Cassava leaves as protein source for pigs in Central Vietnam

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CONTENTS

SCOPE OF THE THESIS

Cassava leaves may contain about 20% of crude protein in the dry matter fraction. The protein is low in the essential amino acids methionine and tryptophan, but is rich in lysine. The biological value of cassava leaf protein can be increased by the addition of synthetic methionine. Cassava leaves are high in fibrous components which is a factor limiting it's exploitation as a source of crude protein for non-ruminant animals. Cassava leaves also contain anti-nutritional factors (ANF's) such as cyanogenic glucosides and tannins. Cyanogenic glucosides can give rise to toxic hydrocyanic (HCN) by the action of either enzymatic activity in damaged plant tissues or within the digestive tract of the animal. However, a major part of the absorbed cyanide is rapidly detoxified by conversion to thiocyanate which is excreted in the urine. Tannins may reduce protein digestibility.

The use of cassava leaves in pig production in Vietnam would be a means of reducing the dependence on traditional, expensive protein sources such as fish meal and soybean meal. However, it is necessary to know the most appropriate way to use the leaves in pig nutrition and the extent to which they can replace other protein sources in the diet. In other words, the question is how to improve the nutritional quality of the pig diets in small-scale farmer households in Central Vietnam using the available leaves of the cassava plant. Methods of processing to reduce levels of HCN cyanide in cassava include: cooking, drying, ensiling, soaking and de-watering. The main purpose of the present studies was to identify the best method, in terms of simplicity and being easy to apply by small-holder farmers. The experiments described in this thesis were carried out in an attempt to contribute to the optimal use of cassava leaves in pig nutrition.

Chapter 1 presents an overview of cassava production and the nutritional value of cassava leaves. Chapters 2 to 6 describe the various experiments that were carried out. In Chapter 7 the outcomes of the studies are integrated, leading to the major conclusions.

The main questions addressed in the various experiments are as follows.

1. What is the effect of using different additives for ensiling cassava leaves on the level of HCN and on the use of the product as a protein source for pigs?
2. What is the effect supplementing the traditional diet with ensiled cassava leaves on growth performance of pigs kept on small-holder farms? The effect of supplementation of ensiled cassava leaves was compared with that of fresh duckweed or sweet potato vines.

3. What is the difference between three simple methods to reduce HCN content of cassava leaves before feeding (washing; chopping and washing; and chopping, washing and wilting) on growth performance of pigs?

4. What is the ileal digestibility of protein from either cassava, sweet potato, duckweed or stylosanthes?

5. What is the effect of different levels of DL-methionine supplementation on growth performance of pigs given diets containing cassava leaves?

Chapter 1

Cassava (*Manihot esculenta* Crantz) production: nutritive value and toxic factors

Cassava cultivation

Cassava (*Manihot esculenta* Crantz) has been grown in Latin America since some 4000 years ago. According to Jalaludin (1977), cassava originated in Guatemala, Mexico and Brazil. At the present time, cassava is grown in more than 90 countries. It is high energy food obtained with low inputs and little effort. The cassava crop can adapt to a variety of climatic conditions though a warm and humid climate is preferred. Cassava is a drought tolerant crop and can be grown in areas where there are occasionally prolonged spans of drought. In certain Asian and African countries cassava is a major crop (Table 1)

Table 1: The area of cassava harvest (*1000 ha) in selected countries (FAO 2006)

	2001	**2002**	**2003**	**2004**	**2005**
World	**17170**	**17314**	**17476**	**18475**	**18630**
Angola	573	593	6434	694	749
China	241	241	251	251	251
Ghana	726	794	807	784	784
India	254	239	240	240	240
Indonesia	1318	1277	1245	1256	1224
Mozambique	834	1020	1046	1069	1050
Tanzania	661	660	66	660	670
Thailand	1049	988	1022	1057	986
Vietnam	292	337	372	384	424

Source: http://faostat.fao.org

In Vietnam, the yield of cassava and the area planted has been increasing recently. Compared with 2000, the area planted in 2005 has increased by 37%, while the yield has increased by 50% (Table 2).

Table 2: The yield, and area planted of cassava in Vietnam

Year	Yield, kg/ha	Area planted (*1000 ha)
2000	8,359	274
2001	12,005	292
2002	13,169	337
2003	14,275	372
2004	14,978	389
2005	15,355	424

Source: http://faostat.fao.org

In Central Vietnam, cassava is the third crop in importance after rice and maize. Cassava products are usually used for human consumption and as animal feed. For the poorest farmers, cassava is usually the staple food. There are 12 provinces in Central Vietnam where cassava is an important crop, accounting for about 42% of the total cassava area in Vietnam (Table 3; Figure 1).

Table 3: The area of cassava harvest (times thousand ha) in provinces of Central Vietnam
(Annual statistics; http://www.gso.gov.vn)

	2001	2002	2003	2004	2005
Total Vietnam	**292.3**	**337.0**	**371.9**	**388.6**	**423.8**
Central provinces	*115.1*	*138.7*	*159.1*	*170.7*	*179.4*
Thanh Hoa	11.9	13.6	15.2	14.5	15.0
Nghe An	10.2	9.9	11.3	12.5	13.9
Ha Tinh	2.6	2.9	3.1	3.7	3.9
Quang Binh	3.8	3.6	4.0	5.0	5.6
Quang Tri	3.2	4.1	5.4	6.8	7.8
Thua Thien Hue	4.5	4.9	5.5	5.9	6.6
Da Nang	0.9	0.7	0.5	0.3	0.2
Quang Nam	11.5	12.6	12.6	13.3	13.2
Quang Ngai	11.7	14	15.7	16.3	17.1
Binh Dinh	10.1	10.6	11.3	11.6	12.0
Phu Yen	3.0	4.0	4.7	5.6	9.9
Khanh Hoa	4.2	4.3	4.4	4.6	5.9

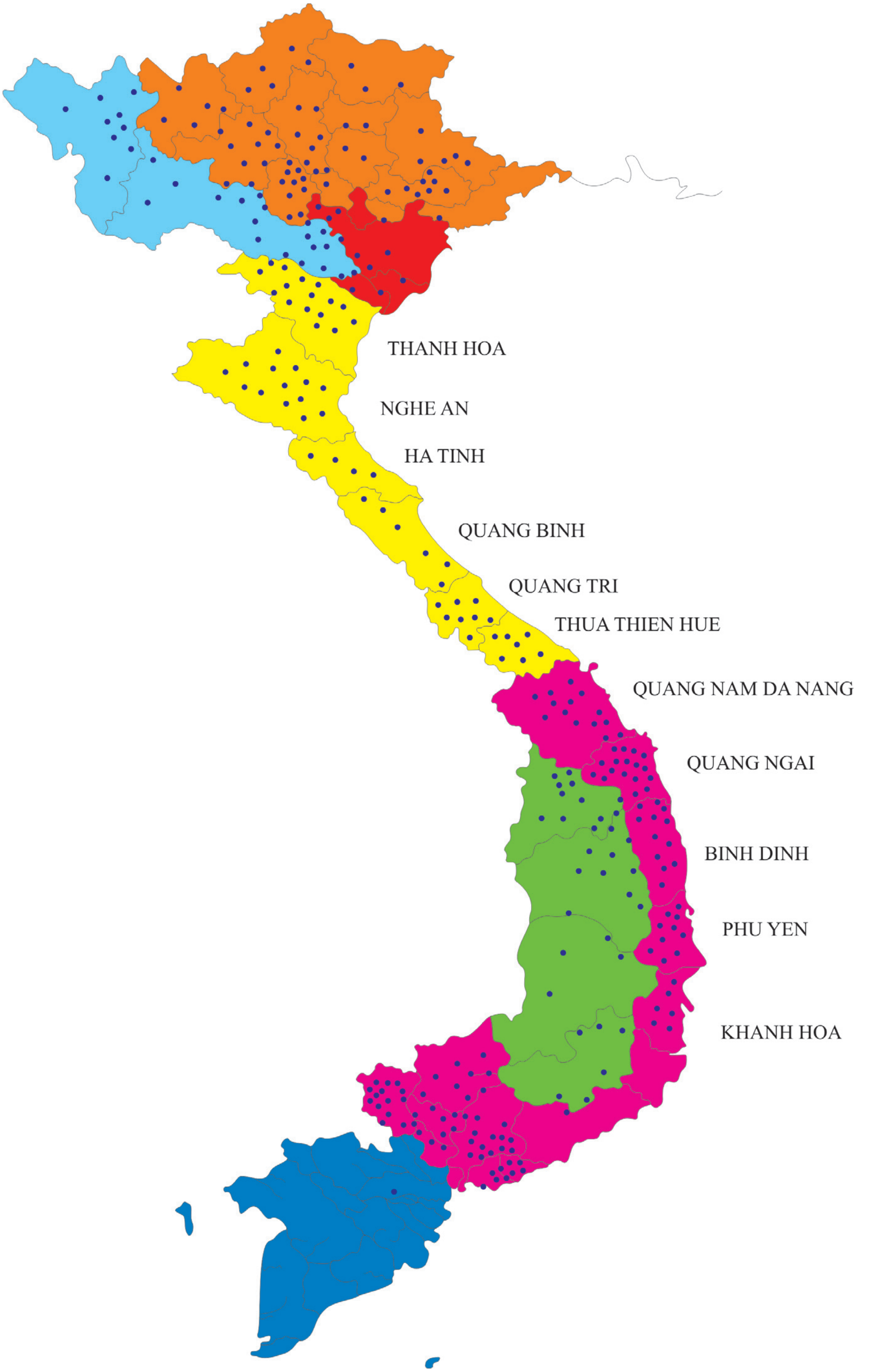

Figure 1. Cassava cultivation in Centre of Viet Nam (each dot = 1000 ha)

In Vietnam, the role of cassava has been changed from a "food crop" to an "industrial crop" for starch processing and the animal feed industry. At the present time, it is planted on 423.8 thousand ha with a total yield of 6646 thousand tonnes for the whole country. In Central Vietnam the area is 121 thousand ha and the total yield is 1634 thousand tonnes (Annual Statistics: http://www.gso.gov.vn). For the total cassava starch production in Vietnam it is estimated that 70% is for export while 30% is used domestically (Hoang Kim et al., 2000).

Cassava is one of seven new agricultural export products which are being promoted by government and local authorities. Vietnam is the second exporter of cassava products after Thailand.

Cassava research in Vietnam has made steady progress. Vietnam's Cassava Program (VNCP), a network of cassava research and extension centres, was set up in 1991. This programme has expanded and has led to significant increases in production. New cassava varieties are being grown on over 95.0 thousand ha. Pilot cassava farms of high productivity, with high economic returns and soil fertility maintenance were set up in various provinces, in close cooperation with CIAT, VEDAN, the Nipon foundation and starch processing factories. The trade of cassava products in the world, Asia and Vietnam is shown in Table 4.

Table 4: Word trade of dried cassava products (metric tonnes)

	2000	2001	2002	2003	2004
World	3961772	5356992	3616958	4749084	6466755
Asia	3535894	5002894	3305716	4334858	6006058
China	26	10	45	9	35
Philippine	581	359	818	850	620
Malaysia	589	933	672	1135	999
Indonesia	151439	177075	70429	21999	234169
Thailand	3246962	4684648	2904153	3677118	5019012
Viet Nam	135057	138500	328277	632006	749666

Source: http://faostat.fao.org

Cassava is traditionally grown for the production of roots. However, the leaves have become increasingly important as a source of protein for monogastric and ruminant animals (Preston 2001; Wanapat 2001). According to Bui Huy Dap (1987), the average yield of cassava leaves in Vietnam is of the order of 7 to 12 tonnes/ha/year, containing from 500 to 1400 kg of crude protein. In Thailand, in a production system in which leaves were harvested three times prior to root harvest, the productivity was 20 tonnes dry matter per ha of leaves with 25% of crude protein (Wanapat, 1995). Preston (2001) described a system in which the cassava foliage was harvested repeatedly at 56 day intervals over a 2-year period; annual yields were of the order of 90 tonnes equivalent to some 4.5 tonnes of protein. High levels of organic manure (120 tonnes/ha/year) were necessary to support these very high levels of production.

Chemical composition

The chemical composition of cassava roots has been widely studied. On a fresh basis, cassava roots have 32% dry matter (DM). In the DM cassava roots contain 89% nitrogen free extract, 0.1% lipids, 5.1% crude fiber, 2.2% crude protein and 2.8% ash. Cassava roots have 64-72% starch with 10-20% amylose, and 80-90% amylopectin (Johnson and Ramond, 1965; Jalaludin 1977).

When cassava leaves are a by-product of root harvesting the DM yield may amount to as much as 4.6 tonnes/ha (Ravindran et al., 1987). The leaves offer a vast scope as a protein source in animal feeding. Cassava leaves can be harvested from 4 months of age in a cycle of 60-75 days. With adequate irrigation and fertilization, annual leaf DM yields of over 21 tonnes/ha can be obtained (Ravindran 1993).

Cassava leaves have been shown to be an excellent source of protein (Wanapat, 1995), the protein level depending on variety, growing conditions and fertilizer application. Eggum (1970) and Allen (1984) reported that the leaves were very rich in protein, ash and vitamins with an average of 21% crude protein (range of 16.7 to 39.0%), of which 85% was true protein.

Rogers and Milner (1963) studied the amino acid composition of a range of varieties and concluded that they were deficient in methionine. Gerloff et al. (1965) concluded that, on a total amino acid 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. By the addition of methionine, the BV of cassava leaf protein could be increased to 80% (Eggum, 1970).

Dried cassava leaves are reported to be low in lipids (0.6% in DM), but rich (24%) in glucose and starch. The crude fiber level was 11% and ash content 6.7% with an amount of xanthophylls of 350 mg/kg (Frochlich et al., 2001). The leaves are rich in macro-minerals according to Tewe (1984) with the following concentrations (% of DM): calcium, 1.75, potassium, 1.28, magnesium, 0.42, phosphorus, 0.45 and sodium, 0.02. Micro-elements levels (mg/kg) were as follows: zinc, 149, manganese, 52, iron, 259 and copper, 12. The nutritive value of cassava leaves was reviewed by Lancaster and Brooks (1983) who showed that the proximate composition compared favourably well with that of other foods, but cassava leaves contained more vitamins and minerals, particularly calcium, iron, vitamin A, riboflavin, thiamin, niacin and vitamin C (Table 5).

Table 5. Mineral and vitamin content (per 100 g fresh material) of cassava leaf, amaranth leaf, soybean and yellow maize grain

	Calcium (mg)	Iron (mg)	Vit.A (μg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vit.C (mg)
Cassava leaves	300	7.6	3,000	0.25	0.60	2.4	310
Amaranth leaf	410	8.9	2,300	0.05	0.42	1.2	50
Soybean	185	6.1	28	0.71	0.25	2.0	0
Maize	13	4.9	125	0.32	0.12	1.7	4

Digestibility of cassava and some forages in pigs

Cassava leaves, like many forages, are rich in protein but high in fibrous components (cell walls). The high fiber content in leaf meal can be a major factor limiting it's exploitation as a source of crude protein and other nutrients for non-ruminant animals (Calvert et al 1985; Just 1982). Fibrous feeds in the diet of pigs leads to increased rate of passage of digesta through the gut and reduced ileal and total tract digestibility (Jorgensen et al., 1996; Kass et al., 1980; An et al., 2004). High-fiber diets affect gut size and development, particularly the large intestine (Jorgensen et al., 1996). Nearly all fiber digestion takes place in the caecum and colon, where bacteria break down fermentable carbohydrates that have escaped digestion in the stomach and small intestine. Volatile fatty acids from fiber fermentation can provide from 5% to 28% of the energy requirements of the growing pigs according to Kass et al. (1980). However, levels of more than 7-10% of crude fiber in the diet will result in decreased growth rates (Kass et al., 1980).

Pigs are able to digest a substantial part of plant fiber pre-caecally (Lindberg et al., 1995). Lindberg and Anderson (1998) reported that levels of 10 and 20% of either meal of white clover, lucerne, red clover and perennial rye grass in barley-based diets reduced the digestibility of organic matter, but increased the digestibility of crude fiber. The authors concluded that forage meals could be used to a limited extent as a protein source for pigs.

Ravindra et al. (1987) observed in pigs a depression in digestibility of DM, ether extract, cell wall components and hemicellulose in diets in which cassava leaf meal (CLM) replaced coconut oil meal. Sarwat et al (1988) also observed a lower digestibility of DM and organic matter (OM) when CLM was included in a sorghum-based diet that was fed to growing pigs. Phuc et al. (1996) reported a significant reduction in apparent digestibility of DM, OM, fiber and ether extract as the level of inclusion of sun-dried CLM increased from zero in the control diet to 216 g/kg at the highest level of inclusion.

The use of cassava leaves in pig production in Vietnam has become increasingly important as a means of reducing the dependence on traditional protein sources such as fish meal and soybean meal, which are very expensive. However, it is necessary to know the most appropriate way to use the leaves in pig production and the extent to which they can replace other protein sources in the diet.

Toxic factors in cassava

Hydrocyanic acid (HCN)

Cyanogenic glucosides are the main anti-nutritional factors (ANF's) in cassava leaves, as they can give rise to toxic cyanide (HCN) (Van Soest, 1994). The release of free HCN is brought about by the action of either the endogenous enzyme linamarase in damaged plant tissues or by βglucosidases within the digestive tract of the animal. The presence of cyanogenic glycosides constitutes a major limitation to the use of cassava in both human and animal food (Tewe, 1995). However, a major part of the absorbed cyanide is rapidly detoxified by conversion to thiocyanate which is excreted in the urine. Owing to this rapid detoxification, it is possible for animals to ingest doses of cyanogenic glycosides only slightly less than the lethal dose over extended time periods, without harm (John et al., 1980). Factors that influence toxicity include the size and type of animal, the rate of ingestion, the type of food ingested simultaneously with the cyanogenic glycoside, presence of active degradative enzymes in the animal digestive tract, and the ability to detoxify cyanide (Clarke and Clarke., 1967). Thiocyanate is a potent goitrogen and has been implicated in the aetiology of goitre in animals (Langer 1966; Shihombing et al., 1971) and humans (Ekpechi, 1973).

$$\text{Cyanogenic glucoside} \xrightarrow{\text{Linamarase}} \text{Glucose} + \text{Aglycon}$$

$$\text{Aglycon} \xleftrightarrow{\text{Hydroxynitrilelyase}} \text{HCN} + \text{Aldehyde or Ketone}$$

$$\text{HCN} + Na_2S_2O_3 \xrightarrow{\text{Rhodanase}} \text{HSCN} + Na_2SO_3$$

$$\longrightarrow \longrightarrow \text{Reduced toxicity and release through the urine}$$

Figure 2: Metabolism of cyanogenic glucosides. The sequential action of the enzymes linamarase and hydroxynitrilelyase is essential for the complete hydrolysis of the cyanogenic glucoside, linamarin, into free cyanide. The enzyme rhodanase converts cyanide into thiocyanate.

Ingestion of cyanide from high cyanide (bitter) cassava varieties may cause death (Akintonwa et al., 1994), exacerbate goitre and cretinism (Delange et al., 1994), and may cause tropical ataxic neuropathy (TAN) in older persons (Osuntokun,1994).

According to Oke (1978), the sulphur needed for the detoxification of cyanide is obtained from dietary methionine. The presence of cyanogenic glucosides could lead to a deficiency of this essential amino acid at poor or marginal supply of natural sources of methionine in the diet, resulting in reduced animal performance. Fish meal, which is a good source of sulphur amino acids, not only can balance the amino acid profile of cassava, but the same time may facilitate to some extent the detoxification process (Tewe, 1992) of the remaining cyanide content in the ensiled cassava foliage. Ly and Pok Samkol (2001) have shown that digestibility of the crude protein of ensiled cassava leaves by pigs can be high when the dietary protein supply is high and rich in sulphur amino acids. Nitrogen (N) balance in young Mong Cai pigs fed very high levels of ensiled cassava leaves was improved by supplementation with DL-methionine (Ly, 2002). The best level of additional DL-methionine in diets of crossbred pigs (Large White x Mong Cai)) fed ensiled cassava root-based diets was 0.2% compared with levels of 0, 0.1, and 0.3 % (Nguyen Thi Loc and Le Khac Huy, 2003).

Acute toxicity studies

Reports on what constitutes a lethal dose of HCN in animals and humans are summarized in Table 6.

Table 6: Lethal dose of HCN in animals and humans

Species	mg/kg BW	References
Humans	1	Oke,1978
	0.5-3	Montgomery 1969
	0.5-3.5	Tewe, 1995
Guinea-pig	1.43	EPA, 1990*
Rabbit	0.66	EPA, 1990*
Cat	0.81	EPA, 1990*
	2	Conn, 1979
Dog	1.34	EPA, 1990*
	4	Conn, 1979
Monkey	1.3	EPA, 1990*
Rat	0.81	EPA, 1990*
Mouse	3.7	Conn, 1979
Cattle	2	Conn, 1979
Sheep	2	Conn, 1979

According to Tewe et al. (1984), when growing pigs ate a cassava-based ration with 96 mg/kg diet of cyanide, the thyroxin level in the blood was reduced. These researchers also indicated that with HCN levels of about 0.5-3.5 mg/kg live weight this would provoke death in humans (Tewe, 1995). However, Iyayi (1994) in a study in which weaner pigs (Landrace X Large White) were given diets containing either 0, 239 or 419 mg HCN/kg, there were no differences in weight gain, feed intake and feed conversion rate. Serum albumin and total protein were not affected, but serum urea increased significantly with increasing cyanide intake. Monogastric animals are less susceptible to cyanide acid poisoning than ruminants according to Robson (2007) as the low pH in the abomsum destroys the enzymes that convert the glucosides to HCN.

Processing methods to reduce HCN level in cassava

Many researchers have reported that HCN in cassava can be reduced by processing. Suspending cassava in water was one way to reduce the level of glucosides and HCN (Cooke and Maduagwu, 1978), because glucosides dissolve easily in water. Soaking of cassava roots normally precedes cooking or fermentation and removes about 20%

of free cyanide in fresh root chips (Cooke and Maduagwu, 1978). After soaking and boiling, the free cyanide of cassava chips is rapidly lost in the boiling water and 90% is removed within 15 minutes (Cooke and Maduagwe, 1978). Gomez and Valdivieso (1988) reported that free cyanide in cassava roots after 26 weeks of ensiling was reduced to 36% of the initial value.

Table.7 The effect of processing methods to reduce HCN content in cassava (mg/kg)

Materials	**Processing method**	**HCN before**	**HCN after**	**% reduction**	**References**
Leaves	Sun-dry	509	86	83.1	Phuc and Lindberg, 2000
Leaves	Cooked	35.9-107	0.3-1.9	99.2-98.2	Ngudi et al.,2003
Meals	Grating,dewatering, fermenting and roasting	-	-	80-90	Padmajag, 1995
Peels	Heap fermentation on soaking	253-1081	48.72	80.7-95	Tweyoungvere and Katongole, 2002
Pulp	Fermentation	-	-	37.3-52.4	Kemdirim et al., 1995
Sliced	Soaking	-	-	38.1-38.4	
Cassava products (Fresh)	Boiling	140	77.6	55.4	Ojo and Deane, 2002
	Baking	140	122	87.1	
	Frying	140	125	89.3	
	Steaming	140	121	86.4	
	Drying	140	99.2	65.9	

Gomez and Valdivieso (1984), Oke (1994) and Westby (1994) reported higher levels of cyanide in ensiled cassava leaves compared with sun-dried leaves. It can be concluded that drying and ensiling are effective ways of reducing the toxicity of cassava products. In their review of effects of ensiling of cassava, Ly and Rodríguez (2001) stated: “From the early study of Bui Van Chinh et al. (1992) to one of the most recent studies (Chhay Ty et al., 2001), there is unequivocal evidence of the positive effect of ensiling on the hydrolysis of linamarin and posterior reduction of cyanide in the leaves” . However, it is well known that the ensiling process by itself does not eliminate all the cyanogenic glucoside components present in the material. It is noteworthy to point out that neither animal deaths nor symptoms of intoxication have been reported in any of the Vietnamese experiments (Bui Van Chinh et al., 1992; Bui Huy Nhu Phuc et al., 1996; Nguyen Van Lai and Rodriguez,1998).

However, most processing methods are costly (need fuel for cooking), require containers (ensiling) or depend on the weather (sun-drying). The question is how to use fresh cassava leaves as a protein source with simple processing methods which are easily applied by small scale farmers?

Tannins

Another anti-nutritional factor in cassava leaf is tannin. Tannins are present in most plant leaves. They are defined as phenolic compounds of moderately high molecular weight containing sufficient phenolic hydroxyl groups capable of forming strong complexes with protein and other macro-molecules (Van Soest et al., 1987) .

Tannins can be divided into two classes: hydrolysable and condensed compounds. Hydrolysable tannins such as tannic acid are present only in low concentrations in commonly consumed foods and therefore most of the focus has been on the condensed tannins (also called proanthocyanidins). Animals respond differently to dietary tannins, because there is variation in the biological activity of the tannins themselves (Hagerman et al., 1992; Reed, 1995). Most biological assays for tannins have relied on protein precipitation (Hagerman and Butler, 1978). Many researchers have shown that tannins and other polyphenolic compounds increase endogenous losses and alter digestibility and the site of digestion of nutrients, including minerals, protein and amino acids in monogastric animals and ruminants (Wang et al., 1994; Tanner et al., 1994; Terrill et al., 1994; Reed, 1995; Yu et al., 1996). Tannins have been considered as anti-nutrients or ANF's due to a range of adverse effects including reduced feed intake and digestibility, poor feed conversion and reduced growth rate (Chung et al., 1998; Reed et al., 1982; Onwuka, 1992). Tannin contents in cassava leaves are reported to increase with maturity and vary between cultivars (Ravindran, 1993). Reported levels in cassava leaves vary from 30 to 50g/kg DM (Ravindran, 1993). Harvesting of cassava leaves at an early growth stage (3 months) reduces the condensed tannin content and increases the protein content, resulting in higher nutritive value (Wanapat et al., 1995).

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Chapter 2

Digestibility and nitrogen retention in fattening pigs fed different levels of ensiled cassava leaves as a protein source and ensiled cassava root as energy source

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Abstract

This study aimed to evaluate different additives for ensiling cassava leaves and the use of the product as a protein source for pigs. Cassava leaves were wilted and ensiled in plastic bags with 5 or 10% "A" molasses and 10 or 15 % rice bran (fresh basis). After 7 days of ensiling the leaves the level of HCN had fallen from 177 mg/kg dry matter in the fresh leaf to 80 mg/kg in the ensiled material. There was a further slight reduction in HCN level up to 14 days but no change at 21 days. There were no differences attributable to kind or level of additive. Digestibility and nitrogen balance trials in a double 4*4 Latin square arrangement were conducted to evaluate substitution of fish meal by ensiled cassava leaf (ECL; levels of 0, 50, 75 and 100 g/day of protein) in diets based on ensiled cassava root (ECR). It was observed that the pigs were reluctant to consume all the ECL, especially when this was at a high level in the diet. Thus the actual intakes of protein from the cassava leaf silage were less than the planned amounts being 36, 63 and 62 g/day compared with the planned quantities of 50, 75 and 100 g/day, respectively. Intakes of fish meal were 134, 92, 69 and 46 g/day and were only slightly less than the planned quantities of 150, 100, 75 and 50 g/day, respectively. The biggest difference between planned and recorded intake was for the intended 100 g/day of protein from ECL with the actual intake being only 62 g/day, thus total protein intake on the highest level of ECL was only 127 g/day compared with the intended level of 150 g/day. There were no differences in total dry matter intake whereas it was expected that this would have increased with increasing levels of ECL in order to compensate for the lower protein content in this feed compared with fish meal. There was an indication ($P= 0.08$) that apparent diet digestibility of dry matter decreased with increasing level of ECL

(from 90.1 to 87.4% for 0 to 100 g/day protein substitution). The decrease in crude protein digestibility (from 86.6 to 79.6% from 0 to 100 g/day protein substitution) was highly significant (P=0.001). Nitrogen retention was 14.5, 13.8, 12.0 and 9.91 g/day for ECL0, ECL50, ECL75 and ECL100 diets, respectively. The differences between ECL0 and ECL50 compared with the higher levels of ECL were highly significant (P=0.001). Nitrogen retention values, as percent of N intake and N digested, were high on all diets (ranges were 57-58% and 66 to 61%, respectively) and did not differ among diets, indicating efficient utilization of the absorbed amino acids. There were no indications of cyanide toxicity on any of the diets.

Key words: Ensiled cassava leaves, ensiled cassava root, protein, digestibility, nitrogen retention, growing pigs

Introduction

In central Vietnam, the main energy sources in pig production are cassava roots, cassava root by-products, rice bran and by-products of crop production. The protein content in these feeds is low and quite high levels of protein supplements are needed to satisfy nutrient requirements. However, conventional sources of protein meals (fish meal, groundnut cake, soya bean meal) are expensive in Vietnam. It is therefore important to identify local sources of protein especially those that can be produced on the small farm.

There are many research papers about using cassava leaf as a protein source for animal production. Bui Huy Nhu Phuc et al. (1996) evaluated levels of substitution of soya bean meal by dried cassava leaves (DCL) and found a depression in N digestibility and retention as the level of DCL increased. Ensiled cassava leaves were compared with duckweed in diets of sugar cane juice fed to growing Mong Cai pigs (Du Thanh Hang et al., 1997). Digestibility of the dry matter of the total diet was 86.0% and that of ECL per se was estimated to be 77%. Satisfactory performance of growing pigs fed final ("C") molasses supplemented with ECL was reported by Bui van Chinh et al. (1992). According to Sarwat et al. (1988) the inclusion of 15% fresh cassava leaves in the diet had no adverse effects on the performance of growing-finishing pigs. Alhassan and Odoi (1982) and Ravindran (1990) reported linear depression in weight gain and feed efficiency when cassava leaf meals were included at up to 30% in diets for growing-finishing pigs.

In Vietnam, cassava (*Manihot esculenta* Crantz) is the major crop after rice. In Hue province, with a total area of 5,009 km² and population density of 207 people/km², the cultivated area of cassava is 5,555 ha compared with 49,490 ha of rice and 823 ha of maize. The average productivity of cassava is estimated to be 3,230 kg of fresh roots/ha (Thua Thien Hue, 1997).

According to Ravindran and Rajaguru (1988) the yield of cassava leaves can be as much as 4.6 tonnes dry matter per ha taken as a by-product at root harvest. In Viet Nam, reported yields are lower: 2.5 to 3 tonnes cassava leaves containing 500-600 kg dry matter and 110 to 130 kg crude protein from 1 ha of cassava prior to root harvesting (Bui Van Chinh et al., 1992). Crude protein in the dry matter of cassava leaves is reported to vary from 16.7 to 39.9 (Allen, 1984) with an average of 21%.. Almost 85% of the crude protein fraction is true protein according to Eggum (1970). Rogers and Milner (1963) and Eggum (1970) reported that cassava leaf protein is deficient in methionine but high in lysine. Cassava leaves are a good source of minerals, particularly Ca, Mg, Fe, Mn and Zn (Ravindran and Ravindran, 1988). Cassava leaves are also rich in ascorbic acid and vitamin A, and contain significant amounts of riboflavin. But considerable losses of vitamins, particularly of ascorbic acid, occur during processing (Ravindran, 1992).

The traditional processing of cassava leaves for feeding to pigs in the Central part of Vietnam is by drying and boiling. Drying is often difficult due to rain at the time of harvesting and boiling takes time and fuel. Ensiling the leaves appears to be a more appropriate way of conservation for conditions at the level of the small farm household. This study aimed to determine the digestibility and nitrogen retention in pigs when ensiled cassava leaves replaced up to 50% of the fish meal protein in diets based on ensiled cassava roots. Two experiments were carried out. The first aimed to evaluate the effects of molasses and rice bran as additives on some quality parameters of cassava leaf silage. The second was a digestibility and nitrogen retention study with pigs fed ensiled cassava leaves to replace fish meal in a diet of ensiled cassava roots.

Experiment 1: Effect of molasses and rice bran as additives in ensiled cassava leaves

Materials and methods

Ensiling procedure

Cassava leaves were collected from farmers' fields immediately prior to harvesting the roots. The leaves with petioles were separated from the stem and chopped into small pieces (1-2cm) with a knife. They were then spread out in the shade with cross ventilation and wilted for one day. The leaves were turned regularly to avoid fermentation and growth of moulds. After wilting, the leaves were mixed with 5 or 10% "A" molasses and 10 or 15% rice bran (fresh basis), put into plastic (polyethylene) bags (about 5 kg per bag) and pressed to exclude the air. The bags were then sealed.

Chemical analysis

Samples of the freshly processed leaves were taken on the day of ensiling and after 7, 14 and 21 days for analysis of dry matter, crude protein, crude fibre, hydrocyanic acid (HCN) and pH (AOAC, 1984).

Results and discussion

Chemical composition of ensiled cassava leaves

The change of HCN concentration during the ensiling period is shown in Figure 1. For all the additives there was a marked reduction in the HCN level after 7 days of ensiling with another slight fall after 14 days and then no further change at 21 days. The slightly lower levels with rice bran can be explained by the greater diluting effect of the bran compared with molasses. Molasses contains less dry matter and was used at lower levels than the rice bran. Similar values for HCN in fresh cassava leaves were reported by Nguyen Van Lai and Rodriguez (1998); however, in their experiment the level of HCN continued to fall beyond 21 days and up to 56 days of the ensiling period. The values obtained by Bui Van Chinh et al. (1992) in cassava leaves ensiled with molasses were 32 - 34 mg/kg DM.

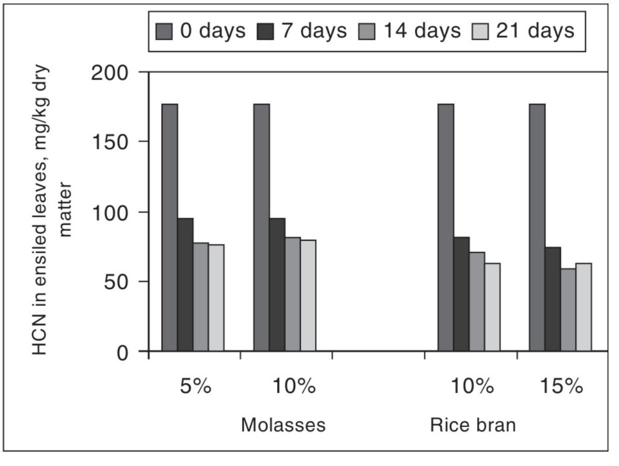

Figure 1: Effect of levels of molasses and of rice bran on HCN content of ensiled cassava leaves after different periods of ensiling

Changes in pH with time of ensiling are shown in Figure 2. There was an immediate fall from pH 7.1 to pH 4 within seven days of initiating the ensiling process and then a more gradual reduction to pH 3.5 after 21 days. There were no differences due to kind or level of additive. These trends are similar to those reported by Nguyen Van Lai and Rodriguez (1998).

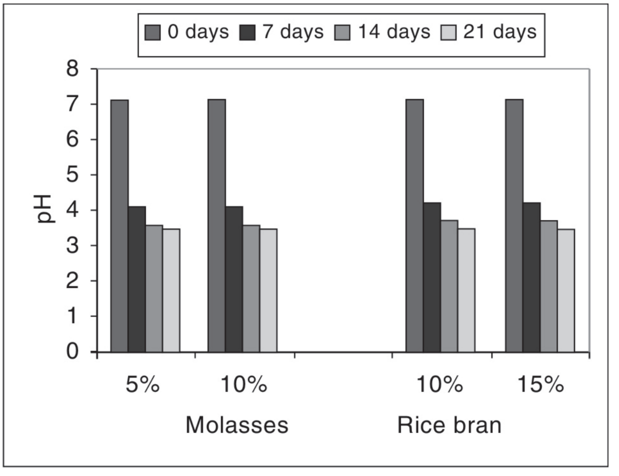

Figure 2: Effect of levels of molasses and of rice bran on pH of ensiled cassava leaves after different periods of ensiling

Experiment 2: Digestibility and nitrogen retention infattening pigs fed different levels of ensiled cassava leaves (ECL) as a protein source and ensiled cassava root (ECR) as energy source

Materials and methods

The study was done at the research farm of Hue University which is located in Hue city, Thua Thien Hue Province in Central Vietnam. There are two distinct seasons: the dry season from April through August, with a characteristic south-west hot wind throughout the season. The hot wind coupled with sunny weather can increase day temperature up to 40 - 41 ºC. The rainy season starts in September and lasts through to March. The season is characterized by an uneven distribution of rainfall which is concentrated from October to December. During this time the average monthly rainfall is around 3,000 mm and the average humidity is between 80 - 85%.

Objective

The objective of the study was to evaluate effects on digestibility and nitrogen balance in fattening pigs of substituting fish meal by ensiled cassava leaves at levels of 0, 50, 70 and 100 g/day of crude protein.

Treatments and design

The four treatments were:

- ECL0 : Fish meal (150 g/CP/pig day) + ensiled cassava root (ECR) ad-libitum
- ECL50: The same as ECL0 but with 50g of the fish meal protein replaced by ECL
- ECL75: The same as ECL50 but with 75 g fish meal protein replaced by ECL
- ECL100: The same as ECL75 but with 100 g fish meal protein replaced by ECL

The experimental design was a double 4*4 Latin square arrangement (Table 1).

Table 1: Allocation of pigs to experimental treatments

Period /Pig	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	ECL0	ECL100	ECL75	ECL50	ECL50	ECL75	ECL100	ECL0
2	ECL100	ECL75	ECL50	ECL0	ECL75	ECL100	ECL0	ECL50
3	ECL75	ECL50	ECL0	ECL100	ECL100	ECL0	ECL50	ECL75
4	ECL50	ECL0	ECL100	ECL75	ECL0	ECL50	ECL75	ECL100

Animals and housing

Eight F1 Large-White x Mong Cai castrated male pigs with initial body weight of 48 - 50 kg and age of four months were randomly allotted to the diets according to the design layout (Table 1). The pigs were housed individually in metabolism cages that allowed the separate collection of urine and faeces. The size of the metabolism cage was 1.5 x 0.55 m (length x width) and they were made of galvanized steel with wooden slatted floor. The experimental periods were of 10 days: five days for adaptation period to allow the pigs to become familiarized with the new diet and a five day period for collection of faeces and urine.

Diets and feeding

The cassava leaves (after removing stems and petioles) were chopped into small pieces (1-2 cm) and ensiled with 5 % “A” molasses and stored for 21 days before feeding. The ensiling of the cassava roots (ECR) was according to the method used by Nguyen Thi Loc et al, (1997). The roots were washed and chipped by hand and ensiled with 0.5% common salt in plastic bags. The ECR was offered at levels of 2.5 to 3 kg at each of three meals daily. After 40 minutes the uneaten feed was collected and weighed. Amounts offered and refused were weighed and samples taken for analysis. The ECL and fish meal were fed separately following the same procedure as with the ECR.

Measurements

Urine and faeces of each pig were collected separately and weighed twice daily and stored at - 20 ºC. Urine was collected in a bucket via a funnel below the cage. To prevent nitrogen losses by evaporation of ammonia, the pH was kept below pH 4 by collecting the urine in 50 ml of 25 % sulphuric acid. The urine and feces from each animal were collected for five days and at the end of the period, the faeces were mixed, dried (in a drying over at 60-65 ºC), ground and representative samples taken for analysis. Dry matter of feed offered and refused, dry matter and nitrogen in faeces and nitrogen in urine were determined according to AOAC (1984).

Results and discussion

Composition of feeds

The composition of the feeds is shown in Table 2.

Table 2: Composition of feeds offered

	Dry matter, %	Nitrogen % of DM	Crude fibre % of DM	HCN mg/kg DM
ECL	34.5	4.1	16.8	60.9
ECR	42	0.36	2.5	95.9
Fish meal	91.2	6.74		

The value for nitrogen content of the fish meal indicates that the protein level is about 42% which is below the average for this ingredient which normally has around 60-70% protein in dry matter (Gohl and Speedy, 1996). The low nitrogen content of the ECR shows that there is little protein (<2% in DM) in this energy source which means that responses in parameters of nitrogen metabolism truly reflect the nutritive value of the protein supplement. This can be a problem when cereal grains are used as the basal diet in view of the 9-10% protein in these energy sources. The values for HCN in the ECR and the ECL are less than the suggested maximum values of this compound in order to avoid toxicity (Gomez and Valdivieso, 1985).

Digestibility and N retention

The data in Table 3 show that the actual intakes of protein from the cassava leaf silage were less than the planned amounts being 36, 63 and 62 g/day compared with the planned quantities of 50, 75 and 100 g/day, respectively. The biggest difference between planned and recorded intake was for the intended 100 g/day of protein from ECL with the actual intake being only 62 g/day. There were no differences in total dry matter intake whereas it was expected that this would have increased with increasing levels of ECL in order to compensate for the lower protein content compared with fish meal.

Table 3: Mean values for intake of feed and protein, coefficients of apparent digestibility and N retention for different levels of substitution of fish meal by cassava leaf silage

	ECL0	**ECL50**	**ECL75**	**ECL100**	**SEM**	**P**
Intake fresh basis, g/day						
ECL	0	409	716	701		
ECR	2,250	2,062	2,000	2,000		
Fish meal	360	240	180	120		
Dry matter intake, g/day	1,264	1,226	1,251	1,191	42.0	0.62
Protein intake, g/day						
Fish meal	134	92.2	69.1	46.1		
ECL	0	36.2	63.3	62.0		
ECR	21.2	19.5	18.9	18.9		
Total	156	148	151	127		
Protein in DM, %	12.3	12.1	12.1	10.7		
Digestibility, %						
Dry matter	90.1	89.5	87.4	89.6	0.73	0.08
Crude protein	86.6[a]	84.9[a]	80.1[b]	79.6[b]	1.30	0.001
N retention						
% of digested N	65.7	68.7	62.9	60.7	2.38	0.13
% of N intake	56.9	60.0	54.0	58.2	2.31	0.31
Per day, g	14.2[a]	13.8[a]	12.0[ab]	9.91[b]	0.74	0.001

[ab] *Means without common subscript are different at P<0.05*

The dry matter intake of each of the diet constituents as a percentage of total dry matter intake is shown in Table 4. It can be seen that with free access to the ECL and ECR, but with fish meal restricted, the pigs reached a ceiling in consumption of ECL at a level of 20% of the total diet dry matter. It is not known if the inability of the pigs to consume the highest levels of ECL was because of low palatability of the ECL or a physical effect due to the fibre content or even the residual level of HCN. The fibre level increased from 3.39 % of DM for the ECL0 diet to 5.17% for the highest level of ECL. A level of 5.17% fibre in the diet is relatively low and would not be expected to be a constraint to intake. It would seem that some other factor is constraining the intake of ECL at levels in excess of 20% of the diet dry matter.

Table 4: Intake of dietary constituents expressed as percentage of total intake (% dry matter basis)

	ECL0	**ECL50**	**ECL75**	**ECL100**
ECL	0	12	20	20
ECR	74	71	67	71
Fish meal	26	18	13	9

Coefficients of apparent digestibility of dry matter were high with only an indication (P=0.08) of lower values with the ECL.

The decrease in the digestibility of the nitrogenous fraction was more marked (P=0.001) with a linear decrease as levels of ECL increased. The trends were similar to those reported by Bui Huy Nhu Phuc et al. (1996) who worked with ensiled cassava leaf and dried cassava root meal diets for growing pigs. The decreases in digestibility were more pronounced in the studies reported by Bui Huy Nhu Phuc et al. (1996), from 94 to 85% for dry matter and from 88 to 66% for crude protein.

Daily nitrogen retention decreased significantly (P=0.001) from 14 g/day on the ECL0 diet to 9.91 g/day on ECL100, a similar fall to that reported by Bui Huy Nhu Phuc et al, (1996). There were no significant differences between levels of ECL in the nitrogen retained as a percentage of nitrogen consumed and nitrogen digested. This finding is similar to the results of Bui Huy Nhu Phuc et al. (1996) although they reported a sharp drop in these values for the highest level of ECL substitution. Furthermore, the values in the present study were some 10 percentage units higher than found by Bui Huy Nhu Phuc et al. (1996).

The higher values for nitrogen retention as percentage of nitrogen digested in the present study, compared with the results of Bui Huy Nhu Phuc et al. (1996), indicate that the dietary amino acid profile was better balanced in the diets used here. It has been shown that the limiting amino acid in cassava leaf is methionine (Eggum, 1970; Rogers and Milner, 1963) but that it is rich in lysine and threonine. Soya bean is also low in methionine while fish meal is a good source of this amino acid, thus a combination of cassava leaf and fish meal, as used in the present study, would be expected to be superior to a combination of cassava leaf and soya bean meal, which was the complementary protein source used by Bui Huy Nhu Phuc et al. (1996).

Conclusions

- Ensiling cassava leaves with 5% molasses or 10% rice bran is an effective method of conserving this feed resource and reducing cyanide levels to non-toxic proportions. There appear to be no advantages from using higher levels of either molasses or rice bran.
- Ensiled cassava leaves can be used at up to 20% of the dry matter in diets of ensiled cassava roots for growing-fattening pigs thus providing some 40% of the total dietary protein equivalent in a cassava root diet of about 50% of the supplementary protein. These levels assume that the complementary protein source is fish meal.

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Chapter 3

Ensiled cassava leaves and duckweed as protein sources for fattening pigs on farms in Central Vietnam

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Abstract

Forty-four crossbred pigs (mainly Large White x Mong Cai) about 3 months old and 23-25 kg initial weight were allocated among two groups of families (4 pigs/household) to compare effects of supplementing the traditional diet with either ensiled cassava leaves or fresh duckweed. In six farm households (trial 1) the comparisons (two pigs per treatment) were: ensiled cassava root, brewery by-product (brewers' grains), rice bran and sweet potato vines (control) or the same as the control but with ensiled cassava leaves replacing the sweet potato vines. The cassava leaves were ensiled with 5 % molasses and stored 21 days before feeding. The cassava roots were ensiled with 0.5% common salt and stored 21 days before feeding. In five farm households (trial 2) the comparisons (two pigs per treatment) were the same as for the trial with ensiled cassava leaves except that fresh duckweed replaced the ensiled cassava leaves. The duckweed (*Lemna minor*) was grown in artificial ponds lined with polyethylene sheets on one farm and in natural ponds in the others. The ponds were fertilized with manure from buffaloes and pigs. The duckweed was harvested daily and fed immediately after harvesting.

In five out of six farms in the first trial, pigs fed ensiled cassava leaves grew faster than the controls receiving sweet potato tops, but the overall difference was not significant. On all five of the farms in the second trial, the pigs supplemented with fresh duckweed consumed more dry matter and grew 35% faster (P=0.001). It is concluded that in pig fattening diets based on ensiled cassava root, rice bran and brewers' grains: ensiled cassava leaves can replace sweet potato vines with no effect on growth or carcass traits; and that fresh duckweed (providing 5% of the diet dry matter) has a stimulating effect on live weight gain which may be partially explained by the additional intake of protein, although other factors are likely to be involved.

Key words; Pigs, fattening, on-farm, cassava, ensiled cassava leaves, duckweed

Introduction

In Central Vietnam, pig production plays an important role in the farming activities especially for small-holders. The pigs provide manure, meat and income. In recent years, the income from pig raising is very low and in some cases is negative because the pigs grow slowly and the cost of feeds is high. The main cause is the imbalance in the nutrients in the ration particularly the energy and protein ratio. The main energy sources are cassava, cassava-by products, rice bran, maize and sweet potato. Those feeds have a low protein content. The conventional protein sources such as fish meal, soya bean meal and groundnut cake are not used by the farmers because they are too expensive and the farmers do not have enough money to buy them. Thus there is a need to identify and promote other protein sources especially those that can be produced on the farm.

Many researchers have shown that the leaves from different plants can be used as protein sources in pig feeding at the small scale level of production. Bui Van Chinh et al. (1993) showed that ensiled soya bean foliage can replace 20-37 % of the protein in the ration without affecting the performance of the pigs. According to Perez (1996), the fresh foliage from immature soya bean, planted at weekly intervals and harvested when the grain is at the milk stage, has considerable potential as a protein source in the tropics that can be available in the wet season, and even year round if irrigation is available.

According to Montaldo (1977), cassava plants can withstand defoliation for several years if they receive adequate fertilization and irrigation, and under such management have the potential to produce up to 4 tonnes of protein per hectare, annually. Ensiling of cassava leaves is an appropriate way to conserve them (Limon, 1992; Bui Van Chinh et al., 1992; Du Thanh Hang, 1998). It has been shown that the ensiled leaves can provide all the protein in a diet of sugar cane juice for native Mong Cai pigs (Du Thanh Hang et al., 1997) and up to 50% of the protein in diets based on ensiled cassava roots (Du Thanh Hang, 1998).

Another plant protein source with high potential is duckweed (Rodriguez and Preston, 1996; Nguyen Duc Anh et al., 1997; Le Ha Chau, 1998). The protein is more digestible than in cassava leaves (Nguyen Van Lai and Rodriguez, 1998) and with appropriate fertilization of the pond water the protein content can be as high as 35-40% in the dry matter (Leng et al., 1995; Rodriguez and Preston, 1996; Nguyen Duc Anh et al., 1997; Le Ha Chau, 1998). The balance of essential amino acids in duckweed is considered to be comparable to that in soya bean meal (Muztar et al., 1976; Rusoff et al., 1980). As with ensiled cassava leaves, pigs have been fed with duckweed as the only source of supplementary protein in diets of: sugar cane juice (Du Thanh Hang et al., 1997; Nguyen Van Lai and Rodriguez, 1998); ensiled cassava roots (Nguyen Van Lai and Rodriguez, 1998); and mixtures of ensiled cassava roots and rice bran (Nguyen Van Lai, 1998). Up to the present, most of the work with duckweed supplementation of pig diets has been done on-station. The trial of Nguyen Van Lai (1998) was done on-farm but with very young pigs (weight range 5 to 20 kg). There appear to be no reports on the use of duckweed with fattening pigs under farm conditions.

Objectives

The aim of this study was to evaluate ensiled cassava leaves and duckweed as protein supplements in diets for growing-fattening pigs fed on local resources at small-holder farm level.

Materials and methods

Location

The study was done in Thuy Xuan village which is located in the upland area of Thua Thien Hue Province. The pig population in the area is estimated to be of the order of 5000. The number of pigs per family is stratified according to income, as follows: high income farmer:12 - 15 pigs, middle income: 4 -6 pigs and poor farmer: 1 -2 pigs. The traditional feeds are dried cassava root, rice bran and brewery by-products. The crude protein in the dry matter of the typical diets was calculated to be less than 9 %. Rice bran is the most expensive ingredient. Cassava root is available in Thuy Xuan constantly. The area planted in cassava is about 65 ha, with a productivity of 3,500 kg/ha. The population density is about 1 person/600 m^2 and there are 30 ha of springs, ponds and lakes. This means that there are opportunities for the farmers to grow water plants.

Choice of farmers

Feeding trials were carried out in farm households in Thuy Xuan village from May to November 1997. The families were selected on the basis of:

- The farmers' willingness to participate in the research
- Availability of a closed pig pen with cement floors of adequate size.
- Cassava and duckweed availability on the farm

Treatments and design

Trials were carried out with two groups of farmers. In each farm, two pigs received the conventional diet; two more pigs were fed the experimental diet which was the conventional diet plus either fresh duckweed (group 1) or ensiled cassava leaves (group 2) in each case replacing fresh sweet potato vines. A total of 9 households took part in the trials. In two households the trial was done with both ensiled cassava leaf and duckweed (with four pens each having 2 pigs).

Animals and feeding

Forty-four crossbred pigs (mainly Large White x Mong Cai) about 3 months old and 23-25 kg initial weight were allocated among two groups of families (4 pigs/household); one group of 6 families and one of 5 families. The pigs were vaccinated against Pasteurella, Erysipelas and Hog cholera, dewormed and ear-notched. An adaptation period of 20 days was allowed before starting the experiment, which lasted for 120 days. The following diets were investigated:

Ensiled cassava (6 families)

- Control: This consisted of ensiled cassava root, brewery by-product (brewers' grains), rice bran and sweet potato vines.
- Ensiled cassava leaves: The same as the control but with ensiled cassava leaves replacing the sweet potato vines

The cassava leaves were ensiled with 5 % molasses as described by Du Thanh Hang (1998) and stored 21 days before feeding. The cassava roots were ensiled with 0.5% common salt (Nguyen Thi Loc et al., 1996) and stored 21 days before feeding.

Duckweed (5 families)

The diets were the same as for the ensiled cassava leaves except that fresh duckweed replaced the ensiled cassava leaves. The duckweed (*Lemna minor*) was grown in artificial ponds lined with polyethylene sheets (area of 10m*2m) on one farm and in natural ponds in the others (average area per pond was about 50m^2). Manure from buffaloes and pigs was applied to the ponds (about 5 kg buffalo manure weekly and 3 to 4 kg pig manure every second day). The duckweed was harvested daily and fed immediately after harvesting.

Procedure

The pigs were weighed in the early morning each month using a 100 kg capacity portable scale with an accuracy of 0.5 kg. Feeds offered and refused were recorded for each meal (3 times a day) using a 20 kg capacity portable scale. The farmers mixed all the ingredients prior to feeding which was done three times per day. Feed samples were taken (two times for cassava leaves and 4 times for duckweed)for analysis of dry matter, nitrogen and crude fibre using the procedures of AOAC (1985).

Carcass measurements

At the end of the experiment, 6 pigs were slaughtered from each treatment and the carcasses were evaluated. Carcass weight was determined directly after slaughtering to calculate hot carcass dressing percentage. Carcass length was measured from the first rib to the pubis bone. Average back fat thickness and loin eye area were determined at the tenth rib. The carcasses were separated into lean meat, fat, bone and skin and the amounts recorded.

Statistical analysis

Mean values for effects of supplementation on weight gain, back-fat thickness and area of loin eye muscle were compared using the GLM option of the analysis of variance, determined with software of Minitab, release 10.2. The sources of variation in the ANOVA was the source of supplement. The analysis was made separately for each group of farmers.

Results

Feed composition

The composition of the feed ingredients is shown in Table 1. The HCN levels in all the cassava products were below 100 mg/kg dry matter which is considered as the safe level to avoid toxicity (Tewe 1992). The protein content of the duckweed was almost twice that in the sweet potato vines; differences in the fibre content were less marked but favoured the duckweed.

Table 1: Composition of feeds

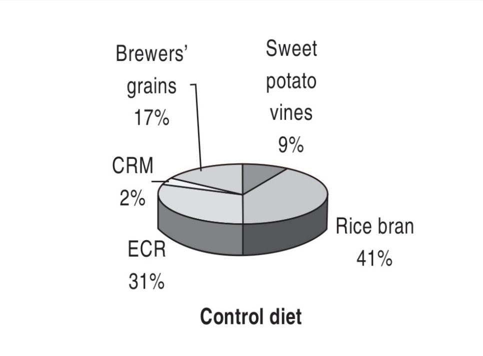
	Dry matter %	N*6.25 % in DM	Crude fibre % in DM	HCN mg/kg DM
Ensiled cassava leaves	35	27	15.3	70.7
Duckweed	6.48	30.1	8.3	
Sweet potato vines	13.6	17.7	10.5	
Rice bran	87.6	14.8	8.45	
Cassava root meal	90	1.4	0.57	26.7
Brewers' grains	21	25.3	12.7	
Ensiled cassava root	40.2	1.5	0.92	65.3

The trial with ensiled cassava leaves

Feed intakes in the trial with ensiled cassava leaves are shown in Table 2. In the experimental diet the ensiled cassava leaves provided 9% of the diet dry matter (Figure 2) and 15.0% of the total protein (calculated from Table 2). The sweet potato vines were 9% of the control diet dry matter (Figure 1) and provided 13.4% of the protein (calculated from Table 2).

Table 2: Intakes (g/animal /day) of dry matter and of protein (from day 0 to day 120) for pigs fed the conventional (control) diet or the control plus ensiled cassava leaves (ECL) replacing the sweet potato vines

	Dry matter		Crude protein	
	ECL	**Control**	**ECL**	**Control**
Ensiled cassava leaves	150	0	41	0
Sweet potato vines	0	190	0	34
Rice bran	910	820	134	110
Ensiled cassava root	620	620	9	5
Brewers' grains	340	340	86	29
Cassava root meal	50	50	0.0	0.0
Total DM	**2.070**	**2.020**		
Total protein			**270**	**250**
Protein, % of DM			**13.1**	**12.4**



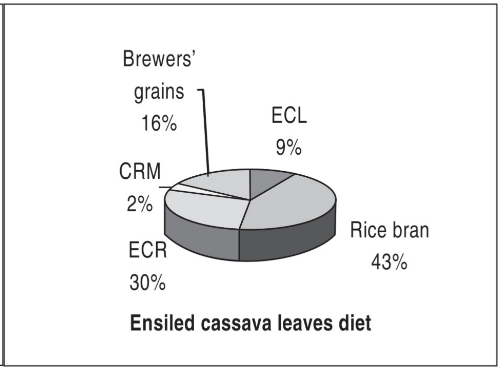


Figure 1: Ingredients in the control diet with sweet potato vines (% as dry matter)

Figure 2: Ingredients in the diet with ensiled cassava leaves (% as dry matter)

There were no differences in growth rate or feed conversion between the pigs fed the conventional diet and those receiving ensiled cassava leaves as replacement of the sweet potato vines. Feed costs were lower with ensiled cassava leaves supplementation.

Table 3: Mean values for growth rate and feed conversion in pigs fed the conventional (control) diet or the control with ensiled cassava leaves replacing the sweet potato vines

	ECL	**Control**	**SEM**	**P**
Live weight, kg				
Initial	25.8	23.3	0.89	0.16
Final	78.1	74.5	1.02	0.02
Daily gain	0.435	0.426	0.01	0.34
DM feed conversion	4.99	4.81	0.16	0.43
Feed cost/pig/day, VND	4,072	4,737	95	0.004
Feed cost/kg gain, VND	9,375	11,143	3.06	0.009

The trial with duckweed

The data on feed intake during the trial are shown in Table 4. The proportions of the diet contributed by the different ingredients are shown in Figures 3 and 4.

Table 4: Intakes (g/animal/day) of dry matter and of protein for pigs fed the conventional (control) diet or the control plus fresh duckweed (DW) replacing the sweet potato vines

	Dry matter		Protein	
	Duckweed	**Control**	**Duckweed**	**Control**
Duckweed	100	0.00	30.1	0
Sweet potato vines	0.00	130	0	23
Rice bran	730	610	108	90.3
Ensiled cassava root	640	530	8.96	7.42
Brewers' grains	330	320	83.5	81.0
Cassava root meal	220	220	3.3	3.3
Total DM	**2,020**	**1,810**		
Total protein			**234**	**205**
Protein, % in DM			**11.6**	**11.3**

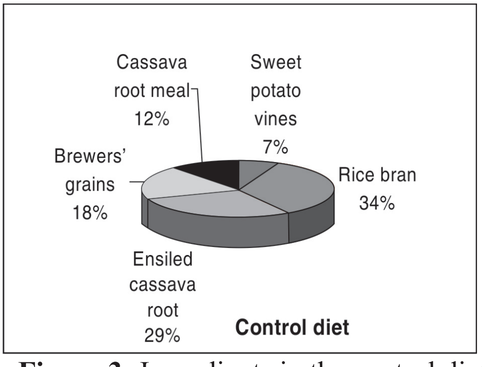


Figure 3: Ingredients in the control diet with sweet potato vines (% as dry matter)

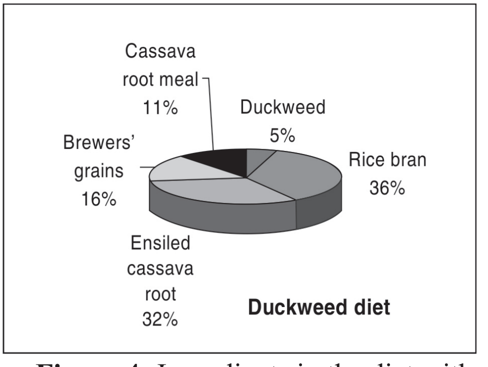


Figure 4: Ingredients in the diet with duckweed replacing sweet potato vines (% as dry matter)

The duckweed contributed 5% of the diet dry matter (Figure 4) and 12.9% of the total protein (Table 4) in the experimental diet. Comparable data for sweet potato vines in the control diet were 7% (Figure 3) and 11.2% (Table 4). The total protein content in the dry matter was the same for both diets (11.6 and 11.3%).

Growth performance

Mean values for initial and final weight, weight gain and feed conversion are given in Table 5 for pigs fed the conventional diet (control) and the control diet with fresh duckweed replacing the sweet potato vines. Pigs fed duckweed as a supplement were significantly heavier at the end of the trial (P=0.001), gained weight more rapidly (P=0.001) and had better feed conversion (P=0.001) than the control pigs fed sweet potato vines as the protein supplement.

Table 5: Mean values for growth rate and feed conversion in pigs fed the conventional (control) diet or the control with fresh duckweed replacing the sweet potato vines

	DW	Control	SEM	P
Live weight, kg				
Initial	20.3	19.0	1.33	0.50
Final	86.31	67.5	2.15	0.001
Daily gain	0.552	0.404	0.01	0.001
DM feed conversion	3.66	4.50	0.10	0.001

The data for growth rates on the individual farms show the consistency of the response to duckweed in the different locations (Figure 5). By contrast, in 5 out of the 6 farms in the trial with ensiled cassava leaf (Figure 6) there was faster growth on this supplement compared with the control receiving sweet potato tops, but the overall effect was not significant.

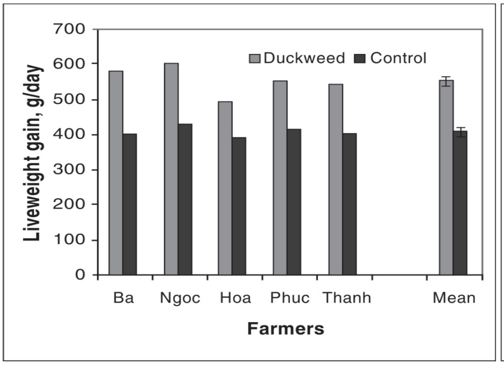


Figure 5: Growth rates of pigs on individual farms fed the conventional diet (control) or with fresh duckweed replacing the sweet potato vines in the control diet

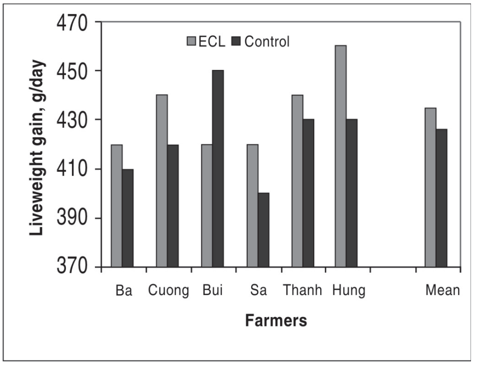


Figure 6: Growth rates of pigs on individual farms fed the conventional diet (control) or with ensiled cassava leaf replacing the sweet potato vines in the control diet

Carcass measurements

Mean values for carcass traits in a sample of pigs from both trials are shown in Table 6.

There were no effects on carcass traits attributable to supplementation with either ensiled cassava leaves or duckweed (Table 6).

Table 6: Mean values for carcass traits in a sample of pigs from both trials

	Ensiled cassava leaves			Duckweed		
	ECL	**Control**	**SEM**	**DW**	**Control**	**SEM**
Carcass yield, %	nd	nd		68.4	68.8	0.56
Carcass length, cm	88.3	80.7	3.12	85.0	80.0	0.85
Back fat, cm	3.1	3.03	0.53	3.2	3.0	0.10
Loin eye area, cm²	29.0	29.2	0.47	32.6	29.2	1.04
Composition of the carcass, %						
Lean meat	40.6	40.3	0.55	42.1	41.3	0.66
Fat	34.7	34.9	1.0	33.8	33.9	0.38
Bone	14.9	15.9	0.52	15.0	15.9	0.68
Skin	9.73	8.86	0.33	9.1	8.87	0.08

nd: Not determined

Discussion

The contrasting feature of the two trials was the highly significant improvement in growth rate and feed conversion when fresh duckweed replaced the sweet potato vines in the traditional diet and the less consistent and non significant effect when ensiled cassava leaves was the alternative supplement. The origin of the pigs was the same and those on the control treatment performed similarly in both trials (404 g/day growth rate and 4.5 conversion in the duckweed trial and 426 and 4.81 in the trial with cassava leaf silage). The contribution to the diet of the protein from duckweed was less than that from ensiled cassava leaves (12.9 versus 15%) and although the former has a slightly better array of essential amino acids (compare the reports of Rusoff et al., 1980 and Ravindran, 1992) it is unlikely that this could be the sole explanation. Working with scavenging native chickens in Cambodia, Hong Samnang (1998) reported a significant improvement in growth rate when the birds had access to duckweed even though the amount consumed was small (contributing the equivalent of less than 1 g protein/day). A stimulating effect of small amounts of duckweed (5% of the diet dry matter) in the diet of broiler chickens was also observed by Haustein et al. (1994).

Conclusions

It is concluded that in pig fattening diets based on ensiled cassava root and brewers' grains:

- Ensiled cassava leaves can replace sweet potato vines with no effect on growth or carcass traits
- Fresh duckweed (average intake 1.5 kg/day) has a stimulating effect on live weight gain (daily gain was increased by 37%)

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Chapter 4

The effects of simple processing methods of cassava leaves on HCN content and intake by growing pigs

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Abstract

The composition of the leaves of twenty cassava varieties, taken from the upper part of the plant at the time of root harvesting, ranged from 23.7 to 31.1% for DM, 23.7 to 29.5% for crude protein (in DM) and 610 to 1840 for HCN (mg/kg DM).

Leaves collected from a high-yielding variety after 60 days of growth and at root harvest (180 days) were wilted for 24 hours for determination of changes in the DM and HCN content. The HCN level was reduced by 58% after 24 hours with no difference between the two sources of leaves.

Three simple methods to reduce HCN content of cassava leaves before feeding (washing, chopping and washing; and chopping, washing and wilting) were compared in a feeding trial with 6 pigs of 25 kg mean live weight (3 Mong Cai and 3 F1 [Large White*Mong Cai]) in a double Latin square arrangement 2(3*3) with 21-day periods. The leaves were offered ad libitum in one feed trough with a mixture of ensiled cassava roots and rice bran (2:1 fresh basis) at 80% of ad libitum level in a separate trough.

The fresh cassava leaves were readily consumed providing 38% of the dietary DM and over 70% of the dietary protein with no effect of processing method on total DM

intake, which ranged from 27 to 31 g/kg live weight. Levels of HCN were reduced slightly (16%) by washing and substantially (82%) by wilting, resulting in intakes of HCN between 6.0 and 15 mg/kg live weight, levels considerably higher than previously reported as safe to avoid toxicity (1.4 to 4.4 mg/kg live weight). There were no apparent symptoms of HCN toxicity.

The results indicate that fresh cassava leaves, chopped and washed before feeding, can be included in ensiled cassava root / rice bran diets for growing pigs at levels of up to 40% of the diet DM, and as the sole source of supplementary protein. The long term effects of this feeding system have still to be determined.

Key words: chopping, feed intake, fresh cassava leaves, HCN, pigs, washing, wilting

Introduction

Cassava (*Manihot esculenta*, Crant) is cultivated in Vietnam in an area exceeding 250,000 ha (Bui van Chinh and Le Viet Ly, 2001). Traditionally the crop is grown for the roots, which are used as human and animal food and industrially as a source of starch. In recent years, attention has been focused on the leaves as feed for ruminants (Preston, 2001;Wanapat, 2001) and pigs (Bui Huy Nhu Phuc et al., 2001).

The main limiting factor to the use of cassava leaves as animal feed is the presence of cyanogenic glucosides, which give rise to hydrocyanic acid (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 with the stage of maturity being perhaps the major source of variation (Ravindran, 1995).

Many researches have demonstrated that the HCN content in leaves can be reduced by sun-drying (Bui Van Chinh and Le Viet Ly, 2001) and ensiling (Ly and Rodríguez, 2001). However, sun-drying is difficult when cassava leaves are harvested in the wet season and ensiling is labour-intensive. The use of simpler methods of processing the fresh leaves, such as chopping, washing and / or wilting would facilitate the use of cassava leaves as animal feed.

Materials and methods

Effect of age of leaves on chemical composition

Twenty varieties of cassava were grown under the same condition in the research garden of the Crop Production faculty in Hue university. The experimental design was a randomized block with plots of 2*5 m and three replications of each variety. Cassava stem cuttings were used as planting material at 50*30 cm spacing between rows and cuttings. Cattle manure was applied at 800 kg/ha prior to planting. Samples of leaves were taken at the time of root harvest (180 days) from the tender green leaves at the top of the stem. Determinations were made of dry matter (DM), crude protein and HCN according to AOAC (1990) procedures.

Effect of chopping and wilting on HCN content

Leaves from the high-yielding cassava variety (Cao san) were collected at 9.00 am. The petioles were removed, and the leaves chopped into small pieces (2-3 cm) and then spread out on a plastic sheet under a roof and allowed to wilt until 9.00 the next morning. Samples were taken for analysis immediately after collecting the leaves (09.00 am) and again at 12.00 am, 15.00 pm, 18.00 pm and at 09.00 am the following morning.

Samples of cassava leaves were taken after 60 days of growth and at the time of harvesting the roots after 180 days. For the 60-day growth (CL60), the leaves were collected at a point on the stem about 2/3 of the height of the plant. The leaves taken at the harvesting of the root (CL180) were from the top of the cassava tree.

Feed intake of cassava leaves by growing pigs

Treatments and design

Three treatments were compared in a double 3*3 Latin square arrangement using 3 pigs of each of two breeds (Mong Cai and F1 Large White*Mong Cai):

- *W:* Fresh cassava leaves were washed and fed immediately
- *CW:* Fresh cassava leaves were chopped into small pieces (2-3 cm) then washed prior to feeding
- *CWW:* Fresh leaves chopped, washed and wilted for 24 hours

The basal diet was a mixture of ensiled cassava root and rice bran (2:1 ratio in fresh basis) with an estimated crude protein content: 6.2% in DM.

Feeds and feeding system:

The cassava leaves without petiole were collected at the time of root harvesting (180 days). For the "W" treatment, they were washed two times in a plastic basin (each time for about 5 minutes with 10 litres water per 2 kg of leaves). For "CW" the leaves were chopped into small pieces (2-3 cm) and then washed as in "W". For "CWW" processing was as for "CW" followed by wilting over-night under a roof.

The ensiled cassava roots were made from whole cassava roots purchased from a local farmer. They were washed, ground and mixed with 0.5% NaCl prior to ensiling in plastic bags under anaerobic conditions for 21 days. Rice bran was purchased from the market. Prior to feeding the ensiled cassava root and the rice bran were mixed in a ratio of 2:1 (fresh basis).

During a 7-day adaptation period, the cassava leaves and the mixture of ensiled cassava root and rice bran were fed ad libitum in separate feed troughs to establish the expected level of intake of the ensiled cassava root and rice bran mixture.

In the subsequent experimental period of 21 days the offer level of the mixture of ensiled cassava root / rice bran was set at 80% of the intake observed during the period of adaptation, while the cassava leaves continued to be fed ad libitum, both feeds in separate feed troughs. The mixture of ensiled cassava root / rice bran was fed three times daily at 06.00, 12.00 and 18.00 hr. The cassava leaves were given 4 times at 08.00, 10.00, 14.00 and 16.00 hr.

Animals

The Mong Cai and F1 (Large White*Mong Cai) pigs had an average initial weight of about 23 kg. They were allocated to the three treatments in an arrangement of two Latin squares (2[3*3]) with periods of 14 days (Table 1).

Table 1: Allocation of diets and animals

Periods/pigs	MC1	MC2	MC3	LM1	LM2	LM3
1	CW	W	CWW	CW	W	CWW
2	W	CWW	CW	W	CWW	CW
3	CWW	CW	W	CWW	CW	W

Measurements

Records were kept daily of amounts of feed offered and refused. Representative samples were taken for analysis of DM, CP and HCN according to AOAC (1990) procedures.

Results and discussion

Chemical composition of twenty cassava varieties

The composition of the leaves of twenty cassava varieties, taken from the upper part of the plant at the time of root harvesting, ranged from 23.7 to 31.1% for DM, 23.7 to 29.5% for crude protein (in DM) and 610 to 1840 for HCN (mg/kg DM) (Table 2). The range for crude protein was less than that reported by Ravindran (1995) (16.7 to 39.9%) but similar to that described by Bui Huy Nhu Phuc et al. (2001) for cassava varieties in the South of Vietnam (21 to 34 %). Other researchers have also shown that there is considerable variation in the chemical composition among leaves of different cassava varieties (Ravindran, 1990; Eggum, 1970).

Table2: Chemical composition of twenty cassava varieties

Variety	DM (%)	Crude protein (% in DM)	HCN (mg/kg FM)	HCN (mg/kg DM)
Ba Trang	30.2	21.1	181	610
Nep Hong Ha	31.1	25.1	268	863
KM140-1	26.2	26.7	233	880
CMR258-2	26.9	26.2	261	971
KM32	26.3	27.6	268	1018
KM94	29.9	25.0	322	1079
KM98-1	28.5	23.7	320	1121
Phu Tho Green	27.4	20.4	317	1158
SM927-36	27.2	26.0	334	1227
KM98-2	27.3	26.3	360	1319
San Do	26.9	25.9	371	1378
SM1447-7	27.5	23.8	397	1411
KM21-10	21.8	23.3	326	1496
HL23	24.4	29.5	368	1509
Cao san #	30.5	29.3	462	1517
KM108-2	25.8	24.5	393	1525
KM95	26.9	25.4	442	1642
KM140-2	25.5	27.2	434	1701
CMR3515-8	26.2	24.0	447	1709
SM1717-12	26.3	27.2	483	1840

 # High yield variety

The protein in cassava leaves has an acceptable array of essential amino acids and compares favourably with alfalfa and soybean meal (Phuc and Lindberg, 2001). However, the major concern with the use of cassava leaves as animal feed is the risk of cyanide toxicity. In this study there was a wide variation in HCN content from 610 to 1840 mg/kg DM. The wide variation observed in the HCN content of cassava leaves has been attributed to genetic, physiological, edaphic and climatic differences (Gomez and Valdivieso, 1985). In this study the environmental conditions (soil, fertilization and climate) were similar, thus it is probable that the major factor causing variation in HCN content was genetic. Pham Sy Tiep and Nguyen Van Dong (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 mature leaves and 14.4 in the oldest leaves. In the present study the leaves taken for analysis were still very green, and the average HCN level (349 mg/kg fresh matter) was similar to that reported by Pham Sy Tiep and Nguyen Van Dong (1998). Duong Thanh Liem (1998) also reported similar values (from 305 to 425 mg/kg fresh leaves).

Effect of chopping and wilting on HCN content

The 58% reduction in the HCN content of the cassava leaves after wilting 24 hours in the shade (Table 3 and Figure 1) is similar to that (60%) reported by Bui Van Chinh and Le Viet Ly (1996), but much less than when wilting is under sunlight when up to 90% reduction in HCN has been reported (Gomez and Valdivieso 1985; Ravindran et al 1987). The faster rate of drying of the leaves sampled from the 60-day growth compared with the leaves from the mature plant was not reflected in the changes in HCN content, which were similar for both sources of leaves.

Table 3: Effect of wilting on DM and HCN content of cassava leaves sampled at the time of harvesting the roots (180 days from planting) and after 60 days of growth

	Time of wilting after harvest, hours						
	0	**3**	**6**	**9**	**24**	**SEM**	**P**
Dry matter, %							
At root harvest#	26.7^{a}	28.8^{b}	29.6^{b}	33.8^{c}	40.4^{d}	0.43	0.001
After 60 days##	30.2^{a}	37.4^{b}	50.7^{c}	57.4^{d}	63.9^{e}	0.37	0.001
HCN, mg/kg DM							
At root harvest#	1197^{a}	1102^{a}	885^{b}	883^{b}	626^{c}	52.4	0.002
After 60 days##	1435^{a}	1081^{b}	932^{b}	814^{b}	393^{c}	100	0.001

 # Leaves at the top of the plant; ## Leaves from the lower part of the plant ^{abcde} *Means without common superscript are different at P<0.05*

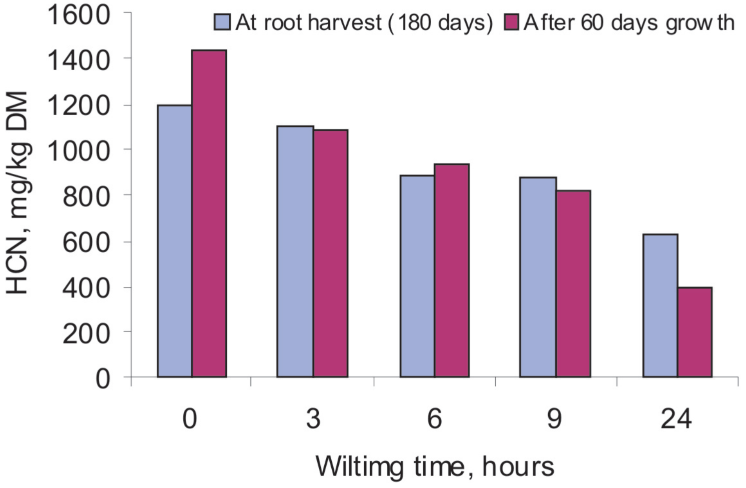

Figure 1: Variation in DM and HCN in cassava leaves, from mature and immature plants, analysed immediately or after wilting 3, 6, 9 and 24 hours

Feed intake of fresh cassava leaves by pigs.

Washing alone or washing after chopping the leaves reduced slightly (by 16 and 21%) the HCN level (Table 4); however, the pink colour in the urine observed when fresh cassava leaves were fed was no longer visible when the leaves were washed prior to feeding. Wilting for 24 hours after chopping and washing reduced the HCN by 82%.

Table 4: The chemical composition of cassava leaves analysed immediately after collection (fresh), or after washing (W), chopping and washing (CW) or chopping, washing and wilting 24 hours (CWW)

	Fresh	W	CW	CWW	SEM	P
Dry matter (%)	26.3^{a}	19.4^{b}	20.1^{c}	40.2^{d}	0.20	0.001
HCN (mg/kg DM)	1427^{a}	1202^{ab}	1124^{b}	252^{c}	110	0.001
% reduction	100	16	21	82		

^{abcd} *Means without common superscript are different at P<0.05*

Although there were major differences in the HCN content of the cassava leaves (Table 4) as between washing with and without chopping and 24 hour wilting, this did not appear to affect the DM intake neither of the ensiled cassava root / rice bran mixture nor the cassava leaves (Table 5). The intake of cassava leaves accounted for over 30% of the total DM intake (Figure 2), resulting in intakes of HCN of 373 and 337 mg/day with washed and chopped / washed leaves, compared with 146mg/day for the wilted leaves. Reported toxic levels of HCN (mg/kg live weight) for pigs are 1.4 (Getter and Baine, 1938), 2.1 to 2.3 (Johnson and Ramond, 1965), 4.4 (Butler, 1973) and 3.5 (Tewe, 1995). In the present study the HCN intakes were much higher (from 6.0 to 15 mg/kg live weight) yet no signs of toxicity were observed.

Table 5 : Mean values for DM intake (g/kg live weight) in Mong Cai and F1 pigs fed ensiled cassava root and rice bran supplemented with fresh cassava leaves after washing, chopping and washing, and chopping, washing and wilting

	Ensiled cassava root + rice bran	Cassava leaves	Total DM
Mong Cai			
Chopped washed	16.7	10.0	26.7
Chopped washed wilted	21.2	10.6	31.8
Washed	25.3	9.5	34.8
F1 (Large White*Mong Cai)			
Chopped washed	17.2	9.9	27.1
Chopped washed wilted	21.3	11.4	32.6
Washed	16.5	10.3	26.8
Average of breeds			
Chopped washed	16.9	9.97	26.9
Chopped washed wilted	21.2	11.0	32.2
Washed	20.9	9.89	30.8
SEM	1.71	0.4	1.67
Prob.	0.15	0.13	0.074

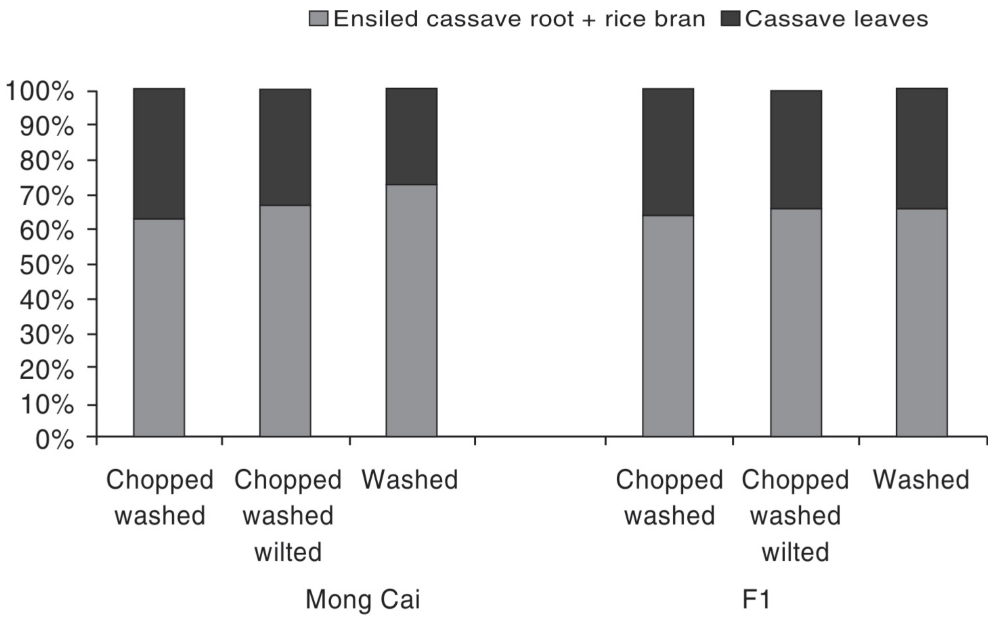

Figure 3. The proportions of cassava leaves and the ensiled cassava root/ rice bran mixture in the overall DM intake of Mong Cai and F1 pigs according to the processing of the leaves

The cassava leaves contributed 37 to 39% of the diet DM and from 71 to 74% of the total protein, providing an overall level of 15% crude protein in the diet DM (Table 6). These levels of dietary crude protein provided by fresh cassava leaves are much higher than has previously been reported in the literature (Bui Huy Nhu Phuc et al., 2001; Ly and Rodríguez, 2001). However, they are comparable with results of a recent experiment in Cambodia (Chhay Ty and Preston, 2005) in which fresh cassava leaves supplied 42% of the diet DM and 70% of the protein in a basal diet of broken rice fed to growing pigs.

Table 6: Mean values for intake of fresh cassava leaves in growing pigs according to the processing of the leaves (CW: chopped and washed), W: washed; CWW: Chopped, washed and wilted)

	CW	W	CWW	SEM	P
Intake of cassava leaves, g/day					
Fresh	1277	1228	687		
DM	248	247	277	13.56	0.22
Contribution of cassava leaves in the diet, %					
Of total DM	38.6	38.27	37.3	2.21	0.91
Of total protein	73.6	71.1	71.2	1.93	0.59
% crude protein in diet DM	14.8	14.7	14.5	0.49	0.91

Conclusions

- Pigs given restricted amounts (80% of observed voluntary intake) of a 2:1 mixture of ensiled cassava roots and rice bran, and free access to fresh cassava leaves, which had been washed, chopped and washed or wilted for 24 hours, consumed similar amounts of total DM (range of 27 to 32 g DM/kg live weight.
- Levels of HCN were reduced slightly (16%) by washing and substantially (82%) by wilting, resulting in intakes of HCN from 6.0 to 15 mg/kg live weight, levels considerably higher than previously reported safe levels to avoid toxicity (1.4 to 4.4 mg/kg live weight).
- The fresh cassava leaves were readily consumed providing38% of the dietary DM and over 70% of the dietary protein.

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Chapter 5

Ileal and total tract digestibility in growing pigs fed cassava root meal and rice bran with inclusion of cassava leaves, sweet potato vine, duckweed and stylosanthes foliage

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Abstract

Four F1 (Large White x Mong Cai), crossbred pigs, surgically fitted with post-valve T-caecum (PVTC) cannulas, were used in a 4*4 Latin square arrangement to determine the nutritive value of feeds in which the foliages from sweet potato, cassava, duckweed and stylosanthes provided 30% of the dry matter of the diet, the remainder of which was a mixture (50: 50) of cassava root meal and rice bran.

The diets with fresh water spinach or fresh cassava leaves had a higher apparent digestibility of organic matter in the total digestive tract, but not at the level of the ileum, when compared with the diets containing sweet potato vines or stylosanthes foliage. Apparent digestibility of crude protein was also higher on the diets with water spinach and cassava leaves but the differences were confounded by different concentrations of crude protein in the foliages, and were not significant when the digestibility coefficients were corrected by covariance for differences in protein content of the diets.

The total tract apparent digestibility of the crude fiber ranged from 44 to 51%, and did not differ among diets. On all the diets, a substantial proportion of the crude fiber (close to 40%) was digested pre-caecally.

Key words: Pigs, Sweet potato vines, Duckweed, Cassava leaves, Stylosanthes foliage, Digestibility, Ileal cannulas.

Introduction

In smallholdings, in remote areas in Central Vietnam, many farmers have not enough money to buy high quality protein sources such as fish meal and soybean meal. But farmers are able to produce plants of which the leaves can be relatively high in protein. Examples of these potential protein sources are the foliages from duckweed, cassava, sweet potato and stylosanthes.

Sweet potato (*Ipomoea batatas* L) is the third important crop in Vietnam after rice and maize and in 2001 occupied 245,000 ha (Statistical Yearbook, 2002). The leaves have a protein content ranging from 26 to 33% in the dry matter (DM) and have been used successfully as supplementary feed for different classes of livestock (Dominguez, 1992; Woolfe, 1992; Moat and Dryden, 1993; Ishida et al., 2000; Le Van An and Lindberg, 2004).

Duckweed (*Lemna* spp) is a small floating aquatic plant which grows on natural pond surfaces. They flourish in different climates and are fast growing and when adequately fertilized may contain up to 40% protein in DM (Porath et al., 1979; Bui Xuan Men, 1995; Skillicorn et al., 1993; Leng et al., 1995). Duckweed protein has a more balanced array of essential amino acidy, than most vegetable proteins and closely resembles animal protein according to Culley and Epps (1978).

Cassava (*Manihot esculenta)* is an annual crop widely grown in tropical countries. The leaves are rich in protein, carotene and minerals, and for this reason they are considered to be a potential source of animal feed in tropical countries (Gohl, 1993; Preston and Leng, 1987; Wanapat et al.,1997).

Stylosanthes guianensis (Stylo) is a legume that has been introduced in many tropical countries (Mannetje and Jones, 1992). It has been reported to produce between 12 and 17 tonnes of DM/ha/yr with 14 to 18 % crude protein in the DM (Satjipanon et al., 1995). According to Mannetje and Jones (1992), the DM digestibility of young plant material lies between 60 to 70%, but with increasing age and lignification this may be reduced to below 40%.

The objective of the research described in this paper was to determine the nutritive value of feeds for growing pigs in which the foliages from the above plants were used as the only protein source for supplementing low-protein energy sources derived from cassava root meal and rice bran.

Materials and methods

Animals and experimental design

Four F1 (Large White x Mong Cai), crossbred pigs (two castrated males and two intact females) with an average live weight of 60 ±1.7 kg were given four diets according to a 4 x 4 Latin Square design. The energy component of the diets was a mixture (1:1) of cassava root meal and rice bran. The four test diets contained the energy component plus dried meals of either sweet potato vine (SPV), duckweed (DW), cassava leaves (CL) or Stylo foliage (SV), all of which were included at the level of 30% of total diet DM. The pigs had been vaccinated against pasteurellosis and hog cholera, and surgically fitted with post-valve T-caecum (PVTC) cannulas to allow collection of ileal digesta (Van Leeuwen et al., 1991).

The four experimental diets were introduced to the pigs two weeks post-surgery. The experimental periods were for 14 d, comprising 5 d for adaptation to each diet followed by 4 d of collection of faeces, 1 d of collection of ileal digesta, 1 d of rest and finally a second day of collection of ileal digesta.

The pigs were housed individually in pens which had a floor area of 3 m^2. During digesta collection the pigs were restricted to a limited space within the pen.

Diets and feeding system

Sweet potato vines (SPV) were harvested 70 days after planting, were chopped into small pieces (1-2 cm long) and then spread out on a concrete floor overnight for wilting to reduce the moisture content. They were then dried in an oven at 45°C for about 12 h. The same procedure was followed for cassava leaves (which were collected at the time of harvesting the roots), for duckweed (collected from natural ponds) and stylo foliage (harvested 60 days after planting). The dried materials from the four sources were then milled through a 1 mm screen and stored in a dry environment.

Chromium oxide was included in the diets as an indigestible marker. All diets were supplemented with a standard mixture of vitamin, minerals and trace elements formulated according to the requirements proposed by NRC (1988).

The test diets were offered to the pigs in two meals daily at 06:00 and 18:00 h). Water was freely available from nipple drinkers.

Sample collection

The samples of ileal digesta were collected for one hour every second hour during a 12 h collection period, using a soft plastic tube connected to the ileal cannula. For each experimental period there were thus 12 samples which were weighed, homogenized and immediately frozen (-18ºC) after collection. At the end of each collection period, the samples were thawed, homogenized, sub-sampled and dried at 60ºC. Faeces were collected daily and stored at 4ºC. At the end of each experimental period the samples were polled and mixed. Sub-samples were taken and dried at 60ºC. The dried samples of ileal digesta and faeces were milled through a 1 mm screen prior to chemical analysis.

Chemical analysis

The chemical composition was determined according to the methods of AOAC (1984). Dry matter (DM) content was measured by drying at 105ºC for 24 h. Nitrogen was determined by the Kjeldahl method and crude protein expressed as N*6.25. For faeces and ileal digesta the nitrogen determination was done on the fresh samples. Ether extract (EE) was determined by Soxhlet extraction. Ash was the residue after ashing the samples at 600ºC. Chromium oxide in feed, faeces and digesta was determined according to the method of Fenton and Fenton (1979).

Statistical analysis

Analysis of variance was performed according to a 4 x 4 Latin Square design using the General Linear Model (GLM) procedure of the Minitab Software (Version 12) (Minitab 1998). Sources of variation were treatment, pigs, periods and error.

Results

There were major differences in the chemical composition of the four protein sources (Table 1). Crude protein was highest in duckweed, followed by cassava leaves with lowest values for sweet potato vines and stylo foliage. Crude fibre was highest in stylo and lowest in duckweed and cassava leaves with intermediate values for sweet potato vines.

Table 1. Chemical composition of feed ingredients (% in DM)

	Dry matter (%)	Crude protein	Crude fat	Crude fiber	Ash
Rice bran	90	11.3	9.7	7.8	8.5
Cassava root	89	2.3	2.1	3.1	2.3
Duckweed	92	31.7	3.2	8.7	3.8
Sweet potato vine	84	17.0	5.4	14.2	5.6
Stylo foliage	87	17.3	2.5	20.5	5.5
Cassava leaves	85	26.5	4.7	8.5	3.4

The composition of the test diets (Table 2) reflected the differences in the composition of the protein sources, with lowest levels of crude protein and highest levels of crude fiber on the Stylo diet, with exactly the opposite trend for the DW diet.

Table 2. Proportions of ingredients and chemical composition of the diets

	DW	CL	SPV	Stylo
Ingredients, % DM basis				
Rice bran	34	34	34	34
Cassava root	34	34	34	34
Sweet potato vine	0	0	30	0
Duckweed	30	0	0	0
Cassava leaves	0	30	0	0
Stylo foliage	0	0	0	30
Mineral-vitamin mix	1.6	1.6	1.6	1.6
Chromic oxide	0.4	0.4	0.4	0.4
#Chemical composition, % in DM				
Organic matter	95	95	94.7	94.7
Crude protein	14.0	13.3	8.18	8.42
Crude fat	5	5.4	5.6	4.8
Crude fiber	5.85	6.23	7.8	9.58
Ash	4.8	4.7	5.3	5.3

Derived from recorded intakes and chemical analyses of samples

The intakes of crude protein also reflected the composition of the four sources of supplementary protein, with highest values for the diet containing duckweed followed by the diet with cassava leaves and with lowest values for the stylo diet (Table 3). DM intakes were relatively low on all diets when expressed as a function of live weight (range from 2.2 to 2.5 g/kg live weight).

There were no differences among diets in ileal apparent digestibility of organic matter. The values for crude protein apparent digestibility mirrored those for crude protein intake with highest values for the duckweed diet and lowest values for the stylo diet. These differences can be explained by the decreasing proportion of endogenous N in the faeces when dietary intake of crude protein increases. Adjusting the apparent digestibility coefficients by covariance using dietary crude protein concentration as the covariate reduced the differences to the point where they were no longer significant (P=0.08). As expected the valued of apparent digestibility of the crude fiber at the level of the ileum were low for all diets with no differences among them. Total tract apparent digestibility of crude fibre was higher for the duckweed and cassava leaf diets than for those with sweet potato vines or stylo foliage. Surprisingly, this difference appeared to reflect a greater proportion of the diet organic matter being digested in the large intestine and caecum in the case of the duckweed and cassava leaf diets, compared with those containing sweet potato vines or stylo foliage. On all the diets the greater part of the digestion of the crude fiber took place post-ileum., with no differences among the diets. Diet crude fiber content was negatively related with total tract digestibility of OM (Figure 2) but not with ileal digestibility of OM (Figure 1). There were indications (P = 0.19 and 0.12) that ileal and total tract digestibility of crude protein was negatively related to the concentration of crude fibre in the diet (Figures 3 and 4). In contrast, ileal and total tract digestibility coefficients for organic matter and crude protein were positively correlated with the crude protein of the diet (Figures 5, 6, 7 and 8).

Table 3. Mean values for feed intake and apparent ileal and total tract digestibility of the experimental diets

	DW	CL	SPV	Stylo	SEM	Prob.
Mean daily intake, g/d						
Dry matter	1373	1177	1583	1422	124	0.24
Crude protein	189	157	129	120	15.4	0.068
Crude fibre	87.3	85.2	103	132	10.3	0.057
Crude protein, g/kg DM	140[a]	133[a]	81.8[b]	84.2[b]	7.9	0.003
Ileal apparent digestibility, %						
Organic matter	81.7	80.9	81.2	79.6	0.52	0.08
Crude protein	73.2[d]	69[c]	65.7[b]	60.7[a]	1.13	0.004
Crude protein#	70.4	66.9	68.3	63	2.3	0.08
Crude fat	59.8[c]	55.9[bc]	52.5[ab]	49.5[a]	1.73	0.01
Crude fiber	21.1	18.3	18.1	17.1	1.2	0.16
Total tract apparent digestibility, %						
Organic matter	87.9[b]	88.4[b]	84.8[a]	83.8[a]	0.63	0.001
Crude protein	75.7[c]	72.8[c]	68.7[b]	63.6[a]	1.1	0.004
Crude protein#	73	70.8	71.1	65.8	2.4	0.12
Crude fat	65.1[a]	62.2[a]	58.7[b]	54.3[b]	1.78	0.01
Crude fiber	49.2[a]	50.76[a]	46.4[b]	43.9[b]	1.47	0.03
Proportion of total tract apparent digestibility that occurred post-ileum, %						
Organic matter	7.05	8.54	4.25	5.01		
Crude protein	3.30	5.22	4.37	4.56		
Crude fat	8.14	8.54	4.25	5.01		
Crude fiber	57.1	63.9	61.0	61.0		

 # Corrected by covariance for differences in the crude protein content of the diets
[a bc] *Means with different superscripts within rows are different at P<0.05*

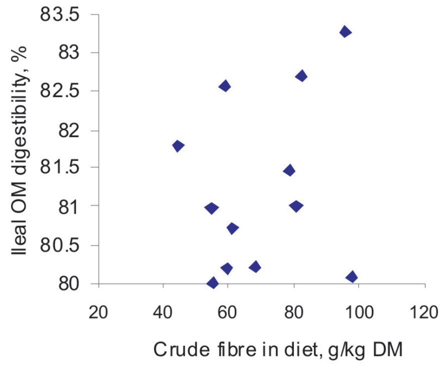

Figure 1: Relationship between crude fiber content of the diet and ileal digestibility of organic matter

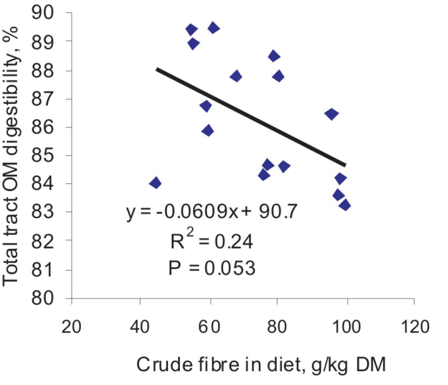

Figure 2: Relationship between crude fiber content of the diet and total tract digestibility of organic matter

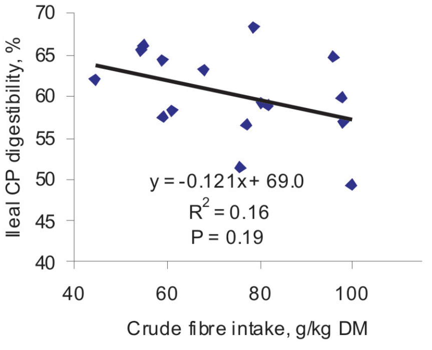

Figure 3: Relationship between crude fiber content of the diet and ileal digestibility of crude protein

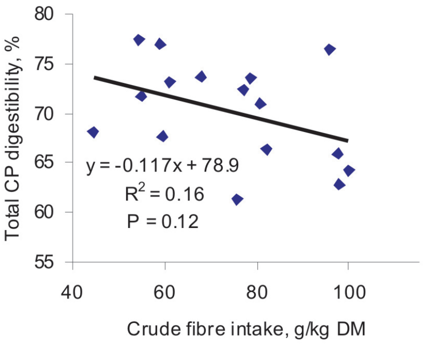

Figure 4: Relationship between crude fiber content of the diet and total tract digestibility of crude protein

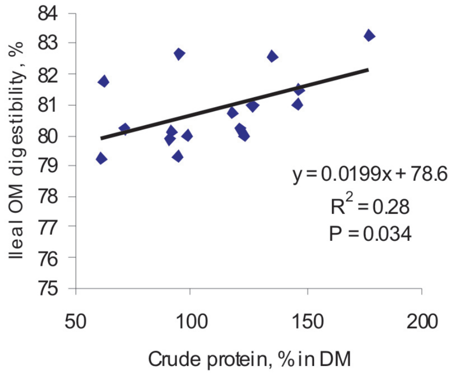

Figure 5: Relationship between crude protein content of the diet and ileal digestibility of organic matter

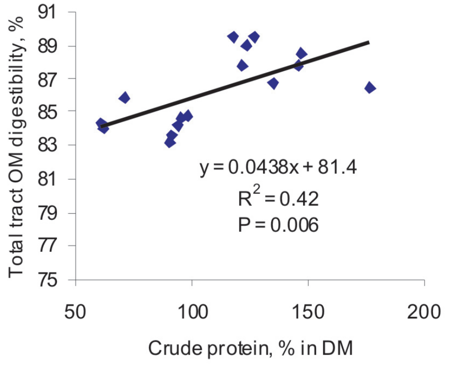

Figure 6: Relationship between crude protein content of the diet and total tract digestibility of organic matter

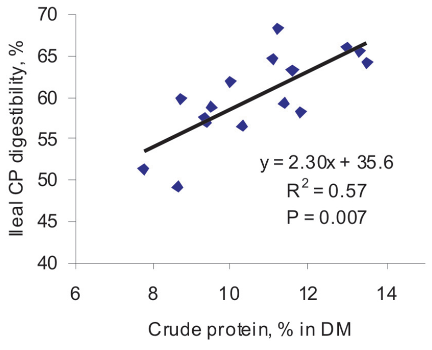

Figure 7: Relationship between crude protein content of the diet and ileal digestibility of crude protein

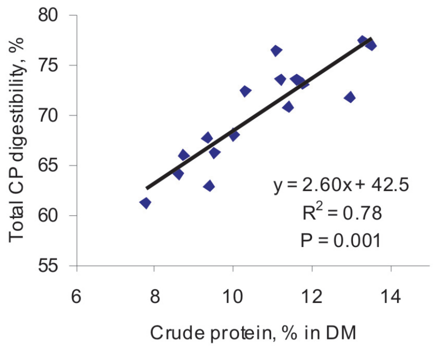

Figure 8: Relationship between crude protein content of the diet and total tract digestibility of crude protein

Discussion

Reports in the literature (Low, 1982; Gramham et al., 1986; Graham and Aman, 1987a,b) show conclusively that addition of fiber to the diet leads to lower apparent ileal digestibility of starch, crude protein, fat and minerals. According to Sauer and Ozimek (1986), the level and source of dietary fiber are the two most important factors influencing the amount of endogenous nitrogen and amino acids present in the ileal digesta. In the present experiment, comparisons among the protein sources were confounded by the major differences in intake of crude protein and crude fibre, which resulted from fixing the inclusion rate at 30% of the total diet DM for all the protein sources. It is therefore difficult to decide whether the better results for duckweed and cassava were due to the lower content of fibre or the higher content of crude protein, as both these elements were (crude protein), or tended to be (crude fiber), correlated with ileal and total tract digestibility of organic matter and crude protein.

The total tract digestibility of the crude fibre ranged from 44 to 51%, and did not differ among diets. Phuc and Lindberg (2000) reported similar values in pigs fed diets with high levels of cassava leaves, groundnut foliage and leucaena leaves.

On all the diets, a substantial proportion of the crude fiber (~40%) was digested pre-caecally, which is in agreement with earlier reports on feeding lucerne, red clover, white clover and perennial ryegrass to pigs (Andersson and Lindberg, 1997a, b). The absence of differences in crude fiber digestibility among the diets, at the level of both the ileum and the total tract, indicates that the nature of the fibrous component in each of the protein sources was similar. It can be concluded therefore that in the case of the four foliar sources of protein used in this experiment, that it was the quantity of fibre, rather than the quality, which contributed to the differences in digestibility of organic matter and crude protein among the four diets.

The crude protein content of the foliages, and therefore of the diets, appears to be the factor that explains most of the differences in ileal and total tract digestibility of organic matter and crude protein. Thus when the digestibility coefficients of crude protein, both at the ileum and for the total tract, were adjusted for differences in the crude protein content of the diets, the differences among the forage sources were no longer significant (Table 3). Bounhong Norachack et al. (2004) found no differences in digestibility of DM and crude protein in pigs fed different proportions of stylo foliage and fresh cassava leaves supplying from 20 to 30% of the DM of a diet based on broken rice. However, in their experiment, N retention was almost three times higher when cassava leaves rather than stylo foliage were the major protein source.

Conclusions

- Inclusion of fresh duckweed or fresh cassava leaves, as the only source of supplementary protein in diets for growing pigs based on cassava root meal and rice bran, increased apparent digestibility of organic matter in the total digestive tract but not at the level of the ileum, when compared with inclusion of similar quantities either sweet potato vines or stylo foliage.
- Apparent digestibility of crude protein was higher on the diets with duckweed and cassava leaves but the differences were confounded by different concentrations of crude protein in the foliages, and were not significant when the digestibility coefficients were corrected by covariance for differences in protein content of the diets.

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Chapter 6

Effect of supplementary DL-methionine in pig diets with cassava leaves as a major protein source

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Abstract

A feeding trial was conducted to evaluate the effect of different levels of DL-methionine supplementation on growth performance of pigs given diets with cassava leaves and cassava root products providing 20 and 30% of the diet dry matter (DM), respectively. Twenty eight crossbred (Large White x Mong Cai) pigs with an average initial weight of 27.2 ± 1.7 kg were housed in individual pens and allocated to 7 treatments in a completely randomized design. Six treatments were arranged as a 2*3 factorial design; the factors being fresh (FCL) or ensiled (RCL) cassava leaves and 0, 0.1 or 0.2% supplementary DL-methionine. The seventh treatment was the basal (control) diet which contained (% in DM) rice bran 29, maize 23, ensiled cassava root 20, cassava root meal 10 and fish meal 15. The 6 diets in the factorial arrangement were prepared by replacing 6 percentage units of fish meal and 14 percentage units of rice bran with either fresh or ensiled cassava leaves (20% in the diet DM), plus the indicated levels of DL-methionine. The experiment lasted 80 days.

Growth rates were higher, and DM and crude protein conversion rates tended to be better, for fresh compared with ensiled cassava leaves. Intakes of DM and crude protein were not affected by processing of the cassava leaves, nor by level

of supplementary DL-methionine. Live weight gains and DM conversion improved linearly with level of supplementary DL-methionine. At slaughter, the weights of the liver and thyroids, the loin eye area and estimated lean meat yield were increased on fresh compared with ensiled leaves. Estimated yield of lean meat increased and back fat decreased as the level of supplementary methionine was increased.

There were no differences in growth performance between the best diet from the cassava leaves-methionine treatments (FCL + 0.2% DL-methionine) and the control diet. Back fat thickness was less and estimated yield of lean meat was greater on the former diet.

Keywords: Cassava leaves, DL-methionine, ensiled, fresh, growth performance, pigs

Introduction

In Vietnam, pig production plays a very important role at the household and at national level. Currently the pig population in Vietnam is estimated at 26 million animals (General Statistical Office, 2004). The majority of the pigs for slaughter are produced on small scale farms. However, under village conditions the use of protein supplements is limited because of the high cost. The animals are mainly fed with cheap local materials and unconventional feed resources in order to minimize the costs. Generally, conventional protein supplements such as soybean and fish meal are not used because they are expensive. This situation has given rise to a range of research reports concerning the feasibility of using protein-rich forages as a means of improving the nutritive value of traditional pig diets (Bui Nhu Phuc, 2006; Preston, 2006). Particular attention has been given to the use of cassava leaves as a protein source as these are widely available in Vietnam at farm level. The availability of this resource has increased with the changing role of cassava from being a “food crop” to an “industrial crop” for starch processing and the animal feed industry. At the present time about 330 000 ha are planted with cassava with a total yield of the order of 4 million tonnes of roots. The total cassava starch production in Vietnam is estimated at about 500,000 tonnes, 70% of which is for export (Hoang Kim et al., 2000).
Cassava is traditionally grown for the production of roots. However, the leaves have become increasingly important as a source of protein for monogastric and ruminant animals (Preston, 2001; Wanapat, 2001). According to Bui Huy Dap (1987), the average yield of cassava leaves in Vietnam is of the order of 7 to 12 tonnes/ha/year, containing from 500 to 1400 kg of crude protein. Cassava leaves are rich in protein but they are low in sulphur amino acids (Gomez et al., 1985). The leaf protein is reported to be limiting in methionine and tryptophan but rich in lysine, with an

overall biological value of 49 to 57% (Frochlich et al., 2001). By the addition of synthetic methionine, the biological value of the protein could be increased to 80% according to Roger and Milner (1963) and Eggum (1970). The advantages of adding methionine and cystine to pig diets based on cassava root have been demonstrated by Maner and Gomez (1973).

Materials and methods

Animals and management

Twenty-eight crossbred pigs (Mong Cai female x Large White male) aged about 80 days and with mean body weight of 27.2 ± 1.7 kg (mean ± SD).were housed in individual cages. They were vaccinated against hog cholera and pasteurellosis, and de-wormed 2 weeks before being randomly allocated to seven dietary treatments.

Experimental design, diets and feeding

The control diet consisted of rice bran, maize, cassava root meal, ensiled cassava root and was supplemented with fish meal (FM) as the sole source of supplementary protein. The cassava root meal and ensiled cassava root provided 50% of the total diet dry matter (DM). The six test diets were formulated by replacing part of the fish meal of the control diet with either fresh or ensiled cassava leaves supplemented with zero, 0.1 or 0.2% DL-methionine (Table 1).

The cassava leaves were from a highly productive variety that is common in central Vietnam. They were chopped and wilted under a roof (fresh leaves) or ensiled for 90 days with 5 % molasses as described by Du Thanh Hang (1998). The cassava roots were ensiled with 0.5% common salt (Nguyen Thi Loc et al., 1996) and stored 21 days before feeding.

The diets were fed in amounts that led to minimum refusals with the cassava leaves given as a separate feed. Feeding times were 8:00, 10:00 and 15:00 h for the cassava leaves and 6:00, 12:00 and 17:00 h for the other components of the diets. The refusals were collected before each new meal was offered. Drinking water was freely available.

Table 1: Ingredients in the experimental diets (% DM basis)

	Control	ECL	ECL-0.1M	ECL-0.2M	FCL	FCL-0.1M	FCL-0.2M
Rice bran	29.0	13.5	13.5	13.5	13.4	13.4	12.0
Maize	25.0	23.0	22.9	22.8	25.0	23.0	23.0
ECR	20.0	20.0	20.0	20.0	20.0	20.0	20.0
CRM	10.0	12.5	12.5	12.5	9.7	11.6	12.9
Fish meal	15.0	9.0	9.0	9.0	9.0	9.0	9.0
FCL	0	0	0	0	20.0	20.0	20.0
ECL	0	20.0	20.0	20.0	0	0	0
Premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-methionine	0	0	0.1	0.2	0	0.1	0.2
Soybean oil	0	1.0	1.0	1.0	1.9	1.9	1.9

FCL: Fresh cassava leaves; ECL: Ensiled cassava leaves; CRM: Cassava root meal; ECR Ensiled cassava roots

Table 2: Chemical composition of the experimental diets (% in DM)

	Control	ECL	ECL-0.1M	ECL-0.2M	FCL	FCL-0.1M	FCL-0.2M
Crude protein	14.2	14.4	14.5	14.5	14.7	14.7	14.6
Lipids	7.6	7.5	7.5	7.5	7.6	7.5	7.3
Crude fiber	3.8	6	6	6	5.9	5.9	5.8
Ash	6.9	6.3	6.3	6.3	6.4	6.4	6.3
Ca	0.63	0.6	0.6	0.6	0.68	0.68	0.68
P	0.63	0.51	0.51	0.51	0.53	0.52	0.51
ME, MJ/kg#	13.8	13.5	13.4	13.4	14.0	14.0	14.0
Lysine#	0.68	0.72	0.72	0.72	0.74	0.74	0.73
Methionine#	0.27	0.27	0.37	0.47	0.29	0.39	0.48
Cystine#	0.21	0.18	0.18	0.18	0.2	0.2	0.19
HCN (mg/kg DM)	27.8	72.7	72.7	72.7	97.2	98.5	99.4

 # Estimated from literature values

Measurements

Feed intake and live weight

Records were kept of amounts of feeds offered and refused. Live weights were taken every 20 days and growth rate calculated from the slope of the linear regression of weight against days on experiment.

Carcass measurements

For the evaluation of carcass traits, 21 representative pigs (3/treatment) were starved for 24 hours and weighed prior to slaughter. Carcass traits were measured immediately after slaughter. The P2 back fat thickness was measured on the partitioned carcass 10 cm from the midline behind the 10th rib using a trace paper and a ruler; the loin area was measured by tracer paper.

Chemical analyses

Samples of feed and refusals were dried at 60 ^{o}C for 24 h and ground through a 1 mm sieve prior to chemical analysis according to the standard methods of AOAC (1984). Dry matter (DM) was measured by drying fresh samples at 105 ^{o}C for 24 h. Crude protein was determined on fresh samples by the Kjeldahl method (N* 6.25). Ether extract (EE) was determined by Soxhlet extraction. Ash was the residue after ashing the samples at 600 ^{o}C. Calcium and phosphorous were determined according to AOAC (1984) using the dry method and alkalimetric ammonium molybdophosphate method, respectively.

Statistical analysis

Analysis of variance was performed using the general linear model (GLM) procedure of Minitab Version 14. Two models were evaluated. The effects of processing of leaves of leaves (fresh versus ensiled) and levels of methionine (0, 0.1 and 0.2%) were analysed as a 2*3 factorial arrangement, the terms in the model being processing of cassava leaves, levels of methionine, the interaction processing of leaves*methionine and residual error. The second model compared the best treatment from the six treatments in the 2*3 factorial component of the experiment with the control. The terms in this model were diet and error. When the data indicated linear relationships between performance traits and inputs (eg: levels of supplementary methionine), regression analysis was applied using the regression function in the Minitab software.

Results

Growth and feed conversion

Main effects of processing of cassava leaves and supplementation with methionine

There were significant effects on pig growth performance and carcass traits due to both cassava leaf processing and DL-methionine supplementation.
The interactions between the two factors were not significant, therefore the results are presented only for the main effects (Tables 3 and 5).

Growth rates were higher, and DM and crude protein conversion rates tended (P=0.1 and 0.18) to be better, for fresh compared with ensiled cassava leaves (Table 3). Intakes of DM and crude protein were not affected by processing of the cassava leaves, or by level of supplementary DL-methionine. Live weight gains and DM conversion improved linearly with level of supplementary DL-methionine (Figures 1, 2 and 3).

Table 3: Mean values for main effects of ensiled versus fresh cassava leaves and levels of supplementary methionine (M)

	Ensiled	Fresh	SEM	P	0.0M	0.1M	0.2M	SEM	P
Live weight, kg									
Initial	26.8	27.6	0.93		27.2	27.4	27.0	1.1	
Final	72.6	78.5	1.2	0.004	69.6^{a}	74.3^{b}	82.7^{c}	1.2	0.001
Daily gain	0.578	0.657	0.010	0.001	0.540^{a}	0.599^{b}	0.712^{c}	0.012	0.001
Feed intake, g/day									
Crude protein	284	299	8.3	0.21	297	291	286	10	0.74
DM	1.97	2.05	0.057	0.36	2.04	2.01	1.97	0.070	0.79
Feed conversion									
Crude protein	0.496	0.466	0.015	0.18	0.552^{a}	0.487^{b}	0.405^{c}	0.019	0.001
DM	3.45	3.19	0.11	0.10	3.79^{a}	3.37^{b}	2.79^{c}	0.13	0.001

^{abc} *Means without common superscript are different at P<0.05*

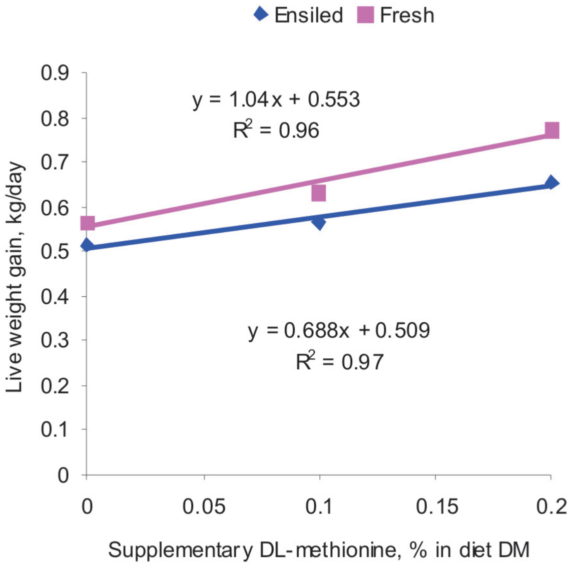

Figure 1. Relationship between live weight gain and level of supplementaryDL-methionine for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves

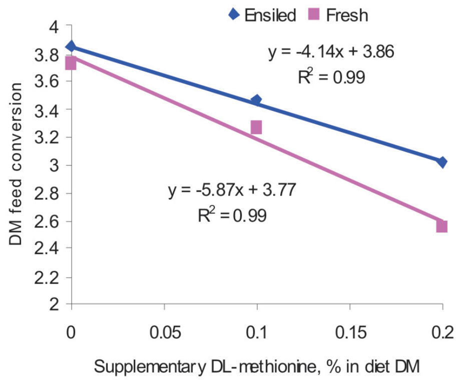

Figure 2. Relationship between DM feed conversion and level of supplementary DL-methionine for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves

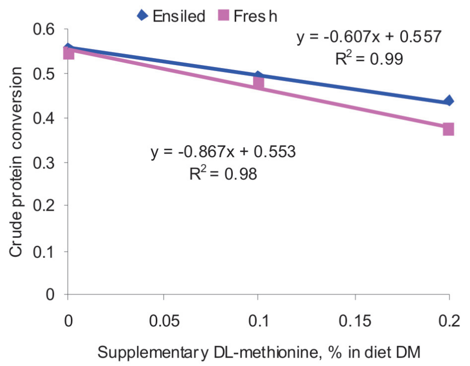

Figure 3. Relationship between crude protein conversion and level of supplementary DL-methionine for pigs fed diets based on cassava roots and fresh or ensiled cassava leaves

Comparison with control diet

There were no differences in performance traits between the control and the best of the treatments with cassava leaves and supplementary methionine (Table 4; Figures 4, 5 and 6).

Table 4: Mean values for growth performance traits of pigs fed the best cassava leaves-methionine treatment (FCL-0.2M) and the control

	Control	**FCL-0.2M**	**SEM**	**P**
Live weight (kg/day)				
Initial	26.9	27.3	0.802	
Final	85.1	86.8	0.865	0.232
Daily gain	0.749	0.772	0.0131	0.27
Feed intake				
DM (kg/day)	2.04	1.97	0.093	0.591
Crude protein (g)	298	287	13	0.59
Conversion (kg)				
DM	2.73	2.56	0.141	0.434
Crude protein	0.397	0.373	0.0205	0.436

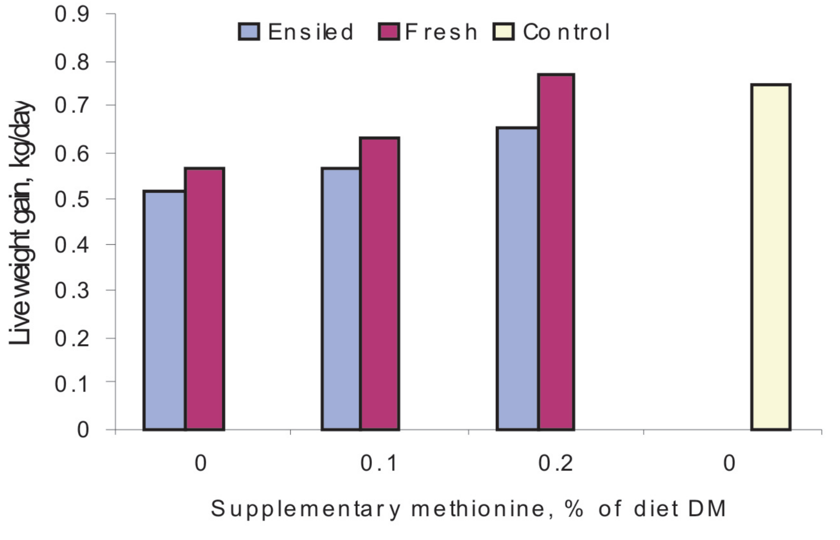

Figure 4. Mean values for growth rate of pigs fed a control diet or the control diet with fresh or ensiled cassava leaves as partial replacement for fish meal each with 3 levels of supplementary methionine

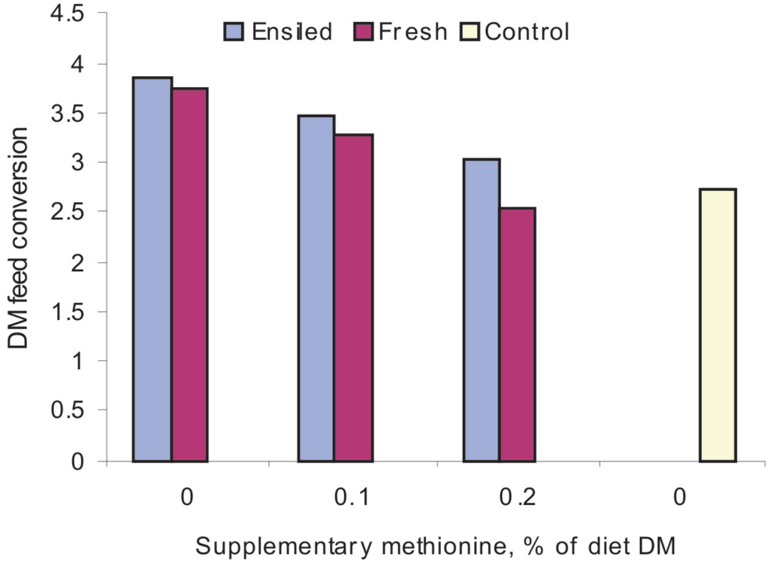

Figure 5. Mean values for DM feed conversion of pigs fed a control diet or the control diet with fresh or ensiled cassava leaves as partial replacement for fish meal each with 3 levels of supplementary methionine

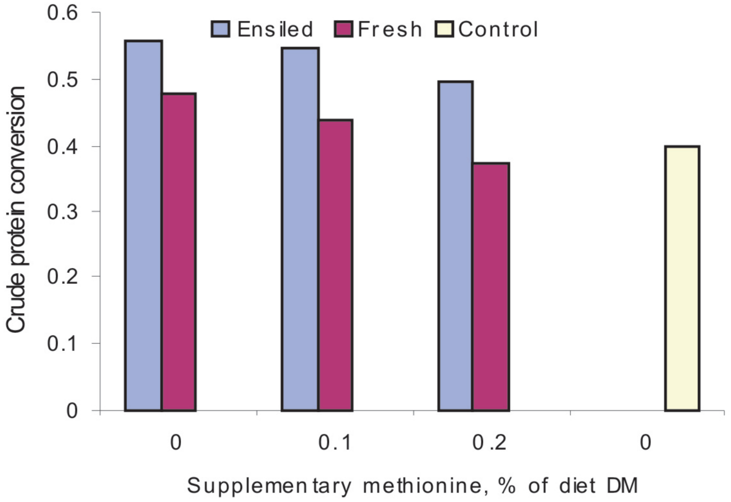

Figure 6. Mean values for crude protein conversion for pigs fed a control diet or the control diet with fresh or ensiled cassava leaves as partial replacement for fish meal each with 3 levels of supplementary methionine

Carcass traits

Main effects of processing of cassava leaves and supplementation with methionine

The only traits affected by processing of the cassava leaves were the weights of the liver and thyroids, the loin eye area and estimated lean meat yield all of which were increased on fresh compared with ensiled leaves (Table 5). There was a suggestion that the liver and thyroids were lighter when DL-methionine was supplemented to the diets (P=0.06 and 0.03). Back fat decreased as the level of supplementary methionine was increased.

Table 5: Mean values for main effects of ensiled versus fresh cassava leaves and levels of supplementary methionine (M) on carcass traits (adjusted by covariance for differences in slaughter live weight)

	Ensiled	Fresh	SEM	P	0.0M	0.1M	0.2M	SEM	P
Carcass									
Hot weight, kg	59.8	60.4	0.442	0.370	59.7	60.2	60.5	0.686	0.280
Trimmed, kg	54.2	54.8	0.472	0.380	53.8	54.5	55.1	0.650	0.570
Length, cm	88	89.11	0.930	0.416	90.3	87.3	88.1	0.135	0.294
Liver, kg	1.39	1.61	0.025	0.006	1.59	1.46	1.46	0.035	0.060
Loin area, cm^2	27.1	28.0	0.221	0.012	27.8	27.2	27.6	0.300	0.350
Back fat, cm	2.99	2.88	0.057	0.196	3.24[a]	2.90[b]	2.66[b]	0.080	0.007
Thyroid, g	32.2	34.6	0.688	0.031	35.6[a]	31.5[b]	33.2[b]	0.900	0.030
Intestines, cm									
Small	15.0	14.9	0.358	0.813	15.7	14.4	14.7	0.500	0.229
Large	5.21	4.85	0.097	0.022	4.97	5.21	4.91	0.114	0.224

[ab] *Means without common superscript are different at P<0.05*

Comparison with control diet

The only differences in carcass traits between the control diet and the best of the cassava leaves – methionine diets was in back fat thickness, which favoured the experimental diet of fresh cassava leaves with 0.2% supplementary DL-methionine (Table 5).

Table 6: Mean values for carcass traits of pigs fed the best cassava leaves-methionine treatment (FCL-0.2M) and the control

	Control	FCL-0.2M	SEM	P
Carcass				
Hot weight, kg	67.0	65.8	0.43	0.162
Trimmed, kg	60.4	60.3	0.76	0.901
Length, cm	86.9	90.4	0.03	0.296
Liver, kg	1.49	1.62	0.039	0.131
Loin area, cm^2	28.3	28.7	0.30	0.509
Back fat. cm	3.70	2.86	0.070	0.005
Thyroid, g	35.0	35.8	0.32	0.145
Intestines, cm				
Small	12.8	15.7	0.96	0.235
Large	3.98	5.02	0.53	0.333

Discussion

The noteworthy features of the results are: (i) the better growth performance of the pigs when cassava leaves were fed fresh rather than ensiled; and (ii) the marked improvement in growth rate (32%) and feed conversion (25%) due to DL-methionine supplementation, the response being similar for the diets with either fresh or ensiled cassava leaves.

Most of the earlier experiments with cassava leaves in pig diets was focused on the need to reduce the risk of toxicity from hydrocyanic acid, the precursors of which (cyanogenic glucosides) are present in high concentrations in the fresh leaves of most cassava varieties. Both ensiling and, to a greater extent, sun-drying have been shown to reduce HCN levels dramatically (Bui Nhu Phuc, 2006). The surprising result in the present study was the better performance on the fresh leaves compared with the ensiled leaves. As far as the authors are aware, there have been no direct comparisons between fresh and ensiled leaves in the same experiment. Chhay Ty and Preston (2005) fed fresh cassava leaves to young pigs at 50% of the DM of the diet, the energy component of which was broken rice, and reported no signs of HCN toxicity even though the levels of HCN (7.4 mg/kg live weight) exceeded what had been considered to be the thresh-hold level (3.5 mg/kg live weight) for toxicity (Tewe, 1992). Du Thanh Hang et al. (2006) showed that DM intake was not affected by the processing method (washing chopping or wilting of the leaves), when pigs were fed diets with 30% cassava root products and 20% cassava leaves (DM basis). Intakes of HCN (from 6 to 15 mg/kg live weight) were considerably above the recommended threshold for toxicity of 3.5 mg/kg live weight (Tewe, 1995). In the present experiment HCN intakes were calculated to be 2.8 and 4.0 mg/kg live weight for the ECL and FCL diets, respectively, and were not related to growth performance.

A possible explanation for the poorer growth performance on ensiled versus fresh leaves could be the negative effects of the ensiling process on the biological value of the cassava protein. The fermentation taking place in ensiled forages converts soluble carbohydrates to organic acids and also promotes proteolysis (McDonald, 1981). Oshima and McDonald (1978) reported that, in general, of the total N (TN) in fresh forages, 75 to 90% would be protein N (PN), and the remainder non-protein N (NPN) After ensiling, NPN may account for as much as 80% of TN according to Papadopoulos and McKersie (1983) and Albrecht and Muck (1991). Owens et al. (1999) found that when alfalfa and red clover were ensiled the NPN content increased from 136 to 353 (g/kg total N) in red clover and from 165 to 625 g/kg total N in alfalfa. There appear to be no comparable data on the ensiling of cassava leaves but it is probable there is some loss in protein quality during the ensiling process.

Positive effects of supplementary DL-methionine on growth performance of pigs fed cassava root meal were first reported by Maner and Gomez (1973). The use of cassava leaves together with cassava root products as the basis of pig diets is a more recent development. Nguyen Thi Hoa Ly (2006) observed a 23% increase in growth rate in pigs when DL-methionine was added at 0.15% of the DM of diets with 17 to 25% ensiled cassava roots and 15% ensiled cassava leaves (DM basis). By contrast, in the present study the growth rate increase was 32% and the levels of cassava roots and leaves were 30 and 20%, respectively. In the experiment of Nguyen Thi Hoa Ly (2006) synthetic lysine was also added and was thus confounded with the level of methionine. Addition of 0.3% DL-methionine to a diet with 50% of the DM as fresh cassava leaves had no effect on growth rate or feed conversion of pigs fed a basal diet of broken rice (Chhay Ty et al., 2005). In this case the contrast with the present study was in the basal diet which did not contain cassava root products. From a theoretical standpoint, it is to be expected that there would be nutritional benefits from DL-methionine supplementation of pig diets rich in cassava leaves because of the relative deficiency of the sulphur amino acids in cassava leaf protein and the fact that a source of sulphur is required for the detoxification of HCN.

There is need for further research on the relative effects of fresh versus ensiled cassava leaves as major protein sources in pig diets as well as the potential benefits to be gained from supplementation with DL-methionine.

Acknowledgements

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Chapter 7

General discussion

In Central Vietnam, cassava is the third crop after rice and maize, with the products being used for human consumption and animal production. The by-products are also important feed resources for animals.

Pig production is usually based on locally available feed resources of which, cassava root meal, rice bran and maize are most important. These feeds are high in energy but low in protein. Small scale farmers rarely use commercial protein sources such as fishmeal and soybean meal, because these are expensive. The question is how to improve the nutritional quality of the pig diets in small-scale farmer households in Central Vietnam using available feed sources. One option that has received much attention recently is the use of the leaves of the cassava plant (Bui Huy Nhu Phuc et al ., 2001).

Cassava nutritive value and HCN toxicity

There has been much research to determine the nutritive value of cassava roots and leaves and ways to reduce the potential toxicity from hydrocyanic acid (HCN). The first study in Vietnam on use of cassava leaves as animal feed was reported in the North by Bui van Chinh et al. (1992, 2001), and in the South by Bui Huy Nhu Phuc et al. (2001).

Rogers (1959) observed that leaves from 11-12 months cassava plants contained from 20.6 to 30.4% crude protein in dry matter (DM). Rogers and Milner (1963) later reported that the protein content of manioc (cassava) leaves in Brazil varied from 17.8 to 34.8% in DM and from 18.5 to 32.4% in DM for Jamaican varieties. With regard to HCN toxicity, there are reports of levels from 200 to 800 mg HCN/kg fresh leaves (Wood, 1965) and as high as 4000 mg/kg fresh leaves (Ravindran and Ravindram, 1988). Ravindran (1995) considered that the wide variability in levels of crude protein and HCN could be due to differences in cultivars, stage of maturity, and soil fertility.

In the present study, the leaves from twenty cassava varieties, taken from the upper part of the plant at the time of root harvesting had from 23.7 to 29.5 % crude protein in DM and from 610 to 1840 mg/kg of DM of HCN. These values were lower than was reported by Ravindran (1995) (16.7 – 39.9%) for crude protein but similar to that described by Bui Huy Nhu Phuc et al. (2001) for cassava varieties in the South of Vietnam (21 to 34%). In this study, the environmental conditions for growth of the different cassava varieties were similar. So the wide variation observed in HCN levels may well be genetic in origin.

Methods of processing to reduce levels of HCN cyanide in cassava include: cooking, drying, ensiling, soaking and de-watering. The main purpose of the present study was to identify the best method, in terms of simplicity and being easy to apply by small-holder farmers in Central Vietnam.

Ensiling

Ensiling is one of the methods recommended for preserving and processing green forages (McDonald et al., 1991). In Central Vietnam, this method is especially appropriate for use in the rainy season. In the experiment reported in Chapter 2, cassava leaves were ensiled with molasses or rice bran at concentrations of 5 or 10% (molasses) and 10 or 15 % (rice bran) (all on fresh basis). The pH of the ensiled leaves declined from 7.1 to 4.0 within 7 days of initiating the ensiling process. After 31 days the pH was 3.5. After 7 days of ensiling, the HCN in the leaves had decreased from 177 mg/kg DM to 80 mg/kg DM. Gomez and Valdivieso (1985) reported a 36% reduction in HCN in ensiled cassava leaves, which is similar to the finding in the present study. There were no differences due to kind or level of the additive, which is in agreement with reports of Abou-Raya et al. (1973), Moseley and Ramanathan (1989) and Sibanda et al. (1997) that molasses and cereal grains are equally useful as silage additives.

Chopping, washing and wilting

Chopping, washing and wilting are methods, which can easily be applied at small-holder farmer level. In the research reported in chapter 4, there was a 58% reduction in the HCN content of the cassava leaves after wilting 24 hours in the shade. Bui Van Chinh and Le Viet Ly (1996) observed a similar reduction (60%) in HCN for wilting in the shade. However, when wilting was in sunlight, there was a 90% reduction in HCN (Gomez and Valdivieso 1985; Ravindran et al 1987). The faster rate of drying of the leaves sampled from 60-day growth cassava, compared with the leaves from the mature plant, was not reflected in changes in HCN content, which were similar for both sources of leaves.

Washing alone or washing after chopping the leaves reduced slightly (by 16 and 21%) the HCN level; however, the pink colour in the urine observed when fresh cassava leaves were fed was no longer visible when the leaves were washed prior to feeding. Wilting for 24 hours after chopping and washing reduced the HCN by 82%.

Feed intake in pigs fed fresh cassava leaves with simple processing methods

Most researchers who have conducted experiments with cassava leaves as feed for pigs used some form of processing prior to feeding. The question to be asked is: what happens if the cassava leaves are fed in the fresh form?

Although there were major differences in the HCN content of the cassava leaves as between washing, with and without chopping, and 24 hour wilting, this did not appear to affect the DM intake of the ensiled cassava root / rice bran mixture or the cassava leaves (chapter 4). The cassava leaves accounted for over 30% of the total DM intake resulting in intakes of HCN of 373 and 337 mg/day with washed and chopped / washed leaves, compared with 146 mg/day for the wilted leaves. Reported toxic levels of HCN (mg/kg live weight) for pigs are 1.4 (Getter and Baine 1938), 2.1 to 2.3 (Johnson and Ramond, 1965), 4.4 (Butler, 1973) and 3.5 (Tewe, 1995). In the present study the HCN intakes were much higher (from 6.0 to 15 mg/kg live weight) yet no signs of toxicity were observed.

The cassava leaves contributed from 71 to 74% of the total diet protein, providing an overall level of 15% crude protein in the diet DM. These levels of dietary crude protein provided by fresh cassava leaves are much higher than has previously been reported in the literature (Bui Huy Nhu Phuc et al., 2001; Ly and Rodríguez, 2001). However, they are comparable with results of a recent experiment in Cambodia (Chhay Ty and Preston, 2005) in which fresh cassava leaves supplied 42% of the diet DM and 70% of the protein in a basal diet of broken rice fed to growing pigs.

The results of this study indicate that farmers can use fresh cassava leaves as a protein source, with the only processing being chopping and washing. This will reduce costs and improve the economic efficiency of using cassava leaves in pig feeding.

Digestibility and nitrogen retention

Cassava leaves are rich in protein but relatively high in fiber. Feeding fibrous diets to pigs is known to reduce ileal and total tract digestibility (Jorgensen et al., 1996, Kass et al., 1980; An et al., 2004). Ensiling not only reduces HCN toxicity but also is a preservation method appropriate for use in the rainy season. In the experiment reported in Chapter 2, ensiled cassava leaves replaced fish meal as the main protein source in a basal diet of ensiled cassava root. The coefficient of apparent digestibility of crude protein and nitrogen retention decreased linearly as the level of ensiled cassava leaves increased. This negative trend is similar to the finding of Bui Huy Nhu Phuc et al. (1996), where ensiled cassava leaves replaced soybean meal. In both studies, N retention as percent of N digested decreased with replacement of fish meal / soybean meal with ensiled cassava leaves, indicating an inferior amino acid balance in the cassava leaves. Eggum (1970) and Rogers and Miner (1963) reported that the limiting amino acid in cassava leaves was methionine but that the leaves were rich in lysine and threonine.

The coefficients of apparent digestibility at the level of the ileum and the total tract were determined with F1 pigs (Large White x Mong Cai) fed diets having 30% of leaf meal from cassava (CLM), duckweed (DW), sweet potato vine (SPV) or stylosanthes (stylo) (Chapter 4). Crude protein intake and ileal digestibility of pigs were similar for CLM and DW diets and higher than for SPV and Stylo diets. There were negative relationship between dietary crude fiber level and total tract and ileal digestibility of organic matter, crude protein, crude fat and crude fibre. Ileal digestibility of crude fiber was in the range 17.1 to 21.1%, showing that pigs are able to digest a substantial part of plant fiber pre-caecally, which is in agreement with earlier reports by Lindberg et al. (1995).

In one trial carried out with farmers in Thuy Xuan village, located in the upland area of Thua Thien Hue Province, the traditional low protein diets (combinations of dried cassava root, rice bran, brewery by-products and sweet potato vines, with about 9% crude protein in DM)) were supplemented with either ensiled cassava leaves or fresh duckweed (Chapter 3). The pigs fed ensiled cassava leaves grew faster than the controls receiving sweet potato vines in five out of six farms. However, in the test with duckweed, this supplement stimulated total DM intake and increased daily gain on average by 35% compared with pigs on the control diet. These differences indicate that the amino acid profile in fresh duckweed is superior to that in ensiled cassava leaves. Culley and Epps (1978) reported that the amino acid balance of the protein in duckweed was similar to that in animal protein. By contrast, cassava leaves have been found to be deficient in methionine (Eggum, 1970).

DL-methionine as a supplement to cassava leaves in pig diets

The relative deficiency of the sulphur amino acids in cassava leaf protein (Eggum, 1970), and the fact that a source of sulphur is required for the detoxification of HCN (Oke, 1978), were the reasons for studying supplementation of cassava leaves with DL-methionine. According to Eggum (1970), addition of DL-methionine to a pig diet, rich in cassava leaf meal , increased the biological value of the protein from 0.49-0.51 to 0.8. Ly (2002) also showed that N balance in young Mong Cai pigs fed very high levels of ensiled cassava leaves could be improved by supplementation with DL-methionine.

Increasing levels of DL-methionine (0, 0.1 and 0.2%) were fed to growing pigs receiving basal diets with fresh or ensiled cassava leaves and cassava root products providing 20 and 30% of the diet DM. (Chapter 6). Growth rates were higher, and

DM and crude protein conversion rates tended to be better, for fresh compared with ensiled cassava leaves. On both sources of cassava leaves, live weight gains and DM conversion improved linearly with level of supplementary DL-methionine. At slaughter, the weights of the liver and thyroids, the loin eye area and estimated lean meat yield were increased on fresh compared with ensiled leaves. Estimated yield of lean meat increased and back fat decreased as the level of supplementary methionine was increased. There were no differences in growth performance between the best diet experimental diet (fresh cassava leaves + 0.2% DL-methionine) and the control diet containing 15% fish meal. These beneficial effects from feeding DL-methionine findings are similar to those reported by Job (1975) and (Portela and Maner, 1972), when cassava root meal was the basal diet.

The surprising result in the present study was the better performance on the fresh compared with the ensiled leaves. As far as the authors are aware, there have been no direct comparisons between fresh and ensiled leaves in the same experiment. Chhay Ty et al. (2005) fed fresh cassava leaves to young pigs at 50% of the DM of the diet and reported no signs of HCN toxicity even though the levels of HCN (7.4 mg/kg live weight) exceeded what had been considered to be the thresh-hold level (3.5 mg/kg live weight) for toxicity (Tewe, 1995). In the present experiment, HCN intakes were calculated to be 2.8 and 4.0 mg/kg live weight for the ensiled and fresh cassava leaf diets, respectively, and were not related to growth performance.

A possible explanation for the poorer growth performance on ensiled versus fresh leaves could be the negative effects of the ensiling process on the biological value of the cassava protein. The fermentation taking place in ensiled forages converts soluble carbohydrates to organic acids and also promotes proteolysis (McDonald, 1981). Oshima and McDonald (1978) reported that, in general, of the total N in fresh forages, 75 to 90% would be protein N, and the remainder non-protein N (NPN). After ensiling, NPN may account for as much as 80% of total N, according to Papadopoulos and McKersie (1983) and Albrecht and Muck (1991). Owens et al. (1999) found that when alfalfa and red clover were ensiled the NPN content increased from 136 to 353 g/kg total N in red clover and from 165 to 625 g/kg total N in alfalfa. There appear to be no comparable data on the ensiling of cassava leaves but it is probable there is some loss in protein quality during the ensiling process.

Positive effects of supplementary DL-methionine on growth performance of pigs fed cassava root meal were first reported by Maner and Gomez (1973). The use of cassava leaves together with cassava root products, as the basis of pig diets is a more recent development. Nguyen Thi Hoa Ly (2006) observed a 23% increase in growth rate in pigs when DL-methionine was added at 0.15% of the DM of diets with 17 to 25% ensiled cassava roots and 15% ensiled cassava leaves (DM basis). By contrast, in the present study the growth rate increase was 32% and the levels of cassava roots and leaves were 30 and 20% respectively. In the experiment of Nguyen Thi Hoa Ly (2006) synthetic lysine was also added and was thus confounded with the level of methionine. Addition of 0.3% DL-methionine to a diet with 50% of the DM as fresh cassava leaves had no effect on growth rate or feed conversion of pigs fed a basal diet of broken rice (Chhay Ty et al 2005). However, according to Nguyen Thi Loc and Le Khac Huy (2003), addition of 0.3% DL-methionine in a basal diet with 20-40% ensiled cassava root slightly reduced growth rate, while a level of 0.2% DL-methionine increased growth rate. From a theoretical standpoint, it is to be expected that there would be nutritional benefits from DL-methionine supplementation of pig diets rich in cassava leaves because of the relative deficiency of the sulphur amino acids in cassava leaf protein and the fact that a source of sulphur is required for the detoxification of HCN.

Conclusions

The most important findings from the research reported in this thesis are:

- Fresh cassava leaves can be fed to pigs at levels up to 30% of the diet DM with no apparent risk of HCN toxicity.
- The fresh leaves appear to a have superior nutritive value to ensiled cassava leaves, when used as a major protein source in diets of growing pigs.
- The limitation to the nutritive value of cassava leaves is the reduced apparent digestibility of the protein and inferior amino acid balance compared with fish meal or soybean meal.
- Feeding supplementary DL-methionine to growing pigs receiving diets with high proportions of cassava leaves and cassava roots improves growth rate, feed conversion and carcass quality.
- There is need for further research on the relative effects of fresh versus ensiled cassava leaves as major protein sources in pig diets as well as the potential benefits to be gained from supplementation with DL-methionine.

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SUMMARY

In Central Vietnam, pig production plays an important role for small scale farmers. Feeds are mainly based on cassava root and rice bran, which have low protein content. Thus the traditional diets are imbalanced in terms of nutrient supply, especially for protein content, that is generally low and of poor quality. Protein sources of high quality, such as fish meal and soybean meal, are rarely used by poor farmers because they are expensive. This situation has been the stimulus for development of feeding systems in which cassava leaves replace all or part of the protein supply in pig diets. Cassava leaves are rich in protein and readily available to small-holder farmers in Central Vietnam as by-products when roots are harvested or as major products when cassava is grown as a semi-perennial forage. Because of the presence of high levels of cyanogenic glucosides in cassava leaves, most of the earlier research focused on ways to reduce the risk of toxicity from hydrocyanic acid (HCN), usually by ensiling or drying the leaves before feeding. There appear to be no reports in the literature of mortality in pigs fed cassava products.

The aim of the studies described in this thesis was to evaluate the use of cassava leaves as protein sources for pigs when used at high levels in the diet, either in fresh form or with simplified methods of processing.

In a study on leaves of twenty cassava varieties taken from the upper part of the plant at the time of root harvesting, the crude protein varied from 23.7 to 29.5 % in DM and the HCN from 610 to 1840 mg/kg DM. In this study the environmental conditions for growth of the cassava plants were similar, which implies that the wide variation reported in the literature for crude protein in cassava leaves (16.7 - 39.9% in DM) may have been the result of differences in climate, soils and / or fertilizer treatments.

The risk of HCN toxicity can be reduced by processing the leaves by drying or ensiling. When cassava leaves were ensiled with molasses (5 or 10% fresh basis) or rice bran (10 or 15% fresh basis), the HCN in the leaves was reduced from 177 mg/kg in DM to 80 mg/kg DM in the ensiled product. There were no differences due to the kind or the level of the additives.

Chopping, washing and wilting the leaves are simple ways to process the leaves and easy to apply at small-holder farmer level. In the study of these processing methods there was a 58% reduction in the HCN content of the cassava leaves after wilting 24 hours in the shade. The faster rate of drying of the leaves sampled from the 60-day growth compared with the leaves from the mature cassava plant was not reflected in the changes in HCN content, which were similar for both sources of leaves. Washing

alone or washing after chopping the leaves reduced slightly (by 16 and 21%) the HCN level. However, the pink colour in the pig's urine observed when fresh cassava leaves were fed, was no longer visible when the leaves were washed prior to feeding. Wilting for 24 hours after chopping and washing reduced the HCN by 82%. Despite these wide differences in HCN levels as a result of these simple methods of processing, this did not appear to affect neither the DM intake by pigs of the basal diet of ensiled cassava root / rice bran mixture nor the cassava leaves. In this study, the intake of cassava leaves accounted for over 30% of the total DM intake resulting in intakes of HCN of 373 and 337 mg/day with washed and chopped / washed leaves, compared with 146 mg/day for the wilted leaves. Reported toxic levels of HCN (mg/kg live weight) for pigs range from 1.4 and 4.4 mg/kg. In the present study the HCN intakes were much higher (from 6.0 to 15 mg/kg live weight) yet no signs of toxicity were observed. The conclusion from these results is that the limiting factor to the use of high levels of fresh cassava leaves is not the risk of cyanide toxicity but rather the level of fiber or some other factor(s) reducing the digestibility of the protein.

That factors other than HCN, were the limitations of cassava leaves as a protein source for growing pigs was evident in an experiment where ensiled cassava leaves replaced fish meal in a diet with ensiled cassava roots as the energy component. The apparent digestibility of the crude protein and the nitrogen retention showed a linear decrease as the ensiled cassava leaves replaced the fish meal.

The ileal and total tract digestibility coefficients of cassava leaves in the form of leaf meal were compared with other foliar sources of protein in the form of duckweed, sweet potato vines and stylosanthes leaves. There were negative relationships between crude fiber levels in the diets and total tract and ileal digestion of organic matter, crude protein and crude fibre. On all the diets, a substantial proportion of the crude fiber (close to 20%) was digested prior to the caecum, showing that pigs are able to digest a substantial part of plant fiber in this part of the digestive tract.
The feeding of ensiled cassava leaves under practical farm conditions was tested in a trial with farmers in Thuy Xuan village, which is located in the upland area of Thua Thien Hue Province . The traditional feeds for pigs are dried cassava root, rice bran, brewery by-products and sweet potato vines. The crude protein in the dry matter of the typical diets was calculated to be less than 9 %. In five out of six farms in the trial, the pigs (mainly crosses of Large White x Mong Cai) fed ensiled cassava leaves grew faster than the controls receiving sweet potato vines. However, in a parallel study, supplementation with small proportions of fresh duckweed (5% of diet DM) increased the growth rates by 35%, implying that duckweed was a better protein source than ensiled cassava leaves.

From a theoretical standpoint, it is to be expected that there would be nutritional benefits from DL-methionine supplementation of pig diets rich in cassava leaves because of the relative deficiency of the sulphur amino acids in cassava leaf protein and the fact that a source of sulphur is required for the detoxification of HCN. A feeding trial was conducted to evaluate the effect of different levels of DL-methionine supplementation (0, 0.1 or 0.2% in diet DM) on growth performance of pigs given diets with fresh or ensiled cassava leaves and ensiled cassava root. Intakes of DM and crude protein were not affected by processing of the cassava leaves, nor by level of supplementary DL-methionine. Growth rates were higher, and DM and crude protein conversion rates tended to be better, for fresh compared with ensiled cassava leaves. With both sources of leaves, live weight gains and DM conversion improved linearly with level of supplementary DL-methionine. At slaughter, the weights of the liver and thyroids, the loin eye area and estimated lean meat yield were increased on fresh compared with ensiled leaves. Estimated yield of lean meat increased and back fat decreased as the level of supplementary methionine was increased. There were no differences in growth performance between the best diet from the cassava leaves-methionine treatments (fresh leaves + 0.2% DL-methionine) and the control diet containing fish meal. Back fat thickness was less and estimated yield of lean meat was greater on the former diet.

The overall conclusions from the studies reported in this thesis are that the limiting factors in cassava leaves as protein sources for pigs are not the cyanogenic glucosides, but other factors which result in decreased avai ability, and/or biological value of the protein component.

SUMMARY (in Vietnamese)

Ở Miền Trung Việt Nam, chăn nuôi lợn là nghề quan trọng trong chăn nuôi nông hộ. Nguồn thức ăn chủ yếu dựa vào cám và sắn củ mà những thức ăn này có thành phần protein rất thấp. Vì vậy, những khẩu phần truyền thống thường mất cân đối về giá trị dinh dưỡng, đặc biệt là thành phần protein, không chỉ nghèo về số lượng mà còn kém về chất lượng. Nguồn protein truyền thống như bột cá, bột đậu tương được sử dụng rất hạn chế trong chăn nuôi nông hộ, lý do chính là do giá thành đắt đỏ. Đây chính là nguyên nhân kìm hãm sự phát triển chăn nuôi lợn ở các nông hộ.

Cây sắn được trồng ở hầu hết các nông hộ Miền Trung. Lá sắn là nguồn thức ăn giầu protein, thường được coi là nguồn phụ phẩm sau thu hoạch lấy củ nhưng cũng có thể là sản phẩm chính cho thu hoạch quanh năm. Do sự hiện diện ở mức cao của cyanogenic glucosides trong lá sắn nên hầu hết các nghiên cứu trước đây tập trung tìm ra biện pháp giảm thiểu tối đa lượng độc tố acid hydrocyanic (HCN) trong lá. Phương pháp được đề xuất là: ủ chua hay phơi khô lá trước khi cho gia súc ăn. Tuy kết quả của các nghiên cứu đã nhấn mạnh sự giảm thiểu độc tố HCN nhưng chưa có công bố nào kết luận chính xác về giá trị liều lượng độc tố gây lợn chết do ăn sắn và các sản phẩm của sắn.

Mục đích của nghiên cứu đặt ra cho luận án này là đánh giá việc sử dụng lá sắn tươi đã qua sơ chế đơn giản như nguồn protein khi dùng với mức cao trong khẩu phần nuôi lợn.

Thành phần dinh dưỡng và độc tố trong lá của 12 giống sắn khác nhau, được lấy ở phần ngọn của cây khi thu hoạch củ. Kết quả phân tích cho thấy, thành phần protein thô biến động từ 23,7 đến 29,5% trong vật chất khô (DM) và hàm lượng HCN từ 610 tới 1840mg/kgDM. Trong nghiên cứu này, các yếu tố điều kiện môi trường, phân bón và đất trồng sắn được khống chế như nhau, vì vậy sự biến động các số liệu protein thô đó chủ yếu là do ảnh hưởng bởi yếu tố giống sắn.

Phương pháp chế biến lá sắn để làm giảm độc tố HCN được dùng là phơi héo và ủ chua. Lá sắn sau khi phơi héo được ủ với 5 hoặc 10% rỉ mật hay 10 hoặc 15% cám gạo (tính theo dạng nguyên trạng). Sau 21 ngày, lượng độc tố HCN trong lá đã giảm từ 177 mg/kgDM xuống 80 mg/kgDM. Không phát hiện thấy sự ảnh hưởng của các chất và các mức phụ gia tới hàm lượng HCN.

Băm, rửa và phơi héo lá sắn là cách chế biến đơn giản dễ áp dụng ở mức nông hộ. Kết quả nghiên cứu sử dụng phương pháp đơn giản này cho thấy: hàm lượng HCN giảm được 58% sau khi phơi héo 24 h dưới hiên nhà. Với hai nguồn lá lấy tại thời điểm 60 ngày sau khi trồng và ngay khi thu hoạch củ, kết quả cho thấy không có sự khác nhau nhiều về nồng độ HCN. Bằng phương pháp rửa lá hay băm rồi rửa, nồng độ HCN giảm khoảng 16 đến 21%. Khi theo dõi trên đàn lợn được nuôi bằng những

loại lá chế biến đơn giản này không thấy xuất hiện mầu hồng ở nước tiểu giống như ở đàn ăn lá sắn tươi chưa rửa hoặc chưa băm và rửa. Phơi héo sau 24 giờ dưới hiên nhà đã làm giảm 82% HCN. Mặc dù có sự khác nhau về hàm lượng HCN trong lá ở các phương pháp chế biến khác nhau, nhưng không thấy ảnh hưởng của nó tới lượng vật chất khô ăn vào của thức ăn cơ sở (cám trộn với sắn ủ) và lá sắn. Trong nghiên cứu này, lượng lá ăn vào ở lợn là trên 30% tính theo DM và lượng HCN ăn vào là 373 mg/ngày với phương pháp rử hay băm rửa và 146 mg/ngày bằng phương pháp phơi héo.

Các mức độ gây độc cho lợn của HCN (mg/kg trọng lượng sống) đã được công bố trước đây là: 1,4 tới 4,4. Kết quả của nghiên cứu này cho giá trị cao hơn nhiều so với các công bố đó: từ 6,0 đến 15 mg/kg trọng lượng sống của cơ thể vẫn không thấy dấu hiệu gây độc ở lợn. Lượng lá sắn đóng góp từ 37 đến 39% vật chất khô và từ 71 đến 74% tổng lượng protein của khẩu phần, nâng tỷ lệ protein của khẩu phần lên 15% tính theo DM. Kết luận của nghiên cứu này là: yếu tố hạn chế lớn nhất khi sử dụng lá sắn tươi nuôi lợn không phải là liều gây độc của HCN mà có thể do các yếu tố khác như mức xơ trong khẩu phần.

Khi sử dụng lá sắn ủ thay thế bột cá trong khẩu phần với nguồn năng lượng là củ sắn ủ chua. Khả năng tiêu hóa protein biểu kiến và tích lũy ni tơ giảm dần theo mức tăng dần của lá sắn ủ trong khẩu phần. Nghiên cứu so sánh hệ số tiêu hóa hồi tràng và tiêu hóa toàn phần ở lợn khi sử dụng lá sắn như nguồn protein với các nguồn thức ăn xanh giầu protein khác như bèo tấm, ngọn lá khoai lang và cỏ stylo ở dạng bột. Kết quả cho thấy có mối tương quan tỷ lệ nghịch giữa tỷ lệ xơ trong khẩu phần với tỷ lệ tiêu hóa toàn phần cũng như tiêu hóa hồi tràng hợp chất hữu cơ, protein và xơ thô. Ở tất cả các khẩu phần, một phần lớn xơ đã được tiêu hóa (gần 20%) ở hồi tràng, chứng tỏ rằng lợn có thể tiêu hóa một phần xơ ở đoạn trước ruột già.

Một thí nghiệm nuôi dưỡng về sử dụng lá sắn ủ trong điều kiện nông hộ tại xã Thủy Xuân thuộc vùng cao của Tỉnh Thừa Thiên Huế đã được thực hiện và trình bày trong trong luận án. Thức ăn cơ sở ở đây bao gồm: bột sắn, cám gạo, bã bia và ngọn lá khoai lang. Thành phần protein trong khẩu phần này ước tính vào khoảng 9%. Thí nghiệm được tiến hành ở 5 trong 6 hộ trên lợn lai F1 giữa Large White và Móng Cái. Kết quả cho thấy khẩu phần sử dụng lá sắn ủ cho tăng trọng nhanh hơn khẩu phần thân, lá khoai lang. Tuy nhiên, song song với nghiên cứu này, một nghiên cứu về sử dụng bèo tấm, với mức 5% trong vật chất khô của khẩu phần cũng được tiến hành đồng thời để so sánh. Kết quả cho thấy khẩu phần cho ăn bằng bèo tấm cho khả năng tăng trọng cao hơn 35%. Điều đó cho thấy nguồn protein trong bèo tấm tốt hơn trong lá sắn ủ.

Theo lý thuyết, việc bổ sung DL-methionine trong khẩu phần của lợn với lá sắn sử dụng như nguồn protein có thể khắc phục sự thiếu hụt acid amin chứa lưu huỳnh trong protein của lá và hạ thấp độc tính do HCN gây ra. Đánh giá hiệu quả do bổ sung các mức DL-methionine khác nhau (0, 0,1 và 0,2% trong DM) tới khả năng sinh trưởng, phát triển của lợn khi sử dụng lá sắn dạng tươi và dạng ủ chua. Kết quả cho thấy, lượng vật chất khô và protein ăn vào không bị ảnh hưởng bởi phương pháp chế biến lá sắn cũng như mức DL-methionine bổ sung. Khả năng tăng trọng và chuyển hóa protein ở khẩu phần sử dụng lá sắn tươi tốt hơn khẩu phần lá sắn ủ. Ở các khẩu phần với hai nguồn lá sắn tươi và lá sắn ủ khả năng tăng trọng và chuyển hóa thức ăn tốt hơn theo mức bổ sung tăng dần của methionine. Đồng thời, kết quả khảo sát khi giết mổ cũng cho thấy trọng lượng của gan, tuyến giáp trạng và diện tích mắt thịt cao hơn ở những khẩu phần lá sắn tươi và thấp hơn ở những khẩu phần sử dụng lá sắn ủ. Khối lượng thịt nạc cũng tăng và độ dầy mỡ lưng giảm theo mức DL-methionine tăng lên. Không có sự sai khác thống kê giữa khẩu phần thí nghiệm tốt nhất (lá sắn tươi + 0,2% DL-methionine) với khẩu phần đối chứng (chỉ dùng bột cá). Một điểm đáng chú ý ở kết quả nghiên cứu này là khả năng tăng trọng có xu thế cao hơn ở các khẩu phần sử dụng lá sắn tươi so với những khẩu phần dùng lá sắn ủ.

Kết luận chung từ các nghiên cứu trong luận án này là nhân tố chính hạn chế về việc sử dụng lá sắn như nguồn protein cho lợn không hoàn toàn do cyanogenic glucosides mà có thể do các nguyên nhân khác như sự mất cân bằng acid amin thiết yếu của protein trong lá hay giá trị sinh vật học protein lá sắn thấp.

SAMENVATTING

In centraal Vietnam is de varkenshouderij belangrijk voor de kleinschalige boeren. Het varkensvoer bestaat vooral uit eiwitarme producten als cassavewortels and rijstevoermeel. De nutriëntenvoorziening in de traditionele rantsoenen is niet gebalanceerd, vooral het eiwitgehalte en de eiwitkwaliteit laten te wensen over. Eiwitbronnen zoals vismeel en sojaschroot worden door de arme boeren zelden gebruikt vanwege de te hoge kosten. Dit vormde de aanleiding tot de ontwikkeling van een voerstrategie waarin cassaveblad een belangrijke rol speelt als eiwitbron. Het blad van de cassaveplant is rijk aan eiwit en is beschikbaar voor de boeren als een bijproduct van de oogst van cassavewortelen. Bovendien kan een deel van het blad van de cassaveplant ook het hele jaar geoogst worden. Omdat cassaveblad rijk is aan blauwzuurhoudende glucosiden, heeft het onderzoek zich vroeger vooral gericht op het verminderen van het risico van blauwzuurvergiftiging door middel van drogen of inkuilen van de bladeren. Opvallend genoeg wordt er in de literatuur geen melding gemaakt van sterfte van varkens door het vervoederen van cassaveproducten.

Het doel van het onderzoek in dit proefschrift was om na te gaan in hoeverre het verse of op eenvoudige wijze behandelde cassaveblad als eiwitbron voor varkens kan dienen.

In een onderzoek met twintig verschillende variëteiten van de cassaveplant werd gevonden dat het ruw eiwitgehalte in de bladen van het bovenste deel van de planten varieerde van 23,7 tot 29,5 % in de droge stof en dat het gehalte aan blauwzuur tussen de 610 en 1840 mg per kg droge stof lag. Omdat de omgevingscondities voor deze cassavevariëteiten in deze proef gelijk waren, kan aangenomen worden dat de grote variatie in eiwitgehalte zoals gerapporteerd in de literatuur (16,7 – 39,9%) vooral veroorzaakt wordt door verschillen in klimaat, bodem of bemesting.

Het risico op blauwzuurvergiftiging kan verminderd worden door het drogen of inkuilen van de bladeren. Het inkuilen van cassaveblad met melasse (5 tot 10% op vers product basis) of met rijstevoermeel (op basis van het verse product 10 tot 15 %) zorgde voor een daling van het blauwzuurgehalte in de silage van 177 naar 80 mg per kg drogestof. Het type en de hoeveelheid toevoegmiddel had geen significant effect.

Het hakken, wassen en voordrogen van de bladen zijn simpele bewerkingen die de kleinschalige boerenbedrijven ook eenvoudig kunnen toepassen. Uit de proeven bleek dat het in de schaduw voordrogen van de bladeren gedurende 24 uur het blauwzuurgehalte met 58% deed afnemen. Het feit dat bladeren geplukt na 60 dagen groei, sneller droogden dan de bladeren geplukt bij het oogsten van de wortels, had geen invloed op de mate van afname van het gehalte aan blauwzuur.

Het wassen alleen of het wassen en het hakken van de bladeren verlaagde het blauwzuurgehalte slechts met 16 tot 21%. Echter, het wassen van de bladeren zorgde er wel voor dat de roze kleur van de urine, die bij het voeren van verse cassavebladeren

wordt waargenomen, verdween.Voordrogen gedurende 24 uur na hakken en wassen verlaagde het blauwzuurgehalte met 82%.
Ondanks de grote verschillen in het gehalte aan blauwzuur ten gevolge van de verschillende behandelingsmethoden, was er geen verschil in de totale droge stof opname.
Het rantsoen in deze proef bestond voor 30 % van de totale droge stof uit cassavebladeren. Dit resulteerde in een opname aan blauwzuur van 373 mg, 337 mg en 146 mg per dag bij respectievelijk gewassen bladeren, gewassen en gehakte bladeren en gewassen, gehakte en voorgedroogde bladeren. In de literatuur wordt opgegeven dat een opname van 1,4 tot 4,4 mg blauwzuur per kg lichaamsgewicht toxisch zou zijn. In deze proef varieerde de opname aan blauwzuur tussen de 6 en 15 mg per kg lichaamsgewicht en maar er werden geen verschijnselen van blauwzuur vergiftiging waargenomen.
Het ontbreken van de symptomen van blauwzuurvergiftiging bij het voeren van rantsoenen met een relatief hoog aandeel vers gehalte cassavebladeren is bevestigd in recente proeven door andere onderzoekers. De conclusie van dit onderzoek is dat de beperkende factor bij het gebruik van verse cassavebladeren niet het risico op blauwzuurvergiftiging is, maar het gehalte aan ruwe celstof of één of meerde andere factoren die de eiwitvertering verlagen.
Het feit dat andere factoren dan blauwzuur zorgen voor de beperkte waarde van cassavebladeren als eiwitbron voor groeiende varkens werd duidelijk in een proef waarbij ingekuilde cassaveblad vismeel verving in een rantsoen met ingekuilde cassavewortelen als energiebron. De schijnbare verteerbaarheid van ruw eiwit en de stikstof retentie namen lineair af met de toename van het aandeel ingekuilde cassaveblad in het rantsoen.
De ileale en fecale verteerbaarheid van cassavebladeren in de vorm van meel werd vergeleken met een aantal andere bladeren, namelijk eendenkroos, loof van de zoete aardappel en stylosanthes blad. Er was een negatief verband tussen het gehalte aan ruwe celstof in het rantsoen en de ileale en fecale verteerbaarheid van organische stof, ruw eiwit en ruwe celstof. Bij alle rantsoenen bleek een deel (ca. 20%) van de ruwe celstof al in de dunne darm te worden verteerd.
Het voeren van ingekuilde cassavebladeren in de praktijk werd onderzocht in een proef bij boeren in het dorp Thuy Xuan in de bergen van de provincie Thua Thien Hue.
In dit gebied bestaat het gebruikelijke rantsoen voor varkens uit gedroogde cassavewortels, rijstevoermeel, bijproducten van de bierbrouwerij en loof van de zoete aardappel. Het eiwitgehalte in de droge stof van een dergelijk rantsoen werd berekend en lag onder de 9%. Op vijf van de zes boerderijen in deze proef groeiden de varkens (voornamelijk een kruising tussen Groot Yorshire en Mong Cai) die ingekuild cassaveblad kregen, sneller dan de controle varkens die het loof van de zoete aardappel kregen. Echter, in een andere, gelijktijdig lopende proef bleek dat het voeren van eendenkroos (5% van de droge stof) de groeisnelheid van de varkens

met 35 % te verhogen. Dit wijst erop dat eendenkroos een betere eiwitbron is dan ingekuild cassaveblad.
Op theoretische gronden is het te verwachten dat het toevoegen van DL-methionine aan varkensrantsoenen die veel cassaveblad bevatten, nutritionele voordelen op zal leveren. Ten eerste, is het eiwit in het cassaveblad relatief arm aan methionine en ten tweede, is het bekend dat de zwavel uit de methionine nodig is voor het ontgiften van blauwzuur. Daarom werd er een voederproef uitgevoerd om het effect van het toevoegen van DL-methionine (0, 0,1 of 0,2 % van de droge stof) op de groei van varkens te onderzoeken. Hierbij bestond het rantsoen uit verse of ingekuilde cassavebladeren naast ingekuilde cassavewortelen.
De opname aan droge stof en ruw eiwit werd niet beïnvloed door de bewerking van het blad (vers of ingekuild) en ook niet door het niveau van toegevoegd DL-methionine. De groeisnelheid was hoger en de voederconversie was lager bij de dieren die met vers cassaveblad werden gevoerd in vergelijking met de varkens die ingekuild cassaveblad kregen. Ongeacht de bewerking van de cassavebladeren, nam de groei lineair toe en de voederconversie lineair af met een toenemende hoeveelheid DL-methionine in het rantsoen. Bij het slachten bleek het gewicht van zowel de lever als de schildklier en de geschatte hoeveelheid mager vlees groter te zijn bij de varkens die met verse cassavebladeren werden gevoerd in vergelijking varkens die ingekuilde cassaveblad kregen.
Er was geen verschil in groeisnelheid tussen de varkens die vers cassaveblad kregen in combinatie met het hoogste gehalte aan toegevoegd DL-methionine (0,2%) en een controle groep waarbij het rantsoen vismeel bevatte. Wel had de controlegroep een geringere spekdikte en een hoger geschat gehalte aan mager vlees.

De conclusie van de proeven die in dit proefschrift worden beschreven, is dat de beperkende factor bij het gebruik van cassaveblad als eiwitbron voor varkens niet de blauwzuurhoudende glucosiden, maar andere factoren die de beschikbaarheid en/of de biologische waarde van het cassave-eiwit verminderen.

CURRICULUM VITAE

Mrs Du Thanh Hang was born 12 May 1961 in Ha Noi, Capital of Vietnam. She attended the Agriculture University No 2, in Ha Bac Province, Vietnam and obtained a BSc degree of Animal Science in 1984.

From 1984 up to now she is lecturer and researcher of Animal Nutrition at the Department of Animal Nutrition of the Faculty of Animal Science, University of Agriculture and Forestry, Hue city, Vietnam (Previously named Agriculture University No. 2).

In 1995, she followed a two-months course on feed analysis at Chiang Mai University, Thailand.

In 2002, she took part in a training course of grass technology in Australia for one month.

In 2004, she followed a two-months course on dairy cattle production in the Netherlands (International Agricultural Centre).

She graduated and obtained two Master degrees. In 1998 she graduated in Integrated Farming Systems in Thu Duc, Ho Chi Minh City at an International Course funded by the Danish Embassy. In 1999 she graduated in Animal Science at Hue University, her study being funded by the Vietnamese Government.

From 1996-1999, she received a grant from the International Foundation for Science (IFS) for a project entitled "Using non-conventional feeds for monogastric animals in Central Vietnam".

From 1996 up to now, she received several funds from the Swedish Agency for Research Cooperation with Developing Countries (SIDA-SAREC and MEKARN) for her studies in the field of using local feed resources, especially for studies on use of cassava leaves as protein source for pigs

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PUBLICATIONS

This thesis

Chapters 2, 3 and 4 of this thesis are papers published in the peer-reviewed, Journal "Livestock Research for Rural Development", the citations to which are as follows:

Du Thanh Hang 1998 Digestibility and nitrogen retention in fattening pigs fed different levels of ensiled cassava leaves as a protein source and ensiled cassava root as energy source. Livestock Research for Rural Development. 10 (3): http://www.cipav.org.co/lrrd/lrrd10/3/hang1.htm

Du Thanh Hang 1998 Ensiled cassava leaves and duckweed as protein sources for fattening pigs on farms in Central Vietnam. Livestock Research for Rural Development. Volume 10, Number 3. http://www.cipav.org.co/lrrd/lrrd10/3/hang2.htm

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

Chapters 5 and 6 have been submitted to the same journal and are presently under review.

Other publications of the author of this thesis

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