indicating that ensiling may be a better treatment in terms of nutritional value. However, it must be noted that in the ensiled products, 5 and 10% rice bran for the CL and SPV was used. Differences may have originated to a certain extent due to the addition of rice bran in the ensiled product.

In the growing pigs, the total tract digestibility coefficients for various fibres range from around zero to 0.9 depending on the fibre source (An, 2004). The variation in digestibility can be explained by differences in the chemical structure and also the part of fibre which is water soluble or water insoluble (Noblet and Le Goff, 2001). A part of the dietary fibre can be digested before the end of the ileum (Graham et al., 1986; Jørgensen et al., 1996; Andersson and Lindberg, 1997; Phuc and Lindberg, 2000) although the main fraction is fermented by micro-organisms in the hindgut. In the present study (Chapter 5), the CIAD of CF ranged from 0.22 to 0.31 and shows that pigs are able to digest a substantial part of plant fibre pre-caecally, which is in agreement with earlier reports by Lindberg and Cortova (1995). The coefficient of total tract digestibility of CF were generally 14 to 20% higher. This increased digestibility in the large intestine will have yielded volatile fatty acids which would have been used by the pigs as an energy source. Also CP fermentation occurred in the large intestine and effected 9-10% of the consumed CP. This nitrogen would have been absorbed across the large intestinal mucosa as ammonia and excreted in the urine as urea.

None of the CIAD of AA were different between the ensiled and dried CL. Tyrosine and aspartic acid CIAD were different between the ensiled and dried SPV products. Combining the ensiled products in a 50:50 mixture resulted in an ileal digestible lysine content which was 10.4% units higher than could be expected from the CIAD data of the two individual ingredients (ensiled CL and SPV). For ensiled CL+SPV, the CIAD of histidine, lysine, threonine, valine, and proline was higher than the expected average value of a mixture of dried CL+SPV. For these AA, the values of the individual ingredients were not additive. A likely explanation could be that the anti-nutritional factors in the CL and SPV were diluted in the CL+SPV diets to an extent that there were smaller physiological effects than when individual ingredients were fed at double the concentration.

As indicated above, CL and SPV have the potential as a dietary protein and AA source for growing pigs due to its relatively high protein and AA content. Compared with other protein sources often used in feeds for growing pigs such as FM and fresh or ensiled shrimp by-products (Ngoan and Lindberg, 2001), CL and SPV showed a lower CIAD of CP and AA (Table 2). The data in Table 2 show that the CIAD of CP of CL and SPV varies between 44.0 to 49.2% while for FM or ensiled shrimp by-product they vary from 76.8 to 78.7% .The present study found that the CIAD of AA ranged from 52.8% for arginine in dried CL to 87.7 for tyrosine in ensiled SPV. The values recorded are in line with the data on FM and ensiled shrimp by-product. Compared to the AA requirement estimates for 50-80 kg lean type pigs (NRC, 1998), the ileal digestible methionine+cysteine content of the ensiled and dried CL, ensiled and dried SPV and ensiled and dried CL+SPV diets (Table 2) only met 36, 38, 45, 33, 36 and 37% of the requirements for growth, respectively. Therefore based on this comparison, the first limiting AA in CL and SVP (dried or ensiled) in the present study were the sulphur-containing AA. These findings (Chapter 5) are in good agreement with reports in the literature (Eggum, 1970; Phuc et al., 2000; Chauynarong et al., 2009; Montagnac et al., 2009) that the concentration of the sulphur-containing AA is rather low in CL. These AA are required for body protein deposition. In addition, methionine has a sulfhydryl group (-SH) which is important for the detoxification of cyanide (Tewe, 1992) by converting HCN to the non-toxic thiocyanate (Oke, 1979). The second most limiting AA in the six diets (Chapter 5) was lysine, meeting 53, 48, 49, 40, 51 and 50% of the requirements of growing lean

pigs (NRC 1998). These data are in contrast to reports of Woolfe (1992) and An et al. (2003) who found that lysine was the first limiting AA in fresh sweet potato leaves. Based on the study reported in Chapter 5, supplementation of diets containing dried and ensiled CL, SPV or a 50:50 mixture on a DM basis of CL+SPV as the sole protein source with both sulphur-containing AA and lysine can therefore be expected to increase protein utilization by growing pigs.

Table 2. Coefficient of ileal apparent digestibility (%) of crude protein and essential amino acids in growing Large White×Mong Cai pigs.

Component	Fish meal ^a	Ensiled			Dried	
		Shrimp by-product ^a	Cassava leaves ^b	Sweet potato vines ^b	Cassava leaves ^b	Sweet potato vines ^b
Crude protein	78.7	76.8	46.8	49.2	44.0	45.3
Arginine	78.6	77.1	52.8	63.0	52.0	57.3
Histidine	75.6	74.4	69.4	69.5	69.0	68.0
Isoleucine	74.4	73.3	64.8	75.5	65.3	75.5
Leucine	75.9	74.8	67.4	79.0	65.0	76.2
Lysine	76.7	75.8	69.7	71.8	68.3	67.0
Methionine+						
Cysteine	74.9	73.3	70.6	73.7	71.2	69.5
Phenylalanine	72.5	69.8	58.4	74.0	61.3	65.3
Threonine	71.2	66.6	65.7	74.8	63.0	71.2
Tyrosine	75.8	74.4	73.6	87.7	75.8	65.3
Valine	75.5	74.2	59.2	66.8	59.2	68.3

^aNgoan and Lindberg (2001).

^bChapter 3.

Methionine and lysine supplementation to cassava leaves in pig diets

It has been known for some time that methionine is the first limiting AA in cassava protein when fed to rats (Eggum 1970). Little research has focused on the supplementation of diets containing ensiled cassava proteins with methionine or lysine on the performance of monogastric production animals. The relative deficiency of the sulphur-containing AA in cassava leaf protein (Chapter 5), and the fact that sulphur is required for the detoxification of HCN (Oke, 1978), were the reasons to

study the economic impact of supplementation of CL with DL-methionine (Chapter 6). Because lysine is the second limiting AA after the sulphur-containing AA, lysine addition was also studied in order to determine the potential gains which can be achieved by supplementing diets with both these two AA (Chapter 6).

The results in Chapter 6 clearly showed that supplementation of diets containing 45% (in the DM) of ensiled cassava (30% ECR+15% ECL) with 0.05%, 0.10% and 0.15% DL-methionine significantly increased the ADG in the pigs by 4.9%, 11.6% and 13.8%, and also gave lower feed costs by 2.6%, 7.2% and 7.5%, respectively (Study 1). Growing pigs fed 15% (in the DM) of ensiled CL in their diet with additional L-lysine and DL-methionine at a medium level had an ADG of 660 g/day. In comparison, without the addition of AA to the diet, ADG of the pigs was only 537 g/day (Study 2). The results confirm the calculation conducted in Chapter 5 and show that methionine is the first and lysine the second limiting AA in ensiled CL. Moreover, methionine is also required for the detoxification of the HCN in cassava. Therefore pig diets with a high inclusion level of cassava root and leaves could benefit from additional supplementation with synthetic methionine. Although DL-methionine and L-lysine are expensive for farmers and difficult to mix in the diet under practical farming conditions in Vietnam, the studies here indicate that farmers could achieve additional benefit by utilising these AA in conjunction with ensiled CL.

An overview of the effects of the use of ensiled CL and the supplementation of ensiled CL diets with DL-methionine and L-lysine on changes in ADG and FCR and feed costs is provide in Table 3. Overall, similar ADG, FCR and DMI values were obtained between studies. Although difficult to compare the studies as diets, conditions and BW were different between the animals, some general conclusions can be drawn from the comparison. Replacing FM by vegetable protein generally resulted in lower ADG and reduced feed costs. The increase in ADG with the use of synthetic AA is much higher compared to the reduced ADG due to the replacement of FM. However, diet costs were much more reduced by replacing fish meal with ensiled CL compared to addition of synthetic AA to ensiled CL diets. In the present study, no comparison was made between the diets formulated with FM as the protein source and diets containing ensiled CL with the addition of synthetic AA. The data in Table 3 where ensiled CL was included at 15% of the DM indicates that it can be expected that ADG would increase, FCR would decrease and feed costs would be reduced in

the order of 15 to 20%. A future study should compare the addition of methionine and lysine to ensiled CL diets to a diet where FM is used as a protein source in order to determine the real benefit of replacing FM.

Conclusions

1. Cassava leaves can be preserved up to three months by common ensiling methods with rice bran or cassava root meal inclusion of 5 or 10% and fresh cassava roots at levels of 20, 30, 40 and 50% (as fresh basis).
2. Ensiling of cassava leaves improves the overall quality of the leaves as an animal feed ingredient markedly by reducing the HCN content up to 80% of the original values in the fresh cassava leaves.
3. The content of amino acids in cassava leaves and sweet potato vines can be somewhat affected by the preservation process.
4. Processed (dried and ensiled) sweet potato vines have a higher nutrient digestibility compared to corresponding cassava leave products. Ensiling results in a higher digestibility of dietary nutrients compared to drying of sweet potato vines. Mixing ensiled cassava leaves and ensiled sweet potato vines may yield additional benefits in terms of increased digestibility of amino acids in pigs compared to feeding these ingredients solely.
5. Using ensiled or dry cassava leaves and sweet potato vines to replace 70% of the crude protein from fish meal in diets of Large White×Mong Cai pigs gave similar performance and carcass traits. Higher financial returns are possible if ensiled or dried cassava leaves and sweet potato vines are used in diets for pigs in Vietnam.
6. Supplementing diets containing ensiled cassava leaves with methionine and lysine can significantly increase the performance of Large White×Mong Cai pigs and the economic benefits for Vietnamese farmers.
7. Hydrogen cyanide concentration of cassava leaves is reduced by both ensiling and drying, with drying being slightly more effective. However, in humid tropical countries where cassava leaves cannot be conserved by sun-drying during the harvesting season, the ensiling method is recommended. Ensiling is a practical solution to conserve cassava leaves and reduce the hydrogen cyanide content during root harvest.

General discussion

Table 3. Changes in average daily gain (ADG), feed conversion ratio (FCR) and diet costs of inclusion of ensiled cassava leaves (ECL) or addition of methionine and lysine in diets for growing (20-80 kg) Large White×Mong Cai pigs.

Parameter	Chapter									
	3	4	4	4	6	6	6	6	6	6
Control diet	FM ^a	FM	FM	FM	ECL	ECL	ECL	ECL	ECL	ECL
Addition	-	-	-	-	Methionine (% DM)			Lysine+methionine (% DM)		
Level	-	-	-	-	0.05	0.10	0.15	Low	Medium	High
ECL inclusion (DM ^b basis)	20	10	15	20	15	15	15	15	15	15
Change in ADG	-3.0	-3.7	-6.7	-8.9	+4.9	+11.6	+13.9	+8.8	+22.9	+12.5
Change in FCR	-3.4	+3.9	+7.8	+7.5	-3.7	-9.4	-10.8	-8.7	-13.4	-9.0
Change in diet costs	-16.1	-9.2	-12.2	-18.4	-2.6	-7.2	-7.5	-3.3	-4.4	-3.2

^aFish meal.

^bDry matter.

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Summary

The general objectives of the work presented here were to evaluate processing methods for the preservations of cassava leaves (CL) and sweet potato vines (SPV) for later feeding during feed shortages in Vietnam. In addition, the nutritional value (including hydrogen cyanide (HCN) contents) of stored and processed CL and SPV as ingredients in diets for pigs were studied to determine their optimal use. Five studies were conducted.

Chapter 1 provides an introduction and a literature review providing information and data on livestock production in Vietnam, cassava and sweet potato cultivation and chemical composition, anti-nutritional factors in CL and SPV and on processing methods to reduce HCN levels in cassava (roots and leaves).

The effects of different levels of various fermentative additives on the quality of CL are reported in Chapter 2. A total of 8 different additions with 18 replicates each using 144 laboratory silos during 0 to 90 days were studied. Silage quality was assessed by changes in pH, dry matter (DM), crude protein (CP) and HCN content. Fresh CL were collected at the time of root harvest and spread out on the floor in the open air during 5 hours for wilting. Cassava leaves were mixed with 0.5% NaCl and the additives rice bran, cassava root meal at 5 or 10% addition and fresh cassava roots at 20, 30, 40 and 50% addition based on pre-wilted weight of CL. Hydrogen cyanide content of the fresh CL was 1,304 mg/kg DM and this was reduced to an average of 251 mg/kg DM after 90 days of ensiling. The inclusion of rice bran or cassava root meal at a level of 5 or 10% and fresh cassava roots at all levels produced good quality silage that can be stored up to three months. The entire ensiling process improves the product markedly by reducing the HCN content by 80% of the original value observed in the fresh CL.

Chapter 3 reports a study to evaluate the effects of replacing 70% of the protein from fish meal (FM) by protein from ensiled or dry CL and SVP on the performance and carcass characters of growing F₁ (Large White×Mong Cai) pigs in Central Vietnam. Twenty-five crossbred pigs (Large White×Mong Cai) with an initial weight of 19.7 kg were allocated randomly to five treatment groups with 5 animals per group (3 males and 2 females). Pigs were kept individually in pens (2.0×0.8 m) and fed one of five diets over 90 days. The control diet was formulated with FM as the protein source while the other four diets were formulated by replacing 70% of FM

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protein by protein from ensiled CL (ECL), dry CL, dry SPV or ensiled SPV. Animals were fed their diets at 4% of body weight (BW). Results showed that final BW, average daily gain (ADG), DM intake and feed conversion ratio (FCR) among the experimental treatments were not significantly different ($P>0.05$). Ensiled CL or dried CL and ensiled SPV reduced feed cost per unit gain by 8-17.5% compared to the FM diet. There were no significant differences in carcass characters among the diets ($P>0.05$). Lean meat percentages and protein deposition ranged 41.5-45.8% and 40.2-52.9 g/d, respectively. Using ensiled or dried CL and SPV can replace at least 70% of the protein from FM (or 35% of total diet CP) without significant effects on performance and carcass traits of growing (20-65 kg) pigs. Including CL and SPV could improve feed cost and therefore has economic benefits.

Chapter 4 reports a study to determine the effect of the inclusion of different levels of ensiled CL (variety KM94) in the diets on performance and carcass characteristics of growing pigs in Vietnam. A total of 40 Large White×Mong Cai pigs with an initial live weight of 23.5 kg were randomly allocated to one of the four pens. Four experimental diets were formulated containing inclusion levels of ensiled CL KM94 diet of 0, 10, 15 and 20% in the DM for two growth periods, 20 to 50 kg (60 days) and 50+ kg (30 days). Diets were formulated based on ileal amino acid digestibility values of the KM94 products and were isonitrogenous and isocaloric on a ME basis. Dry matter intake and final weight tended to decreased with increasing levels of ensiled CL (KM94) in the diet while there was a significant ($P=0.02$) decrease in ADG. Protein depositions of the F_1 pigs tended ($P=0.09$) to decrease with increasing inclusion levels of ensiled CL (KM94). There was no significant difference in feed conversion ratio, carcass quality and fat gain between the groups of pigs. There were clear reductions in feed costs among the experimental diets ($P=0.001$) with increasing levels of ensiled CL (KM 94) in the diet. It was concluded that, in diets for growing pig, inclusion of ensiled CL reduces growth rate of pigs in Vietnam but increases profitability as measured by feed costs per unit gain.

Chapter 5 describes a study to determine the ileal and total tract apparent digestibility of crude protein (CP) and AA in ensiled and dried CL and SPV as a single ingredient or in a 50:50 mixture of CL+SPV in growing (>60 kg BW) pigs. Coefficients of ileal (CIAD) and total tract (CTTAD) apparent digestibilities of organic matter (OM), CP, AA, crude fibre (CF) and neutral detergent fibre (aNDFom)

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were determined in growing pigs fed practical diets. The CP in the diets originated mainly from ensiled and dried CL, SPV or CL+SPV with the main energy source originating from ensiled cassava root which provided less than 9% of dietary CP. The six diets were formulated to contain 120 g CP/kg DM, 13 MJ ME/kg DM and were fed to 60 kg growing pigs in a 6×6 Latin square design. Daily intake of OM, CF, aNDFom and ME differed ($P<0.001$) among diets while for DM and CP a trend was observed. There were significant differences among diets ($P<0.05$) for the CIAD and CTTAD of DM, OM, CP and CF and in the CTTAD of aNDFom. There were differences ($P<0.05$) among diets for the CIAD of most AA except methionine+cysteine, glycine, glutamic acid and serine. The CIAD of AA for the ensiled CL, SPV and CL+SPV were in most cases not different from the corresponding CIAD of AA of the dried ingredients. The use of a combination of CL and SPV in diets of growing pigs resulted in higher CIAD for CF and several AA compared to expected values from the individual ingredients. The first and second limiting AA in ensiled and dried CL and SPV were found to be the methionine+cysteine and lysine. Cassava leaves and sweet potato vines have the potential to improve protein and amino acid supply in diets for pigs especially when combined with ingredients containing high concentrations of the first two limiting amino acids.

The final research chapter in this thesis (Chapter 6) reports two studies to determine the impact of supplementation of diets containing ensiled CL as the main protein source with synthetic amino acids (DL-methionine alone or with L-lysine). A total of 40 Large White×Mong Cai pigs with an average initial BW of 20.5 kg were randomly assigned to four treatments. The basal diet contained 45% (DM basis) ensiled CL and ensiled cassava root and was supplemented with 0%, 0.05%, 0.10% and 0.15% DL-methionine. Results showed a significantly improved performance and protein gain by extra methionine. This reduced the feed cost by 2.6%, 7.2% and 7.5%, respectively. In study 2, Large White×Mong Cai pigs with an initial BW of 20.1 kg were randomly assigned to the four treatments. The four diets were as follows: a basal diet containing 15% ensiled CL supplemented with different amounts of the amino acids L-lysine and DL-methionine to the control diet. The results showed that diets with 15% of DM as ensiled CL with supplementation of 0.2% lysine+0.1% DL-methionine and 0.1% lysine+0.05% DL-methionine at the 20-50 kg and 50+ kg,

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respectively, resulted in the best performance, protein gain and lowest costs. Ensiled CL can be used as a protein supplement for feeding pigs and additional amounts of synthetic methionine and lysine improves performance.

A general discussion (Chapter 7) provides explanation and interpretation of the results as well as the implication for using CL and SPV as a protein sources for pigs. This chapter also discussion the chemical composition and HCN toxicity of CL and preservation method and its effect on HCN. After the discussion, the general conclusions are provided.

Overall the work presented here shows that CL and SPV vines are good economic alternatives for the traditionally used protein sources (e.g. FM, soybean meal) in feed for pigs in Vietnam. Ensiling appears to be a practical solution to conserve SPV and CL (reduce HCN content) and provide a solution for the rainy season when sun-drying to preserve CL and SVP is often not feasible.

Samenvatting

Het doel van de proeven beschreven in dit proefschrift was als eerste om te evalueren hoe het drogen of ensileren van cassave bladeren (CL) en van het loof van zoete aardappelen (SPV), de voedingswaarde van het geconserveerde product voor varkens beïnvloedt. Speciale aandacht wordt besteed aan het effect van beide conservatiemethoden op het cyanide gehalte (HCN) van CL.

Hoofdstuk 1 (de inleiding) omvat een literatuuroverzicht waarin informatie en data wordt verstrekt over de dierlijke productiesector in Vietnam, de productieomvang van CL en SPV, anti-nutritionele factoren in CL en SPV en methoden om het cyanide gehalte van CL terug te dringen.

Hoofdstuk 2 beschrijft een studie waarbij het effect van verschillende koolhydraat-bronnen op de silagekwaliteit van CL is onderzocht. In totaal werden 8 verschillende behandelingen met in totaal 144 silos gedurende 0 tot 90 dagen gevolgd. De CL werden eerst gemengd met 0.5% NaCl en daarna werden rijstevoermeel (rice bran, RB), cassave wortels meel (cassava root meal, CRM) in een concentratie van 5 of 10% toegevoegd met als extra toevoeging verse cassave wortels in concentraties van respectievelijk 20, 30, 40 en 50% op basis van het voordrooggewicht van de CL. Het HCN gehalte van de verse bladeren was 1304 mg/kg droge stof (DM) en dit was gereduceerd tot gemiddeld 251 mg/kg DM op 90 dagen na begin van ensileren. De toevoeging van 5 of 10% RB of CRM, met als extra toevoeging van 20, 30, 40 en 50% verse cassave wortels op basis van vers gewicht, produceert een goede kwaliteit silage die zeker tot 3 maanden lang bewaard kan worden en in het voer opgenomen kan worden.

In hoofdstuk 3 wordt een groeiproef beschreven waarbij 70% van het in centraal Vietnam traditioneel gebruikte vismeeleiwit in voer voor Large White×Mong Cai varkens vervangen werd door eiwit uit silage en uit gedroogde CL en SPV. De groei van dieren werd vergeleken met dieren gevoerd met een vismeel controle voer. De dieren kregen 4% van hun lichaamsgewicht aan voer per dag. Resultaten lieten zien dat het slachtgewicht, groei, DM opname en voederconversie tussen de rantsoenen niet significant ($P>0.05$) verschilden. Echter de voerkosten waren significant lager per kg slachtgewicht of per kg varkensvlees met de voerbehandelingen CL en SPV. Per kg groei waren de voerkosten 8-17.5% lager in vergelijking met het vismeelvoer. Er waren geen verschillen in karkascompositie en

eiwitdepositie tussen behandelingen. Het gebruik van CL en SPV kan tot op 70% van het vismeleeiwit vervangen in het voer voor varkens van 20 tot 65 kg. Het gebruik van CL en SPV kan resulteren in lagere voerkosten.

Hoofdstuk 4 gaat nader in op de vervanging van vismeel door CL variant KM94 in voer voor Large White×Mong Cai varkens. Veertig varkens (23.5 kg) werden verdeeld over 4 groepen waarbij iedere groep één van vier voeders werden gevoerd. De vier experimentele voeders bestonden uit 0, 10, 15 en 20% geënsileerde bladeren van de cassave variant KM 94 en werden geformuleerd voor twee groeiperiodes, 20 tot 50 kg (~60 dagen) en 50+ kg (~30 dagen). De voeders werden geformuleerd op basis van schijnbare ileale verteerbaarheid van aminozuren (AA) en hadden vergelijkbare eiwit- en metaboliseerbare energiegehalten. De resultaten lieten zien dat met een toename van het aandeel CL KM94 in het voer, de groeieresultaten ($P=0.02$) en eiwitaanzet ($P=0.09$) enigszins achterbleven bij die van de dieren op het vismeel, basis rantsoen. Er was een significant effect van CL KM94 toevoeging op de voederconversie ($P=0.001$). Ondanks de lagere groei waren de voerkosten per kg groei op CL KM94 voer ook lager. Met 10, 15 en 20% CL KM94 silage waren de voerkosten respectievelijk 9, 12 en 18% lager dan met vismeleeiwit in het voer. Opnemen van CL KM94 silage in voer van varkens in Vietnam resulteert in een lagere groei maar verhoogd de winstgevendheid.

Hoofdstuk 5 beschrijft een studie omtrent de schijnbare ileale en fecale ruw eiwit (CP) en AA verteerbaarheid zowel in silage als in gedroogde CL. Dit werd ook bepaald in gedroogde en geënsileerde SPV en ook in een 50:50 mengsel (op DM basis) van silage van zowel CL en SPV in voer van groeiende (>60 kg) varkens. De coëfficiënt voor ileale (CIAD) en faecale (CTTAD) verteerbaarheid van organische stof (OM), CP, AA, ruwe vezel (CF) en voedingsvezels (NDF) werden bepaald in varkens in een 6×6 Latijnse vierkant proefopzet. De zes voeders werden geformuleerd zodat ze 120 g CP/kg DM en 13 MJ ME/kg DM bevatte. Dagelijkse OM, CF, NDF en ME verschilden ($P<0.001$) tussen voeders en er was een tendens voor verschillen tussen voeders in DM en in CP opname. Verschillen tussen voeders met betrekking tot CIAD en CTTAD voor DM, OM, CP en CF en met betrekking tot CTTAD voor NDF werden aangetoond. De CIAD voor methionine+cysteïne, glycine, glutaminezuur en serine waren significant ($P<0.05$) verschillend tussen voeders.

De CIAD voor AA van de geënsileerde CL, SPV en CL+SPV producten waren in de meeste gevallen gelijkwaardig met de waarden van de gedroogde ingrediënten. Het gebruik van een mix van 50:50 CL en SPV resulteerde in iets hogere CIAD waarden voor de vezelfractie voor verscheidene AA vergeleken met de berekende waarden van de individuele ingrediënten. De meest limiterende essentiële AA in zowel geënsileerde als gedroogde CL en SPV zijn methionine+cysteïne en lysine. Cassave bladeren en SPV hebben duidelijk een goede potentie als ruw eiwit- en aminozuurbron in voeders voor varkens vooral in combinatie met ingrediënten met een hoog gehalte aan de limiterende aminozuren methionine+cysteïne en lysine.

De studie die beschreven is in hoofdstuk 6 werd uitgevoerd conform de studie in hoofdstuk 4 maar nu met toevoeging van de synthetische AA DL-methionine en L-lysine of met beiden. Varkens van ongeveer 20 kg kregen één van vier behandelingen waarbij het controle rantsoen 45% geënsileerde CL op DM basis bevatte. De CL silage was gemaakt met toevoeging van cassave wortels. Aan het rantsoen waarin deze silage opgenomen was werd verder nog 0.05, 0.10 of 0.15% DL-methionine (op DM basis) toegevoegd. Resultaten lieten duidelijk zien dat door extra methionine in het voer op te nemen, een hogere dagelijkse lichaams- en eiwitgroei werd bereikt met respectievelijk 2.6, 7.2 en 7.5% minder voerkosten. In een andere behandeling in deze proef waarbij 15% DM in de vorm van CL silage verstrekt werd, werd 0.2% lysine+0.1% DL-methionine aan voer voor varkens van 20 tot 50 kg lichaamsgewicht toegevoegd en boven 50 kg lichaamsgewicht was de toevoeging 0.1% lysine+0.05% DL-methionine. De toevoeging van beide AA resulteerde in de hoogste lichaams- en eiwitgroei. Er werd geconcludeerd dat dit rantsoen het beste is voor kruisingsbiggen (Large White×Mong Cai). Suppletie van rantsoenen voor mestvarkens met CL silage en met methionine en lysine kan de groei van deze varkens flink verbeteren bij duidelijk lagere voerkosten.

In de algemene discussie (hoofdstuk 7) wordt ingegaan op de verklaring van de effecten die met conservering van CL en SPV via ensileren en drogen op eiwitgehalte en op eiwitsamenstelling werden gevonden. Ook wordt ingegaan op de waarden voor verteerbare nutriënten en op de verklaring van de bevindingen. De achtergrond van de resultaten van proeven met betrekking tot het opnemen van geconserveerde CL en SPV als hoofdeiwitbron in het voer op de productieresultaten worden besproken. In deze algemene discussie wordt ook de chemische samenstelling

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en de HCN gehalten van de CL voor en na conservering bediscussieerd en op het eind worden de algemene conclusies geformuleerd.

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Nguyen Thi Hoa Ly

Curriculum Vitae

Mrs Nguyen Thi Hoa Ly was born on the 11th of January 1958 in Quang Binh province, Vietnam. She attended the Agriculture University No 2, in the Ha Bac Province, Vietnam and obtained her BSc degree in Animal Science in 1980. From 1981 to 2012 she was a lecturer and researcher in the Animal Nutrition at the Department of Animal Nutrition in the Faculty of Animal Science at the University of Agriculture and Forestry, Hue city, Vietnam (Previously named Agriculture University No. 2). She is since 2000 a senior lecture at the same University. In 1996, she obtained her MSc in Animal Science at Hue University, Vietnam and in 1995 followed a two-months course on feed analysis at Chiang Mai University, Thailand. In 2001, she obtained a Diploma in Animal Feed Science at Barneveld, The Netherlands and in 2002, she took part in a three week training course into grass technology in Australia. From 2005 to 2006, she attended the “training-of-trainer” course on “Cassava Production, Processing, Animal Feeding and Farmer Participatory Research” held in Laos, Indonesia and East Timor which was organised and sponsored by the Cassava project in Asia of the International Center for Tropical Agriculture (CIAT).

From 2000 to 2006, she received several funds from CIAT and the Swedish Agency for Research Cooperation with Developing Countries (SIDA-SAREC and MEKARN) for her studies in the field of using local feed resources, as protein source for pigs in Vietnam.

Besides her studies and career as a lecturer, Mrs Nguyen Thi Hoa Ly is married and has three children.

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