Feeding cassava foliage to sheep

Nutrient properties and hydrogen cyanide toxicity

Khuc Thi Hue
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2012

Acta Universitatis Agriculturae Sueciae
2012:4

Cover: Cassava foliage in sheep feeding system in Vietnam
(photo: Khuc Thi Hue, 2011)

ISSN: 1652-6880
ISBN: 978-91-576-7688-7

Print: SLU Service/Repro, Uppsala 2012

Feeding cassava foliage to sheep: Nutrient properties and hydrogen cyanide toxicity

Abstract

The potential of cassava foliage (*Manihot esculenta* Crantz) as a protein-rich feed in sheep production in Vietnam was examined by studying cassava foliage yield, hydrogen cyanide (HCN) content, toxicity and performance of lambs fed the foliage as a supplement. Cassava foliage fed *ad libitum* as a protein supplement to a basal diet of urea-treated rice straw gave similar lamb live weight gain (LWG) as diets supplemented with commercial concentrate or protein-rich foliage of stylosanthes (*Stylosanthes guianensis*) or jackfruit (*Artocarpus heterophyllus*). Field studies showed that higher harvesting frequency gave higher yield of cassava foliage. A K94 cassava variety always gave the highest foliage yield. Content of dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and total tannins in the cassava foliage increased with plant and leaf maturation, while crude protein (CP) and HCN content decreased. Total tannin content was highest at first harvest and decreased slightly at subsequent harvests, while the reverse was true for HCN content. Different cassava varieties and degrees of processing (fresh, wilted or hay) resulted in differences in foliage HCN content. Feeding cassava foliage at 2% of body weight (BW) from the start led to wasted feed, but prolonging the adaptation period to 21 days resulted in lower LWG. A 7-day period for adaptation to cassava foliage was a good compromise for lamb performance and health. Feeding cassava foliage as fresh, wilted or hay did not result in any difference in LWG, but hay had lower DM and CP digestibility. Inclusion of cassava in the diet of sheep had a positive nematode control effect, but HCN consumption and thiocyanate concentration in urine increased with increasing foliage intake.

Keywords: Adaptation, cassava foliage, digestibility, growth, harvest, hydrogen cyanide, lamb, *Manihot esculenta* Crantz, processing, thiocyanate, variety.

Author's address: Khuc Thi Hue, SLU, Department of Animal Nutrition and Management, P.O Box 7024, SE-750 07 Uppsala, Sweden.
E-mail: hienhue02@yahoo.com

Dedication

To my family with my respectful gratitude,

To my husband Nguyen Van Hien,

and my son Nguyen Minh Hieu.

Contents

List of Publications

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

I Hue, K.T., Van, D.T.T. and Ledin, I. 2008. Effect of supplementing urea treated rice straw and molasses with different forage species on the performance of lambs. *Small Ruminant Research* 78, 134-143.

II Hue, K.T., Van, D.T.T., Ledin, I., Wredle, E. and Spörndly, E., 2011. Effect of harvesting frequency, variety and foliage age on nutrient composition, hydrogen cyanide content and yield of cassava foliage. *(Submitted to the Asian-Australasian Journal of Animal Science)*.

III Hue, K.T., Van, D.T.T., Ledin, I., Spörndly, E. and Wredle, E. 2011. Effect of different adaptation strategies when feeding fresh cassava foliage on intake and physiological responses of lambs. *Tropical Animal Health and Production 44 (2) 267-276.*

IV Hue, K.T., Van, D.T.T., Ledin, I., Spörndly, E. and Wredle, E. 2010. Effect of feeding fresh, wilted and sun-dried foliage from cassava (*Manihot esculenta* Crantz) on the performance of lambs and their intake of hydrogen cyanide. *Livestock Science* 131, 155-161.

Papers I, III and IV are included with the permission of the publishers.

Abbreviations

ADF	Acid detergent fibre
BW	Body weight
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FCR	Feed conversion ratio
HCN	Hydrogen cyanide
LWG	Live weight gain
ME	Metabolic energy
NDF	Neutral detergent fibre
OM	Organic matter
SCN^-	Thiocyanate

Introduction

Vietnam is a tropical country located in South East Asia with a total area of 33.1 million hectares and a population of 86 million. About 67% of the total labour force works in agriculture. The area used for agricultural purposes is estimated to be about 25 million ha. The value of the agricultural output accounts for 25% of GDP, and is made up of 77% food crops, 20% livestock products (mainly pigs, cattle, poultry) and 3% other products (GSO, 2011).

The population of sheep in Vietnam increased rapidly in the period 1996-2006, from 3,000 to 56,800 head (BAH, 2007). However, from 2008 the sheep population declined, to around 47,000 head in 2010 (BAH, 2011). There are many reasons behind the reduction in the sheep population. The main problem in sheep production in Vietnam is providing feed of sufficient quantity and quality all year round. For many years, the feed resources for almost all sheep have depended on natural grazing and fodders. Recently, sheep producers have experienced a decrease in grazing land owing to demand for land for industrialisation and for human population increase. The dependence on natural feed resources means that sheep production in Vietnam is variable and has low efficiency.

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub of the family Euphorbiaceae. It originated in South America and is extensively cultivated as an annual crop in the tropics and sub-tropics for the dual purposes of tuberous roots as a source of energy for humans and animals and foliage as a feed for animals. Cassava foliage is recognised as a source of undegraded protein with a high content of digestible nutrients for both non-ruminants and ruminants (Wanapat, 2001). The foliage can be used as a supplement for animals in either fresh or wilted form or as hay (Phengvichith & Ledin, 2007; Wanapat *et al.*, 1997). At root harvesting, 9 to 10 months after planting, the foliage production can be about 5 tonnes dry matter (DM)/ha (Mui, 1994). It is estimated that about 2.5 million tonnes of cassava foliage are produced in Vietnam as a by-product of root harvesting.

A limitation when feeding cassava foliage is the content of cyanogenic glucosides, mainly linamarin and lotaustralin (Alan & John, 1993). Hydrolysis of these cyanogenic glucosides liberates hydrogen cyanide (HCN) (Poulton, 1988) and causes toxicity symptoms in animals when they exceed the tolerated dose. Thus, understanding the dietary effects of cassava foliage so that animals could be fed the optimal amount to give the best performance and to minimise the incidence of HCN toxicity would be beneficial for sheep production.

Background

Sheep production in Vietnam

The local Phan Rang is the most common breed of sheep in Vietnam. This breed was originally introduced from India, Pakistan and Africa during French colonial times. Phan Rang sheep have brown wool, a long, thin tail and low average mature body weight (BW) of about 50 kg for rams and 40 kg for ewes. The sheep are used for meat production only. Phan Rang sheep are concentrated mainly in the south-central region of Vietnam and are well adapted to the local climate, feed and management conditions.

There are three main systems for management of small ruminants in Vietnam, intensive, semi-extensive and extensive (Binh & Lin, 2005). The semi-extensive rearing system is commonly applied in practical sheep production, especially in south-central Vietnam. In this system, the sheep are allowed to graze on common grazing land for 4-8 hours/day, and are brought back home at night. During the night, the sheep may be provided with supplementary feeds such as agricultural by-products, grasses, plant foliage, *etc*. The diet of sheep in the semi-extensive system is up to 90% dependent on natural grazing and fodder. Farmers pay very little attention to managing the available feed resources for sheep during the year or disease control, which leads to variable performance and fluctuating prices for products.

Ongoing problems due to the limited land area and lack of availability of natural feeds are causing some sheep farmers to change their rearing system to a more intensive form whereby the sheep are grazed 2 times/day (morning and afternoon) for about 4 hours in total. To supplement this, grasses and fodder crops are cultivated, harvested and carried to feed the animals 2 to 4 times/day.

According to Binh *et al.* (2005), who studied actual sheep production in Ninh Thuan, the herd size of small ruminants in Vietnam can be divided into three classes: small-scale (less than 50 head), medium-scale (50 to 100 head) and large-scale (100 to 500 head), with medium-scale being the most common. Secondary cassava cultivation for foliage is usually carried out on smallholder farms. After harvesting cassava for root production, the cassava stems are re-planted at a higher density and the shoots used as a feed supplement for animals. In medium- and large-scale animal production systems, cassava foliage is used as feed supplement fed mostly as hay or silage. In those cases, cassava foliage is collected in large amounts at root harvesting, sun-dried to hay or ensiled and fed to animals, especially during the dry season.

Cassava cultivation in Vietnam

Cassava is one of four most important crops in Vietnam, but it has always been a secondary crop after rice. In last decades, especially since the late 1970s, cassava has played an important role in national food security. Recently, cassava has become an important source of cash income for farmers, who use it for animal feed and/ or for sale as substrate to starch factories (Kim *et al.*, 2008).

Table 1. *Area (1000 ha) of cassava cultivation in different regions of Vietnam, 2006-2010*

	Year				
Region	2006	2007	2008	2009	2010
Red River Delta	8.4	8.8	7.9	7.5	7.3
Northern Midlands and Mountains	93.7	96.5	110.0	101.4	104.6
North Central and Central Coastal	140.3	151.2	168.3	157.2	155.0
Central Highlands	125.9	129.9	149.1	137.7	133.2
South East	100.9	102.9	111.4	97.7	90.1
Mekong River Delta	6.0	6.2	7.3	6.3	6.0
Total	*475.2*	*495.5*	*554.0*	*507.8*	*496.2*

Source: GSO (2011).

The area of cassava cultivation and total root production are increasing annually throughout the country (Fig. 1), *e.g.* from about 230,000 ha and 2 million tonnes, respectively, in 2000 to more than 510,000 ha and 8.5 million tonnes in 2010 (GSO, 2011). The cassava cultivation areas are mainly concentrated to the regions of Northern Midlands and Mountains, North Central and Central Coastal, Central Highlands and South East, where about 80% of total production is situated (Table 1).

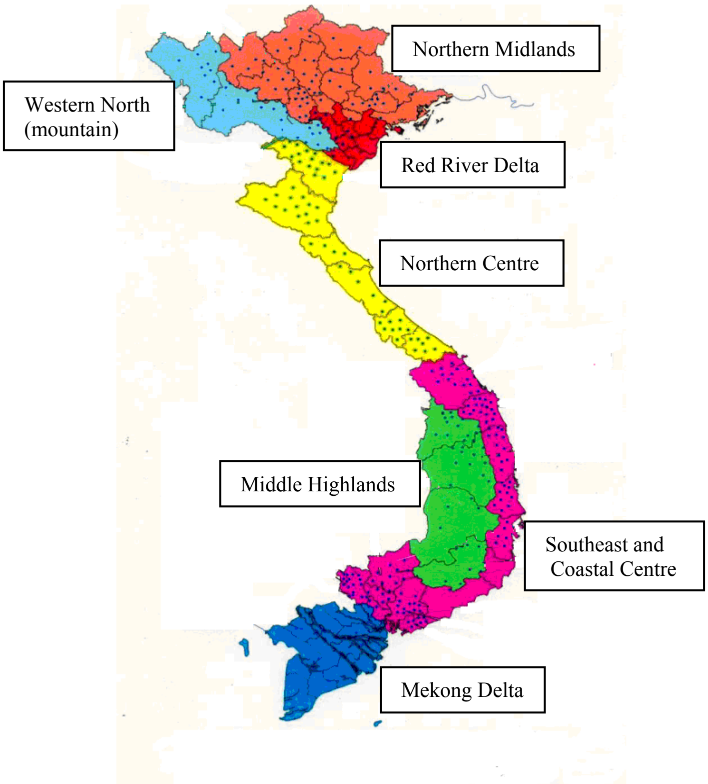


Fig. 1. Map of cassava cultivation in Vietnam, each dot represents 1000 ha (Hang, 2007).

The great increase in cassava yield is the result of successful implementation of new cassava varieties with high yield potential, such as KM94, KM140, KM98-5, KM98-7, *etc*. (Khanh, 2007), more sustainable production practices and expansion of area. According to Kim *et al.* (2008), total cassava starch production in Vietnam is 0.8-1.2 million tonnes/year, 70% of which is exported to China, Taiwan, Japan, Singapore, Malaysia, South Korea and countries in Eastern Europe. The remaining 30% of cassava starch produced is used by food and feed companies or for home consumption.

The foliage produced as a by-product of cassava root production comprises a large amount of biomass which can be used as a feed supplement for animals. Based on the areas of cassava cultivation in Table 1, the amount of cassava foliage produced in the period 2006-2010 was an estimated 2.5 million tonnes (on DM basis) per year (Table 2), containing about 0.5 million tonnes of crude protein (CP).

Table 2. *Estimated cassava foliage production (1000 tons) at cassava root harvesting in Vietnam, 2006-2010*

Region	Year 2006	2007	2008	2009	2010
Red River Delta	42.0	44.0	39.5	37.5	36.5
Northern Midlands and Mountain	468.5	482.5	550.0	507.0	523.0
North Central and Central Coastal	701.5	756.0	841.5	786.0	775.0
Central Highlands	629.5	649.5	745.5	688.5	666.0
South East	504.5	514.5	557.0	488.5	450.5
Mekong River Delta	30.0	31.0	36.5	31.5	30.0
Total	*2376.0*	*2477.5*	*2770.0*	*2539.0*	*2481.0*

Source: Recalculated from GSO (2011).

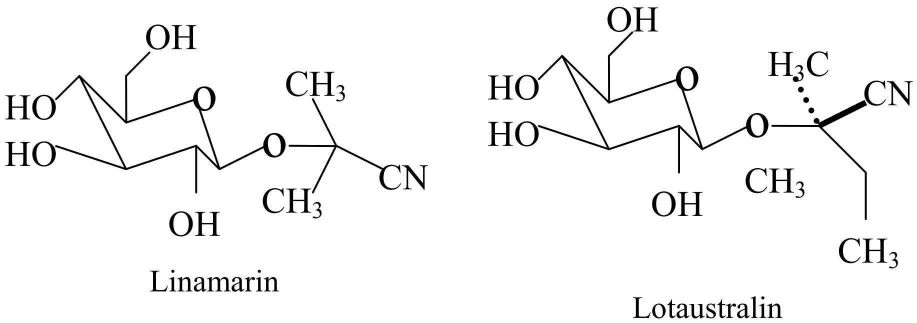
Cassava foliage as a feed for ruminants - nutrients and benefits

Cassava foliage is recognised as a locally available animal feed resource with a high edible biomass yield (Dung *et al.*, 2005a; Khang *et al.*, 2005), a valuable source of protein, varying from 2.24 to 2.84 tonnes/ha (Dung *et al.*, 2005; Khang *et al.*, 2005) and a high concentration of minerals and vitamins (Chadha, 1961). The CP concentration of cassava foliage is around 200 g/kg DM (Khang *et al.*, 2005).

Many studies have focused on cassava foliage as a feed for animals, especially for ruminants. Fresh cassava foliage or cassava hay has been fed to cattle, both beef and dairy, with good results (Thang *et al.*, 2010; Wanapat, 2009; Wanapat *et al.*, 1997). Cassava foliage is also a good source of protein for small ruminants. Sokerya & Rodriguez (2001) found that the growth rate of goats was higher when a diet based on brewer’s grain was supplemented with cassava leaves than when it was supplemented with three other types of foliage. Increasing the proportion of cassava foliage DM fed to goats from 0 to 47% of total DM feed offered resulted in increased DM intake (DMI), organic matter (OM) digestibility and nitrogen retention (Do *et al.*, 2002). The optimal level of wilted cassava foliage and cassava hay feed for growing goats in terms of intake, live weight gain (LWG), digestibility and feed cost was estimated to be 30-40% of total DM intake by Phengvichith & Ledin (2007) and around 22% of total DM intake by Tien Dung *et al.* (2005).

Cassava foliage - hydrogen cyanide formation

Cassava contains two cyanogenic glucosides, linamarin and lotaustralin (Fig. 2), which are synthesised with the amino acids valine and isoleucine as the respective precursor (Bokanga, 1994). Koch *et al.* (1992) demonstrated that the cyanogenic glucosides in cassava are biosynthesised in the leaves, then translocated and accumulated in plant tissues in varying proportions. Cassava leaves, including petioles, usually contain the highest concentration of cyanogenic glucosides, which may be 5 to 20 times higher than the concentration of cyanogenic glucosides in the root (Bokanga, 1994). Within the cells, linamarin and lotaustralin are mostly stored inside the vacuoles (Vetter, 2000; Bokanga, 1994), while the enzymes linamarase, lotaustralinase, which are capable of hydrolysing cyanogenic glucosides, are located in the cell wall (Mkpong *et al.*, 1990).



Linamarin

Lotaustralin

Fig. 2. Structure of the cyanogenic glucosides linamarin and lotaustralin (Morant *et al.*, 2008).

The breakdown of cyanogenic glucosides will therefore not occur when the cells are intact. When the cells are damaged, either by chewing by animals (or humans) or by technical processing, the enzymes and the cyanogenic glucosides come into contact and the reactions are initiated, resulting in the formation of HCN (Vetter, 2000).

The hydrolysis of linamarin and lotaustralin (Fig. 3) leads to the formation of sugar and α-hydrocynitrile. If the pH is above 5, α-hydrocynitrile spontaneously breaks down into a keto compound and HCN (Zagrobelny *et al.*, 2004; Bokanga, 1994). At lower pH values, the dissociated reaction is directly catalysed by an α-hydrocynitrile lyase into a keto compound and HCN (Zagrobelny *et al.*, 2004).

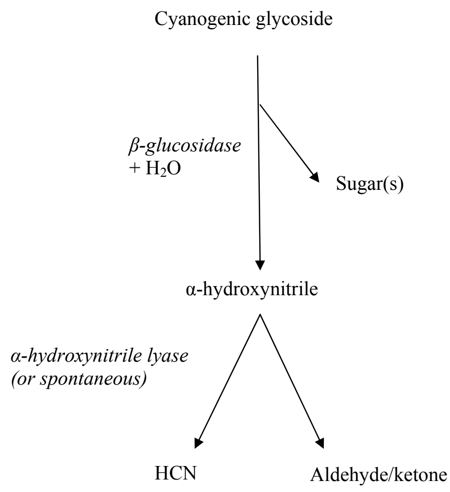


Fig. 3. Degradation of cyanogenic glycosides and cyanohydrins (Poulton, 1988).

Hydrogen cyanide in cassava foliage - toxic effects and detoxification

When the tissues are disrupted, cyanogenic glucosides are brought into contact with β-glucosidases and α-hydrocynitrile lysases, which hydrolyse them to release HCN. Cyanide is toxic to humans and animals due to linking with iron, manganese or copper ions, which are the functional groups of many enzymes involved in the reduction of oxygen in the cytochrome respiratory chain. Consequently, ATP formation ceases and tissues suffer energy deprivation (Zagrobelny *et al.*, 2004). Acute HCN toxicity symptoms include saliva excretion, vomiting, excitement, staggering, paralysis, convulsions, coma and death. The signs of toxicity may occur within seconds or minutes following consumption of pure HCN, but there may be no symptoms of cyanide poisoning when cyanogenic plants are eaten slowly or over a period of time (Burritt & Provenza, 2000).

Hydrogen cyanide is detoxified by two main reactions. The first route involves the formation of β-cyanoanaline synthase, which converts β-cyanoanaline to asparagine (Miller & Conn, 1980). The second route proceeds by conversion of HCN into thiocyanate (SCN^-) and is catalysed by rhodanese (Bordo & Bork, 2002). The detoxification route involving β-cyanoanaline is common in plants and insects, while the thiocyanate pathway occurs mainly in higher animals (Zagrobelny *et al.*, 2004). Rhodanese probably serves a variety of other functions, the most important of which is to donate sulphur to protein (Bordo & Bork, 2002). The amount of proteins in the diet affects the degree of cyanide tolerance, particularly proteins high in cysteine, as they provide the sulphur essential for thiosulphate production (Gleadow & Woodrow, 2002).

Tannins in feeds and the effect on internal parasites

According to Hoste *et al.* (2006), tannins are secondary compounds present in plants and comprise polyphenols of great diversity. The physical and chemical properties of tannins vary with different plants and in different plant parts and seasons (Waghorn *et al.*, 1990). Within plants, tannins have been proposed to act as a chemical defence against invasion by pathogenic microrganisms or foraging by insects or herbivores (Barry & McNabb, 1999). Based on their structure, Haslam (1989) categorised tannins into two major groups, hydrolysable tannins and condensed tannins.

Hydrolysable tannins consist of gallic and ellagic acid esters of sugars, which are more soluble in the water and more susceptible to enzymatic and non-enzymatic hydrolysis (Haslam, 1989). When hydrolysable tannins are consumed by ruminants, they can be degraded into gallic and ellagic acid, and absorbed in the digestive tract. Hydrolysable tannins are thus considered to have a negative physiological effect on ruminants, almost comparable to a toxic effect (Hoste *et al.*, 2006).

Condensed tannins are polyphenols of higher molecular weight and consist of oligomers or polymers of catechin, which mainly produce cyanidin and delphinidin when depolymerised (Waterman, 1999). According to Hoste *et al.* (2006), only a small amount of condensed tannins is absorbed in the digestive tract of ruminants, because they are not susceptible to hydrolysis.

Condensed tannins bind strongly to protein and this reactivity is pH-dependent (Reed, 1995). The condensed tannin-protein complexes are formed at pH 3.5-7.5 and are dissociated at pH <3.5 and the protein is thus released (Jones & Mangan, 1977). The major beneficial effect of tannins is the protection of plant protein from digestion in the rumen, making the protein available for digestion and utilisation in the lower gut (Waghorn *et al.*, 1990). However, higher tannin levels (above 50 g/kg DM) in plant material can become an anti-nutritional factor and can result in reduced feed intake and digestibility in animals (Barry & McNabb, 1999).

According to Mui *et al.* (2005), forages containing condensed tannins have the potential to help control antihelminthic-resistant gastro-intestinal parasites by direct or indirect biological effects. The direct effect may be mediated through interaction between condensed tannins and parasites, in which tannins may affect the physiological functions of gastro-intestinal parasites, through interference with parasite hatching and development of infective stage larvae (Molan *et al.*, 2002; Athanasiadou *et al.*, 2001). The indirect effect of condensed tannins may be enhanced resistance to gastro-intestinal parasite infection through increased protein supply, which is of high priority for tissue repair and immune response (Nieze *et al.*, 2002).

Objectives

The overall aim of this thesis was to improve the use of cassava foliage as a protein-rich feed in sheep production in Vietnam by studying cassava foliage yield, HCN content and toxicity and the performance of lambs when using the foliage as a supplement. Specific objectives were to:

- Study the effect of feeding cassava foliage as a supplement compared with other protein-rich forage species on digestibility and the performance of growing lambs.
- Study the effect of harvesting interval, variety and stage of plant maturity on yield, chemical composition and HCN content in cassava foliage.
- Investigate the effect of different adaptation periods when introducing fresh cassava foliage into the diet on intake and physiological responses in lambs.
- Estimate the effect of feeding fresh, wilted and sun-dried foliage from cassava on digestibility, the performance of lambs and their intake of HCN.

Hypotheses

- Supplementing the diet with cassava foliage has similar effects on the digestibility and performance of growing lambs to supplementation with other protein-rich foliages or commercial concentrates (Paper I).
- Higher harvesting frequency results in higher yields of cassava foliage and increases the HCN content of the foliage compared with harvesting foliage only once, at root harvesting (Paper II).
- A K94 variety of cassava commonly grown in Vietnam gives the highest foliage yield and HCN content in the foliage and a local cassava variety the lowest yield and HCN content (Paper II).

- A prolonged adaptation period of up to 21 days results in higher intake of cassava foliage and higher daily gain compared with a 7-day period or no adaptation period (Paper III).
- Introducing cassava foliage to sheep without an adaptation period induces physiological responses such as increased heart rate, breathing and other signs of acute toxicity in some individuals (Paper III).
- Feeding lambs wilted or hay cassava foliage gives lower HCN intake, increased foliage intake and higher growth rate than with fresh foliage (Paper IV).

Materials and methods

Overall layout of the study

The study comprised four experiments: In the first of these (Paper I), fresh cassava foliage of a medium bitter variety harvested on average at 3 months was compared with two other protein-rich foliages, jackfruit (*Artocarpus heterophyllus*) and stylosanthes (*Stylosanthes guianensis*), and a commercial concentrate as a feed supplement for lambs. The effect of the four different supplements on growth rate, feed conversion ratio and diet digestibility were studied. The results showed that lambs fed cassava foliage had similar LWG to those supplemented with a commercial concentrate, but at a lower feed cost.

Very bitter (K94) | Medium bitter (K98-7) | Sweet (local)

Fig. 4. Appearance of foliage of K94 (very bitter), K98-7 (medium bitter) and local (sweet) cassava varieties available in Vietnam.

Northern Vietnam has two main seasons, dry and rainy. The dry season is quite long, almost 6 months. When growing cassava foliage for forage production, it is important to establish the best varieties and harvesting schedules in order to improve foliage yield and chemical composition and to allow foliage to be supplied all year round. Therefore, our second experiment (Paper II) studied the yield, chemical composition and HCN content of cassava foliage of three different varieties at three different harvesting schedules. These were: three harvests, at 3, 6 and 9 months after planting; two harvests, at 6 and 9 months after planting; or one harvest, at 9 months after planting. The cassava varieties used were: K94 (very bitter), K98-7 (medium bitter) and a local (sweet) (Fig. 4).

Due to the high content of HCN in cassava foliage, animals may need to have a gradual introduction to the material to allow physiological adaptation to HCN present. Therefore, when feeding high amounts of cassava foliage it is important to know how long this adaptation period should be. There is a general opinion that at least 14 days are necessary, but no actual research results concerning this question were found. Thus, the third experiment (Paper III) tested different adaptation strategies when introducing cassava foliage into the diet of lambs. Fresh cassava foliage was supplemented to up to 2% BW using three different strategies. The first treatment group was offered the entire amount on the first day of the experiment. The introduction of cassava foliage in the second and third treatment groups was gradual, reaching 2% supplementation on day 7 and day 21 of the experiment, respectively.

Processing may lead to changes in chemical composition and nutritive value of cassava foliage, which in turn may result in differences in feed intake, growth rate, digestibility and intake of HCN. In the fourth experiment (Paper IV), lambs were supplemented with cassava foliage at 1.5% BW (medium bitter variety harvested at around 3 months) in three different forms: fresh, or processed as wilted or hay.

Study sites

The studies were conducted at the Goat and Rabbit Research Centre, Sontay town, Hanoi capital in Northern Vietnam. The centre is located in the hilly area, 40 km from Hanoi capital to the west, (N21^{o}06’N, 105^{o}25’E), and at 220 m above sea level. The climate is tropical monsoon with a wet season from April to October and a dry season from November to March. The mean temperature ranges from 15 to 30°C and average annual rainfall is 1870 mm.

Field crop study: Land preparation, design, planting, data collection and management

The field crop study (Paper II) was conducted from March to November, 2009, and was repeated in 2010, on an acidic soil with low fertility. The study was set up in a field of 2300 m^2, 1800 m^2 of which was used for planting and 500 m^2 as border areas around the whole planting area. The experimental areas received goat manure corresponding to 200 tonnes/ha, 500 kg NPK (8:10:14)/ha and 300 kg urea/ha and year. All manure and half the NPK and urea were applied at planting. The remaining NPK and urea were divided equally and applied on all plots every 3 months.

A randomised design was used in the study. Treatments in the experiment corresponded to the harvesting frequency at 3, 6 and 9 months (three harvests), at 6 and 9 months (two harvests), and at root harvesting at 9 months (one harvest) after planting. In each treatment, three cassava varieties were used (K94, K98-7 and local corresponding to very bitter, medium bitter and sweet taste), with four replicates of each combination of treatment and variety (plots), giving a total of 36 plots.

Stem cuttings, 20-25 cm long, were planted at 15-20 cm depth in rows, and with 60 cm between rows and 40 cm between plants. In total, 3600 stems were planted (100 stems per plot).

Fresh cassava foliage from each plot was weighed to estimate the fresh yield. At each harvest, the fresh cassava foliage from each of the harvested plots was sampled for further analysis of DM, ash, CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), total tannins and HCN content.

Experimental animals, feeds, designs and management

The lambs used for the experiments were of the local Phan Rang sheep breed. All lambs in the feeding trials were kept in individual cages. The animals had free access to clean water. Mineral lick blocks were available *ad libitum.* Before starting all experiments, the lambs were treated for parasites and were vaccinated against pasteurellosis, enterotoxaemia, foot and mouth disease and sheep pox.

For the fresh cassava, stylosanthes, jackfruit foliage and guinea grass (*Panicum maximum*) was harvested from the field 1-2 hours before feeding (Papers I, III and IV). To prepare wilted cassava foliage, fresh cassava foliage was left for 16 to 18 hours in the shade, while for cassava hay it was sun-dried for 2-3 days before feeding (Paper IV). The rice straw used in Paper I was treated with 4% urea, 1% salt and 1% lime for 10 to 15 days before feeding.

The concentrate was a commercial concentrate produced by the Guyomach feed company (Paper I) or by the CP company (Paper IV), and was based mainly on fish meal, soybean meal, maize and rice bran. Cassava chips (Paper IV) and molasses (Paper I) were bought in the local market.

All signs of physiological suffering such as saliva excretion and abnormal behaviours during the experimental period were recorded (Papers

I, III and IV). In Paper III, heart rate, respiration rate and rumen movement were measured on the day before feeding fresh cassava foliage and at day 1, 2, 3, 4, 5, 6, 7, 13, 14, 20, 21 and 42 after feeding. On each observation day, all three physiological parameters were measured at 1, 2, 4, 8 and 12 hours after feeding fresh cassava foliage.

Samples of feed offered and feed refusals were taken once per week for determination of DM content, then pooled monthly for further analysis. Faecal samples were taken from individual animals (Paper I) for enumeration of gastro-intestinal parasite eggs (nematodes, coccidia oocysts and cestodes).

A summary of animal feeds, designs and management regimes (Papers I, III and IV) is shown in Table 3.

Table 3. *Summary of materials and methods used in Papers I, III and IV*

	Paper I	Paper III	Paper IV
Experimental animals	- *Feeding trial:* 32 lambs, average BW 14.9 kg. - *Digestibility trial:* 8 male lambs	24 lambs, average BW 19.6 kg	- *Feeding trial:* 24 lambs, average BW 15 kg. - *Digestibility trial:* 6 male lambs
Experimental diet	- *Control:* Urea-treated rice straw plus 20% molasses + concentrate at 1.5% of BW on DM basis. - *UTR-C:* Urea-treated rice straw plus 20% molasses + fresh cassava foliage. - *UTR-S:* Urea-treated rice straw plus 20% molasses + stylosanthes forage. - *UTR-J:* Urea-treated rice straw plus 20% molasses + jackfruit foliage	- *Control:* Guinea grass + concentrate at 1.5% of BW on DM basis. - *FCF-0:* Guinea grass + fresh cassava foliage at 2% of BW on DM basis at the beginning. - *FCF-7:* Guinea grass + fresh cassava foliage reaching 2% of BW on DM basis at day 7. - *FCF-21:* Guinea grass + fresh cassava foliage reaching 2% of BW on DM basis at day 21.	- *FCFd:* Guinea grass + 100g cassava chips + fresh cassava foliage at 1.5% of BW on DM basis. - *WCFd:* Guinea grass + 100g cassava chips + wilted cassava foliage at 1.5% of BW on DM basis. - *CHd:* Guinea grass + 100g cassava chips + cassava hay at 1.5% of BW on DM basis.

Experimental time	- *Feeding trial:* 84 days. - *Digestibility trial:* 112 days (4 periods * 28 days per period) and started 2 weeks after the feeding trial.	42 days	- *Feeding trial:* 84 days. - *Digestibility trial:* 84 days (3 periods * 28 days per period) and started 2 weeks after the feeding trial.
Experimental design	- *Feeding trial:* Randomised with 8 replications. - *Digestibility trial:* A replicated 4 x 4 Latin square.	Randomised with 6 replications.	- *Feeding trial:* Randomised with 8 replications. - *Digestibility trial:* A replicated 3 x 3 Latin square.
Feeding schedules	- *Urea-treated rice straw:* fed *ad libitum* and 4 times/day. - *Concentrate and foliages:* supplemented twice per day. The first supplementation was after the first meal of feeding urea-treated rice straw.	- *Guinea grass:* divided and fed 4 times per day at 20:20:20:40% of total amount offered. - *Fresh cassava foliage:* supplemented once only after the first meal of feeding guinea grass.	- *Guinea grass:* divided and fed 4 times per day at 20:20:20:40% of total amount offered. *Fresh, wilted cassava foliage or cassava hay:* supplemented twice per day. The first supplementation was after the first meal of feeding guinea grass. - On days of sampling urine for analysing SCN^-, cassava foliage: supplemented once only.
Measure-ments	- Feed intake - Nutrient intake - Live weight gain - Feed conversion ratio - Digestibility - Nitrogen retention - Nematode eggs counted in faeces	- Feed intake - Fresh cassava intake - Nutrient intake - HCN consumed - Daily gain - Heart rate, respiration rate, rumen movement. - Thiocyanate concentration in urine.	- Feed intake - Nutrient intake - HCN consumed - Live weight gain - Feed conversion ratio - Thiocyanate concentration in urine - Digestibility - Nitrogen retention

The amounts of faeces and urine (Papers I and IV) excreted by individual animals were recorded twice daily, at 7.00 h and 18.00 h, and samples were taken and preserved for further analysis (Chen & Gomes, 1992).

The urine samples for analysing thiocyanate (Paper III) were taken on the day before feeding fresh cassava foliage and on day 1, 2, 3, 4, 5, 6, 7, 13, 14, 20, 21 and 42, at 8 and 12 hours after feeding on each day. In Paper IV, on day 7 of every data collection period, the urine was sampled before and 1, 2, 4, 8, 12, 18 and 24 hours after feeding cassava foliage.

Sample analysis

Samples of feed offered and refused (Papers I, III and IV) and fresh cassava foliage samples (Paper II) were analysed for DM, ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), total tannins and HCN content (only in Papers II, III and IV). Faeces were also analysed for DM, ash, CP, NDF and ADF. Urine samples were analysed for DM, nitrogen and SCN^- content. The DM, ash and HCN content were determined according to the standard methods of AOAC (1990). Nitrogen was determined by the Kjeldahl procedure. Neutral detergent fibre and ADF concentration were analysed according to the procedure of van Soest *et al.* (1991). Total tannins were measured according to AOAC (1975). Thiocyanate content was determined by using flow injection analysis (Patel & Singh Patel, 1999).

Statistical analysis

Data in Papers I, III and IV and thiocyanate data (Paper III) were analysed statistically by ANOVA using the general linear model (GLM) procedure of Minitab Software version 13 (Minitab, 2000) for Paper I, version 15 (Minitab, 2008) for Paper IV, and the GLM procedure in SAS (SAS, 2008) for Papers II and III. Treatment least squares means which showed significant differences at the probability level $P<0.05$ were compared using Tukey’s pairwise comparison procedure.

The number of nematode eggs (Paper I) was analysed by variance analysis using $\log_{10}$ transformation. The results were back-transformed by taking anti-logarithms of the least squares means and standard error of nematode numbers and presented as geometric means. All statistical tests were applied to the transformed data.

Thiocyanate content (Paper IV), heart rate, respiration rate and rumen movement (Paper III) were analysed as a mixed linear model (Littell *et al.*, 2006) using the mixed procedure of SAS software (SAS, 2008).

Results

Effect of different harvesting periods and varieties on cassava foliage yield

The total DM and CP yield of the K94, K98-7 and local cassava varieties increased with increasing harvesting frequency (Paper II). Three harvests resulted in the highest DM and CP yield, while yields were lowest in the single cut at root harvest, 9 months after planting. For the different harvesting schedules, (3, 2 and 1 harvest), the DM yield range of cassava foliage was 5.6-6.3, 5.4-6.1 and 4.5-5.3 tonnes/ha, respectively

Among the cassava varieties, the K94 cassava variety had higher foliage yield than the local on every harvesting occasion. The K98-7 cassava variety always provided the lowest amount of foliage.

Effect of different harvesting periods, varieties, stages of leaf maturity and processing methods on chemical composition and hydrogen cyanide content of cassava foliage

The DM, CP, NDF, ADF and content of total tannins in cassava foliage were affected by harvesting interval (Paper II). The longer the cutting interval, the higher the DM, NDF, ADF and total tannin contents in cassava foliage. In contrast, CP and HCN content were lower at later harvesting time, *i.e.* at 9 and 6 months after planting compared with 3 months. The chemical composition of the foliage differed for the three different cassava varieties in all respects except for the CP content, which was similar. The DM, NDF and ADF content were generally higher in the foliage of the local cassava variety than in that of the K94 and K98-7 varieties, while the total tannin content did not differ between varieties. The HCN content was significantly highest in K94 cassava variety (Table 4).

As regards stage of maturity, the younger leaves of K94, K98-7 and local cassava varieties showed lower concentrations of DM, NDF, ADF and content of total tannins than the older leaves, while the CP and HCN concentrations were higher.

The cassava foliage used as a feed supplement for lambs in Papers I, III and IV was from the K98-7 variety and its chemical composition is presented in Table 4. The CP content of fresh cassava foliage did not change when the material was wilted for 16-18 hours in the shade, while it was clearly reduced by sun-drying for 2 to 3 days. The HCN content was 35 and 81% lower in wilted cassava foliage and cassava hay, respectively, compared with fresh cassava foliage (Paper IV).

Table 4. *Chemical composition of cassava foliage of different varieties and processed forms*

Cassava foliage		DM	CP	NDF	ADF	Total tannins	HCN[1]
		%	% on DM basis			mg/kg	
Paper II	K94	19.1-28.2	18.6-20.7	36.4-39.4	20.7-28.5	2.86-4.36	489-730
	K98-7	19.4-27.8	18.6-20.5	36.5-39.4	26.8-28.3	2.76-4.20	359-607
	Local	20.3-29.2	18.5-20.8	38.1-43.2	28.2-31.6	2.74-4.13	307-552
Paper I, III and IV	Fresh	18.4-20.4	20.2-20.8	35.8-43.1	27.0-31.3	2.30-3.5	333-341
	Wilted	37.2	20.5	41.7	30.2	2.3	217
	Hay	95.3	17.6	53.9	34.2	1.5	60

[1] Mg/kg on fresh basis

Feed intake of cassava foliage

Different combinations of cassava foliage with basal feed consisting of urea-treated rice straw (Paper I), guinea grass (Paper III) and guinea grass + cassava chips (Paper IV) had effects on total DM intake (DMI) of the lambs, but no effect on the DMI of cassava foliage was found. The total DMI of the lambs ranged from 33 to 44 g/kg BW (Paper I). In Paper III, the total DMI varied from 28 to 33 g/kg BW when cassava was fed at different adaptation periods. At the fixed amount of 1.5% BW in different form (fresh, wilted or sun-dried), the total DMI was not significantly different the diets and ranged from 34 to 36 g/kg BW (Paper IV).

The cassava foliage intake as DM varied around 1.5% of BW, even when the foliage was supplemented in *ad libitum* amounts (Paper I) or at 2% (Paper III) or a fixed amount at 1.5% of BW (Paper IV) (Table 5).

Table 5. *Total dry matter intake (DMI) and DMI of cassava foliage by lambs on different combination diets*

Basal feeds	Cassava supplementation level	Total DMI in % of BW [b]	DMI of cassava foliage [a]	
			in % of BW [b]	*g DM/day*
Paper I: Urea-treated rice straw	*Ad libitum*	3.3	1.6	345
Paper III: Guinea grass	2% BW at beginning	2.8	1.4	299
	Reaching 2% BW at day 7	3.0	1.5	322
	Reaching 2% BW at day 21	2.8	1.4	274
Paper IV: Guinea grass + cassava chips	Fresh, 1.5% of BW	3.4	1.5	306
	Wilted, 1.5% of BW	3.6	1.5	366
	Hay, 1.5% of BW	3.4	1.4	309

[a] Overall experimental means; [b] calculated on DM basis

Physiological responses

No signs of toxicity, such as saliva excretion, vomiting, excitement, staggering, paralysis, convulsions, coma and death, were noted among the lambs fed cassava foliage during all experiments (Papers I, III and IV). Heart rate, respiration rate and rumen movement in lambs fed fresh cassava foliage were not affected by different adaptation periods (Paper III).

Digestibility and nitrogen retention

The apparent digestibility of DM and OM in the lambs supplemented with fresh cassava foliage was similar to that of concentrate, and significantly higher than for the diets supplemented with stylosanthes or jackfruit foliage (Paper I). No differences in digestibility of CP and NDF were found between the diets supplemented with concentrate, stylosanthes and cassava foliage, while digestibility was significantly higher than for the diet supplemented with jackfruit foliage.

When supplementing cassava foliage in different forms (Paper IV), the DM, OM, NDF and ADF digestibility were similar between the diets supplemented with fresh and wilted cassava foliage, but were significantly higher than for the diet supplemented with cassava hay.

Nitrogen retention was similar and varied from 9.9 to 10.8 g/day between diets supplemented with concentrate or different protein-rich forages of cassava, stylosanthes and jackfruit. Supplying the cassava supplement for lambs in fresh form or processed as wilted or hay resulted in no difference in nitrogen retention, which ranged from 3.2 to 3.8 g/day.

Growth performance of animals fed cassava foliage

The LWG was 77 g/day when the lambs were fed the basal diet of urea-treated rice straw, supplemented with cassava foliage *ad libitum* (Paper I). However, in different adaptation strategies when feeding fresh cassava foliage, lambs seem to respond better to an adaptation period of 7 days, with faster growth (76 g/day) compared without and with 21-day periods (65 and 69 g/day, respectively) (Paper III). Supplying the cassava foliage in different forms, as fresh, wilted or hay (Paper IV), did not affect the LWG (73-77 g/day).

Thiocyanate in urine

When introducing fresh cassava foliage into the diet of lambs using different adaptation periods (Paper III), the SCN^- concentration in urine increased following the increase in fresh cassava foliage intake over time, being lower in the beginning and higher towards the end of the 42-day experiment.

In Paper IV, the lambs were fed cassava foliage once a day. The SCN^- concentration in the urine from lambs fed fresh and wilted cassava foliage gradually increased from the level before feeding, reaching the highest level at 12 h after feeding before decreasing again. No significant effect of time was observed for the diet with cassava hay. The SCN^- concentration in the diet with cassava hay was significantly lower at all observation times compared with the diets with fresh and wilted cassava foliage.

Internal parasites

Supplementing cassava foliage to lambs, with a basal feed of urea-treated rice straw, had no significant effect on the number of eggs from cestodes or coccidian oocysts in the faeces, but reduced the number of nematode eggs compared with feeding concentrate or stylosanthes (Paper I).

General discussion

Cultivation and processing of cassava foliage

The potential yield of cassava foliage varies and depends on various factors such as cultivar, age of plant, plant density, soil quality, fertilisation, harvesting frequency and climate (Ravindran, 1992). The foliage production of cassava is generally lower when foliage is only harvested as the agricultural by-product at root collection, but higher DM yield of foliage can be obtained when cassava is harvested more than once.

In this study, the yield was highest when cassava foliage was harvested three times, at 3-month intervals, and was lowest with only one harvest at 9 months after planting (Paper II). These results were similar to previous findings, as can be seen in Table 6. The ranges for harvesting frequencies of foliage in the literature were between one and four harvests over a 9-month cultivation period. Thus, within the ranges shown in Table 6, it seems that total yield of cassava foliage increased with increasing number of harvests. This result was consistent irrespective of variety or season of harvesting or planting.

Frequent cutting of certain tree forages such as *Leucaena leucocephala* has been reported to reduce DM yield on each harvest occasion, because overcutting can lead to stem death (Karim *et al.*, 1991). Foliage production of cassava at a second harvest, 5 to 6 months after planting, was higher than at the first cut according to Wanapat (2008), Phengvichith *et al.* (2006) and Paper II. In these studies, cassava was cultivated during the period from March to December, which means that 5 to 6 months after planting time would be the rainy season in Asian countries. Frequent harvests may lead to a reduction in the yield of foliage at each harvest but provide a more even food supply over the season and give a larger total DM yield. However, it must also be emphasised that there are other factors not studied here, such as climate (temperature, rainfall), soil quality and fertilisation, which are also important for the yield of cassava foliage.

Table 6. *Foliage production of cassava foliage at different harvesting schedules*

Harvesting schedules	DM foliage production	Source
• Two different harvesting schedules: - One foliage harvest at root harvesting after 10 months - First harvest at 3 months and subsequent harvests at 56 to 75 days thereafter.	• Foliage yield at root harvesting was only 1.2 tonnes/ha, while was 10.09 tonnes/ha with more intensive harvests	(Dung *et al.*, 2005a)
• First harvest at 105 days and subsequent harvests at every 45, 60, 90 or 285 days thereafter	• Increased following increased harvesting frequency: from 0.64 tonnes DM/ha at 285 days to 4.57 tonnes/ha at harvesting every 45 days	(Khang *et al.*, 2005)
• First harvest at 3 months and subsequent harvests every 2 months from March to December	• Increased from 0.88 and 1.03 tonnes/ha at no subsequent harvest to 3.38 and 4.13 tonnes/ha with 3 subsequent harvests in KS50 and local varieties, respectively	(Phengvichith *et al.*, 2006)
• Two different harvesting schedules: - 3 harvests: The first harvest at 4 months and at every 2 months thereafter. - 4 harvests: The first harvest at 2 months and at every 2 months thereafter.	• Higher yield with 4 harvests compared with 3 harvests (7.1 vs 6.5 tonnes/ha)	(Phengvilaysouk & Wanapat, 2008)
• Three different harvesting schedules: -Three harvests, at 3, 6 and 9 months after planting - Two harvests, at 6 and 9 months after planting One harvest, at 9 months after planting	• The highest DM yield was with 3 harvests (5.6-6.3 tonnes/ha). It was intermediate with 2 harvests (5.4-6.1 tonnes/ha) and lowest with 1 harvest (4.5-5.3 tonnes/ha)	Paper II

In the present study (Paper II), no or minor differences in CP content were found between varieties and harvesting frequencies but significant differences were found in the concentration of secondary compounds. The content of total tannins in the cassava foliage was not different among the cassava varieties, but was affected by different harvesting schedules and stages of leaf maturity. It was higher at the first harvest and in old leaves, and lower in the subsequent harvests and in young leaves. A possible reason for the slight reduction in total tannin content in cassava foliage at subsequent harvests is the high carbohydrate demand generated by rapid re-growth limiting the substrate of carbon-based secondary metabolite. The content of HCN was affected by harvesting schedule, being lower at the first harvest, and increasing at subsequent harvests, irrespective of whether this harvest took place at 6 or 9 months after planting. The increase in HCN content in subsequent harvests could be due to the role of cyanogenic glucosides in protection of plants from damage by herbivores, insects, *etc*., as has been suggested by Zagrobelny *et al.* (2004). There was a higher HCN content in young leaves compared with old leaves. Among the cassava varieties tested here, very bitter cassava foliage showed the highest HCN concentration and the sweet variety the lowest. The variation in HCN content in cassava foliage depends on variety, stage of maturity, soil fertility and environment (Mlingi *et al.*, 1995) and HCN content normally decreases with leaf maturation (Vetter, 2000).

Variety also has a strong effect on the yield of cassava foliage (Phengvichith *et al.*, 2006). In Paper II, the K94 cassava variety always had a higher yield of foliage compared with the K98-7 and local varieties. The difference in foliage production between varieties could be due to differences in genotype and growing period.

Based on the discussion above, it can be recommended that cassava should be planted in early March each year in northern Vietnam, in order to get the highest foliage production during the rainy season (April to September). A harvesting schedule with the first harvest 3 months after planting and subsequent harvests at to 2- to 3-month intervals thereafter would be the best way to improve foliage production. Foliage from the medium bitter cassava variety K98-7 was used in a safe way for sheep in Papers I, III and IV, by feeding at 2% of BW after other feeds had been offered. This variety, as well as the sweet local cassava variety, which had a lower HCN content, is definitely suitable for use as a feed supplement for sheep. Foliage from the very bitter cassava variety K94 may also be used as a feed supplement, but processing to reduce the HCN content before feeding is strongly recommended.

The different environmental temperatures to which the foliages were subjected during processing could be the cause of variations in HCN reduction. According to Achidi *et al.* (2008), free cyanide evaporates at a temperature of 25°C, and the cyanogenic glucosides in the cassava leaves were reduced by almost 50% when heated at 60°C for 30 minutes. In Paper IV, wilting and sun-drying reduced the HCN content in fresh cassava foliage from 333 mg/kg to 217 and 60 mg/kg (35% and 82%), respectively. Studies by Hang & Preston (2005) and Phengvichith & Ledin (2007) showed that wilting cassava foliage in the shade reduced the HCN content by 58% and 45%, respectively. Reductions in HCN levels of up to 63% by sun-drying in the open air have been reported by others (Khieu Borin *et al.*, 2005). Ensiling has also been shown to reduce HCN content in fresh cassava by 78% (Khang & Wiktorsson, 2006; Man & Wiktorsson, 2001).

Cassava foliage in feeding management

According to Crush & Caradus (1995), hungry animals are generally less discriminating in their choice of feed. The concept of never feeding cassava foliage to sheep when they are hungry needs to be followed. Eating and chewing activities disrupt the plant tissues, and this causes the cyanogenic glucosides to come into contact with the hydrolysing enzyme β-glucosidase (Zagrobelny *et al.*, 2004). After chewing, the amount of HCN released will be absorbed quickly into the blood when the animal stomach is empty.

According to Sousa *et al.* (2003), sheep and goats are highly susceptible to HCN toxification. Chewing behaviour and the neutral pH (5-6) in the rumen are optimal for the hydrolysing activity of β-glucosidase (Essen, 1993). In the experiments presented in this thesis, the lamb diet was supplemented with cassava foliage after a meal in the early morning (20% of total daily feed offered) of urea-treated rice straw (Paper I) or guinea grass (Papers III and IV). The HCN consumed with fresh cassava foliage was 6.3 mg/kg BW (Paper I), 5.1-5.4 mg/kg BW (Paper III) and 6.7 mg/kg BW (Paper IV). Those values exceed the tolerance level of sheep (2.0-4.0 mg HCN/kg BW) suggested by Conn (1979) and Kuma (1992). However, no signs of poisoning such as saliva excretion, vomiting, excitement, staggering, paralysis, convulsions, coma or death were noticed when cassava foliage was supplied either once or twice a day. This lack of toxicity symptoms could be due to the feeding regime, where the cyanogenic glucosides in cassava foliage were mixed with the other feeds (urea-treated rice straw or guinea grass). In this way, the toxicity of cyanogenic glucoside was perhaps diluted to below the threshold value. It is also possible that when eating cassava foliage, sheep adapt their chewing behaviour to minimise the tissue damage that they inflict on leaves when masticating in order to limit exposure of cyanogenic glucosides to degradative β-glucosidases (Gleadow & Woodrow, 2002).

The results of Paper III indicate that it is beneficial for sheep to have an adaptation period of approximately 7 days during which cassava foliage is gradually introduced into the diet, in order to avoid negative effects on intake, LWG and physiological responses. When cassava foliage was introduced into the diet without an adaptation period, a lot of feed was wasted as animals did not consume the entire amount offered. However, compared with a 7-day adaptation period, a prolonged adaptation period of 21 days gave a lower LWG of lambs (Paper III). It is probable that the 10-day period of adaptation to cassava foliage before starting the experiment that was applied in Papers I and IV also contributed to the lack of signs of poisoning.

The thiocyanate concentration in urine increased following an increase in intake of fresh cassava foliage (Paper III). When lambs were fed fresh or wilted cassava foliage once daily, the thiocyanate content in urine gradually increased from the basal level before feeding, reaching the highest level at 12 hours, after which the level decreased (Paper IV). When cassava hay was fed to lambs, thiocyanate concentration in urine did not change during the 24 hours of data collection. The changes in thiocyanate concentration in the urine (Papers III and IV) during the hours after feeding cassava foliage support the suggestion that higher feeding frequency (split dose) of cassava foliage can reduce the risk of HCN toxicity by preventing the lethal HCN threshold being exceeded on any one occasion.

Self-regulation of intake

The DMI of sheep normally ranges from 15 to 30 g/kg BW, depending on feed quality and feeding management (NRC, 1985). However, under good feeding conditions and using high quality feeds (high palatability), the DMI of sheep can reach 40-50 g/kg BW.

In our experiments the total DMI varied, *e.g.* 33 g/kg BW (Paper I), 28-30 g/kg BW (Paper III) and 34-36 g/kg BW (Paper IV), when the lamb diet was supplemented with cassava foliage in combination with urea-treated rice straw (Paper I) or guinea grass (Papers III and IV). However, different supplementation levels of cassava foliage, *e.g. ad libitum* (Paper I), at 2% BW (Paper III) and at 1.5% BW (Paper IV), had no effect on the overall mean cassava foliage intake (about 1.5% BW). Self-regulation of intake with feed containing cassava foliage as a secondary compound was expressed very clearly in Paper III. The DM cassava intake increased gradually during first 7 days to 1.5% BW and varied around this amount until the end of the experiment. Thus, a supplementation level of cassava foliage at 1.5% of BW (DM basis) for lambs could be appropriate in respect of the economics of sheep production.

One important factor that has been known to affect the feeding behaviour and self-regulation of intake of small ruminants is feed preferences. Post-ingestive learning and sensory recognition of different feeds allows animals to predict the nutritional and physiological consequences of intake (Baumont *et al.*, 2000). According to Provenza (1995), small ruminants are very sensitive to the four primary tastes: sweet, salty, bitter and sour. When given a familiar feed, sheep do not control intake (Pfister *et al.*, 1990). With a novel flavour, in general, animals tend to take small amounts of the feeds and learn whether it is safe, the normal rate of intake being resumed if no adverse effects are experienced (Provenza *et al.*, 1994). Thus, the bitter taste of the cassava foliage could be the primary reason for the limited intake of fresh cassava foliage by lambs during the first 7 days of the experiment.

The fairly constant intake of fresh cassava foliage at around 1.5% BW could be also due to the sensory recognition of malaising, caused by exposition to HCN in the rumen. In this case, the lambs might sense the malaise signals in the rumen wall and/or in the liver, enabling them to avoid consuming more fresh cassava foliage, thus demonstrating diet awareness as suggested by Forbes (1998).

Effect of feeding cassava foliage on production and digestibility

According to Binh *et al.* (2005), Phan Rang sheep are very well adapted to the local environment in Vietnam. The annual growth rate at 3 to 6 months of age is about 68-73 g/day when lambs are fed a diet with sufficient nutrients according to their requirements.

When the lambs in the present study were fed fresh cassava foliage *ad libitum* (Paper I), the LWG was 77.7 g/day. A similar LWG was obtained by supplementing the diet with a commercial concentrate or with other protein-rich foliage such as jackfruit and stylosanthes. The form in which the cassava foliage was supplied at 1.5% of BW (fresh, wilted or hay) did not result in any difference in LWG (73-77 g/day), and no difference was obtained compared with their potential growth performance as reported by Binh *et al.* (2005). Based on standard values of nutritive requirements in sheep, as suggested by Paul *et al.* (2003), the lambs used in the present experiments consumed sufficient nutrients, especially CP and ME. Thus, no difference in LWG of the lambs could be expected.

Apparent digestibility of cassava foliage DM in lambs was 60% when fresh cassava foliage was supplied *ad libitum* in combination with urea-treated rice straw (Paper I), and 67.4% when fresh cassava foliage was supplied at 1.5% BW in combination with guinea grass (Paper IV). The lower DM digestibility obtained when cassava foliage was fed together with urea-treated rice straw could be due to the low nutritive value of rice straw (low CP, high fibre and low digestibility), as discussed in Paper I.

Processing fresh cassava foliage into wilted form did not result in a reduction in apparent DM digestibility (68.7%), but this decreased to 60% when cassava foliage was provided in the form of hay (Paper IV). The CP digestibility was similar irrespective of whether fresh cassava foliage was combined with urea-treated rice straw or guinea grass, but was lower with cassava hay. The difference in digestibility could relate to the lower CP and higher NDF and ADF content in cassava hay compared with fresh and wilted cassava foliage (Paper IV). This in turn could be due to some of the leaves being lost during processing of cassava hay, resulting in a higher proportion of stems and fibrous material.

As regards the nutritive value of the feed, feeding cassava foliage in a combination diet with guinea grass could be better than combining it with urea-treated rice straw. However, cultivating guinea grass as a feed for animals would require its cultivation as a crop on arable land and most sheep producers need land, seeds, fertiliser, *etc*. for cultivation of other crops destined for human consumption. In contrast, rice straw is considered as agricultural by-product of low value. It is somewhat bulky and is produced in large amounts after each rice harvest in Vietnam. The alternative uses of rice straw are limited and farmers usually burn it after rice harvesting. This is a waste of resources and also has adverse environmental effects through the large amounts of smoke produced. The technique of urea treatment to improve the nutritive value of rice straw is very well-known, cheap and easy to apply and offers an interesting and cheap alternative if it is combined with a protein-rich feed. Thus, feeding cassava foliage in combination with urea-treated rice straw could be beneficial in terms of both economics and the environment.

The LWG of lambs was similar in Papers I and IV, but the nitrogen retention was very different (9.8-10.9 g/day and 3.2-3.8 g/day, respectively). In fact, nitrogen retention in Paper I was higher than the requirement for growth according to Paul *et al.* (2003). The very high value obtained in Paper I could be due to nitrogen being lost as ammonia during the day, but the urea-treated rice straw-molasses only being sampled at feeding and at 24 hours after feeding. This may have resulted in the difference between nitrogen content in the feed and in refusals being overestimated and calculated intake being higher than the true intake.

Effect of feeding cassava foliage on animal health

Feeding cassava foliage to sheep could reduce the number of nematode eggs counted in sheep faeces, as demonstrated in Paper I. The positive antiparasitic effect of cassava foliage has also been shown in goats (Sokerya *et al.*, 2009; Seng *et al.*, 2007; Dung *et al.*, 2005) and in buffaloes and cattle (Netpana *et al.*, 2001). Condensed tannins in the cassava foliage seem to play an important role in reducing gastro-intestinal nematodes (Mui *et al.*, 2005).

According to Hoste *et al.* (2006), there are two possible mechanisms behind the effect of tannins on nematodes in ruminants, one indirect and one direct. The indirect effect, via protein-binding ability, comes about through condensed tannins protecting proteins from degradation in the rumen, thus making true protein and amino acids available, which are absorbed in the small intestine. This in turn may improve the nutrient status and resistance of the host animal (Min *et al.*, 2003; Waghorn *et al.*, 1990). The direct effect also relates to the ability for binding protein, whereby condensed tannins bind to the nematode cuticle, buccal cavity, oesophagus, cloaca and vulva, which are known to be proline- and hydroxyproline-rich structures (Thompson & Geary, 1995). This leads to a change in the physical and chemical properties of the nematodes (Hoste *et al.*, 2006). According to Athanasiadou *et al.* (2001), the protein-binding ability of condensed tannins can also inhibit enzymatic activity by interfering with enzymes secreted or excreted by the worms or with enzymes involved in metabolic pathways that are essential in nematode functions.

Hydrogen cyanide in cassava foliage is known as a potent toxic compound that can have major detrimental effects on animal health. In Papers I, III and IV, there were no physiological responses or signs of toxic symptoms. However, these experiments did not study the effects of feeding cassava foliage in terms of changes in the internal organs of the animals in a short- or long-term perspective. According to Burritt & Provenza (2000), cases of cyanide poisoning due to ingestion of cassava foliage can occur and have an adverse effect on animal health and production. The specific symptoms of cyanide poisoning include saliva excretion, vomiting, excitement, staggering, paralysis, convulsions, coma or death. Rosly *et al.* (2010) indicated that feeding cassava foliage to goats at 7 mg/kg BW resulted in periportal necrosis.

According to Andersson *et al.* (2007), long-term exposure to cyanide is associated with lesions of the central nervous system, giving rise to ataxia in animals, namely sheep, cattle and horses. Thiocyanate is less toxic than HCN, but according to Wemheuer (1993), thiocyanate interferes as a competitive inhibitor for iodine intake and hormone synthesis, with iodine accumulation in the thyroid gland as a result.

Reflections

Going back to the starting point for these investigations, in many cases the experimental results confirmed our original hypotheses.

The hypothesis that there would be no differences in the digestibility and performance of growing lambs when commercial concentrate was replaced with cassava foliage was supported by the results obtained (Paper I). It was also confirmed that lambs supplemented with cassava foliage performed as well as those supplemented with other protein-rich foliages.

In the field crop study (Paper II), the results verified our starting hypotheses in almost all aspects, with higher yield and higher HCN content at higher harvesting frequencies, higher yield and HCN content in the very bitter cassava variety K94 and the lowest HCN content in the local sweet variety.

In experiments where different adaptation strategies were tested (Paper III), our hypothesis was that a prolonged adaptation period of up to 21 days would give a higher intake of cassava foliage and a higher LWG compared with a 7-day period or no adaptation period. However, the results indicated that an intermediate adaptation period of approximately 7 days gave the best results. Prolonging the adaptation period to 21 days even reduced the LWG of lambs compared with a 7-day period. Furthermore, introducing cassava foliage to lambs without an adaptation period did not induce a negative physiological response, as had been expected in some individuals, so the original hypothesis regarding the physiological response was rejected.

Finally, feeding lambs wilted or hay cassava foliage (Paper IV) resulted in lower HCN content in the feed and gave a lower HCN intake, in accordance with the original hypothesis. However, this lower HCN content did not increase foliage intake and did not give the expected higher growth rate compared with lambs fed fresh cassava foliage.

Conclusions

- Cassava foliage can be used as a protein source, as a replacement for commercial concentrate, without decreasing LWG of sheep and could improve the cost-effectiveness of sheep production by reducing feed costs and controlling nematodes.
- Foliage yield of cassava was greater at higher harvesting frequency, being highest at three harvests and lowest at one. A K94 cassava variety consistently had the highest foliage yield. A local cassava variety had higher DM, NDF and ADF content in the foliage than K94 and K98-7 varieties, but lower CP and ash content. At different harvesting intervals, DM, NDF and ADF content in cassava foliage increased continuously with plant and leaf maturation, while CP content decreased. The HCN content was affected by variety and declined with plant and leaf maturation, *i.e.* it was lower at first harvest and increased somewhat at subsequent harvests. The content of total tannins did not differ between varieties but increased with increasing plant and leaf maturity. It was slightly lower in the foliage at multiple harvests.
- Feeding 2% of BW of fresh cassava foliage to lambs without any adaptation period led to wasted feed, as the animals would not consume all feed offered. An adaptation period of 21 days led to a lower live weight gain, so an adaptation period of 7 days may be a good compromise when feeding cassava foliage. A feeding level of 1.5% of BW, when fresh cassava foliage was fed in the morning after another feed was offered, did not result in any changes in heart rate, respiration rate or rumen movement.
- Processing cassava foliage through wilting and sun-drying can reduce the HCN content. There was no difference in intake or lamb

performance when supplied with wilted or sun-dried cassava foliage from a medium bitter variety K98-7 compared with the fresh material.

- Feeding cassava foliage only once daily increased the HCN consumed and the thiocyanate concentration in the urine of lambs. When the lambs were supplied with a fresh or wilted cassava foliage supplement, the thiocyanate concentration increased to a peak 12 h after feeding and then decreased.

Implementation and future research

The findings of this study can be applied to optimise the use of cassava foliage as a feed supplement in sheep production. The experiments showed that cassava foliage is a high quality feed with regard to nutrient content, especially protein content and digestibility. The hydrogen cyanide content is fairly high but several factors, such as wilting, drying and frequent harvesting, can lead to lower hydrogen cyanide content in the foliage, as demonstrated here.

Using an adaptation period of 7 days and offering cassava foliage at 1.5% of BW (on DM basis) in a combined diet can give live weight gain of approximately 75 g/day in lambs. The feeding regime should be adapted to the fact that cassava foliage contains hydrogen cyanide, and it should therefore be fed in small amounts several times daily rather than giving the whole amount on one feeding occasion. Furthermore, it is recommended that cassava foliage be offered after other feeds to minimise the risk of toxicity. If this feeding regime is applied, cassava foliage can be a promising feed supplement for lambs and can lead to better growth performance, less wasted feed and minimisation of the negative toxic effects of hydrogen cyanide on animal health and production.

Implementing the knowledge obtained here with regard to processing, harvesting and feeding management can decrease the risk of toxicity of animals and optimise the positive production effects of using cassava foliage as a supplement in the diet of lambs. Cassava foliage therefore has potential for improving sheep productivity in Vietnam.

Cassava for foliage production should be cultivated in a system where the foliage is harvested at 3 months after harvesting and every 2 to 3 months thereafter. This will enable farmers to provide protein-rich foliage as a supplementary feed for animals all year round.

In the present study, cassava foliage was only used as a supplement for growing lambs. Therefore the effects of supplementing cassava foliage on the reproductive performance of sheep should be investigated in future studies. Long-term feeding of cassava foliage to ruminants may result in tissue damage, goitre and lesions of the central nervous system, due to hydrogen cyanide content. Thus, further studies should examine the effects of a cassava foliage supplement on rumen environment parameters, thyroid gland hormones, liver enzymes, tissues *etc*. Since tannins seem to reduce methanogenesis in the rumen, it would also be interesting to study the effect of feeding cassava foliage on methane production by ruminants, as part of a strategy to decrease anthropogenic greenhouse gas emissions.

Acknowledgements

I gratefully acknowledge the Swedish International Development Agency, Department for Research Cooperation (Sida-SAREC) for their financial support through the Mekong Basin Animal Research Network (MEKARN) project, which made my PhD studies possible.

I am thankful to the Department of Animal Nutrition and Management, SLU, for providing a good academic environment and facilities for study in animal sciences and the Board of Directors of the Goat and Rabbit Research Centre, National Institute of Animal Sciences, for granting me the permission to do my PhD study, providing good research facilities, experimental animals, feeds and animal houses.

This thesis would not have been possible without the guidance, help and encouragement of my supervisors:

The most special thanks I would like to express to Prof. Inger Ledin, who first accepted me as a MSc student in 2006, continuously supported me as a PhD student at SLU and always supervised me until the thesis was completed. Thanks very much for believing in me and the ways you taught and encouraged me to enjoy research.

I would also like to express my gratitude to my main supervisor, Assoc. Prof. Eva Spörndly, for great efforts in helping me, in correcting and giving valuable comments for this thesis, and especially for understanding my feelings when I stayed in Sweden and caring about my study work in Vietnam.

I would like to acknowledge Dr. Ewa Wredle, my co-supervisor, for all encouragement during her trips in Vietnam when I carried out the experiments and during the times when I stayed in Sweden, for valuable comments and helps in so many ways to make the thesis possible.

Sincere thanks to Dr. Do Thi Thanh Van, my co-supervisor in Vietnam, who always motivated and guided me, both as a supervisor in study and as an older sister in life.

I also would like to express my thanks to:

All professors and teachers at the Department of Animal Nutrition and Management, SLU, who gave lectures and advised me in many fields of animal science; this knowledge will be valuable to me throughout my professional life as a researcher. Special thanks to Assoc. Prof. Maria Neil for organising seminars and following up on my study progress.

Thanks to Dr. Ngo Quang Man (former regional cordinator), Dr. Duong Nguyen Khang (present regional cordinator) and Mr. Nguyen Van Cong (administration officer) of the MEKARN project in Nong-Lam University, Vietnam, for helping in financial management and delivery of research funds.

To my colleagues and staffs at the Goat and Rabbit Research Center, Mrs. Tran Thi Hanh, Ms. Can Thi Hop, Mrs. Dinh Thi Dao, Mrs. Truong Thi Le, Mrs. Nguyen Thi Thu, Mrs. Nguyen Thi Hang, Mr. Le Hong Sam, Mrs Can Thi Kiem, Mr. Tran Van Nghia, Mr. Nguyen Van Sao, Mr. Nguyen Duc Tuong, Mr. Trinh Duy Linh etc. and the students from Thai Nguyen University of Agriculture and Forestry and Hanoi University of Agriculture, who were involved in taking care of the animals and collecting the data for the experiments.

To many friends from Vietnam, Laos, Cambodia, China, Burkina Faso, Nicaragua and Sweden, who became a part of my life – I will never forget the time spent with you all.

To my family for their support and believing in me. To my husband, Nguyen Van Hien, for all his love, unfailing support, unceasing encouragement and patience during these study years. To my son, Minh Hieu for the warmness and inspiration he passes on to me.

Lastly, I would like to express my appreciation to all those who in one way or another contributed to this study but are not mentioned here.

References

Achidi, A.U., Ajayi, O.A., Maziya-Dixon, B. & Bokanga, M. (2008). The effect of processing on the nutrient content of cassava *(Manihot esculenta Crantz)* leaves. *Journal of Food Processing and Preservation* 32, 486-502.

Alan, J.D. & John, A.M. (1993). Effect of oral administration of brassica secondary metabolites allyl cyanide, allyl isothocyanate and dimethyl disulphide, on the voluntary food intake and metabolism of sheep. *Britian Journal of Nutrition* 70, 631-645.

Andersson, H.C., Brimer, L., Cottrill, B., Fink-Gremmels, J., Jaroszewski, J. & Sørensen, H. (2007). Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to cyanogenic compounds as undesirable substances in animal feed. *The EFSA Journal* (434).

AOAC (1975). *Official Methods of Analysis.* 12th Ed. Association of Official Analytical Chemists, Washington DC.

AOAC (1990). *Official Methods of Analysis*. 15th Ed. Association of Official Analytical Chemists, Washington, DC.

Athanasiadou, S., Kyriazakis, I., Jacson, K. & Coop, R.L. (2001). Effects of short-term exposure to condensed tannins on an adult Trichostrongylus colubriformis. *Veterinary Parasitology* 146, 728-732.

BAH (2007). *Bureau of Animal Husbandry, Vietnam*.Http://www.cucchannuoi.gov.vn/Statistical.aspx.

BAH (2011). *Bureau of Animal Husbandry, Vietnam*. Http://www.cucchannuoi.gov.vn/Statistical.aspx.

Barry, T. & McNabb, W. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* 81(04), 263-272.

Baumont, R., Prache, S., Meuret, M. & Morand-Fehr, P. (2000). How forage characteristics influence behaviour and intake in small ruminants: a review. *Livestock Production Science* 64(1), 15-28.

Binh, D.V. & Lin, N.K. (2005). *Research and the development of improved small ruminant production systems in Vietnam*. Report of The Goat and Rabbit Research Centre - NIAH-MARD Vietnam.

Binh, D.V., Mui, N.T. & Norton, B. (2005). *Survey findings of goat production in Ninh Thuan, Binh Thuan and Lam Dong at the second year implementing CARD project (VIE 009).*

Binh, D.V., Nha, H.T., Thiem, N.T., Van, D.T.T. & Hue, K.T. (2005). *Predicting performance of Phan Rang sheep in North Vietnamese conditions*. In: Proceedings of the International Workshop on Small Ruminant Production and Development in South East Asia, Hanoi, Vietnam.

Bokanga, M. (1994). Distribution of cyanogenic potential in cassava germplasm, *Agronomic Research,* pp. 117-124.

Bordo, D. & Bork, P. (2002). The rhodanese/Cdc25 phosphatase superfamily. *EMBO reports* 3(8), 741-746.

Burritt, E. & Provenza, F. (2000). Role of toxins in intake of varied diets by sheep. *Journal of Chemical Ecology* 26(8), 1991-2005.

Chadha, Y.R. (1961). Source of starch in commonwealth territories. 111. Cassava. *Tropical Science* 3, 101-113.

Chen, X.P. & Gomes, M.J. (1992). *Estimation of microbial proteins supply to sheep and cattle based on urinary excreation of purine derivatives. An overview of the techical details. International Unit.* Rowett Research Institute, Aberdeen, Occasional publication.

Conn, E.E. (1979). Biosynthesis of cyanogenic glycosides. *Naturwissenschaften* 66(1), 28-34.

Crush, J. & Caradus, J. (1995). Cyanogenesis potential and iodine concentration in white clover (Trifolium repens L.) cultivars. *New Zealand Journal of Agricultural Research* 38(3), 309-316.

Do, H.Q., Son, V.V., Hang, B.P.T., Tri, V.C. & Preston, T.R. (2002). Effect of supplementation of amoniated rice straw with cassava leaves or grass on intake, digestibility and N retention by goats. *Livestock Research for Rural Development* 14(3).

Dung, N.T., Ledin, I. & Mui, N.T. (2005). Intercropping cassava (Manihot esculenta Crantz) with Flemingia (Flemingia macrophylla); effect on biomass yield and soil fertility. *Livestock Research for Rural Development* 17(1), 1-13.

Dung, N.T., Mui, N.T. & Ledin, I. (2005). Effect of replacing a commercial concentrate with cassava hay (Manihot esculenta Crantz) on the performance of growing goats. *Animal Feed Science and Technology* 119(3-4), 271-281.

Essen, A. (1993). ß-Glucosidases: overview. *ACS symposium series (USA)- Fao.org. Series 533,1-14.*

Forbes, J.M. (1998). Dietary awareness. *Applied Animal Behaviour Science* 57(3-4), 287-297.

Gleadow, R.M. & Woodrow, I.E. (2002). Mini-Review: constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology* 28(7), 1301-1313.

GSO (2011). *General Statistics Office of Vietnam.* Http://www.gso.gov.vn/default_en.aspx?tabid=469&idmid=3.

Hang, D.T. (2007). *Cassava leaves as protein source for pigs in Central Vietnam.* MsC thesis at the Department of Animal Nutrition and Management, Swedish University of Agriculture Sciences.

Hang, D.T. & 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* 17(9).

Haslam, E. (1989). *Plant Polyphenols - Vegetable Tannins Revisited*. [online]. Cambridge University Press, Cambridge, U.K.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. & Hoskin, S.O. (2006). The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* 22(6), 253-261.

Jones, W.T. & Mangan, J.L. (1977). Complexes of the condensed tannins of sainfoin (Onobrychis viciifolia Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* 28(2), 126-136.

Karim, A., Rhodes, E. & Savill, P. (1991). Effect of cutting height and cutting interval on dry matter yield of Leucaena leucocephala. *Agroforestry Systems* 16(2), 129-137.

Khang, D.N., H. Wiktorsson & Preston., T.R. (2005). Yield and chemical composition of cassava foliage and tuber yield as influenced by harvesting height and cutting interval. *Asian-Australalian Journal of Animal Sciences* 18, 1029-1035.

Khang, D.N. & Wiktorsson, H. (2006). Performance of growing heifers fed urea treated fresh rice straw supplemented with fresh, ensiled or pelleted cassava foliage. *Livestock Science* 102(1-2), 130-139.

Khanh, T.C. (2007). *Cassava cultivar selection for high fresh root yield and starch content, short growing period, and suitable for the South Eastern and Central Highland regions.* MSc. Thesis. Nong Lam University, Ho Chi Minh, Vietnam. 115 pp.

Khieu Borin, Lindberg, J.E. & Ogle, R.B. (2005). Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. *Animal Science* 80(03), 319-324.

Kim, H., Ngai, N.V., Howeler, R. & Ceballos, H. (2008). *Current situation of cassava in Vietnam and its potential as a bio-fuel*. Http://cassavaviet.blogspot.com/2008_09_01archive.html.

Koch, B., Nielsen, V.S., Halkier, B.A., Olsen, C.E. & Moller, B.L. (1992). The biosynthesis of cyanogenic glucosides in seedlings of cassava (Manihot esculenta Crantz). *Archives of Biochemistry and Biophysics* 292(1), 141-150.

Kuma, R. (1992). Anti-nutritonal factors, the potential risks of toxicity and methods to alleviate them. Speedy, A., Pugliese, P. (Eds.). *In: Legume Trees and Other Fodder Trees as Protein Sources for Livestock*, *FAO, Rome,* pp.145-160.

Littell, R.C., Milliken, A.A., Stroup, W.W. & Wolfinger, R.D. (2006). *SAS for Mixed Models*. Cary, N.C., (2nd Ed.). SAS Institute, Inc.

Man, N.V. & Wiktorsson, H. (2001). Cassava tops ensiled with or without molasses as additive effects on quality, feed intake and digestibility by heifers. *Asian-Australalian Journal of Animal Sciences* 14(5), 624-630.

Miller, I.M. & Conn, E.E. (1980). Metabolism of hydrogen cyanide by higher plants. *Plant Physiology* 65, 1199-1202.

Min, B.R., Barry, T.N., Attwood, G.T. & McNabb, W.C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology* 106(1-4), 3-19.

Minitab (2000). *Minitab Release 13.1 for windows.* Minitab Inc, Pennsylvania, USA.

Minitab (2008). *Minitab Release 18.1 for windows.* Minitab Inc., Pennsylvania, USA.

Mkpong, O.E., Yan, H., Chism, G. & Sayre, R.T. (1990). Purification, characterization, and localization of linamarase in cassava. *Plant Physiology* 93(1), 176.

Mlingi, N.L.V., Bainbridge, Z.A., Poulter, N.H. & Rosling, H. (1995). Critical stages in cyanogen removal during cassava processing in southern Tanzania. *Food Chemistry* 53, 29-33.

Molan, A.L., Waghorn, G.C. & McNabb, W.C. (2002). Effect of condensed tannins on egg hatching and larval development of Trichostrongylus colubriformis in vitro *Veterinary Parasitology* 150(3), 65-69.

Morant, A.V., Bjarnholt, N., Kragh, M.E., Kjærgaard, C.H., Jørgensen, K., Paquette, S.M., Piotrowski, M., Imberty, A., Olsen, C.E. & Møller, B.L. (2008). The -glucosidases responsible for bioactivation of hydroxynitrile glucosides in Lotus japonicus. *Plant Physiology* 147(3), 1072.

Mui, N.T. (1994). Economic evaluation of growing Elephant grass, Guinea grass, Sugarcane and Cassava as animal feed or as cash crops on Bavi high land. *In: Proceeding on Sustainable Livestock Production on Local Feed Resources.* Agricultural Publishing House, 16-19.

Mui, N.T., Binh, D.V. & Orskov, E.R. (2005). Effect of foliages containing condensed tannins and on gastrointestinal parasites *Animal Feed Science and Technology* 121, 77-87.

Netpana, N., Wanapat, M., Poungchompu, O. & Toburan, W. (2001). Effect of condensed tannins in cassava hay on fecal parasitic egg counts in swamp buffaloes and cattle. *In: Proceedings International Workshop on Current Research and Developmenton Use of Cassava as Animal Feed*, Khon Kean, Thai Land.

Nieze, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., & Waghorn, G.C., Green, R. (2002). The effect of feeding sulla (Hedysarum coronarium) or lucerne (Medicago sativa) on lamb parasite burndens and immuity to gastrointestinal nematodes. *Veterinary Parasitology* 105, 229-245.

NRC (1985). *Nutrient requirements for sheep.* National Academy Press, Washington DC, USA.

Patel, R. & Singh Patel, K. (1999). Simple and specific method for flow injection analysis determination of cationic surfactants in environmental and commodity samples. *Talanta* 48(4), 923-931.

Paul, S.S., Mandal, A.B., Mandal, G.P., Kannan, A. & Pathak, N.N. (2003). Deriving nutrient requirements of growing Indian sheep under tropical condition using performance and intake data emanated from feeding trials conducted in different research institutes. *Small Ruminant Research* 50(1-2), 97-107.

Pfister, J.A., Müller-Schwarze, D. & Balph, D.F. (1990). Effects of predator fecal odors on feed selection by sheep and cattle. *Journal of Chemical Ecology* 16(2), 573-583.

Phengvichith, V. & Ledin, I. (2007). Effect of feeding different levels of wilted cassava foliage (Manihot esculenta, Crantz) on the performance of growing goats. *Small Ruminant Research* 71(1-3), 109-116.

Phengvichith, V., Stig Ledin, S., Horne, P. & Ledin, I. (2006). Effect of different fertilizers and harvest frequencies on foliage and tuber yield and chemical composition of foliage from two cassava (Manihot esculenta Crantz) varieties. *Tropical and Subtropical Agroecosystems* 6, 177-187.

Phengvilaysouk, A. & Wanapat, M. (2008). Study on the effect of harvesting frequency on cassava foliage for cassava hay production and its nutritive value. *Livestock Research for Rural Development, 20.*

Poulton, J.E. (1988). Localization and catabolism of cyanogenic glycosides. *In: Cyanide Compounds in Biology*, pp. 67-91. DOI: 10.1002/9780470513712.ch6.

Provenza, F.D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management* 48(1), 2-17.

Provenza, F.D., Lynch, J.J. & Nolan, J.V. (1994). Food aversion conditioned in anesthetized sheep. *Physiology & Behavior* 55(3), 429-432.

Ravindran, V. (1992). Preparation of cassava leaf products and their use as animal feeds. *FAO Animal Production and Health Paper*, 111–125.

Reed, J.D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science* 73(5), 1516.

Rosly, S., Liang, J., Nordin, M., Somchit, N. & Jelan, Z. (2010). Tissues Thiocyanate (SCN) Concentration and Liver Pathology of Sheep and Goats Fed on Cassava Forages. *Pertanika Journal of Tropical Agricultural Sciences* 33(1), 127-133.

SAS (2008). *SAS/Stat 9.2 User's Guide*. SAS Institute Inc., Cary, N.C.

Seng, S., Waller, P.J., Ledin, I. & Höglund, J. (2007). The effects of short-term feeding of fresh cassava (Manihot esculenta) foliage on gastrointestinal nematode parasite infections in goats in Cambodia. *Tropical Biomedicine* 24(1), 47-54.

Sokerya, S. & Rodriguez, L. (2001). Foliage from cassava, Flemingia macrophylla and bananas compared with grasses as forage sources for goats: effects on growth rate and intestinal nematodes. *Livestock Research for Rural Development* 13(2).

Sokerya, S., Waller, P., Try, P. & Höglund, J. (2009). The effect of long-term feeding of fresh and ensiled cassava (Manihot esculenta) foliage on gastrointestinal nematode infections in goats. *Tropical Animal Health and Production* 41(2), 251-258.

Sousa, A.B., Manzano, H., Soto-Blanco, B. & Górniak, S.L. (2003). Toxicokinetics of cyanide in rats, pigs and goats after oral dosing with potassium cyanide. *Archives of Toxicology* 77(6), 330-334.

Thang, C., Ledin, I. & Bertilsson, J. (2010). Effect of feeding cassava and/or Stylosanthes foliage on the performance of crossbred growing cattle. *Tropical Animal Health and Production* 42(1), 1-11.

Thompson, D.P. & Geary, T.G. (1995). The structure and function of helminth surface. Marr, J.J., *et al.* (Eds.). *In Biochemistry and Molecular Biology of Parasites.*Academic Press.

Tien Dung, N., Thi Mui, N. & Ledin, I. (2005). Effect of replacing a commercial concentrate with cassava hay (Manihot esculenta Crantz) on the performance of growing goats. *Animal Feed Science and Technology* 119(3-4), 271-281.

van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science* 74(10), 3583-3597.

Vetter, J. (2000). Plant cyanogenic glycosides. *Toxicon* 38(1), 11-36.

Waghorn, G., Jones, W., Shelton, I. & McNabb, W. (1990). Condensed tannins and the nutritive value of herbage. In: *Proceedings of the New Zealand Grassland Association* 51, 171-176.

Wanapat, M. (2001). Role of cassava hay as animal feed in the tropics. Preston, T.R. *et al.* (Eds.). *In: Proceeding of the International Workshop on Current Research and Development on Use Cassava as Animal Feed.* Khon Kean University, Thailand.

Wanapat, M. (2009). Potential uses of local feed resources for ruminants. *Tropical Animal Health and Production* 41(7), 1035-1049.

Wanapat, M., Pimpa, O., Petlum, A. & Boontao, U. (1997). Cassava hay: A new strategic feed for ruminants during the dry season. *Livestock Research for Rural Development* 9(2), 1-5.

Waterman, P.G. (1999). The tannins - an overview. *In: Proceeding of International Workshop on Tannins in Livestock and Human Nutrition*. Australian Centre for International Agriculture Research, Adelaide, Australia.

Wemheuer, W. (1993). Relation between iodine requirement/thyoid metabolism and ferility problems in cows. *Reproduction in Domestic Animals* 28(7), 385-394.

Zagrobelny, M., Bak, S., Rasmussen, A.V., Jørgensen, B., Naumann, C.M. & Lindberg Møller, B. (2004). Cyanogenic glucosides and plant-insect interactions. *Phytochemistry* 65(3), 293-306.