The Effects of Cassava Foliage (*Manihot esculenta*) on Gastrointestinal Parasites of Small Ruminants in Cambodia

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2009

Acta Universitatis agriculturae Sueciae
2009:43

Cover: Goat feeding with cassava foliage
Photo: Lylian Rodrigez

ISSN 1652-6880
ISBN 978-91-86195-90-8

Tryck: SLU Service/Repro, Uppsala 2009

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Abstract

The overall aim of this project was to evaluate whether cassava foliage mitigates gastrointestinal (GI) nematode infections in young goats in Cambodia. The nematocidal activity of fresh cassava foliage was investigated in four trials with experimentally infected pen-fed goats. In the first experiment, the activity on incoming larvae and established adult worms were tested following short-term feeding of fresh foliage for three weeks. Goats (4-6 months) were first treated with ivermectin. After six weeks, they were re-infected with ~2,000 mixed infective third-stage GI nematode larvae (L3). There was only a slight reduction in the faecal egg counts (FEC) during the cassava feeding periods. In the second experiment, the effects of prolonged feeding of fresh (CaF) or ensiled (CaS) cassava foliage for ten weeks using the same type of set up as in experiment one. The inoculations were in accordance with the first trial, except that ~3,000 L3 were administrated. It was found that CaF only reduced worm fecundity, while feeding CaS also reduced the worm burdens, but only of *Haemonchus contortus*. Although this was promising, it was realized that the possible effects of a high protein content in cassava silage was a confounder. Accordingly, a third trial was conducted. The aim was to compare the antiparasitic effects in goats fed CaS, and a supplement of urea molasses and soybean meal (UM). The results indicated that the UM supplementation supported only resilience (weight gain, anaemic state) against GI parasite infection, whereas parasite fecundity and worm burden were more or less unaffected. In the final experiment special attention was paid on the antiparasitic effects of CaS. Both cassava foliage and paragrass (*Brachiaria mutica*) were ensiled with two fermentative additives (sugar palm syrup or rice bran) and were then fed in a similar fashion to the previous experiments. CaS still provided consistent results irrespective of the fermentative additive used. It is concluded that cassava foliage can be used to reduce the adverse effects of GI nematode infections in goats, in particular when offered as silage.

Keywords: Goat, Parasite control, Cassava foliage, Silage, Nematocidal effect, *Haemonchus contortus*, Protein supplementation

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Dedication

To the memory of my father
and Peter Waller

To my mother
and my husband Pok Samkol

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 Seng Sokerya, Waller, P.J., Ledin, I., Höglund, J. (2007). The effect of short-term feeding of cassava (*Manihot esculenta*) foliage on gastrointestinal nematode infections of goats. *Tropical Biomedicine* 24(1), 47-54.

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

III Seng Sokerya, Chandrawathani, P., Suy, M., Höglund, J. The effects of ensiled cassava (*Manihot esculenta*) foliage compared to protein supplementation on gastrointestinal nematode infections in goats (manuscript).

IV Seng Sokerya, Chandrawathani, P., Touch, V., Butbun, M., Rydzik, A., Höglund, J. A comparison of feeding silage from cassava foliage and paragrass in goats experimentally infected with gastrointestinal nematode parasites (manuscript).

Abbreviations

ADG	Average daily weight gain
AR	Anthelmintic resistance
BW	Body weight
BZ	(pro)benzimidazoles
Ca	Cassava foliage
CF	Crude fibre
CP	Crude protein
CT	Condensed tannin
DM	Dry matter
epg	Egg per gram of faeces
FEC	Faecal egg count
GDP	Gross Domestic Products
GI	Gastrointestinal
GIN	Gastrointestinal nematode
GLM	General Linear Model
Gr	Grass
ha	Hectare
HCN	Hydrogen cyanide
L3	Third stage infective larvae
LWt	Live weight
MAFF	Ministry of Agriculture, Fishery and Forestry
ML	Macrocyclic lactones
NRA	Nicotinic receptor agonists
PCV	Packed cell volume
Rb	Rice bran
S	Sugar palm syrup
SEA	Southeast Asia

1 Introduction

Gastrointestinal nematode (GIN) infection is a major cause of wastage and decreased productivity in livestock worldwide (Waller *et al.*, 1996). This parasitism is particularly a major constraint of grazing livestock in the humid tropics, including Southeast Asia (SEA) (Sani & Gray, 2004). Singly the most important nematode pathogen of small ruminants is *Haemonchus contortus,* which results in enormous socio-economic losses to smallholder farmers throughout the entire region (McLeod, 2004), including Cambodia (Sorn & Muirden, 2002). The need for alternative means of parasite control is urgent, because of the serious problems associated with parasites, the availability of sub-standard anthelmintics, and sometimes also because of high levels of anthelmintic resistance (Waller, 1997; Chandrawathani *et al.*, 2004; Hood, 2004).

Cassava (*Manihot esculenta*) is a crop commonly grown in the tropics/subtropics for the production of tubers for human consumption. Large quantities of vegetative material are the by-product after root harvesting, which would be discarded if not used for livestock feeding. This can prove to be a problem, because cassava contains both anti-nutritional and chemo-toxic substances, such as tannins and hydrocyanic acid (Ravindran, 1992). However, it has been shown that cattle and goats can tolerate the undesirable phytochemical effects (Theng *et al.*, 2003; Seng *et al.*, 2001; Seng & Rodriguez, 2001). The crude protein (CP) content of cassava leaves is in the range of 22–29 % of dry matter (DM) (AFRIS, 2007) and between 21–24 % in the foliage (Seng *et al.*, 2001). Furthermore, it has been proven that cassava leaves are rich in amino acids, comparable to soybean meal (Eggum, 1970).

Cambodia's first ethanol plant (bio-energy) project was initially announced in 2006, which resulted in a dramatic increase of cassava plantation. The Ministry of Agriculture, Fishery and Forestry of Cambodia reported a huge annual increase in cassava production, from 500,000 up to 2,500,000 metric tons in 2005 and 2006, respectively (http://www.maff.gov.kh/eng/statistics/crops.html#a6). Thus this created an opportunity for using the by-product for livestock production. Recently, there has been an increasing number of studies undertaken to investigate the possibility of using cassava leaves/foliages, as a feed resource both for small and large ruminants, as well as mongastric livestock throughout the SEA region (Granum *et al.*, 2007; Khang *et al.*, 2004; Khieu *et al.*, 2005; Phengvichith & Ledin, 2007b; Nurulaini *et al.*, 2007).

A major shortcoming of feeding regimes based on fresh cassava foliage is that it is not always a practical option for small-holder farmers, often located in remote agriculture based regions. Conservation of cassava foliage by silage production is a recommended option in livestock production in Cambodia (Chhay *et al.*, 2001). Ingredients such as sugar or molasses, grains and processed by-products such as maize or sorghum meal, rice bran and cassava root meal can be used as silage additives in feed to provide a fermentable substrate (Mühlbach, 1999). In Cambodia, rice bran and sugar palm juice are available, feasible and suitable under small-scale conditions, because rice production occupies approximately 90 % of the total cultivated land (Chea, 2004), whereas sugar palm is a product that farmers produce during the period of December-May (Khieu *et al.*, 1996).

The initial evidence for the anthelmintic properties of cassava foliage was observed when it was realized that the nematode faecal egg counts (FEC) were reduced in animals fed this material (Seng & Rodriguez, 2001; Seng & Preston, 2003; Lin *et al.*, 2003). There is an expanding interest for more traditional health practices as well as nutraceuticals in both industrialized and developing countries during the last decade (Waller, 1997; Githiori *et al.*, 2003; Bendixen *et al.*, 2005; Athanasiadou *et al.*, 2005; Chandrawathani *et al.*, 2006). Similar benefits have been found from research into other non-chemotherapeutic approaches to nematode parasite control in cassava foliage.

2 Background

2.1 Cambodia, agriculture and livestock production

2.1.1 Geography and climate

Cambodia covers 181,035 square kilometers in the southwestern part of the Indochina peninsula. It lies completely within the tropics, and its southernmost points are only slightly more than 10 ° above the equator. Roughly square in shape, the country is bounded in the north by Thailand and by Laos, in the east and southeast by Vietnam, and in the west by the Gulf of Thailand and its mainland. Much of the country's area consists of rolling plains. Dominant features are the large, almost centrally located great lake, Tonle Sap and the Mekong River, which traverses the country from north to south. As a tropical country, Cambodia is bathed in almost all-year sunshine.

The climate is monsoonal, with two distinct seasons, one dry season and a monsoon or rainy/wet season. The wet season usually lasts from May to October with southwesterly winds ushering in the clouds that bring 75 to 80 % of the annual rainfall. The dry season runs from November to April with temperatures averaging from 27 to 40 °C. Minimum temperatures rarely fall below 10 °C. January is the coldest month, whereas April is the warmest. The total annual average rainfall in Cambodia is between 100 and 150 cm, and the heaviest amounts fall in the Southeast.

2.1.2 Agriculture and livestock sector

Cambodia is predominantly agrarian, with agriculture representing 35 % of GDP in 2003 (AusAID, 2006), and at least 70 % of the rural population are directly dependent on agriculture for their livelihood. Of the total cropped area of about 3 million hectares (ha), 2.5 million ha were planted with rice in 2006 (MAFF, 2007), which is the primary staple crop, providing about 70 % of nutritional intake (ADB, 2003). Within agriculture, crops and fisheries are the most important sub-sectors, with 46 % and 30 % of agricultural GDP, respectively, over the period 1994-2003. This is followed by the livestock (14 %) and forestry sectors (10 %) (AusAID, 2006). According to Wint *et al.* (2006), there are figures on livestock species, mapped as biomass in standard 250 kg livestock units, showing that there are 0.766 bovines, 0.306 pigs, 0.08 small ruminants and 0.0056 chickens distributed throughout Cambodia.

2.1.3 Small ruminant production

Livestock plays an important role for Cambodian farmers, and in particular pig and poultry production are important sectors. Small ruminants are only considered a secondary source of income by Cambodian farmers, and therefore, information on them is scarce. Although there are goats in most provinces of Cambodia, most animals are kept and eaten by the Muslim community. There is no data on the distribution of goats within the country, although a recent estimate of the goat population in Cambodia is around 5,000 (San, 2006). However, this figure is likely to be only around 60 % of the actual population. About half of the total goat population in Cambodia is concentrated in the south-eastern part, close to the Vietnamese border (Kampong Cham and Prey Veng provinces). Goats are mainly kept by village farmers that usually have between 10-50 animals, comprising almost exclusively meat-type goats. Semi-free grazing is the most common management practice in many areas. Thus, many goats are allowed to graze during daytime, often in a restricted area, such as on communal land around lakes, or in the cropping fields after harvesting. In the daytime they are usually herded by women or children (Figure 1). Flocks that consist of 20 to 30 goats, are considered as big (Theng *et al.*, 2003). About 90 % of goat feed comes from off-farm sources. In general, the main feed is native grass, that varies in nutritive value and composition. However, natural grass as a source of roughage is normally restricted to the wet seasons. In addition to grasses, some farmers also provide tree leaves and agricultural by-products as feed supplements (Theng *et al.*, 2003; San, 2006).

Foot and Mouth disease, was the most reported disease by farmers in goats in Cambodia in 2001 (San, 2006). Gastrointestinal (GI) parasites, in combination with poor nutrition, were also identified as important problems (Sani *et al.*, 2004). The development of small ruminants in Cambodia is limited, as a result of several major constrains as described by Sani *et al.*

(2004) & Theng *et al.* (2003). These constraints include lack of knowledge on goat husbandry (including health and disease control) by owners and shortage of feed during the dry season. However, at the same time it has been shown that available crop residues and fodder trees are underutilized. A high mortality rate is one of the chief constraints to the growth of herd size in tropical goats, whereas opportunistic harvesting prevents smallholders from developing medium-scale enterprises (Hood, 2004). For small ruminants in the tropics, nutrition and the control of parasitism are key technological innovations that must be improved in order to sustain viability. However, appropriate markets and economic structures must also be put in place to help smallholders.

Figure 1. Goat herder at Lvea Em village in Cambodia. These goats are housed at night time and grazed on communal pastures outside the village during the day. Photo: Seng Sokerya.

2.2 Gastrointestinal nematode parasites of small ruminants in SEA

Goats are infected with numerous gastrointestinal (GI) parasites, many of which are shared with sheep. The most important include protozoan (e.g. coccidia), and helminth parasites (e.g., cestodes, trematodes and nematodes). Among the latter, GI strongyle infection caused by nematodes in different genera within the superfamily Trichostrongyloidea (e.g. *Teladorsagia,* *Haemonchus, Marshallagia, Trichostrongylus, Nematodirus* and *Cooperia*) present the greatest potential problem (Taylor *et al.*, 2007). Some species inhabit the abomasum (e.g. *H. contortus, T. circumcincta, M. marshalli* and *T. axei*), whereas the majority (e.g. *T. colubriformis, T. vitrinus, T. longispicularis, T. rugatus, T. falculatus, T. capricola, T. retortaformis, C. curticei, C. surnbada, N. battus, N. filicollis, and N. spathiger*) are found in the small intestine.

The trichostrongyloids all have in common a direct life-cycle with alternating free-living and parasitic phases that are dependent on a faecal-oral route of transmission (Anderson, 1992). First, male and female worms mate in the GI tract. Second, the females produce eggs that are passed out to the external environment with the faeces. Interestingly, a *H. contortus* female has a tremendous egg laying potential of >5,000 eggs per day, whereas worm fecundity is much more restricted in the other species. The external development will then occur in the faecal pats and in most species the nematode eggs embryonate and consecutively hatch into first stage (L1) and second stage larvae (L2). Both stages are actively feeding on microorganisms (e.g. fungi and bacteria) and are sensitive to severe drought and extreme temperatures. However, the L2 will soon moult into non-feeding third stage larvae (L3) that retain their cuticle from the previous moult. Under favorable (e.g. moist and warm) conditions the development from the egg to the L3 stage may take place in less than a week. The only exception to this basic pattern among the trichostrongyloids is *Nematodirus* spp., where larval development until the L3 stage occurs inside the egg shell (Taylor *et al.*, 2007). In most trichostrongyloids the L3 migrates from the faecal pat and is usually resistant to extreme environmental conditions (e.g. temperate and drought) and can survive on pasture sometimes for more than a year. Thus, grazing animals are often exposed to parasites and their likelihood of re-infection is greater than in those animals kept in confinement. After ingestion by the host the L3 exsheat in the rumen, and in case of *H. contortus*, then penetrates the mucosa of the abomasum where the transformation into the fourth larval stage (L4) takes place. The final moult into the fifth stage (L5) or adult stage takes place 10-15 days later and upon re-emergence of the parasite into the abomasal lumen. The prepatent period (e.g. the time between ingestion of L3 until the appearance of eggs in the faeces) for *H. contortus* in sheep is 2-3 weeks and 4 weeks in cattle. (Taylor *et al.*, 2007). However, the L4 also have the ability to undergo larval arrestment or hypobiosis in response to various environmental and immunological cues (Blitz & Gibbs, 1972). This is an adaptation found in many trichostrongyloid nematodes to survive adverse weather conditions, and is the reason why the transmission of seasonal under extreme weather conditions.

The humid tropical climate, including that of the southeastern Asia region, provides a more or less permanently favorable environment for the survival and development of the external larval stages (Pal *et al.*, 2001). In most part of SEA, small ruminants usually graze, because of the sizeable grasslands available and their function of weed control in many tree-crop industries (Devendra *et al.*, 1997). According to Pathak & Pal (2008), the prevalence of GI parasites was highest in the wet season, due to the favorable environment in the monsoon, which promotes the development of the larval stages on pasture, and/or due to a reduced prepatent period. With distinct wet and dry season conditions, as in SEA, the majority of L3 acquired by the grazing animals undergo arrested development at the end of the wet season, and FEC decline and remain consistently low during the dry season (Chiejina *et al.*, 1989; Eysker & Ogunsusi, 1980). At the time the wet season begins, when pasture larval challenge and intake of L3 are high, there is a sharp increase in the egg output. While the pattern described is the most common, there are at the same time limited areas surrounding drinking places and permanent ponds, which may be sufficiently humid to maintain optimum larval development and transmission all year around (Cheijina *et al.*, 1989).

Parasitic gastro-enteritis and anemia were reported as the major health problems among small ruminants in Vietnam (Binh & Lin, 2005). In the Philippines, the most important parasite that affect was *H. contortus* (Venturina *et al.*, 2002). In Thailand, it has been shown that *H. contortus* affect the productivity (Kochapakdee *et al.*, 1991). The GI parasite species identified in Cambodia have been ranked according to their impact on the host. In this respect, the most pathogenic species are *H. contortus*, followed by *Trichostrongylus* spp., and *Strongyloides* spp. (Sani & Gray, 2004). The dominance of *H. contortus* is in agreement with later findings by Seng *et al.* (2007), who conducted post mortem examinations of young goats artificially infected with the L3 of the nematode parasite. This can be translated as regional identification, because the sources of L3 were cultured from pooled faeces from several goat farms in Kandal Province. The proportion of the different species reported is in the control treatment, that was offered cut grass and supposed not to receive any effects from the experimental treatment, and were *H. contortus* (66 %), *T. colubriformis* (23 %), *T. axei* (7 %) and *T. circumcinta* (4 %).

2.3 Impact of nematode parasites on livestock production

2.3.1 Nutrient utilization/requirements

Feed intake

In general GI nematodes reduce nutrient availability to the host through both reductions in voluntary feed intake and/or reductions in the efficiency of utilization of absorbed nutrients (Dynes *et al.*, 1998). The disturbance of nutrients absorption allows a considerable flow of proteinacious material to the caecum, thus decreasing the availability of amino acids relative to energy (Steel, 1978). Preston & Leng (1987) suggested that, any condition that affects the absorption of amino acids from the small intestine is likely to have an effect on feed intake. Reduction in voluntary feed intake is one of the major factors contributing to the reduced performance commonly reported in parasitized ruminants (Coop *et al.*, 1982). A reduction in voluntary feed intake of up to 50 % is commonly observed during infection with GI nematodes, and can severely influence the protein economy of the host by substantially reducing the total nutrient availability for anabolic processes (Sykes & Greer, 2003). Inappetence may be less obvious in lighter infections and the improvement in resilience and expression of immunity to GI parasites (Coop & Holmes, 1996; Coop & Kyriazakis, 2001). It is suggested that diets lacking in phosphorus (Coop & Field, 1983) and protein (van Houtert *et al.*, 1995) affected the feed intake in parasitised animals as well.

Gut function

GI parasitism may have an overall effect on gut functions, including a depressed feed digestion, as well as an abnormal absorption and rate of digesta passage. Rowe *et al.* (1988) reported a reduction in apparent digestibility of organic matter across the whole digestive tract, but particularly in the abomasum of sheep infected with *H. contortus*. Reduction in energy digestibility has been observed in relation to both abomasal (Coop *et al.*, 1982) and intestinal parasitism (Sykes *et al.*, 1979) in sheep. However, the extent of reduction of course depends upon the worm burden and the reduction is sometimes small and often transitory in its nature (Sykes, 1983). In the small intestine, nematode worms interfere due to damage of the epithelium lining with the secretion of enzymes, which contributes to depressing the ability to absorb amino acids (Symons & Jones, 1975). The decreased rate of digesta passage was associated with elevated levels of gastrin, which is capable of inhibiting feed intake in hungry parasitized sheep (Grovum, 1981). It has also been shown that abomasal parasites, such as *H. contortus* (Bueno *et al.*, 1982), cause a marked decrease in gut motility and digesta flow in infected sheep.

Nutrient requirement

Nematodes in the GI tract often depletes body proteins and increases the animal's need for absorbed amino acids, which will increase the requirements of protein relative to energy (Preston & Leng, 1987). Koski & Scott (2001) suggested that infection with GI nematodes in livestock both increases the protein needs of the host at the same time as they reduce the effectiveness of the immune response. Steel *et al.* (1980) observed an increased leakage of plasma protein into the GI tract in *T. colubriformis* infected lambs. The main effects of GI parasites were associated with high fractional rates of removal of albumin as a result of elevated enteric loss of protein at the site of infection. The effect of this leakage of protein into the gut was to increase the rate at which hemoglobin and albumin were synthesized and compensated for the losses in blood plasma (Symons & Steel, 1978).

Nutrient metabolism

Gastrointestinal nematode may redirect protein synthesis away from skeletal muscle growth to repair of gut tissues, leading to reduced nitrogen retention (MacRae, 1993). Kyriazakis *et al.* (1994) found that sub-clinical GI parasitism in growing sheep was associated with an impairment of protein metabolism. Loss of plasma protein through the faeces and/or urine is a common feature in sheep heavily infected with *H. contortus* (Rowe *et al.*, 1988). An impaired energy metabolism is another concern in the GI parasitized host. MacRae *et al.* (1979) found that lambs infected with *T. colubriformis* showed a decrease of 17 % in metabolizability of dietary energy by the fourth week of infection compared with pair-fed controls. Several studies have also shown the effects of parasitism on trace mineral metabolism. For example, nematode infections in the small intestine have been linked to a reduction in the absorption of phosphorus (Wilson & Field, 1983). The elevation of abomasal pH, which resulted from parasite infection in the abomasum, reduced the absorption of copper (Bang, 1990). It has also been demonstrated that chronic internal parasitic infection can have a serious negative impact on calcium and phosphorus status (Sykes *et al.*, 1979).

2.3.2 Effects on production

As discussed earlier, GIN both cause disorder of gut functions and impair the nutrient metabolism. The most common and primary losses are due to endogenous protein in the form of whole blood, plasma, sloughed epithelial cells and mucus (Rowe *et al.*, 1988; Kimambo *et al.*, 1988; Coop & Holmes, 1996). Extreme protein deficiency results in severe digestive disturbances, weight loss, anemia, edema, and sometimes also reduced resistance to a range of other diseases. In addition, GIN also interferes with the uptake of trace minerals, which may have a huge impact on the growth and reproduction of the host. For example, it has been shown that a lack of calcium and/or phosphorus resulted in abnormal bone development. Also lambs may be born weak and die of maternal copper deficiency (Anonymous, 1985).

Growth performance

The slow rate of attaining maturity in ruminants, due to chronic sub-clinical parasitism in combination with malnutrition and poor management, will have a major impact on the overall performance. Information on the impact on production caused by GI parasites in humid tropical climates is described by Sani & Gray (2004). Kimambo *et al.* (1988) reported that five-month old cross-bred lambs had a growth inhibition from six weeks after primary infection with 2,500/day *T. colubriformis*. During first 20 of the 34 weeks experimental period, infected lambs gained on average 0.4 kg, compared to 3.7 kg in uninfected controls. The growth depressions induced by GI parasites are easily eliminated when infected animals are offered supplementation, particularly if the diet is enriched in protein. For example, *H. contortus* infected young goats on a high protein diet had better live weight gains than those offered a low protein diet (Blackburn *et al.*, 1991). It has also been shown that supplementary feeding of young sheep with fish-meal substantially reduced the production losses (weight gain and wool growth) attributable to infection with *T. colubriformis* (van Houtert *et al.*, 1995). Similarly, the supplementation of urea molasses blocks mitigated the effect of GIN through the improvement of weight gain and herbage digestibility of grazing lambs (Anindo *et al.*, 1998).

Reproductive performance

The most important determinant of the efficiency livestock production system is the females of reproductive age (Sani & Gray, 2004). If the main product is meat, then the critical measures are reproductive and mortality rates. The more and heavier offspring weaned and the sooner she becomes pregnant after birth the more efficient will be the herd. Flock productivity can be expressed in terms of potential offtake (e.g. number of sheep sold per year). In Maharashtra, India, Ghalsasi *et al.* (2002) compared flock productivity after using an intraruminal capsule containing a macrocyclic lactone that prevented incoming larvae from establishing with others treated every three months with albendazole, and other flocks left untreated. It was shown by complete suppression of the worm population that the annual offtake per female in the flock increased by 22 %. Similarly, a reduction (46 %) in offtake and mortality (6 %) was reported in groups of grazing goats dewormed 3 times per month compared to untreated goats (Osaer *et al.*, 2000). Also in Fiji, field studies have shown that supplementation with urea-molasses blocks can result in increased LWt of lambs at weaning, increased reproduction rates in maiden ewes and reduction in faecal egg output in grazing sheep (Knox & Steel, 1996). During lactation, improved dietary protein supply of West African dwarf goats, infected with *H. contortus*, enhanced resistance to parasite establishment and resilience in terms of kidding performance, birth weight and survival of neonates (Nnadi *et al.*, 2009).

Morbidity and mortality

In highly pathogenic nematode parasites of small ruminants, *H. contortus* is capable of causing acute disease associated with high mortality in all age classes (Allonby & Urquhart, 1975). Studies of helminths in small ruminants in SEA have indicated high incidence and mortality in situations where grazing is the predominant husbandry practice (Dorny *et al.*, 1995). Estimates of annual mortalities of small ruminants due to GINs range from 25 to 50 %. In the Philippines, mortalities, especially in the pre-weaning stage, reached its peak during the wet season when the health of the animals is compromised by a lack of feed resources available and extensive pasture contamination with infective larvae (Venturina *et al.*, 2002). In particular *H. contortus,* which is a blood-sucking nematode, is associated with anemia, which in many cases results in the death of young animals in these regions. In high-risk waterlogged areas, this parasite has also been associated with pneumonia due to *Pasteurella* spp. with mortalities that reached up to 80 %, especially in poorly managed farms (Sani & Gray, 2004). Anaemia, poor growth, low milk supply in ewes, weakness and mortalities caused by *H. contortus* were also reported in Indonesia. Among a flock of grazing goats from birth to 14 months of age and not treated with dewormers, postmortem examination showed a mortality rate of 32 % due to *Trichostrongyles* (Daud *et al.*, 1991). Likewise, Symoens *et al.* (1993) found a mortality rate of 74 % for animals up to one year old, whereas it was 34 % in the adults. Also in this case postmortem examination confirmed the major cause of death as a combination of pneumonia and haemonchosis.

2.3.3 Economic losses

The greatest losses associated with GIN are sub-clinical, and economic assessments have shown that the financial costs are enormous in Australia (McLeod, 1995). In developing countries it has been estimated that *H. contortus* and *O. columbianum* have the largest clinical and economic impact on sheep and goat production (Over *et al.*, 1992). In particular *H. contortus* has a very high biotic potential and at times when transmission is favoured, losses can occur in all classes of animals. Annual treatment costs due to this parasite alone have been recently estimated to be $46 million and $103 million in South Africa (Waller & Chandrawathani, 2005) and India (McLeod, 2004), respectively.

Parasitologists often report the effects of GIN on physiological parameters, growth rates, reproduction and mortality rates, but it is relatively rare to see these effects translated to farming systems and the decisions made by smallholders (Hood, 2004). However, evaluation of the economic impacts of GIN in Asia is often confounded by a lack of accurate estimates of disease prevalence and the differing characteristics of small ruminant production systems throughout the region (McLeod, 2004). Estimation of the aggregate economic value of losses is conducted by using national production losses multiplied by livestock product prices. Hood (2004) reported that, the aggregated costs of GIN were greatest for Indonesia, where it was estimated that the costs for the associated diseases were $13 million among 6 countries investigated in Southeast Asia in 1999. Of this total roundworm-inflicted production loss, an estimated $7.1 million was attributable to goat production and $5.6 million to sheep production. At present there is no estimation of economic impacts due to GIN of small ruminants in Cambodia. However, the Department of Animal Health and Production reported that the loss from an estimated 10 % prevalence of parasites in cattle and buffaloes in Cambodia corresponded to approximately AU$ 23,000,000 in 2001 (Tum *et al.*, 2004).

2.4 Control approaches

The basket options for alternative controls of GIN has been long described and modified according to the situation. These options include:

2.4.1 Chemotherapy

Anthelmintic treatment is a very common and in general most efficient control method. To date there are three major drug classes, separated on the basis of similar chemical structure and mode of action, available for use against nematode in small ruminants (i.e. (pro)benzimidazoles, nicotinic receptor agonists, and macrocyclic lactones) (Taylor *et al.*, 2007). However, anthelmintics are not always economically and/or sustainable option.

The problem of anthelmintic resistance (AR), which is when the target organism does not respond to treatment, needs to be recognized globally as one of the greatest threats to grazing livestock production. There has been a rapid development of the small ruminant industries in SEA since the 1990's in an effort to increase locally produced meat (Waller, 1997). The humid tropical/sub-tropical climate conditions that prevail across the region have necessitated the intensive use of anthelmintics in these flocks to control nematode parasitism. Most of the sheep and goat producing countries in the region have reported some degree of AR. In Indonesia, which has the largest population of small ruminants, only low levels of AR to BZ have been reported from Java (Beriajaya *et al.*, 2003). In the Philippines, BZ resistance was first reported in *H. contortus* in Mindanao (Van Aken *et al.*, 1994), and it has since then been reported from many locations throughput the Philippine islands (Ancheta & Dumilon, 2000; Venturina *et al.*, 2003). Studies on the small numbers of goats present in Thailand indicate that BZ resistance is present, but that resistance has not yet emerged to the NRA levamisol or the ML (Kochapakdee *et al.*, 2002). In Malaysia, BZ resistance was confirmed first in 1991 (Dorny *et al.*, 1993) and then also levamisole and ivermectin resistance was confirmed by Sivaraj & Pandey (1994). Subsequently, national surveys found that 50 % of sheep farms and 75 % of goat farms had BZ resistance, and resistance to levamisole, closantel and ivermectin was also detected (Dorny *et al.*, 1994). More recently, Chandrawathani *et al.* (2004) confirmed a total failure of all available substances to control *H. contortus* infected sheep and goats in government breeding farms in east Malaysia. Up to the present time, there has not been any study dedicated to AR in livestock in Cambodia. Ivermectin is the only anthelmintic reported to be used in the regions where goats are raised (Theng *et al.*, 2003). Although no regular faecal egg count reduction test (FECRT) has been conducted in Cambodia, ivermectin seemed to be 100 % effective when the experimental animals used in the current project were treated before the feeding experiments with cassava were started.

2.4.2 Grazing management

Grazing management is the most widely used complement to chemotherapy to significantly reduce the number of anthelmintic treatments in grazing ruminants (Barger, 1996). Effectiveness in grazing management requires knowledge of seasonal larval availability, and climatic requirements for worm egg hatching, larval development and survival (Barger, 1999). Control measures based on this knowledge often combine strategic anthelmintic treatments and various forms of grazing management. While these measures can reduce the frequency of anthelmintic treatment required, their effect on selection for drench resistance is more problematical, unless they can be combined with other forms of control to reduce dependence on anthelmintics.

2.4.3 Ethnoveterinary practices

This approach is still applicable in some countries, and there are examples both from Africa (Githiori *et al.*, 2005) as well as Southeast Asia, and particularly Indonesia (Subandriyo *et al.*, 2004) and the Philippines (Gray *et al.*, 2004). Several indigenous plants in Vietnam have also been investigated, both *in vitro* and *in vivo,* with varying success (Bendixsen *et al.*, 2005). Although this approach may sound attractive, it has to be borne in mind that it is crucial to test the concept before it is used more widely. Ideally this should be conducted with the same parasite, which is the target for the plant preparation in question. For example, only one out of ten concoctions that were based on various Kenyan plant substances had the purported action and reduced nematode FEC. This was so despite that some preparations were tested both in sheep infected with *H. contortus* and in *Heligmosoides polygyrus* infected mice (Githiori *et al.*, 2005).

2.4.4 Immunonutrition

Nutritional supplementation has long been a means to reduce the requirement for chemotherapeutic control of GI nematode infection of small ruminants. Supplementary feeding, particularly with additional dietary protein, can assist resilience to infection during times when resources are being directed towards dealing with the pathophysiological effects of infection and away from production (Knox *et al.*, 2006). The main effect of protein supplementation is to increase the rate of acquisition of immunity and increase resistance to re-infection through an associated enhanced cellular immune response in the GI mucosa (Coop & Holmes, 1996).

It has been shown that both mineral and non-protein nitrogen supplements lead to greater feed intake and increased microbial protein production, which in turn increases protein digestion and absorption in the small intestine (Knox & Steel, 1996). This process provides a better capacity to the animal to withstand parasite exposure, as well as supporting a higher growth rate with reduced loss of productivity and mortality (Waller, 1999). Enhanced nutrition can affect the ability of the host to cope with the consequences of parasitism and to tolerate or overcome parasitism. However, it can also affect the parasite population through the intake of anti-parasitic compounds (Coop & Kyriazakis, 2001). A more recent update of the implications and interactions of nutrients and parasites in small ruminants has been discussed by Kahn *et al.* (2003), Jackson (2008), Athanasiadou *et al.* (2008), as well as with an emphasis in goats by Hoste *et al.* (2005, 2008).

2.4.5 Bioactive plants

One of the novel control approaches is represented by the use of plants with anthelmintic properties. Several examples obtained from controlled *in vivo* and *in vitro* studies have been accumulated, supporting the hypothesis for a role of plant secondary metabolites, and particularly condensed tannins (CT), that have a detrimental effect on GIN of ruminants (Athanasiadou *et al.*, 2001; Molan *et al.*, 2003; Paolini *et al.*, 2004; Bahuaud *et al.* 2006; Barrau *et al.*, 2005; Heckendorn *et al.*, 2007). The tannin role in inhibiting the presence of GIN has been explained as either a direct or indirect mode of action by cooperating with the improvement of the efficiency of protein utilization (Kahn & Diaz-Hernandez, 2000; Makkar, 2003). Based on the biological activity of tannins, a number of tropical fodder crops, including the cassava plant, and their *in vitro* biological activity was evaluated and summarized (Mui *et al.*, 2005; López *et al.*, 2004a; 2004b).

2.4.6 Integrated control

Any specific parasite control method may be unsustainable when used in isolation. The more choices and the greater variety of controls used in combination, rather than relying almost solely on anthelmintics, the longer effective worm control can be expected (Waller, 2006).

It is agreed among parasitologists that anthelmintics will remain an important part of most parasite control strategies in the foreseeable future, but that they should be integrated with other control measures. Parasite control programs for smallholder farming systems in tropical Asia include strategic drenching in combination with confined and grazing systems, improved nutrition and controlled breeding (Sani *et al.*, 2004). Therefore, it is imperative that animal health workers be educated on the "do's and don'ts" of anthelmintic use for the sustainable conservation of present-day drugs (Sani & Gray, 2004). The countries which have epidemiological information on nematode infections in sheep and goats are thus in a stronger position to plan and implement parasite control programs. Currently, the only practical option for reducing reliance on anthelmintics depends on enhancing resistance of the animal to larval establishment by improved protein nutrition and minimizing exposure to parasites.

2.5 The availability and potential of cassava foliage as animal feed

2.5.1 Opportunity for using cassava foliage as animal feed

Cassava (*Manihot esculenta*) is a productive tropical/subtropical crop that is traditionally cultivated to produce roots for human consumption or industrial extraction of starch. The Ministry of Agriculture, Fishery and Forestry (MAFF) reported that Cambodia produced about 500,000 metric tons in 2005 for local use and export to the neighboring counties. However, this figure increased five times in just in one year, to 2,500,000 metric tons in 2006 (http://www.maff.gov.kh/eng/statistics/crops.html#a6). The dramatic increase observed between 2005 and 2006 was mainly due to local demand with the first ethanol plant (bio-energy) project initially announced at this time. Previously, cassava had been characterized as an "exploitive" crop, destructive of soil fertility. However, when cassava is grown as a component of a farming system, in which livestock and crops are closely integrated, its capacity to "exploit" the nutrients in livestock manure becomes a valuable asset (Preston, 2002). The leaves/foliage are by-products, which are rich in protein (Reed *et al.*, 1982), and since the 1970's have been recognized and adopted for use in livestock feeding (Eggum, 1970; Ffoulkes *et al.*, 1977). The other approach to cassava cultivation is to manage it as a semi-perennial forage crop with repeated harvesting at two to three month intervals (Preston *et al.*, 2000).

2.5.2 Nutritional value of cassava foliage

There are two main cassava varieties in Cambodia, characterized by different length of production (Khieu *et al.*, 2005). The major significant difference between the two varieties in terms of nutrition is the hydrogen cyanide (HCN) content (Khieu *et al.*, 2005; Chhay *et al.*, 2007), as it was reported to be higher in the long-term (bitter) variety. Cassava leaves generally contain more that 20 % CP (Reed *et al.*, 1982; AFRIS, 2007) and the foliage contains between 21–24 % (Seng *et al.*, 2001; Seng & Preston, 2003). Cassava leaves have also been reported to have a good essential amino acid content, comparable to soybean meal (Eggum, 1970). Cassava leaves are also a good source of minerals, (e.g. Ca, Mg, Fe, Mn, Zn) as well as vitamins, such as A and B2 or riboflavin (Ravindran, 1992). Cassava leaf protein is low in methionine and tryptophan, but is rich in lysine (Eggum, 1970). The amino acid content of the leaves varies with stage of maturity, sampling procedure and ecological conditions. Ravindran & Ravindran (1988) showed that in mature leaves, the amino acid concentrations tend to decrease; lysine and histidine showed the greatest decrease among all the essential amino acids.

2.5.3 Anti-nutritional factors in cassava foliage

Hydrogen cyanide

The normal range of cyanide content in cassava leaf is from 20 to 80 mg HCN per 100 g fresh leaf weight, or from 800 to 3,200 mg/kg dry matter (DM). This is substantially higher than the normal range of HCN reported for fresh cassava roots (Ravindran, 1992). Variety and stage of maturity are the major factors causing variations in the cyanide content in cassava leaves (Chhay *et al.*, 2001). The HCN toxicity in monogastric animals has been discussed in many reports. However it does not appear to be a problem for ruminants, as the substance can be detoxified by microorganisms in the rumen (Preston, 1995). In small ruminants a diet rich in sulphur (S) appears to be vital for cyanide (CN) detoxification into thiocyanate, also known as sulphocyanate, thiocyanide or rhodanide (Onwuka *et al.*, 1992). Thiocyanate, which is the anion SCN^- is formed in the rumen, carried into serum and lost slowly via the urine. A level of 0.5 % elemental S ensured adequate cassava CN detoxification in sheep and goats (Onwuka *et al.*, 1992).

Tannins

Tannins are polyphenolic substances with various molecular weights and a variable complexity, with the ability to bind proteins in aqueous solution. Tannins are considered to have both adverse and beneficial effects, depending on their concentration and nature, besides other factors such as animal species, physiological state of the animal and composition of the diet (Makkar, 2003). Brooker *et al.* (2000) indicated that the protein-complexing action of tannins may have a more profound effect on intestinal rather than rumen function, and that microbial interactions in the rumen may reduce but not eliminate tannin toxicity. Evidence for the beneficial effects of tannin-rich plants on internal nematodes in ruminants was recently reviewed by Hoste *et al.* (2006), and it was suggested to act through direct antiparasitic activity or indirectly by increasing host resistance. This promotive effect may be of special benefit, e.g., stimulation of the non-specific immune system selectively at the site of infection and when needed. Reed *et al.* (1982) stated that CTs may be the most important factor limiting the use of cassava. As with HCN the concentrations of tannins in the plant depend on the maturity and vary according to methods of cultivation. The tannin content in cassava has been reported to vary from 30 to 50 g/kg DM (Ravindran, 1993). This range of tannin levels has been explained as being beneficial to ruminants, as it enhances the use of the protein as well as playing an anthelminthic role for the control of nematode parasites (Barry & McNabb, 1999; Butter *et al.*, 2001). A similar range of CTs was reported in fresh cassava top (31.4 g/kg) (Khang & Wiktorsson, 2004), in fresh cassava leaves (41.5 g/kg) (Seng & Preston, 2003), as well as in cassava hay from Vietnam (23.0 g/kg) (Dung *et al.*, 2005), and Thailand (40.0 g/kg) (Granum *et al.*, 2007). López *et al.* (2004b) analysed different compounds of CT in some humid tropical fodder crops and showed that cassava forage contained 53.5 g/kg free CT, 9.3 g/kg bound to protein, 18.8 g/kg bound to fibre, and totally 81.6 g/kg.

2.5.4 Preservation as silage

Ensiling is one among several techniques recommended for practical conditions to preserve the quality of feed materials during periods of excess (McDonald *et al.*, 1991). An advantage of this method is that plant materials can be preserved at any time of the year, even when weather conditions are not suitable for sun-drying. The ensiling process ensures not only increased shelf life and microbiological safety, but it also makes most food resources more digestible (Caplice & Fitzgerald, 1999). It has also been shown that fermentation of cassava leaf and foliage reduces toxicity levels of HCN (Chhay *et al.*, 2001; Sokerya *et al.*, 2009). Tropical crops/grasses often have low levels of fermentable carbohydrates compared to temperate forage crops (Catchpoole & Henzell, 1971). Additives are being recommended to improve fermentation and nutritive value of silages, and 4 to 5 % of molasses was suggested to be used (Henderson, 1993), as well as in tropical crops/grasses of low DM content. In the case of Cambodia 5 % sugar palm syrup has been used in the preparation of silage from cassava leaves (Chhay *et al.*, 2001). Besides easily fermentable ingredients such as sugar or molasses, grains and processed by-products such as maize or sorghum meal, rice bran and cassava meal can also be used as silage additives to provide a fermentable substrate (Mühlbach, 1999). Based on the agricultural activities, rice production occupies 90 % of the total cultivated land in Cambodia (Chea, 2004). Therefore, the use of rice bran as additive would be another alternative for silage production in Cambodia, as it is readily available, feasible and practical. It was shown by Phuc *et al.* (2000), that a pH of about 4 would normally have preserved the silage materials satisfactorily. Result from paper IV demonstrated that, cassava foliage/grass ensiled with either sugar palm or rice bran had similar chemical composition, although the silage with rice bran tended to have a higher DM because of the high DM from rice bran itself.

2.5.5 Antiparasitic potential of cassava foliage

The antiparasitic properties of cassava foliage/leaf was initially indicated by observations of FEC reduction both in goats (Seng & Rodriguez, 2001; Seng & Preston, 2003), as well as in dairy cattle and buffalo (Netpana *et al.*, 2001; Granum *et al.*, 2007). However, whether this as due to an association between the tannins present in the cassava leaf or the relatively high protein content, and how this may directly interact with nematode parasite has as yet not been clearly demonstrated. Results from *in vitro* studies with CT extracts from cassava forage showed a nematocidal efficacy on third stage *H. contortus* at 0.3 ppm but no evidence for ovicidal efficacy on this nematode was recognised (López *et al.*, 2004b). Seng *et al.* (2007) exposed an equally-artificial parasitized goat to fresh cassava foliage for 3 weeks as replacement of a normal grass diet. In this experiment a reduction of FEC was shown, but only during the time of cassava feeding, and the worm population recovered during autopsy was not affected. When the feeding period was extended to ten weeks, FEC was stable at a moderate level, while no effect on the adult worm establishment was detected compared to control animals (Sokerya *et al.*, 2009). In contrast, more dramatic negative effects, both on FECs and total *H. contortus* burdens, were observed when the goats were offered ensiled cassava foliage. This result was confirmed in 3 studies (paper II, III and IV), while the total worm burden of the other nematode species originating from the mixed trichostrongyloid larval infection were unaffected.

3 Aims of the thesis

- To investigate the direct effect of cassava foliage following short- and long-term feeding to goats experimentally subjected to mixed GI nematode infections and
- To validate the nematocidal effect of fresh cassava foliage on incoming larval infections and patent worm burdens
- To investigate the nutritional effect of cassava foliage on nematode parasites in goats experimentally subjected to mixed GI nematode infections
- To assess the anthelmintic efficacy of cassava foliage and paragrass silage, using sugar palm or rice bran as additives, on GI nematode parasites in goats.

4 Summary of materials and methods

4.1 Location

All experiments were conducted at the research farm of the Center for Livestock and Agricultural Development (CelAgrid), between October 2004 and April 2008. The research station is located in Kandal Province, approximately 25 km southwest of Phnom Penh, Cambodia. The climate is tropical monsoonal, with two distinct seasons: dry season (November-April) and wet season (May-October).

4.2 Animals and Management

The experimental goats used in the project were purchased from local farmers in Lvea Em village, located in Kandal Province along the Mekong River in Central Cambodia. Only male kids, between 4 to 6 months of age, weighing approximately 14 kg were used in the studies presented in papers I, II and III, whereas they were slightly younger (3-5 month-old) and lighter (about 11 kg) in paper IV. They were housed individually in pens with raised slatted floors and adapted/adjusted to pen feeding (Figure 2).

One week after their arrival to the experimental farm, the kids were subcutaneously injected with ivermectin (Ivomec® Merial, New Jersey, USA) at a dose rate of 200 μg per kg body weight. In paper IV, 15 of 24 goats were treated twice, as positive FECs were still observed after the first dose. This was done to remove previously acquired nematode infections. Six weeks after the last ivomectin injection, the goats were artificially inoculated with a mixture of third stage infective larvae (L3) of GI nematodes cultured from the faeces obtained from naturally infected goats.

Each goat in the study of paper I received approximately 2000 L3, inoculated in four doses of 500 L3 per head and day at 3-4 day intervals during two consecutive weeks. In paper II, III and IV the larval dose per goat was increased to 3000 L3, that were administrated in three equal doses of 1000 L3 for three consecutive days. Three goats on the cassava silage diet in paper III were re-inoculated at week 4 after the first inoculation, as negative FECs were still observed.

Figure 2. The animal holding facility at the experimental farm of CelAgrid in Kandal Province. Photos: Seng Sokerya and Johan Höglund.

4.3 Source of infective larvae

Larvae for the experimental inoculations were obtained from naturally infected goats from small-holder farms in Kandal Province. Fresh faeces were collected and larvae were then cultured in moist conditions for 10 days at room temperature. The methods used for culturing and harvesting infective larvae were according to the established method of Hansen & Perry (1994) and Anonymous (1986).

4.4 Experimental design

The animals were stratified according to live weights and then block randomized into the different feeding treatment groups in each individual experiment. A control group (CO), which was fed on grass, along with the other experimental groups, was included in all experiments presented in paper I, II and III, but not in paper IV. In the study presented in paper I, all groups were fed on a grass diet. In this trial the design was essentially based on a three-week substitution of fresh cassava foliage for grass at different stages of nematode development after ingestion by the goats. Thus, there were three treatments in this study, which included six animals per group. These were: 1) a control group (CO), 2) a cassava foliage group substituted for grass commencing at the time of larval administration (CaL), and 3) a cassava foliage group substituted for grass three weeks after the larval dosing period (CaA).

As an extension from the study presented in paper I, paper II was conducted with a longer-term of feeding fresh cassava foliage (CaF) as the main diet, which was offered to the goats in one group on a daily basis for 10 weeks. In addition to the grass- fed control group, there was also a third group, which received cassava foliage silage (CaS). Thus, the trial presented in paper II was based on three feeding regimes i.e. 1) CO, 2) CaF and 3) CaS, each consisting of six animals.

In the study in paper III, twenty four animals were divided into three equal groups according to a randomised two-block design of four animals in each experimental group. In this trial the goats were subjected to a high protein diet of grass supplemented with 50 g of urea molasses and 200 g of roasted soybean meal/head/day (UM), which provided a protein level comparable to the one found in CaS. Thus, this experiment was composed of three treatments, i.e. 1) CO, 2) CaS and 3) UM. The idea was to investigate the effect of different protein sources compared to the protein in CaS on GIN infection in goats.

In the final study, presented in paper IV, the effects of silage feeds were investigated using the same number of animals per treatment as in the experiment of paper I and II (e.g. six goats). In this experiment, the animals were randomly allotted according to a 2 x 2 factorial design after being balanced for body weight within the group. The treatments consisted of the following combination of factors:

- Forage type, cassava foliage (Ca) versus paragrass (Gr)
- Fermentative additive, sugar palm syrup (S) versus rice bran (Rb)

Accordingly, in this trial four treatments of six goat in each were compared, i.e. 1) CaS, cassava foliage ensiled with sugar palm syrup, 2) CaRb, cassava foliage ensiled with rice bran, 3) GrS, paragrass ensiled with sugar palm syrup and 4) GrRb, paragrass ensiled with rice bran. The feeding period for paper II, III and IV was ten weeks after the last inoculation of L3.

4.5 Feeds and feeding

Both cassava forage and grass used in the paper I was derived from the CelAgrid research farm. Here cassava is grown as a semi-perennial crop with repeated harvesting at 2-3 month intervals, by cutting the stem at 50 to 70 cm above the ground. Fresh foliage, containing mainly stem, petiole and leaf, was offered to the goats on a daily basis after harvesting. The grass sward consisted primarily of three species, with the main component being paragrass (*Brachiaria mutica*). In paper II, III and IV, a monoculture of paragrass collected from an area kept free from livestock grazing or their faecal contamination in Kandal Province was used as the grass source. Cassava foliage used in paper II was obtained daily from 5-6 months old plants, of several small cultivations located in Kandal Province for fresh feeding purpose. In experiment III and IV cassava foliage was only used after it had been ensiled. The raw material was harvested at similar age and as described in paper II from several medium-scale plantations in Takeo and Kampot Provinces.

The silage consisted of forage (either grass or cassava) sun-wilted for a few hours, mixed with 5 % of sugar palm syrup (raw sugar palm diluted in water 1:1). The silage made with rice bran required 10 % rice bran and 0.5 % NaCl. After thorough mixing, the materials were stored anaerobically in sealed plastic bags for at least 21 days prior to being fed to the goats. Only silage made with sugar palm syrup was used in the studies presented in paper II and III, whereas the experiment in paper IV was conducted with silages that were made with either sugar palm syrup or rice bran. The urea molasses used in paper III comprised mainly of rice bran, sugar palm syrup and urea (Appendix 1, in paper III) and was processed according to Seng *et al.* (2001).

In all experiments the goats started on the experimental diet at least three weeks before inoculation with L3, which was considered to be the adaptation period to allow the animal to adjust to the experimental diet. Rations were always provided *ad libitum* in a daily amount equivalent to approximately 4 % of the live weight for each goat. In the study presented in paper I, II and III, the daily ration was divided and offered twice per day (at around 8 and 15 h), while in paper IV it was offered on three occasions at 8, 12 and 17 h. Wheat bran, 200 g/head/day, was supplemented in the afternoon to all treatment groups in paper I, II and III as an extra energy source. However, in paper IV, wheat bran was only offered to the goats fed on grass silage. In addition, these goats were also provided with 0.5 kg of fresh cut grass to maintain their intake quantity, after severe weight depressions had been observed to occur for several weeks. In general, the feed offered in all experiments was adjusted weekly, so that the refusal rate was maintained at 10–20 % of the amount offered. The experimental feeding period lasted for ten weeks. Ample drinking water was provided individually to each goat in water containers.

4.6 Measurements

4.6.1 Analytical procedures of feeds

The amounts of feed offered and rejected by the animals were recorded daily and representative samples were taken fortnightly or monthly to determine DM (Undersander *et al.*, 1993) and CP, expressed as N x 6.25 (Anonymous, 1990). HCN contents (Anonymous, 1990) were determined only in paper I and II. In addition to this, diets in paper III and IV were further analysed for crude fibre (CF) according to Anonymous (1990). In Paper IV pH was recorded by a glass electrode, according to Chhay *et al.* (2001).

4.6.2 Measurements of animal performance and parasitism

Individual LWt of the goats was measured weekly before the morning feeding. Blood and faecal samples were collected directly from the jugular vein and rectum, respectively, at weekly intervals. Nematode FEC was estimated using the McMaster standard procedure (Hansen & Perry, 1994) with a detection level of 50 epg. The red blood packed cell volume (PCV) was determined using the micro-hematocrit method. At the end of the experiment, all goats were slaughtered and the abomasum and small intestine of individual animals were removed for worm recovery, identification and enumeration in at least two aliquots of 20 ml, as described by Hansen & Perry (1994).

4.7 Statistical analysis

Data of FEC were transformed to log(y+1) prior to statistical analysis in paper I and II while FEC were transformed as log(y+50) according to the sensitivity of the McMaster method in the data analysed in paper III and IV. All statistical analyses were conducted in MINITAB 13.31 software (2000), except for the analysis of FEC, PCVs and LWt in paper I and paper II. These were subjected to the repeated measures analysis of variance (ANOVA) of the StatView® 5.0 (SAS Institute) and of the SPSS 11.5 software (2002) correspondingly to compare the dynamics of the treatment effect against time. In paper III and IV, FEC, PCVs and LWt were analysed using the General Linear Model (GLM) in MINITAB. Treatment and time (length of experiment) effects and their interactions, were used as model of this analysis. Regression coefficients of FEC and LWt over time were calculated for each goat to obtain the rate of FEC increase and the average daily weight gain (ADG). The effects on the dependent variables ADG, final

FEC and its increased rate, PCV lost, species of worm counts and feed intake over ten weeks were subjected to GLM of treatment effect (paper I, II and III) or of a factorial design with the initial values of foliage type, silage additive and their interaction as the model (paper IV). When the differences among treatment means were significant at the probability level of $P<0.05$, the means were compared by using Tukey's pair-wise test.

5 Summary of results

5.1 Feed characteristics

The main nutritive values of feeding materials were analyzed during the experiments in all four papers. However, these data are only reported in paper II, II and IV, and are presented in Table 1.

Table 1. *Nutritional value of feeding materials in the experiment of paper II, III and IV*

	Source	DM (%)	CP (%)	CF (%)	pH	HCN (mg/kg)
Grass						
Fresh	II	18.5	15.7	-	-	-
Fresh	III	20.4	15.1	21.8	-	-
Fresh	IV	20.2	17.9	21.8	-	-
Silage (S)*	IV	20.3	8.7	26.6	4.3	-
Silage (Rb)**	IV	25.2	9.6	26.3	4.5	-
Cassava foliage						
Fresh	II	25.7	21.8	-	-	585.8
Silage (S)	II	29.9	24.1	-	-	171.5
Silage (S)	III	30.4	23.9	13.0	-	-
Silage (S)	IV	26.1	20.56	15.09	4.3	-
Silage (Rb)	IV	30.6	22.2	16.7	4.4	-
Wheat bran	II	86.3	14.8	-	-	-
Wheat bran	III	88.6	17.4	4.6	-	-
Wheat bran	IV	88.0	16.9	5.7	-	-
Soybean meal	III	96.7	40.0	5.3	-	-
Urea molasses	III	70.7	38.6	10.6	-	-

DM, Dry matter; CP, Crude protein; CF, Crude fibre; HCN, Hydrogen Cyanide
(-) Means no data. * S, sugar palm syrup; **Rb, rice bran

5.2 Feed intake and growth performance

During the time of cassava foliage substitution, the goats in the CaL and CaA groups had a significantly increased feed intake, both compared to the other period and to other groups (paper I). Feed intake reached 3.0-3.6 % of body weight (BW) in CaL and CaA, while it was only between 2.0-2.4 % of BW in goats without cassava substitution in the experimental period. The control goats in paper II and paper III had similar levels of DM intake of 3.5 and 3.3 % of BW. The inclusion of urea molasses and soybean meal in the grass diet (UM) increased the DM intake up to 4.1 % of BW, as shown in paper III. In paper II the goats on CaF were able to consume 3.4 % of BW and between 3.0-4.4 % of BW when they were given the ensiled forms of cassava (CaS and CaRb), as shown in paper II, III and IV. Goats that were on the grass silage diets (GrRb and GrS) in paper IV consumed approximately 3.5 and 3.9 % DM of BW.

There was a significant interaction of LWts with time ($P<0.05$), although there were no differences in cumulative weight gain between treatments in paper I. The ADGs were between 2.9 and 5.5 g in the control goats. CaL gained 10.5 and 11.9 g/day during and after the period of cassava substitution, while CaA had an ADG of -5.9 and 17.8 g before and after cassava substitution, respectively. In contrast, the CP intakes were more or less stable for the goats fed the grass diets (either in fresh or silage form in paper II, III and IV) and varied between 4.6-5.4 g/kg BW. However, all of these groups lost weight, with the highest loss in GrRb (paper IV), of -23.6 g/day. The inclusion of urea molasses supplement in the UM goats in paper III, increased not only the DM intakes, but also the CP intake (9.3 g/kg BW) and the ADG (6.7 g/day) compared to goats in the control groups. On the diet with fresh cassava (paper II), goats received 7.4 g/kg BW of CP and gained 42.5 g/day.

When cassava foliage was processed into silage, it consistently led to an increase both in the DM and CP intakes, except for in the CaS goats in paper IV, which had the lowest intake, that was comparable to the CaF goats in paper II. The CP intakes of the CaS goats in paper II, III and IV were 8.8, 11.2 and 7.0 g/kg BW, respectively, whereas in the CaRb goats in paper IV it was 11.5 g/kg BW. All goats fed cassava foliage silage gained weight. However, the ADG varied somewhat between the three experiments. The lowest ADG was observed in the CaS goats (20.0 g/day), followed by the CaRb goats (33.9 g/day) in paper IV, while the CaS goats in paper II and paper III on average gained around 60 g/per head and day.

5.3 Packed cell volume

The pre-trial PCVs in all papers were around 30 %, except in paper III where the initial PCV were between 30-34 %. As a result of the parasite induced anemia from *H. contortus*, there was a gradual reduction in the PCVs in all goats, in all experimental groups in paper I, II, III and IV. Overall, at the end of the trials the PCVs were on average below 22 % for all groups fed on grass, includeing the CO groups in paper I, II, and III, as well as the GrRb and the GrS groups in paper IV, whereas the PCVs both in the UM, CaF and CaS groups in paper II, III and IV, were always above 25 %, except for CaRb, that had 22 % PCV at the end of the trial.

There were no differences in PCV values between the experimental groups, in paper I and paper II. However, after 3-4 weeks, when the FECs started to increase rapidly, there was a corresponding decrease in the PCV trajectories in all groups. On the other hand, there was a trend towards a slight increase in PCV levels in the CaS goats in paper II, from week six until the end of the trial, with a final PCV of 29 %. This value was significantly higher than the PCV in the CO group (22 %), which remained consistently low throughout the experiment, and also compared to 25 %, which was the average in the CaF group ($P<0.005$). There was no difference in the cumulative PCVs between treatments in paper III. However, the development of the PCVs over time showed a difference among the groups ($P<0.05$). The PCVs at the termination of this trial were 28, 26 and 21 % ($P\leq0.001$) in the CaS, UM and CO groups, respectively. In paper IV, the cumulative PCVs over the period of the experiment were significantly different in goats fed the different forage types ($P<0.001$). However, there was no difference between silage additives and their interactions (forage x additive) over this time. In this experiment the PCV decrease was greater in the goats fed both types of grass silages (8.8 and 7.8 %) than in the goats on the two different cassava foliage silages (3.7 and 1.2 %; $P<0.005$).

5.4 Faecal egg count

In general, all experimental groups in all studies had positive mean FECs at two to three weeks after the L3 inoculations, which showed that all animals were successfully infected. The FEC trajectories in paper II, III and IV (Figure 3) were much more consistent compared to the pattern observed in paper I. There was a significant increase in the FECs over time between treatment groups in all papers. All groups exposed to the grass diets, both fresh and as silage, had the highest FEC levels, with a maximum of around

4,000 epg observed in the CO group in paper I, II and III, and in the GrS and GrRb group in paper IV. Fresh cassava apparently always depressed the FECs in all goats on this diet. In paper I, this reduction was only observed during the cassava feeding period of the CaL and CaA goats. It was also observed in the CaF goats in paper II, when cassava foliage was fed throughout the experiment. In this trial, the FECs of the CaF group stabilized 5 weeks post infection and remained around 1,500 egp, while the FECs in all the CaS groups in paper II, III and IV, were always below 500 epg. In particular, the results presented in paper IV indicate that the ensiled form of cassava foliage had the capacity to reduce FECs, whereas neither sugar palm syrup nor rice bran that were used as additives had any influence on FEC.

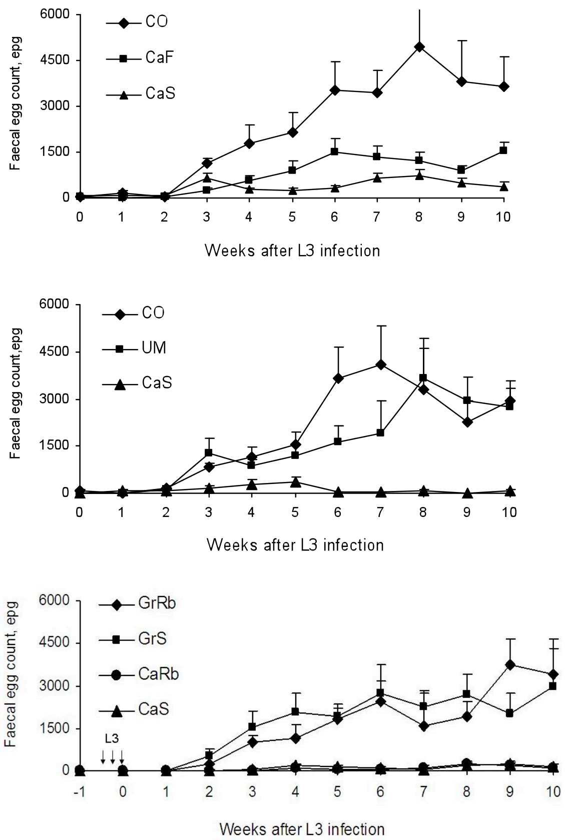

Figure 3. Faecal egg count of goat fed fresh grass (CO), grass ensiled with sugar palm syrup (GrS) or with rice bran (GrRb), fresh cassava foliage (CaF), cassava foliage ensiled with sugar palm syrup (CaS) and with rice bran (CaRb) for 10 weeks. The goats were inoculated with a mixture of 3,000 L3 at the start of experiments.

5.5 Total worm burdens

The most common worm species found in all experiments were *Haemonchus contortus*, *Trichostrongylus axei* and *Trichostrongylus colubriformis*. These species were always identified at worm recovery in paper I, II, III and IV. In addition, minor populations of *Cooperia* spp were found in paper I and IV, and of *Strongyloides* spp. in paper II and III. As a result of inoculation with 2,000 mixed L3 in paper I, and 3,000 L3 in papers II, III and IV, the average total worm burdens (TWB) of the control groups were 779, 1589 and 820 in paper I, II and III, respectively. The intestinal worm, *T. colubriformis*, was highest in paper II, with as many as 878 worms, and where this species contributed to more than 50 % of the TWB. There were only 183 and 160 *T. colubriformis* found in paper I and paper III, respectively. However, the *H. contortus* in the control groups were more evenly distributed in papers I, II and III, with 513, 627 and 640 worms, respectively. In papers I, III and IV, *H. contortus* was the most abundant species, accounting for >50 % of the worm burdens in all treatment groups, whereas in paper II, its proportion was only between 19-40 %.

There was no difference in establishment rates of all worm species in paper I, although CO showed the highest values. In paper II and III, the establishment rates in the treatment groups were consistently more pronounced in all grass fed groups. Of the worm species identified at the termination of each of these trials, an effect was only seen on *H. contortus,* which seemed to be somewhat reduced in all the CaS goats examined at the termination of the experiments presented in paper II and III. In both of these experiments, the number of adult *H. contortus* ranged between 200 and 300. This level was also found in paper IV, which was focused on goats that were fed silage, either made from cassava or grass. All worm species, including *H. contortus*, of the CaF in paper II and the supplemental grass-group (UM) in paper III seemed to be unaffected by the diet as compared to the CO. Furthermore, there were no differences between groups in any other parasite species, neither in relation to forage type, silage additive nor in their interactions in paper IV. Despite the fact that the parasites were inoculated ten weeks earlier in this study, there was still some fourth larval stage (L4) of *H. contortus* found in all groups. However, the proportion of L4 (30 %) in cassava silage goats was found to be significantly (P=0.008) higher than that in the grass silage fed goats (10 %), whereas the remaining worm species identified in this experiment were recovered as adults only.

Table 2. *Number of major worm species recovered at slaughter of goats, 10 weeks after inoculation with a mixture of 3,000 mixed nematode L3*

		Haemonchus contortus	*Trichostrongylus axei*	*Trichostrongylus colubriformis*
Grass				
Fresh[(a)]	CO	513	54	183
Fresh[(b)]	CO	627	73	878
Fresh[(c)]	CO	640	17	160
Fresh + supplement[(c)]	UM	456	38	219
Silage with sugar palm[(d)]	GrS	246	6	79
Silage with rice bran[(d)]	GrRb	202	8	44
Cassava foliage				
Fresh-larval establishment[(a)]	CaL	379	38	208
Fresh-adult establishment[(a)]	CaA	304	75	221
Fresh[(b)]	CaF	658	64	883
Silage with sugar palm[(b)]	CaS	264	47	960
Silage with sugar palm[(c)]	CaS	117	67	188
Silage with sugar palm[(d)]	CaS	244	21	44
Silage with rice bran[(d)]	CaRb	302	15	23

(a) Paper I, the goats were inoculated with a total of 2,000 L3/head and
(b) Paper II, (c) Paper III, (d) Paper IV, with 3,000 L3/head at the start of each experiment.

6 Summary of discussions

6.1 Acceptance of diets by the experimental animals

Tewe (1994) guaranteed the safety of cassava for livestock if it contains a cyanogen level less than 100 mg HCN equivalent per kg BW. In our study, cassava foliage was found to contain HCN 580 mg/kg (paper I), 585 mg/kg DM (paper II) and 172 mg/kg DM in the silage form (paper II). In general, the experimental goats were able to consume these diets in a range of 25 g/kg BW in fresh from (paper II) and between 20-40 g/kg BW of silage (paper II, III and IV). In this quantity of feed, the goats fed fresh and ensiled foliage would ingest about 15 mg/kg and <10 mg/kg BW of HCN per day, respectively. Thus, the experimental animals could be excluded from the possible toxicity risk according to the recommendation above.

During the first 2 weeks, when both CaL and CaA (paper I) were exposed to cassava foliage (switching from a grass diet), the goats showed a reduction in live weight. Ho & Preston (2005) conducted a digestibility trial with the same breed of goats used in this trial and they suggested that at least ten days were required for the animals to gradually adapt to feeding on cassava foliage.

6.2 Feed intake and growth performance

In paper I, all goats showed little or no gain in live weight. This could be attributed to the fact that the daily DM intake was less than the normal intake required for growing goats, namely 3-5 % of BW (Haenlein, 1995). However, although goats in the control groups in paper II and III, and on grass silage in paper IV, appeared to receive a sufficient nutrient level to sustain their growth, according to Devendra & McLeroy (1982), they still had poor performance and lost weight throughout the experimental period. This may be attributed to the effect of parasitism. While the infection rate was lower (2,000 L3) in paper I, the goats still maintained their BW, compared to those in paper II, III and IV that were inoculated with more parasites (3,000 L3). Phengvichith & Ledin (2007a) reported a similar range of the DM and CP consumption by goats on as high a diet level as the CO (paper II, III) and GrS and GRRb goats (paper IV). However, their goats gained around 90 g/day with the FEC level below 600 epg, whereas the goats in paper II, III and IV lost between 5-20 g/day with the level of FEC above 3,000 epg at the end of experiment. The impairment of production due to parasite infection, as a result of inefficient food utilization, has been explained (Parkins & Holmes, 1989; Sykes, 1994; Roy *et al.,* 2003).

When, fresh cassava was substituted for the grass diet for three weeks there was no beneficial effect noticed on growth performance. In contrast, to the short-term feeding (paper I), goats fed cassava in both fresh and silage form, over the ten weeks of the experiment gained weight (paper II, III, IV). Firstly, this result can be explained by the higher level of protein obtained from cassava foliage during ten weeks compared three weeks feeding, and also compared to grass based animals. However, when the grass group was offered additional protein in the form of urea molasses and soybean meal (UM, in paper III) the protein level was comparable to the cassava fed goats. However, still not much improvement in weight gain was noted. The potential effect of cassava foliage, in addition to supplying additional protein, is likely to be associated with the available bypass-protein (Ffoulkes & Preston, 1978; Ravindran, 1992; Wanapat, 2003). It has also been reported that, the CP intake from cassava foliage stimulates the bacterial population in the rumen, thereby increasing the availability of fermentable nitrogen, and later leading to an improved digestion of fibre in the rumen (Khang & Wiktorsson, 2004). The increase in feed intake and hence its influence on the growth of goats was reported when cassava hay (Dung *et al.*, 2005), fresh cassava (Seng & Preston, 2003), wilted cassava (Phengvichith & Ledin, 2007b) or ensiled cassava foliage (Ho & Preston, 2006), were supplemented to low quality feeds.

In parasitism conditions, with the FEC level of ~2,000 epg, CaF goats (paper II) had a similar growth rate of ~40 g/day to those in Seng & Preston (2003) that used the same origin and breed of goat, and were fed exactly the same diet. This ADG was also comparable to goats studied in Phengvichith & Ledin (2007b) that were fed on gamba grass (*Andropogon gayanus*) and supplemented with 40 % of wilted cassava foliage. However, when parasite-free, they required lower amounts of nutrient (78 g/day of crude protein), whereas CaF goats utilised almost twice this protein level to achieve the same growth rate. The ADG of CaS goats were consistent in paper II and III, at ~60 g/day. This growth was in agreement with the results from the parasitized goats in Ho & Preston (2006) with similar FEC (~500 epg). Meanwhile, the CaRb and CaS goats in paper IV had almost the same DM and CP intake as the CaS goats in paper II and III, although they gained only half of the weight compared to the goats in paper II and III. This could have been due to the effect of stress at weaning and different growing stage of the experimental goats in paper IV, that were smaller in size and were about two months younger than the goats in paper II and III.

6.3 Parasitological aspects

In paper I, the reductions in FEC in CaL and CaA groups compared to the control group were 35 % and 50 %, respectively, from only three weeks of feeding. In the investigation described in paper II, both cassava fed groups (CaF and CaS) showed beneficial effects of the diet, compared with the grass fed control group. The reduced FEC noted in both cassava foliage treatments (CaF and CaS) could have been due to the higher protein intake relative to those fed grass (CO). Although the voluntary protein intake from the supplementation of 200 g/d of soybean meal and 50 g urea molasses in the UM goats (paper III) failed to reach the CP content in the CaS treatment, this amount was almost twice as high as that in the CO goats. It seems that the additional protein for the UM goats was just enough to maintain their BW, at the same time as this feed ration also compensated for the parasite-induced blood losses. However, the reduced *H. contortus* burden in CaS compared with CaF and UM suggests a direct effect of some factors in the ensiled cassava foliage, which were not present in fresh cassava foliage and obviously not just an effect of the improved protein level.

6.3.1 Cassava foliage reduce faecal egg count

It is almost a decade since cassava foliage in various forms was reported to have a negative effect on FEC of GINs in goats (Seng & Rodriguez, 2001; Seng & Preston, 2003; Lin *et al.*, 2003). However, in most studies where this effect has been indicated it has only been based on FECs of naturally infected animals, and where the FEC are inherently highly variable. When young naturally infected goats were fed fresh foliage in a ration of either 50 % or 100 % cassava, there was a significant reduction in FEC after a three months feeding period compared to grass fed animals that had FEC approximately five times higher (Seng & Preston, 2003). Lin *et al.* (2003) also found a possible antiparasitic effect of cassava in naturally infected goats. When they offered foliage including cassava *ad libitum* for five months, FEC were lower than in goats fed grass in addition to rice bran and a molasses urea block only. Furthermore, when Dung *et al.* (2005) replaced concentrate with cassava hay at rates from 250 to 1000 g/kg DM, in a diet that contained guinea grass and dried cassava tuber slices, FEC of naturally infected goats were gradually reduced with the increasing levels of cassava hay. A further study in two different geographical regions of Cambodia with grazing goats showed that dried cassava foliage had no influence on FEC in the highland area, but FEC was reduced by 35 % in the lowland area, while ensiled cassava foliage reduced FEC by 25 % and 70 %, in these respective areas (Ho & Preston, 2006). The beneficial effects of feeding cassava foliage to GI nematode infected goats have been suggested to be associated with enhanced immunity in response to the improved protein level. This is in agreement with other studies on parasitized goats on various feed sources (Blackburn *et al.*, 1991; Knox & Steel, 1996; Nnadi *et al.*, 2007; 2009). According to these studies, the protein content has a marked influence on the acquisition of immunity, and especially in malnourished animals where the expressions of acquired immunity will mitigate the detrimental effects of gastrointestinal nematodes in several ways (van Houtert & Sykes, 1996). However, this also includes immunological effects on the establishment rate of the late larval stages inside the host, but also negative effects on parasite development and survival, as well as on the fecundity of the female worms (Coop & Kyriazakis, 2001; Kyriazakis & Houdijk, 2006; Jackson, 2008; Hoste *et al.*, 2008; Athanasiadou, 2008).

6.3.2 Ensiled cassava foliage reduced *H. contortus* population

The "*Haemonchus* specific" effect indicated by the reduction of the actual worm numbers could not be just as result of the crude protein consumption level, as it was not found in the UM groups (paper III). There is no simple explanation for this, but it may be related to the protein composition of cassava foliage in combination with the ensiling process, which possibly makes it different from the proteins in the supplementation that was used in the UM group. Cassava leaves have a high CP content and almost 85 % of the CP fraction is true protein (Ravindran, 1993). Evidence for "bypass" protein characteristics in cassava leaves was reported by Ffoulkes & Preston (1978), who showed that fresh cassava leaves could completely replace soybean meal. Wanapat (2001) suggested that the CTs contained in cassava leaves could have a potential role in forming tannin-protein complexes that escape rumen degradation to reach duodenal digestion. Alternatively, if ensiled cassava foliage contains any direct antiparasitic compound, as was suggested by the reduction of FECs and the *H. contortus* population, this will allow the availability of protein in the CaS treatment to be fully used for growth. Meanwhile, some phyto-chemicals (e.g. tannins, HCN, and their bio-metabolites) are well known in this foliage. Thus the direct effects on parasite infections in animals fed these diets could be due to a direct biochemical effect and the indirect effect due to an improvement in protein intake or both. However, it is unlikely that the anti-parasitic effect of cassava foliage observed herein was due to HCN, because in this study the ensiling process caused a reduction in HCN content from 585 in fresh foliage to 170 mg/kg. Tannin levels could unfortunately not be measured, but it is assumed that these compounds were present in the fresh cassava material used in this study, as it has been shown to be invariably present in cassava foliage (Seng & Preston, 2003). However, at the same time it is important to recognize that tannin levels in the foliage of plants can be dramatically altered by degradation following harvesting (Makkar, 2003). In this respect it has been recorded that the process of ensiling significantly reduced the amount of tannins in cassava foliage (Khang & Wiktorsson, 2006).

6.3.3 Effect of the ensiling process

For other reasons, in the grass silage fed goats (paper IV) neither protein improvement nor any phyto-chemical was noted, as in cassava foliage. However, the total worm burdens were similar to the CaS goats and they also harbored approximately similar numbers of *H. contortus*. In this study, the total establishment of adult worms was uncertain, as it was presented with no proper control treatment (i.e. CO) that can display the result of infection without any experimental effect. From the same rate of infection (3,000 L3), in paper II a three times higher of *H. contortus* was shown in goats fed fresh cassava (CaF), that was similar to fresh grass (CO). However, the CaF goats received a significantly higher level of protein than the CO goats. This confirmed the previous indication that it was not only the high protein from cassava foliage that affected the *H. contortus* burden. Thus, the combined results from paper II, III and IV could suggest that FEC of GI nematodes can be reduced in goats fed various forms of cassava foliage. When ensiled forage (both grass and cassava) was used it also seemed to have an effect on the *H. contortus* burden (Table 3). The reason(s) for this are obscure, but it is proposed that something associated with the processes of silage production of cassava or grass induces the formation of anti-*H. contortus* substance(s).

Table 3. *The effect of feeding fresh or ensiled grass and cassava foliage on faecal egg count (FEC) and packed cell volume (PCV) changes and the* Haemonchus contortus *(Hc) burden in relation to protein intake in goats infected with GI nematode parasites.*

Diet		Final FEC, mean epg	FEC rate[a], mean epg	PCV change[b], %	Hc	CP, g/kg BW
Grass						
Fresh[(1)]	CO	10000	–	–	513	2.1
Fresh[(2)]	CO	4500	70	-10.8	627	4.6
Fresh[(3)]	CO	3000	37	-12.2	640	5.2
Silage[(4)]	GrS	2975	41	-7.5	246	5.4
Silage[(4)]	GrRb	3400	51	-8.6	202	5.0
Cassava foliage						
Fresh[(1)]	CaL	8000	–	–	379	4.8
Fresh[(1)]	CaA	5000	–	–	304	4.8
Fresh[(2)]	CaF	1500	22	-7.4	658	7.4
Silage[(2)]	CaS	500	7	-4.1	264	8.8
Silage[(3)]	CaS	250	1	-2.7	117	11.2
Silage[(4)]	CaS	167	3	1.2	302	7.0
Silage[(4)]	CaRb	117	3	1.0	244	11.5

[a] The rate of FEC increase throughout the experimental period on a daily basis
[b] The amount of PCV decrease or gain at the end of experiment
[(1), (2), (3)] and [(4)] the experimental groups in paper I, II, III and IV, respectively. Each goat in the experiment was inoculated with a total of 2,000 L3 of a mixed nematode species in paper I or 3,000 L3 in paper II, III and IV.

Although there was a sign of larval arrestment in the ensiled cassava fed groups, that was indicated by the significantly higher L4 *H. contortus* than the grass silage group in paper IV, this result was not supported in either paper II nor paper III. Nevertheless, this finding suggested that the rate of larval development is unaffected by the dietary regime in young animals. For example, Wallace *et al.* (1996) found no difference in the prepatent period of *H. contortus* in experimentally infected lambs when either fed a basal diet or if supplemented with soybean. Similarly, studies on the effects of supplementary feeding of sorghum and soybean meal in combination with copper oxide needles did not show any negative influence on the rate of larval arrestment from the improved diet against natural GI nematode infections, neither in kids (Torres-Acosta *et al.*, 2004; 2006), or in browsing adult goats (Martínez-Ortiz-de-Montellano *et al.*, 2007). However, in studies conducted with pubertal 9-12 months old goats and with pregnant goats (Nnadi *et al*., 2007; 2009), a delay was found in the patency period of *H. contortus* in those animals that were on a diet with a high plane of nutrition. Several mechanisms of expression of immunity have been described to act either on the establishment of incoming larvae, or on the adult worm survival and fecundity, that often are reduced in supplemented well-nourished hosts (Balic *et al*., 2000). Jackson (2008) stated that the expression of immunity is also influenced by a range of other factors, such as the age of the host and their experience to previous infection.

7 General conclusion

This study has demonstrated that feeding goats with cassava foliage will reduce the FEC of their GI nematodes. It seems that this effect was mainly in response to a reduced fecundity of the adult female worms, as no clear direct anthelmintic effect of cassava on nematode total worm populations could be verified. The slight anthelmintic effect that was observed seemed to be specific, i.e. it only affected *H. contortus*, and was pronounced when the goats were fed with the silage of cassava foliage. The process of ensiling this material appears to bring about changes that result in increased mortality in *H. contortus*. Although it seems likely that this effect was partly in response to a high nutrition level that most likely improved host immunity, there must have been other additional unknown factors of the ensiling process that acted in combination to reduce the *H. contortus* burden. While a direct anthelmintic effect has yet to be elucidated, bio-active principles in the ensiled cassava cannot be excluded. It is also speculated that the result of L4 arrestment in the goats on the cassava diets in paper IV arose rather from a direct antiparasitic effect than from the improved nutrition.

The ensiling process of cassava foliage, using sugar palm syrup or rice bran as additive, substantially provides both improved resilience and resistance in pen-fed goats experimentally infected with GI nematode parasites. The effect of grass silages (paper IV) can not be ensured, based on the result of fresh feeding from the other experiments (paper I, II, III). Variability from one experiment to another is still a concern. The establishment of the adult worm burden in paper IV creates hesitation in confirming the effect of the ensiling process on the *H. contortus* burden in this trial.

8 Implications and futher research

In these studies we have not reported the results from an *in vitro* study using rumen fluid from goats fed cassava foliage in the egg hatch and larval migration test, because of technical complications in dealing with the rumen fluid. However, the result of a larval feeding inhibition test using different dilutions in water of dried cassava leaf demonstrated a negative effect at 1.25 g/ml on *H. contortus*, *T. circumcinta* and *T. colubriformis* L3.

Further work on cassava foliage is needed to determine in what way the ensiling process leads to a reduction in the number of *H. contortus*. Results of an *in vitro* study are necessary to confirm this finding, while an on-farm trial on the application of cassava feeding will make use of the results from research to benefit farmers in practice.

References

ADB (2003). Report and Recommendation of the President to the Board of Directors on Proposed Loans and Technical Assistance Grant to the Kingdom of Cambodia for the Agriculture Sector Development Program. Asian Development Bank (ADB) RRP: CAM 34380, pp 1–2.

AFRIS (2007). Animal Feed Resources Information Systems, Food and Agriculture Organization. Available from: http://www.fao.org./ag/AGa/agap/FRG/AFRIS/DATA/535.htm [Accessed 2007-05-31].

Allonby, E.W. & Urquhart, G.M. (1975). The epidemiology and pathogenic significance of haemonchosis in a merino flock in East Africa. *Veterinary Parasitology* 1, 129–43.

Ancheta, P.B. & Dumilon, R.A. (2000). Benzimidazole resistance of some nematodes in small ruminants. *Philippine Journal of Veterinary and Animal Sciences* 26, 147–52.

Anderson, R. C., (1992). Nematode parasites of vertebrates. Their development and transmission 2nd ed., CAB International, pp 110–112.

Anindo, D., Toé, F., Tembely, S., Mukasa-Mugerwa, E., Lahlou-Kassi, A. & Sovani, S. (1998). Effect of molasses-urea-block (MUB) on dry matter intake, growth, reproductive performance and control of gastrointestinal nematode infection of grazing Menz ram lambs. *Small Ruminant Research* 27, 63–71.

Anonymous (1985). Nutrient Requirements of Sheep 6th ed., National Academy Press, Washington, pp 2–25.

Anonymous (1986). Manual of Veterinary Parasitological Laboratory Techniques. MAFF/ADAS Reference Book 418 3rd ed. Her Majesty's Stationery Office, London, pp 160.

Anonymous (1990). Official Methods of Analysis 15th ed. AOAC International, Washington DC, pp 69–90.

Anthanasiadou, S., Kyriazakis, I., Jackson, F. & Coop, R.L. (2001). Direct anthelmintic effect of condensed tannins towards different gastrointestinal nematodes of sheep *in vitro* and *in vivo* studies. *Veterinary Parasitology* 99, 205–19.

Athanasiadou, S., Tzamaloukas, O., Kyriazakis, I., Jackson, F. & Coop, R.L. (2005). Testing for direct anthelmintic effects of bioactive forages against *Trichostrongylus colubriformis* in grazing sheep. *Veterinary Parasitology* 127, 233–43.

Anthanasiadou, S., Houdijk, J. & Kyriazakis, I. (2008). Exploiting synergisms and interactions in nutritional approaches to parasite control in sheep production. *Small Ruminant Research* 76, 2–11.

AusAID (2006). Cambodia Agriculture Sector Diagnostic Report. Agrifood Consulting International and CamConsult, pp 35.

Bahuaud, D., Martinez-Ortiz-de-Montellano, C., Chauveau, S., Prevot, F., Torres-Acosta, F., Fouraste, I. & Hoste, H. (2006). Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology* 132, 545–54.

Balic, A., Bowles, V.M. & Meeusen, Els N. T. (2000). The immunobiology of gastrointestinal nematode infections in ruminants. *Advances in Parasitology* 45, 181–241.

Bang, K.S., Familton, A.S. & Sykes, A.R. (1990). Effect of copper oxide wire particle treatment on establishment of major gastrointestinal nematodes in lambs. *Research in Veterinary Science* 49, 132–37.

Barger, I.A. (1996). Prospects for integration of novel parasite control options into grazing systems. *International Journal for Parasitology* 26, 1001–7.

Barger, I.A. (1999). The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal for Parasitology* 29, 41–7.

Barrau, E., Fabre, N., Fouraste, I. & Hoste, H. (2005). Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia Scop.*) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. *Parasitology* 131, 531–8.

Barry, T.N. & McNabb, W.C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* 81, 263–72.

Bendixsen, T., Hanh, T.H., Dong. V.V., Binh, D.V. & Lien, P.S. (2005). A preliminary investigation of Vietnamese indigenous plants for anti-parasite activities in goats. In: Ledin, I. (Ed.), Proceedings of International workshop of small ruminant production and development in SE Asia, Hanoi, pp 97–9.

Beriajaya, Haryuningtyas, D., Husein, A., Hood, G.M. & Gray, G.D. (2003). Transmission of anthelmintic resistance in sheep in West Java, Indonesia. In: Proceedings of the World Association for the Advancement of Veterinary Parasitology 19th International Conference, New Orleans, Louisiana, pp 226.

Binh, D.V. & Lin, N.K. (2005). Research and development of small ruminant production in Vietnam. In: Ledin, I. (Ed.), Proceedings of International workshop of small ruminant production and development in SE Asia, Hanoi, pp 5–17.

Blackburn, H.D., Rocha, J.L., Figueredo, E.P., Berne, M. E, Viera, L.S., Cavalcante, A.R. & Rosa, J.S. (1991). Interaction of parasitism and nutrition and their effects on production and clinical parameters in goats. *Veterinary Parasitology* 40, 99–112.

Blitz, N.M. & Gibbs, H.C., (1972). Studies on the arrested development of *Haemonchus contortus* in sheep. I. The induction of arrested development. *International Journal of Parasitology* 2, 5–12.

Brooker, J.D., O'Donovan, L., Skene, I. & Sellick, G. (2000). Mechanisms of tannin resistance and detoxification in the rumen. In: Brooker, J.D. (Ed.), Tannins in Livestock and Human Nutrition, ACIAR Proceedings no. 92 (253), pp 117–22.

Bueno, L., Dakkak, A. & Fioramonti, J. (1982). Gastro-duodenal motor and transit disturbances associated with *Haemonchus contortus* infection in sheep. *Parasitology* 84, 367–74.

Butter, N.L., Dawson, J.M., Wakelin, D. & Buttery, P.J. (2001). Effect of dietary condensed tannins on gastrointestinal nematodes. *Journal of Agricultural Science* 137, 461–9.

Caplice, E., & Fitzgerald, G.F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology* 50, 131–49.

Catchpoole, V.R. & Henzell, E.F. (1971). Silage and silage-making from tropical herbage species. *Herbage Abstracts* 41, 213–21.

Chandrawathani, P., Yussof, N. & Waller, P.J. (2004). Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, east Malaysia. *Veterinary Research Communications* 28, 479–89.

Chandrawathani, P., Chang, K.W., Nurulaini, R., Waller, P. J., Adnan, M., Zaini, C.M., Jamnah, O., Khadijah, S. & Vincent, N. (2006). Daily feeding of fresh neem leaves (*Azadirachta indica*) for worm control in sheep. *Tropical Biomedicine* 23, 23–30.

Chea, S. (2004). Rice Industry in Cambodia, Special Report. Economic Institute of Cambodia (EIC), Phnom Penh, pp 1–4.

Chhay, T., Ly, J. & Rodriguez, L. (2001). An approach to ensiling conditions for preservation of cassava foliage in Cambodia. *Livestock Research for Rural Development* 13(3). Available from: http://cipav.org.co/lrrd/lrrd13/3/chhayty.htm [Accessed 2007-07-17].

Chhay, T., Preston, T.R. & Borin, K. (2007). Effect of variety and wilting on HCN content of cassava leaves and on intake, digestibility and N retention by growing pigs. *Livestock Research for Rural Development* 19 (9). Available from:
http://www.cipav.org.co/lrrd/lrrd19/9/chha19135.htm [Accessed 2009-04-27].

Chiejina, S.N., Fakae, B.B & Eze, P.I. (1989). Development and survival of free-living stages of gastrointestinal nematodes of sheep and goats on pasture in the Nigerian derived savanna. *Veterinary Research Communications* 13, 103–12.

Coop, R.L., Sykes, A.R. & Anjus, K.W. (1982). The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, feed intake and body composition of growing lambs. *Journal of Agricultural Science* 98, 247–55.

Coop, R.L. & Field, A.C. (1983). Effect of phosphorus intake on growth rate, food intake and the quality the skeleton of growing lambs infected with the intestinal nematode *Trichostrongylus vitrinus*. *Research in Veterinary Science* 35, 175–81.

Coop, R.L. & Holmes, P.H. (1996). Nutrition and parasitic interaction. *International Journal for Parasitology* 26, 951–62.

Coop, R.L. & Kyriazakis, I. (2001). Nutrition–parasite interaction. *Veterinary Parasitology* 84, 187–204.

Daud, I.A., Sani, R.A. & Halim, R.A. (1991). Gastrointestinal parasitism in goats. In: Proceedings of the International Seminar on Goat Production in the Asian Humid Tropics, Prince Songkhla University, Hat Yai, pp 201–05.

Devendra, C., Thomas, D., Jabbar, M.A. & Kudo, H. (1997). Improvement of Livestock Production in Rain-Fed Agro-Ecological Zones of South-East Asia. ILRI, Nairobi.

Devendra, C. & McLeroy, G.B. (1982). Goat and sheep productions in the tropics. Intermediate Topical Agriculture Series, Longman, London, pp 271.

Dorny, P., Claerebout, E., Vercruysse, J., Jalila A. & Sani, R. (1993). Benzimidazole resistance of *Haemonchus contortus* in goats in Malaysia. *Veterinary Record* 133, 423–4.

Dorny, P., Claerebout, E., Vercruysse, J., Sani, R. & Jalila, A. (1994). Anthelmintic resistance in goats in peninsular Malaysia. *Veterinary Parasitology* 55, 327–42.

Dorny, P., Symoens, C., Jalila, A., Vercruysse, J. & Sani, R. (1995). Strongyle infections in sheep and goats under the traditional husbandry system in peninsular Malaysia. *Veterinary Parasitology* 56, 121–36.

Dung, N.T., Nguyen, T.M. & Ledin, I. (2005). Effect of replacing a commercial concentrate with cassava hay (*Manihot esculenta* Crantz) on the performance of growing goats. *Animal Feed Science Technology* 119, 271–81.

Dynes, R.A., Poppi, D.P., Barrell, G.K. & Sykes, A.R. (1998). Elevation of feed intake in parasite-infected lambs by central administration of a cholecystokinin receptor antagonist. *British Journal of Nutrition* 79, 47–54.

Eggum, O.L. (1970). The protein quality of cassava leaves. *British Journal of Nutrition* 24, 761–9.

Eysker, M. & Ogunsusi, R.A. (1980). Observations on epidemiological and clinical aspects of gastrointestinal helminthiasis of sheep in North Nigeria during the rainy season. *Research in Veterinary Science* 28, 58–62.

Ffoulkes, D., Done, F. & Prston, T.R (1977). Cassava forage as a cattle feed: Apparent digestibility and consumption of the whole forage. *Tropical Aminal Production* 3, 234–6.

Ffoulkes, D. & Preston, T.R. (1978). Cassava or sweet potato forage as combined sources of protein and roughages in molasses based diet: Effect of supplementation with soybean meal. *Tropical Animal Production* 3, 186–92.

Ghalsasi, P.M., Nimbkar, C., Kahn, L.P. & Walkden-Brown, S.W. (2002). Effects of different levels of worm control on meat production of deccani sheep in shepherds' flocks in Maharashtra India. In: Proceedings of the 10th International Congress of the Asia–Australasian Animal Production Societies (AAAP), New Delhi, pp 152–6.

Githiori, J.B., Höglund, J., Waller, P.J. & Baker, R.L. (2003). The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. *Veterinary Parasitology* 116, 23–34.

Githiori, J.B., Höglund, J., Waller, P.J. & Baker, R.L. (2005). Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. *Veterinary Parasitology 118,* 215–26.

Granum, G., Wanapat, M., Pakdee, P., Wachirapakorn, C. & Toburan, W. (2007). A comparative study on the effect of cassava hay supplementation in swamp buffaloes (*Bubalus bubalis*) and cattle (*Bos indicus*). *Asian-Australian Journal of Animal Science* 20, 1389–96.

Gray, G.D., Yee, C.A.T. & Villar, E.C. (2004). Worm control for small ruminants in the Philippines. In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 121–49.

Grovum, W.L. (1981). Factors affecting the voluntary intake of food by sheep: The effect of intravenous infusions of gastrin, cholecystokinin and secretin on motility of the reticulo-rumen and intake. *British Journal of Nutrition* 45, 183–201.

Haenlein, G.F.W. (1995). Tropics of profitable feeding and milking of dairy goats. A.S. & A.B. Dairy extension bulletin. pp 110–8.

Hansen, J. & Perry, B. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants: A handbook of International Livestock Research for Animal Disease, Kenya.

Heckendorn, F., Haring, D.A., Maurer, V., Senn, M. & Hertzberg, H. (2007). Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticei*. *Veterinary Parasitology* 146, 123–34.

Henderson, N. (1993). Silage additive. *Animals Feed Science Technology* 45, 35–56.

Ho, B. & Preston, T.R. (2005). The effect of ensiling or sun-drying cassava leaves on feed intake, digestibility and N retention of goats fed a basal diet of paragrass (*Brachiaria mutica*). In: Ho B., Cassava foliage as supplement for goats fed paragrass (*Brachiaria mutica*) in full confinement, or with grazing in semi-confinement. *MSc thesis, Swedish University of Agricultural Sciences, Sweden.*

Ho, B. & Preston, T.R. (2006). Growth performance and parasite infestation of goats given cassava leaf silage, or sun-dried cassava leaves, as supplement to grazing in lowland and upland regions of Cambodia. *Livestock Research for Rural Development* 18 (2). Available from: http://www.cipav.org.co/lrrd/lrrd18/2/buny18028.htm [Accessed 2008-07-17].

Hood, G.M., 2004. Anthelmintic resistance in small ruminant parasites: Implications for smallholders in Southeast Asia. In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 51–61.

Hoste, H., Gaillard, L. & Le Frileux, Y. (2005). Consequences of regular distribution of sainfoin hay on gastrointestinal parasitism with nematodes and milk production in dairy goats. *Small Ruminant Research* 59, 265–71.

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, 253–61.

Hoste, H., Torres-Acosta, J.F.J. & Aguilar-Caballero, A.J. (2008). Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? *Parasite Immunology* 30, 79–88.

Jackson, F. (2008). Nutrition and immunity of nematodes of livestock. *Parasite Immunology* 30 (2), 61–2.

Kahn, L.P. & Diaz-Hernandez, A. (2000). Tannins with anthelmintic properties. In: Brooker, J.D. (Ed.), Tannins in Livestock and Human Nutrition, ACIAR Proceedings no. 92 (253), pp 140–9.

Kahn, L.P., Knox, M.R., Gray, G.D., Lea, J.M. & Walkden-Brown, S.W. (2003). Enhancing immunity to nematode parasites in single bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* 112, 211–25.

Khang, D.N. & Wiktorsson, H. (2004). Effect of fresh cassava tops on rumen environment parameters, thyroid gland hormones and liver enzymes of local yellow cattle fed urea-treated fresh rice straw. *Tropical Animal Health and Production* 36, 751–62.

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, 130–9.

Khieu, B., Preston, T.R. & Lindberg, J.E. (1996). Juice production from the sugar palm tree (*Borassus flabellifer*) in Cambodia and performance of growing pigs fed sugar palm juice. In: Khieu, B., A study on the use of the sugar palm tree (*Borassus flabellifer*). *MSc. thesis, Swedish University of Agricultural Sciences, Sweden.*

Khieu, B., Lindberg, J.E. & Ogle, R.B. (2005). Effect of variety and preservation methods of cassava leaves on diet digestibility by indigenous and improved pigs. *Animal Sciences* 80, 319–24.

Kimambo, A.E., MacRae, J.C., Walker, A., Watt, C.F. & Coop, R.L. (1988). Effect of prolonged subclinical infection with *Trichostrongylus colubriformis* on the performance and nitrogen metabolism of growing lambs. *Veterinary Parasitology* 28, 191–203.

Knox, M.R. & Steel, J.W. (1996). Nutritional enhancement of parasite control in small ruminant production systems in developing countries in South-East Asia and the Pacific. *International Journal of Parasitology* 49, 236–46.

Knox, M.R., Torres-Acosta, J.F.J & Aguilar-Caballero, A.J. (2006). Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Veterinary Parasitology* 139, 385–93.

Kochapakdee, S., Pralomkarn, W., Choldumrongkul, S., Saithanoo, S. & Norton, B. W. (1991). Prevalence of internal parasite in Thai native female goats. In: Saithanoo, S. & Norton, B.W. (Eds.), Proceedings of workshop in Goat Production in the Asian Humid Tropics, Hat Yai, pp 206–12.

Kochapakdee, S., Saithanoo, S. & Choldumrongkol, S. (2002). Endoparasite in small ruminants in Thailand. In: Biological control of nematode parasites of small ruminants in Asia. Final proceedings of FAO technical co-operation project in Malaysia TCP 0065(7). Food and Agriculture Organization. pp 59–70.

Koski, K.G., Scott, M.E. (2001). Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. *Annual Review Nutrition* 21, 297–321.

Kyriazakis, I., Oldham, J.D., Coop, R.L. & Jackson, F. (1994). The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *British Journal of Nutrition* 72, 665–77.

Kyriazakis, I. & Houdijk, J. (2006). Immunonutrition: Nutritional control of parasites. *Small Ruminant Research* 62, 79–82.

Lin, N.K., Preston, T.R., Binh. D.V. & Ly, N.D. (2003). Effects of tree foliages compared with grasses on growth and intestinal nematode infestation in confined goats. *Livestock Research for Rural Development,* 15 (6). Available from: http://www.cipav.org.co/lrrd/lrrd15/6/lin156.htm [Accessed 2009-04-22].

López, J., Tejada, I., Vásquez, C., Juan de Dios Garza, J.de.D. & Shimada, A. (2004a). Condensed tannins in humid tropical fodder crops and their in vitro biological activity. *Journal of Science of Food and Agriculture* 84, 291–4.

López, I., Cantó, V. & Tejada, S. (2004b). *In vitro* effect of condensed tannins from tropical fodder crops against eggs and larvae of the nematode *Haemonchus contortus*. *Food, Agriculture and Environment* 3, 191–4.

MacRae, J.C. (1993). Metabolic consequences of intestinal parasitism. *Proceeding of Nutrition Society* 52, 121–30.

MacRae, J.C., Sharma, G.A.M., Smith, J.S., Easton, J.F. & Coop, R.L. (1979). Preliminary observations on the effects of *Trichostrongylus colubriformis* infestation on the energy and nitrogen metabolism of lambs. *Animal Production* 28, 456.

MAFF (2007). Annaual Conference 2007 on Agriculture, Forestry and Fisheries (Taking stock of 2006-2007 performance and setting direction for 2007-2008). MAFF. Phnom Penh, p 2.

Makkar, H.P.S. (2003). Effect and fate of tannins in ruminant animals, adaption to tannins and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research* 49, 249–56.

Martínez-Ortiz-de-Montellano, C., Vargas-Maga a, J.J., Aguilar-Caballero, A.J., Sandoval Castro, C., Cob-Galera, L., May Martinez, M., Miranda-Soberanis, L., Hoste, H. & Torres-Acosta, J.F.J. (2007). Combining the effects of supplementary feeding and copper oxide needles improves the control of gastrointestinal nematodes in browsing goats. *Veterinary Parasitology* 146, 66–76.

McDonald, P., Henderson, N. & Heron, S. (1991). The Biochemistry of the Silage. 2nd ed. Chalcombe Publications, Marlow, pp 340.

McLeod, R. S. (1995). Costs of major parasites to the Australian livestock industries. *International Journal for Parasitology* 25, 1363–67.

McLeod, R.S. (2004). Economic impact of worm infections in small ruminants in South East Asia, India and Australia. In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 23–33.

MINITAB (2000). Statistical software version 13.31. User's guide to statistics, Minitab Inc. USA.

Molan, A.L., Meagher, L.P., Spencer, P.A. & Sivakumaran, S. (2003). Effect of flavan3-ols on *in vitro* egg hatching, larvae development and viability of infective larvae of *Trichostrongylus colubriformis*. *International Journal for Parasitology* 33, 1691–8.

Mühlbach, P.R.F. (1999). Additive to improve the silage making process with tropical forages. In: Proceeding FAO E-Conference on Tropical Silage. FAO Plant Production & Protection Paper 161. pp 151–64.

Mui, N.T., Binh, D.V., & Orskov, E.R. (2005). Effect of foliages containing condensed tannins on gastrointestinal parasites. *Animal Feed Science 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: Preston, T.R., Ogle, B. & Wanapat, M. (Eds), Proceedings International Workshop on

Current Research and Development on Use of Cassava as Animal Feed, Khon Kaen, pp 41–43.

Nnadi, P.A., Kamalu, T.N. & Onah, D.N. (2007). The effect of dietary protein supplementation on the pathophysiology of *Haemonchus contortus* infection in West African Dwarf goats. *Veterinary Parasitology* 148, 256–61.

Nnadi, P.A., Kamalu, T.N. & Onah, D.N. (2009). The effect of dietary protein on the productivity of west african dwarf (wad) goats infected with *Haemonchus contortus*. *Veterinary Parasitology* 161, 232–8.

Nurulaini, R., Khadijah, S., Adnan, M.J., Zaini, C.M., Jamnah, O., Waller, P.J. & Chandrawathani, P. (2007). Potential use of cassava leaves for worm control for small ruminants in the tropics. In: Claerebout, E. & Vercruysse, J. (Eds.), From EPG to Genes, The 21st International Conference of the World Association of Advanced Veterinary Parasitology. Genes, pp 264.

Onwuka, C.F.I., Akinsoyinu, A.O. & Tewe, O.O. (1992). Role of sulphur in cyanide detoxification in ruminants. *Small Ruminant Research* 8, 277–84.

Osaer, S., Goossens, B., Eysker, M. & Geerts, S. (2000). The effects of prophylactic treatment on the productivity of traditionally managed Djallonke sheep and West African Dwarf goats kept under high trypanosomiasis risk. *Acta Tropica* 74, 13–24.

Over, H.J., Jansen, J. & van Olm, P.W. (1992). Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health Paper no. 96. pp 221.

Pal, S., Roy, S. & Pathak, A.K. (2001). Prevalence of gastro-intestinal parasites in cattle and buffaloes from Chattisgarh region. *Journal of Veterinary Parasitology* 15, 155–6.

Paolini, V., Fouraste, I. & Hoste, H. (2004). *In vitro* effects of three woody plants and sainfoin on third-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology* 129, 69–77.

Parkins, J.J. & Holmes, P.H. (1989). Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Review* 2, 227–46.

Pathak, A.K. & Pal, S. (2008). Seasonal prevalence of gastrointestinal parasites in goats from Durg district of Chattisgarh. *Veterinary World*, 136–7.

Phengvichith, V., Ledin, I. (2007a). Effect of a diet high in energy and protein on growth, carcase characteristics and parasite resistance in goats. *Tropical Animal Health and Production* 39, 59–70.

Phengvichith, V. & Ledin, I. (2007b). Effect of feeding different levels of wilted cassava foliage (*Manihot esculenta,* Crantz) on the performance of growing goats. *Small Ruminant Research* 71, 109–16.

Phuc, B.H.N. (2000). Tropical forages for growing pigs-digestion and nutritive value. *Doctoral thesis, Swedish University of Agricultural Sciences*.

Preston, T.R. (1995). Tropical animal feeding, A manual for research workers. FAO animal production and health paper no.126. pp 68–71.

Preston, T.R. (2002). Production and utilization of cassava in integrated farming systems for smallholder farmers in Vietnam and Cambodia. In: Proceeding of the 7th Regional

Workshop on Cassava Research and Development in Asia: Exploring New Opportunities for an Ancient Crop, Bangkok, pp 48–57.

Preston, T.R. & Leng, R.A. (1987). Matching ruminant production systems with available resources in the tropics and sub-tropics. Armidale: Penambul Books, New South Wales, pp. 181–90.

Preston, T.R., Rodríguez, L. & Khieu, B. (2000). Associations of cassava and legume trees as perennial forage crops for livestock. In: Preston, T.R. & Ogle, B., (Eds.), Proceedings National Workshop-Seminar on Sustainable Livestock Production on Local Feed Resources. Ho Chi Minh City, pp 22–4.

Ravindran, G. & Ravindran, V. (1988). Changes in the nutritional composition of cassava (*Manihot esculenta*, Crantz) leaves during maturity. *Food Chemistry* 27, 299–309.

Ravindran, V. (1992). Preparation of cassava leaf products and their use as animal feed. In: Roots, tubers, plaintains and bananas in animal feeding. Food and Agriculture Organization, APHP Paper 95. pp 111–25.

Ravindran, V. (1993). Cassava leaves as animal feed: Potential and limitations. *Journal of Science Food and Agriculture* 61, 141–50.

Reed, J.D., McDowell, E.R., Van Soest, J.P. & Horvath, J.P. (1982). Condensed Tannins: A factor limiting the use of cassava forage. *Journal of Science Food and Agriculture* 33, 213–20.

Roy, N.C., Bermingham, E.N., Sutherland, I.A. & McNabb, W.C. (2003). Nematodes and nutrient portioning. *Australian Journal of Experimental Agriculture* 43, 1419–26.

Rowe, J.B., Nolan, J.V., de Chaneet, G., Teleni, E. & Holmes, P.H. (1988). The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *British Journal of Nutrition* 59, 125–39.

San, S. (2006). Distribution, management, marketing and constraints of goat production in Cambodia. In: Goats - undervalued assets in Asia. Proceedings of the APHCA-ILRI regional workshop on goat production systems and markets. Luang prabang, Lao PDR. 24 – 25 October 2006. APHCA, Bangkok, pp 21–25.

Sani, R.A. & Gray, D.G. (2004). Worm control for small ruminants in Southeast Asia. In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 3–21.

Sani, R.A., Binh, D. V., Ly N. D., San S. & Phimphachanhvongsod V. (2004). Goat production, parasites and testing of control options in Lao, Cambodia and Vietnam. In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 211–18.

Seng, M., Preston, T.R. & Leng, R.A. (2001). Response of young cattle fed rice straw to supplementation with cassava foliage and a single drench of cooking oil. *Livestock Research for Rural Development,* 13 (4). Available from: http://www.cipav.org.co/lrrd/lrrd13/4/seng134.htm [Accessed 2007-07-06].

Seng, 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). Available from: http://www.cipav.org.co/lrrd/lrrd13/2/soke132.htm [Accessed 2007-07-06].

Seng, S. & Preston, T.R. (2003). Effect of grass or cassava foliage on growth and nematode parasite infestation in goats fed low or high protein diets in confinement. *Livestock Research for Rural Development,* 15 (8). Available from:
http://www.cipav.org.co/lrrd/lrrd15/8/kery158.htm [Accessed 2007-07-06].

Seng, S., Waller, P.J., Inger, L. & 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, 47–54.

Sivaraj, S. & Pandey, V.S. (1994). Isolation of an ivermectin-resistant strain of *Haemonchus contortus* from sheep in Malaysia. *Veterinary Record* 135, 307–8.

Sokerya, S., Try, P., Waller, P.J., Höglund, J. (2009). The effect of long-term feeding of fresh and ensiled cassava (*Manihot esculenta*, Crantz) foliage on gastrointestinal nematode infections in goats. *Tropical Animal Health Production* 41, 251–58.

Sorn, S. & Muirden, A. (2002). Survey of the Distribution, Management, Marketing and Constraints of Goat Production in Cambodia. Goat Project report for ILRI-IFAD TAG 433. National Animal Health and Production Investigation Center (NAHPIC), Phnom Penh, Cambodia.

SPSS (2002). Statistical software, standard version, Release 11.5. SPSS Inc., Chicago, Illinois, USA.

Steel, J.W. (1978). Inter relationship between gastrointestinal helminth infection, nutrition and impaired productivity in the ruminant. In: Farrell, D.I. (Ed.), Recent Advances in Animal Nutrition, University of New England, Armidale, pp. 98–109.

Steel, J.W., Symons, L.E.A. & Jones, W.O. (1980). Effects of level of larval intake on the productivity and physiological and metabolic responses of lambs infected with *Trichostrongylus colubriformis*. *Australian Journal of Agricultural Research* 31, 821–38.

Subandriyo, S., Suhadono T. & Gray, G.D. (2004). Worm control for ruminants in Indonesia. In: In: Sani, R.A., Gray D.G. & Baker, R.L. (Eds), Worm control for small ruminants in tropical Asia. ACIAR Monograph, no. 113, pp 151–88.

Sykes, A.R., Coop, R.L. & Anjus, K.W. (1979). Chronic infection with *Trichostrongylus vitrinus* in sheep. Some effects on food utilization, skeletal growth and certain serum constituents. *Research in Veterinary Science* 26, 372–7.

Sykes, A.R. (1983). Effects of parasitism on metabolism in the sheep. *Nottingham Easter School of Agricultural Science* 35, 317–34.

Sykes, A.R. (1994). Parasitism and production in farm animals. Animal Production 59, 155–72.

Sykes, A.R. & Greer, A.W. (2003). Effects of parasitism on the nutrient economy of sheep. *Australian Journal of Experimental Agriculture* 43, 1393–98.

Symons, L.E.A. & Jones, W.O. (1975). Skeletal tissue, liver and wool protein synthesis by sheep infected with nematode *Trichostrongylus colubriformis*. *Australian Journal of Agricultural Research* 26, 1063–72.

Symons, L.E.A. & Steel, J.W. (1978). Pathogenesis of the loss of production in gastrointestinal parasitism. In: Donald, A.D., Southcott, W.H.& Dineen, J.K. (Eds), The epidemiology and control of gastrointestinal parasites of sheep in Australia.

Commonwealth Scientific and Industrial Research Organization (CISRO), Melbourn, pp 9–22.

Symoens, C., Dorny, P., Alimon, R., Jalila, A., Hardouin, J. & Vercruysse, J. (1993). Productivity of goats in smallholdings of Peninsular Malaysia. In: Proceedings of the Workshop on Development of Sustainable Integrated Small Ruminants–Tree Cropping Production Systems, University of Malaya, Kuala Lumpur. pp 129–36.

Taylor, M. A., Coop, R. L., Wall, R. L., (2007). Parasites of sheep and goats. In: Veterinary Parasitology, 3rd Edition. Blackwell Publishing, 151–258.

Tewe, O. (1994). Indices of cassava safety for livestock feeding. In: Bokanga, M., Essers, A.J.A., Poulter, N., Rosling, H. & Tewe, O. (Eds) Cassava safety. *ISHS Acta Horticulturae*. pp 375.

Theng K., Nam, N. & Preston, T.R. (2003). Observations on goat raising system of farmers along the Mekong river. In: Theng K., Studies on feeding behaviour in goats fed tree foliages. *MSc. thesis, Swedish University of Agricultural Sciences*.

Torres-Acosta, J.F.J., Jacobs, D., Aguilar-Caballero, A.J., Sandoval-Castro, C., May-Martinez, M. & Cob-Galera, L.A. (2004). The effect of supplementary feeding on the resilience and resistance of browsing Criollo kids against natural gastrointestinal nematode infections during the rainy season in tropical Mexico. *Veterinary Parasitology* 124, 217–38.

Torres-Acosta, J.F.J., Jacobs, D.E., Aguilar-Caballero, A.J., Sandoval-Castro, C., Cob-Galera, L. & May-Martinez, M. (2006). Improving resilience against natural gastrointestinal nematode infections in browsing kids during the dry season in tropical Mexico. *Veterinary Parasitology* 135, 163–73.

Tum, S., Puotinen, M.L. & Copeman, D.B. (2004). A geographic information systems model for mapping risk of fasciolosis in cattle and buffaloes in Cambodia. *Veterinary Parasitology* 122, 141–49.

Undersander, D., Mertens, D.R., Theix, N. (1993). Forage Analysis Procedures. National Forage Testing Association, Omaha, pp 154.

Van Aken D., Lagapa J.T., Dargantes A.P., Yebron M.A. & Vercruysse J. (1994). Benzimidazole resistance in a field population of *Haemonchus contortus* from sheep in the Philippines. *Philippine Journal of Veterinary and Animal Sciences* 20, 73–8.

van Houtert, M.F.J., Barger, I.A., Steel, J.W., Windon, G. & Emery, D.L. (1995). Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Veterinary Parasitology* 56, 163–80.

van Houtert, M.F.J. & Sykes, A.R. (1996). Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology* 26, 1151–67.

Venturina, V.M., Cruz, E.M., Alo, A.M.P., Lambio, E.T., Villar, E.C., Gray, D.G. & Gabunada, F.G. Jr. (2002). Assessment of farmer-developed worm control options in goats under smallholder management systems. In: Recent developments in animal production-2002. In: Proceedings of the Philippine Society of Animal Science 39th National and 20th Visayas Chapter Scientific Seminar and Annual Convention, Cebu City, the Philippines.

Venturina, V.M., Ancheta, P.B., Dobson, R.J., Eduardo, S.L. & Gray G.D. (2003). Use of a larval development assay to estimate anthelmintic efficacy in goats in smallholder farming systems. *Philippine Agriculturalist* 86, 134–9.

Wallace, D.S., Bairden, K., Duncan, J.L., Fishwick, G., Gill, M., Holmes, P.H., Mckeller, Q.A., Murry, A., Parkins, J.J. & Stear. M.J. (1996). Influence of soyabean meal supplementation on the resistance of Scottish blackface lambs to haemonchosis. *Research in Veterinary Science* 60, 138–43.

Waller, P. J., Echevarria, F., Eddi, C., Maciel, S., Nari, A. & Hansen, J. W. (1996). The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: General overview. *Veterinary Parasitology* 62, 181–7.

Waller, P.J. (1997). Sustainable helminth control of ruminants in developing countries. *Veterinary Parasitology* 71, 195–207.

Waller, P.J. (1999). International approaches to the concept of integrated control of nematode parasite of livestock. *International Journal for Parasitology* 29**,** 155–64.

Waller, P.J. & Chandrawathani, P. (2005). *Haemonchus contortus*: Parasite problem no. 1 from Tropics - Polar Circle. Problems and prospects for control based on epidemiology. *Tropical Biomedicine* 22, 131–37.

Waller, P.J. (2006). Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. *Animal Feed Science and Technology* 126, 277–89.

Wanapat, M. (2001). Role of cassava hay as animal feed in the tropics. In: Preston, T.R., Ogle, R.B., Wanapat, M. (Eds), Proceedings of the International Workshop on Current research and development on use of cassava as animal feed, Khon Kaen, pp. 13–20.

Wanapat, M. (2003). Manipulation cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. *Asian-Australian Journal of Animal Science* 16, 463–72.

Wilson, W.D. & Field, A.C. (1983). Absorption and secretion of calcium and phosphorus in the alimentary tract of lambs infected with daily doses of *Trichostrongylus colubriformis* or *Ostertagia circumcincta* larvae. *Journal of Comparative Pathology* 93, 61–71.

Wint, W., Bourn, D. & Hendrickson, C. (2006). Livestock, Land, People and Poverty in the Mekong Region of South-East Asia: Cambodia, Lao PDR, Thailand and Vietnam. In: Livestock: note and data sources. Food and Agriculture Organization. Available from: http://ergodd.zoo.ox.ac.uk/mekong [Accessed 2009-03-12].

Acknowledgements

This study was funded by The Swedish International Development Authority/Department for Research Cooperation (Sida/SAREC) through the regional program, Mekong Basin Animal Research Network (MEKARN). I gratefully acknowledge having access to the experimental facilities at the experimental farm of the Center for Livestock and Agriculture Development (CelAgrid) and the Department of Biomedical Science and Veterinary Public Health, Section for Parasitology (SWEPAR), Swedish University of Agricultural Sciences for providing the facilities for my work in Sweden.

I would like to express my sincere thanks and gratitude to those who I am in debt, in making the studies and the completion of this thesis possible:

My main supervisor, professor Johan Höglund, for his guidance during my studies and for his very useful and constructive advice. I am sincerely thankful for all your efforts and support throughout these years and for encouraging me not to be disappointed with the research results and helping me to express them scientifically.

My former supervisor, Dr. Peter Waller, for the memory of his kindness, supportive advice and his interest in contributing his knowledge to the research carried out in the developing countries.

My associate supervisors, professor Inger Ledin and Dr. Chandrawathani Panchadcharam, for their inputs, support and for providing comfortable living conditions during my stays in Sweden and in Malaysia. I will always remember your kindness, care and efficient organization.

Professor Brian Ogle, coordinator of the MEKARN program, for his support and arrangements to ease all my trips and for making it possible for me to study in Sweden. In particular thanks for editing the manuscripts and thesis.

Gratitude is expressed to "never ending researcher" Dr. Thomas Reg Preston for his knowledgeable support and for keeping me updated with respect to related research results.

My appreciation to Dr. Frank Jackson and his colleagues at MRI, Edinburgh, Drs. John Githiori and Tuan Bendixen, for their contribution and advice concerning the *in vitro* studies, even though the results of these could not be presented in this thesis. Professor Stig M. Thamsborg for half time revision of the project and constructive criticism and inputs.

I wish to thank Dr. David Morrison for his kind help and crucial advice on the statistical aspects and editing of manuscripts. Dr. Anna Lunden for her kindness, and for keeping me updated on the student situation at the department. Gunilla Lindgren and Anna Rothman for facilitating and helping with social and administrative activities. Jan Chirico, for reminding me not to work too hard.

Anna Rydzik, I can't thank enough for her help in making possible the work in the laboratory, and for keeping me company in the laboratory work in Sweden and Scotland.

I am very grateful for the help from my students: Piseth, Morn, Vuthy and Makara in carrying out my experiments successfully. Staff of the CelAgrid, particularly Dr. Khieu Borin, Chhay Ty, Chiv Phiny, Keo Sath and Pheng Buntha who always coordinated work during my periods in Sweden. Staff of the Veterinary Research Institute (VRI), Ipoh, Malaysia, Nuril, Dijah, Prema, Khor and Zavida for their hospitality during my visits.

Thanks are extended to former PhD students and colleagues at the department: Karin, Jackie, Sissay, Sunan, Malin, Marlene, Sofia and Sofia, Eva O., Annie, Casia and especially Eva M. and Sara for their understanding and sharing working conditions in the office. Also thanks to former and fellow MEKARN PhD students, An, Khang, Len, Thieu, Hong, Udom, Vanthong, Thuy, Kim Dong, Alice, Milogo and Xuan, for the happy times together in the courses and in daily life in Sweden.

I have to thank Nalinee, Giang and Hadja, individually, for the great times we had when shopping, cooking in the corridor and companying dinners in Sweden. Thanks for having shared all your experiences from study and family life. This thesis could not have been possible without your encouragement of "Try it!".

However, this work also could not have been accomplished successfully without the support from my family. Therefore I would like to convey my sincere gratitude to my mother, my sisters and brother and also my parents in law for their encouragement and understanding. Last but not least to my husband, Pok Samkol, for his love, that brings me everything.