Nutrient Utilisation in Growing Cambodian Cattle

Effect of Different Feed Sources and Feed Conservation Techniques

Keo Sath
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2012

Acta Universitatis agriculturae Sueciae

2012:94

Cover: Local Yellow cattle in metabolic cages
(photo: Keo Sath)

ISSN 1652-6880
ISBN 978-91-576-7741-9
© 2012 Keo Sath, Uppsala
Print: SLU Service/Repro, Uppsala 2012

Nutrient utilisation in growing Cambodian cattle: Effect of different feed sources and feed conservation techniques

Abstract

Natural forages are an important part of the diet of cattle in Cambodia, but their nutrient value, particularly in terms of macro-minerals, may be inadequate, leading to potential mineral deficiencies in grazing cattle.

This thesis compared the nutrient composition of six forage types (rice bran, rice straw, para grass, cassava foliage, leucaena leaves, water hyacinth leaves) from two regions in Cambodia against faecal and urinary excretion of macro minerals by cattle. The analyses showed that Ca and P levels varied markedly between the different forages and that several of the commonly used forages in Cambodia have a mineral composition that does not cover the requirements of cattle.

In an experimental study examining the effects of different levels of dietary supplementation with sun-dried cassava foliage (*Manihot esculenta*) total dry matter (DM) intake and nitrogen retention in cattle was found to increase with increasing intake of cassava foliage, but DM and fibre digestibility decreased. It was concluded that cassava foliage is a good Ca source which compensates for the low Ca content in rice straw and para grass, but P deficiency appears to be exaggerated in cattle with higher cassava intake.

Two experiments to find an appropriate way of using sugar palm syrup when preserving para grass showed that applying at least 2% (fresh matter basis) sugar palm syrup to para grass at ensiling improved the fermentation quality of the resulting silage compared with a rice straw with rumen supplement diet. Rumen pH was slightly lower and feed conversion more efficient in cattle consuming silage. Para grass silage was a good source of digestible nitrogen.

Keywords: cassava foliage, para grass/silage, sugar palm syrup, macro-mineral, intake, digestibility, retention, fermentation quality.

Author's address: Keo Sath, SLU, Department of Animal Nutrition and Management,
P.O. Box 7024, SE-750 07 Uppsala, Sweden
E-mail: keosth@gmail.com

Dedication

To my parents with my respectful gratitude
My wife Much Chanmuny

Gain knowledge through education, gain wealth through seeking!

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 Sath, K., Pauly, T. and Holtenius, K. 2012. Mineral balance of Cambodian cattle based on their faecal and urinary excretion. (Accepted: *Journal of Animal and Veterinary Advances*)

II Sath, K., Pauly, T. and Holtenius, K. 2012. Mineral status in cattle fed rice straw and para grass combined with different levels of protein derived from cassava foliage. (Accepted: *Asian-Australian Journal of Animal Science*)

III Sath, K., Sokun, K., Pauly, T. and Holtenius, K. 2012. Feed intake, digestibility, and N retention in cattle fed rice straw and para grass combined with different levels of protein derived from cassava foliage. *Asian-Australian Journal of Animal Science* 25(7), 956-961.

IV Sath, K., Khen, K., Holtenius, K. and Pauly, T. 2012. Ensiling of para grass (*Brachiaria mutica*) supplemented with sugar palm syrup at ensiling or feeding on silage quality, feed intake and growth performance of local Cambodian cattle. (manuscript)

Papers I-III are reproduced with the permission of the publishers.

Abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
BW	Body weight
Ca	Calcium
CP	Crude protein
DM	Dry matter
FCR	Feed conversion ration
FM	Fresh matter
GDP	Gross domestic product
HCN	Hydrogen cyanide
K	Potassium
L	Litre
LWG	Live weight gain
Mg	Magnesium
MK	Mekong river in Cambodia
Mn	Manganese
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NRC	National Research Council, USA
OM	Organic matter
P	Phosphorous
RS	Rice straw
S	Sulphur
TS	Tonle Sap lake in Cambodia
WSC	Water-soluble carbohydrates

1 Introduction

The basic reason for poor performance of livestock in developing countries is seasonal scarcity and qualitative fluctuations in feed. This impairs growth and reproduction of animals and results in an increased morbidity and mortality rate in animals, particularly during the dry season. However, provision of feeds with optimum quality, thus optimising animal growth, has not been the primary objective for smallholder livestock producers and instead profit maximisation has been prioritised. Many feed resources which could improve livestock production continue to be unused, undeveloped or poorly utilised. For example, locally available feeds or plant resources would minimise the production costs and therefore improve the profitability of livestock production (Wanapat, 2009).

Ruminant animals meet their nutrient requirements mainly from the products of rumen metabolism (*i.e.* microbial protein and VFA) and from nutrients escaping rumen digestion (Leng, 1997). Ruminants can utilise most carbohydrate sources in plant materials and efficiently utilise non-protein nitrogen through microbial fermentation in the rumen (Preston & Leng, 1987). Crop residues and pasture biomass (weeds/grasses from wasteland and fallow cropping land), which are characterised by high fibre content and low content of most essential nutrients, are commonly used for cattle in many areas of the world. In many developing countries, rice straw is by far the most available feed-stuff. It is available almost throughout the year and is fed to cattle especially on small farms. A major problem, for instance in Cambodia, is the long dry season with a scarcity of available forages and the difficulty in storing forages grown during the wet season in order to use them during the following dry season. Thus cattle usually respond with a slow growth rate and reduced reproductive capacity.

There are several possibilities for improving animal performance through simple dietary manipulation. Various supplements consisting of carbohydrates, protein and non-protein nitrogen, minerals and vitamins are used to supply or to balance nutrients in the diets of ruminants. Leng (1997) found that fodder trees play a role in ruminant diets and can be seen as N and mineral supplements, sources of post-ruminal protein for digestion and total feed to supply the nutrients needed. Available feeds for cattle need to be identified and their potential as nutrients investigated. Differences in nutrient value for ruminants of foliage from different trees and shrubs or forages/tree crop foliages have been reported in number of studies (Huyen *et al.*, 2012; Kang *et al.*, 2012; Mendieta-Araica *et al.*, 2011; Kongmanila & Ledin, 2009; Thang *et al.*, 2009; Hue *et al.*, 2008; Soliva *et al.*, 2005).

An alternative approach to overcome the feed shortage periods is to preserve feedstuffs when they are abundant. Sun-drying and ensiling are considered to be the most promising preservation techniques. Some protein leaves from foliage and grasses have been preserved and studied with the aim of solving feed protein shortages and feed scarcity and their potential as feed to ruminants has also been studied (Wanapat, 2009; Touqir *et al.*, 2007; Bertilsson & Murphy, 2003; Man & Wiktorsson, 2002; Man & Wiktorsson, 2001; Yokota *et al.*, 1998; Wanapat *et al.*, 1997).

Apart from energy and protein, minerals also are essential for livestock growth, so mineral nutrition must be taken into consideration. However, there is little information on the mineral status of typical forages for cattle in South East Asia.

The efficiency of utilisation of nutrients is of vital importance for maximising animal production from available feeds. Leng (1997) underlined the importance of proper digestibility evaluations of available tropical feed resources, especially taking into account the variability of the fibre content of the ruminant feeds. Thus, understanding the nutrient utilisation of some different feed resources and feed preservation techniques would be beneficial for improving the performance of Cambodian cattle.

2 Objective

The overall aim of this thesis was to generate information on how different feed resources and feed preservation techniques can be used to improve the performance of growing cattle in Cambodia.

The specific objectives were:

- To evaluate the nutrient value of common forages and grain by-products, with the focus on macro minerals, and to identify potential mineral deficiencies based on faecal and urinary excretion by grazing cattle.
- To evaluate the effects of different levels of cassava foliage in the diet on apparent nutrient digestibility in growing cattle.
- To determine an appropriate level of sugar palm syrup for ensiling para grass.
- To evaluate the response in feed intake and growth performance in cattle when sugar palm syrup is applied either at ensiling or at feeding.

Hypotheses examined in the thesis were:

- The nutrient and mineral content of feeds varies between different collection zones.
- Many commonly used diets are imbalanced in terms of mineral content.
- The apparent DM digestibility and nitrogen balance in growing cattle may be improved by cassava foliage supplementation.
- Cassava foliage supplementation of the diet improves the mineral status of growing cattle.
- Sugar palm syrup can improve the fermentation quality of para grass silage and addition of sugar palm syrup to para grass silage at ensiling or feeding can improve the growth rate of cattle.

3 Background

3.1 Cambodia's geography and climate

The Kingdom of Cambodia, which is located in the Indochina peninsula of South East Asia, occupies 181 035 km^2 and lies between 10^o-15^oN and 102^o-108^oE. Roughly square in shape, Cambodia is bounded by three countries: Thailand to the west and north-west, Lao PDR to the north, Vietnam to the east and south-east and the gulf of Thailand to the south-west. Based on the geography, the country is divided into four main agro-ecological zones: the Coastal zone, the Mekong floodplain, the Tonle Sap floodplain and the plateau/mountain zone. The Mekong floodplain and Tonle Sap floodplain almost traverse the country from north to south and predominantly feature flat or lowland plains.

Cambodia has a tropical monsoon climate with pronounced wet and dry seasons. During the wet season, from May until early October, rainfall is largely derived from the south-west monsoon drawn from the Indian Ocean. The dry season, from November to April, is associated with the north-east monsoon dominated by dry and cool winds and temperatures ranging from 27 to 40 ^oC. January is the coldest month and April the warmest. The wet season accounts for 80% of the annual rainfall. The mean annual rainfall varies between 1 000 and 2 500 mm across the country.

3.2 Livestock production in Cambodia

Livestock contribute about 21% of agricultural GDP in Cambodia (FAO, 2005). Livestock, particularly pigs and poultry, thus play an important role for Cambodian farmers and are considered to represent a family bank for daily petty cash needs and sometimes relatively large expenses. In general, famers understand the importance of having animals as a means of accumulating and maintaining financial reserves. In many cases, however, limited resources, including those of land area and shortage of capital to purchase animals and in particular large ruminants, prevent poor farmers from improving their smallholder livestock production. Cattle have long played an important role in the Cambodian farming system as draught power and a source of useful by-products, and also for returning nutrients to the soil as manure, while selling beef has been a secondary benefit of cattle. The cattle population was an estimated 3.4 million head in 2011 and has not changed much over the past decade (MAFF, 2012). Along the Mekong river region and elsewhere where feed resources are available all year around, farmers keep Haryana or Brahman breeds primarily as draught power. These breeds were introduced into Cambodia in the period from around 1960 until the 1980s. Cattle ownership is spread across the country and the average cattle herd usually comprises 3-5 head per household (Sath *et al.*, 2008b; Borin, 2007). The "Local Yellow" breed is found in most regions of Cambodia. It is primarily used for meat production and has been observed to survive better than Haryana or Brahman under harsh conditions with food shortages and dense parasite populations (personal observation).

Semi-intensive and intensive rearing systems, particularly for pig and chicken production, have increased around population centres during the last decade in order to satisfy the demand for meat for the increasing urban population. Pork and chicken meat is cheaper and thus more often consumed. There are a few farms with semi-intensive or intensive cattle production in Cambodia. The herds are generally traditionally managed and substantially affected by the seasonal variations in climate. The cattle are herded out in the early morning into open fields, public communal grazing areas or along roadsides and on rice stubble in the early dry season. As the dry season progresses, the amounts of these feeds gradually decline and rice straw becomes an essential feed resource during this critical period. In the rainy season, cattle are tethered to graze on areas adjacent to rice fields or self-fed on rice straw before being herded out into paddy fields. However, the grazing areas are gradually decreasing since the land is used for rice crop cultivation. Supplementary feeding is not practised, except on rare occasions when cut-and-carry of native grasses from nearby fields may be used.

3.3 National policies related to livestock

The government of Cambodia has launched a number of activities to develop livestock production with the aim of increasing the national revenue and family income (MAFF, 2007). These activities are: 1) control and prevention of infectious diseases in animals, 2) reducing morbidity and mortality rates of animals through vaccination programmes, 3) improving animal breeds, nutrition and animal feeds, and 4) strengthening and expansion of village animal health workers. However, the highest priority issues for the Cambodian government are animal health services and disease prevention and control. Animal vaccination programmes have been implemented both for cattle and buffalo and the establishment of training programmes for village animal health workers and private veterinary service operations is being strongly supported, with the emphasis on small livestock producers.

3.4 Potential feed resources and feeding strategies for ruminants

Recent progress in ruminant nutrition is the result of empirical feeding experiments and increased knowledge of the events occurring in the rumen, intestines and tissues.

The availability of feed resources is determined by land utilisation. On small farms in South East Asia, and particularly in Cambodia, the economic returns are prioritised and therefore improving the availability of feed is secondary to improving profit. Within the developing countries the basic resources for ruminant production are crop residues, pasture biomass (weeds/grasses from wasteland and fallow cropping land) and other cellulosic biomass. These are generally low in total protein and other metabolisable N sources and often deficient in a range of minerals (Devendra & Sevilla, 2002; Leng, 1997).

Several strategies have been tested by researchers to improve the use of local feed resources. These strategies involve the concept of balancing nutrients for feeding both the rumen microbes and the host ruminant animals. The concepts of optimising feed resource utilisation are based the principles of 1) maximising rumen function by providing macro and micro minerals and nitrogen, 2) optimising the balance of nutrients for metabolism (providing a protein meal that is relatively slowly degraded by microbial action, reducing nutrient losses through control of internal and external parasites *etc*.), and 3) modifying the rumen microbial ecosystem to minimise protozoa that prey on bacteria and reduce protein flow to the intestine. Improving the potential intake of nutrients and utilisation of the basal feed through chemical treatment would also improve the feeding system (Leng, 2004; Preston & Leng, 1987).

In order to identify the best ways to use the available forages, there is a need to develop principles that can be applied in different situations which deal with the solutions of supplementary feeding. The low CP and high fibre content of straw and stovers from grain crops must be taken into account, since these feeds are the main material available for livestock in smallholder mixed crop and livestock systems. Treating or supplementing the feeds with urea or supplementation with high quality grasses/tree leaves and concentrates has been investigated for this reason. Using straw treated with ammonia to improve both its digestibility and non-protein nitrogen content or urea molasses cake supplementation has shown promising results for ruminants (Thu & Udén, 2001; Onwuka, 1999; Dolberg & Finlayson, 1995; Sansoucy, 1995; Wanapat *et al.*, 1985). However, these have not been widely practised in Cambodia. In some areas, cost and availability of urea is a factor and the complexity of the technique may also be a deterrent to use. Limited farmer knowledge, an appropriate place for treating straw and labour and credit requirements are other constraints explaining the limited use of the technique.

However, it is important to ensure that the requirement of specific nutrients is covered, and for this supplementary feeding is generally needed. Leng (1997) has drawn attention to the potential of tree foliages in ruminant nutrition, since they can represent a biomass resource with high digestibility and protein that escapes rumen degradation. They could therefore serve as vitamin and mineral resources that reduce deficiencies in the basal diet and enhance microbial growth and fermentative digestion. The potential of the protein in leaves from different foliages of trees, shrubs and forage crops has been studied in basal protein diets or seed protein replacements (Huyen *et al.*, 2012; Kang *et al.*, 2012; Kongmanila *et al.*, 2012; Thang *et al.*, 2009; Hue *et al.*, 2008; Phengvichith & Ledin, 2007; Hao & Ledin, 2001; Mui *et al.*, 2001). Kongmanila *et al.* (2012) suggested that crude protein from *Erythrina variegata* can replace up to 60% of protein from a mixed diet with soy bean meal without any negative effect on growth rate. Cassava foliage fed at up to 28% of total dry matter intake has been reported to increase the concentration of total rumen volatile fatty acids and ammonia and give a positive response in live weight gain and intake of heifers fed urea-treated straw as the basal diet (Khang & Wiktorsson, 2006; Khang & Wiktorsson, 2004). Phengvichith and Ledin (2007) found that inclusion of cassava foliage to up to 40 % of the total diet gave rise to improved intake, digestibility of DM, OM, NDF, N retention and body weight gain of goats.

Protein escaping rumen degradation is important from a nutritional viewpoint. Proteins of different origin are degraded to varying degrees in the rumen depending on their solubility and rate of passage to the small intestine. Some proteins are naturally protected against microbial attack by tannins from plant cells released during chewing. However, at high concentrations, above 5% of diet DM, the effects of tannins are anti-nutritional, since they reduce feed intake and CP digestibility (Onwuka, 1992; Reed *et al.*, 1982; McLeod, 1974). Barry *et al.* (1986) suggested that a concentration of condensed tannins ranging from 2-4% of dietary dry matter appears to protect the protein from microbial attack in the rumen. Other proteins, for instance those in oilseeds, are denatured by the heat during extraction of oils or are protected against microbial attack by treatment with chemicals such as formaldehyde. In general, the proteins in tropical grasses appear to be part of plant structures that are not readily fermented. There are also proteins associated with lignin that appear to escape attack in the digestive tract and, as grasses become more highly lignified with increasing maturity, the proportion of protein protected against microbial attack presumably also increases.

3.5 Feed conservation

Forage, crop residues and by-products are preferentially consumed in their fresh forms by domestic animals. However, it is possible to conserve these too, ensuring feed availability during future periods of feed shortage. The two most common techniques, drying and ensiling, have been suggested for the preservation of feedstuffs during periods of excess. To improve forage production on smallholder farms, research should focus on suitable times to cut the forage (quality versus quality) and on costs. Rice straw needs to be complemented with other forages such as cassava foliage to boost production during the long dry season.

3.5.1 Sun drying

The aim of sun drying is to reduce the moisture content of fresh forages to a level at which the action of plant and microbial enzymes is inhibited (McDonald *et al.*, 2002). For satisfactory storage of dried forage, the maximum moisture content should not exceed 150-200 g/kg FM. Chopping before sun drying of cassava foliage leads to quicker evaporation and a markedly reduced content of cyanide (HCN) of up to 63% (Hue *et al.*, 2010; Borin *et al.*, 2005). Drying is the traditional conservation method for small-scale farmers in Cambodia, who preserve their grain and cassava tubers by sun drying. It is the cheapest method if high temperatures in combination with low humidity are prevalent. Thus this method is suitable only during the dry season in Cambodia. During the drying process chemical changes arise, resulting in inevitable nutrient losses. A prolonged drying process during bad weather can also encourage the growth of unfavourable bacteria and fungi, which can compromise the health of farm animals (McDonald *et al.*, 2002) and farm workers. In addition, it would be difficult to produce high-quality forage hay during the early wet season, which is the time when most forages reach suitable yield and quality for conservation. If hay making is delayed to the period after the rains, the ongoing process of plant maturation (lignification) would result in decreased forage quality.

3.5.2 Ensiling

Ensiling is less dependent on weather conditions than hay making and forages can be ensiled at any time of the year. Therefore, ensiling would be a more appropriate choice to produce a good quality forage. If cut and ensiled in the early wet season, forages can be preserved at their peak nutritive value. However, to produce high-quality silage, proper techniques and skills are needed and large investments are required.

The essential steps in the ensiling process are to maintain anaerobic conditions, discourage the activities of undesirable microorganisms such as clostridia, enterobacteria and yeasts, and stimulate the growth of lactic acid bacteria (LAB). When LAB produce lactic acid and acidify the forage, the development of other undesirable microorganisms is decreased or inhibited (McDonald *et al.*, 2002). The sugar (WSC) content of the forage plays an important role for the dominance of LAB in the ensiling process. Sugars are usually the first limiting substrate for LAB (Bolsen *et al.*, 1996). LAB require sufficient WSC to produce enough lactic acid to reduce the silage pH to a level that inhibits other competing microorganisms. Pettersson & Lindgren (1990) and Wilkinson *et al.* (1981) found that when the WSC level is too low (<2.0% WSC of FM); undesirable microorganisms can develop rapidly and break down nutrients (Oude Elferink *et al.*, 2000; Henderson, 1993) or produce metabolites, which may reduce forage intake. If used sensibly, silage additives can be useful in leading fermentation into a desirable direction (faster acidification), thus limiting undesirable fermentation and improving the nutritional quality of silage. Considerable research has been conducted on the use of silage additives (Heinritz *et al.*, 2012; Arbabi & Ghoorchi, 2008; An & Lindberg, 2004). However, due to the dynamic nature of silage fermentation, the results have been variable. In tropical areas, the fermentable sugars in forage legumes have been reported to be limiting, ranging from 32 to 77 g/kg DM (Heinritz *et al.*, 2012). A source of readily fermentable carbohydrates is necessary to improve the fermentation of WSC-deficient forages and is frequently used in ensilage of most tropical forages (Henderson, 1993). Wilkinson *et al.* (1981) and Pettersson & Lindgren (1990) suggested that a critical concentration of at least 20-25 g/kg FM of WSC in fresh grasses or unwilted crop could avoid the incidence of inadequate substrate for stimulation of LAB growth. In the case of Cambodia, sugar palm syrup is an easy accessible additive produced by farmers in the palm growing regions and it has an adequate WSC concentration (60-75%).

The efficacy of a silage additive is usually evaluated by its effect on typical fermentation criteria, *i.e.* pH and content of ammonia-nitrogen (NH_3-N) and lactic, acetic and butyric acids. The fermentation acid profile may vary since the dry matter content of crops is different. Silage pH determines the overall effectiveness of fermentation; low pH means greater acid production, restriction of undesirable microbial activity and the prevention of butyric acid fermentation (Weissbach, 1996). If legume or grass silage has undergone proper fermentation, the expected pH ranges from 3.7 to 4.7 (McDonald *et al.*, 2002; Kung & Muck, 2000). However a higher pH may sometimes be acceptable, for instance in pre-wilted ensiled forages (Weissbach, 1996). A badly preserved silage is characterised by high pH and elevated levels of acetic or butyric acid (McDonald *et al.*, 2002), due to exposure to oxygen during storage (Oude Elferink *et al.*, 2000). Oxygen entering the silo is used by aerobic microorganisms, causing increases in yeast and mould populations (Bolsen *et al.*, 1996). A low level of NH_3-N, on the other hand, is a desirable from a silage quality point of view. A high level of NH_3-N indicates that deamination of amino acids is still occurring, which may be associated with the ingress of oxygen (Henderson, 1993). Well-preserved grass silage should have NH_3-N content below 100-120 g/kg N (McDonald *et al.*, 2002; Kung & Muck, 2000).

3.6 Nutrient intake, digestibility and utilisation

Feed intake in ruminants is limited by a number of factors, including feed factors such as fibre content and inhibitors and animal factors such as physiological state, capacity to withstand stress and sensitivity to feed palatability. Feed particle size relates to physical dimensions consistent with passage further down the tract and reflects the effectiveness of chewing and microbial fermentation. Thus the regulation of intake depends on many interactions between animal and feed. Intake will be at a maximum if the feed provides all the nutrients required by the appropriate rumen microbes and by the tissues of the animal.

Plant cell walls are generally the main constituents of forages. They are composed mostly of structural carbohydrates and lignin, accounting for 35-80% of the organic matter in forage crops (Buxton, 1996; Jung & Allen, 1995). The cell wall content is negatively related to intake by contributing to ruminal fill, since cell walls are slowly degradable and thus pass slowly from the recticulorumen. The relatively low digestibility of the fibre fraction contributes to a general reduction in DM digestibility of high forage diets. A reduction in the slowly digestible cell wall fraction is beneficial because it decreases rumen fill and increases DM digestibility. The stems of most forages usually have lower digestibility than the leaves due to their high proportion of cell wall constituents. Differences between leaf and stem digestibility are normally greater in forage legumes than in grasses (Buxton, 1996). Lignin is the indigestible fraction and its content in forages typically ranges from 5 to 25 % of the plant cell wall. Lignin increases with plant maturity and the content is generally higher for legumes than for grasses (Allen & Mertens, 1988). It has been reported that the maximum dietary NDF concentration that will not hinder intake can be as high as 750 g NDF/kg DM for mature beef cows (Buxton, 1996). On the other hand, in high-yielding dairy cows an NDF content less than 250 g/kg DM can limit feed intake (Allen, 2000). Some anti-nutritional factors in forages are also reported to affect intake and digestibility, for instance tannin, as already mentioned above. In addition, forages containing less than 10 g N/kg DM can be assumed to be deficient in nitrogen for rumen microbes and thus negatively intake affect (Hugan, 1996).

An optimal level of rumen ammonia helps to improve feed intake and digestibility and the flow of microbial cells, and therefore protein, to the intestine (Leng, 2004; Leng, 1997). The growth of microbial cells in the rumen requires fermentable carbohydrates and N compounds for synthesis of protein, minerals and vitamins. Preston & Leng (1987) and Hugan (1996) concluded that in most diets based on agro-industrial by-products or low-digestibility forages, the primary limitation to the growth of rumen micro-organisms is probably the concentration of ammonia in the rumen fluid. Therefore, the concentration of ammonia in rumen fluid should be maintained above a critical level. For rumen microbes to be adequately fed requires optimum levels of rumen ammonia in the range 200-250 mg NH_3-N/L rumen fluid. However, the levels of nutrients available to the rumen microbes may range from excessive to inadequate during the intervals between episodes of eating. Moloney *et al.* (1994) found that rumen NH_3-N and proportions of total volatile fatty acids (VFA) in steers fed grass silage and barley or molasses supplements are inconsistent at any time after post-feeding and sampling times of rumen fluid. The ammonia source can be met from urea, other nitrogenous compounds or soluble proteins (such as leaf protein and seed protein).

Protein digestion by ruminants is associated with loss of nitrogen from the rumen and its degradation. Under some circumstances, excessive amounts of protein nitrogen are excreted as urea in the urine under regulation by the kidney. Kaitho *et al.* (1998) and Groff & Wu (2005) found that increasing protein level in excess of requirements increased urinary N and faecal N excretion. When protein is too rapidly or too extensively degraded to ammonia in the rumen, synthesis of microbial protein is restricted by limited available energy (Buxton, 1996). Therefore it is better to have a large proportion of the forage protein pass from the rumen undegraded, so that it can be degraded in the intestines, where absorption is more efficient. On average, 75% of forage protein is degraded in the rumen and only about 25% escapes ruminal fermentation and passes to the intestine (Merchen & Bourquin, 1994; Minson, 1990).

3.7 Dietary minerals and requirements

Limestone, sodium chloride, calcium or magnesium phosphate is often offered as a mineral lick, with other ingredients or as mixtures included in concentrates. However mineral blocks or concentrate supplements are not widely used by small-holder farms in Cambodia and thus mineral intake is solely related to forage/grass intake.

Seven essential minerals (Na, K, Ca, Mg, P, S and Cl) are generally required in quite large amounts (>1g/kg DM of feed) and are called major minerals or macro minerals (Underwood & Suttle, 1999). These minerals are all potentially limiting for animal performance if intake does not meet the requirements. Durand & Komisarczuk (1988) calculated that *in vivo*, about 5 g of P and 1.8 g of S per kg of digestible organic matter should be available in the rumen, while dietary Mg concentration should be in the range 1.5-2.5 g per kg digestible organic matter. Calcium is the most abundant mineral in the body of cattle, as in other mammals. Approximately 99% of the Ca in the body is found in bone, mainly as hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. However, Ca also plays an important role in soft tissue metabolism. Phosphorus is abundantly present in bone too, but is also located in every cell of the body and is an essential component of organic compounds in almost every aspect of metabolism. Calcium and/or P deficiency or imbalance impairs bone mineralisation, especially in growing animals, but may also cause acute diseases. Phosphorus deficiency in cattle may deplete rumen microorganisms of P, which in turn impedes feed intake and DM digestibility, especially fibre digestibility (Ramírez-Pérez *et al.*, 2009). Sodium is the major cation in extracellular fluids and is electrochemically balanced by the anion Cl. However, more than 50% of the body´s Na is stored in bone (Coenen, 2005). Sodium deficiency reduces feed intake and may cause general weakness of cattle (NRC, 2000). Potassium is the most abundant intracellular cation in cells of both animal and plant origin. Cattle consuming forage-based diets seldom experience K deficiency. On the other hand, high K intake can negatively interact both with Mg and Ca metabolism in cattle (NRC, 2000). Magnesium is an essential part of a large number of physiological processes, such as synthesis of DNA and proteins, regulation of muscle functions, cofactor in many enzyme reactions, and it also influences bone strength (Saris *et al.*, 2000; Wilson, 1980). Inadequate intake of Mg may reduce feed intake and impede rumen function in cattle (Wilson, 1980; Ammerman *et al.*, 1971). Sulphur is part of the amino acids methionine, cysteine and thus essential for rumen microbial protein synthesis. The S requirement may increase in ruminants fed cassava and other feeds containing cyanogenic glucosides, since dietary organic sulphur may be required to detoxify these glucosides (Promkot & Wanapat, 2009; Onwuka *et al.*, 1992).

3.8 Dietary minerals: absorption and excretion

Absorption and excretion of macro minerals are largely functions of the animal´ s requirements relative to the potential availability for absorption of the specific mineral, since the ability to store surplus macro minerals is limited. The potential availability of a mineral varies between different feed-stuffs and mineral compounds. Ruminants are generally rather efficient in utilising dietary Ca of different origins (Dryden, 2008). Calcium is mainly absorbed in the small intestine, although it can also be absorbed from the rumen when the rumen concentration is high (Schroeder & Breves, 2006). The net uptake of Ca seems to be largely regulated in the intestines, since urinary excretion appears to be low even when the dietary Ca greatly exceeds the requirement (Kronqvist *et al.*, 2012). Thus the apparent digestibility is low when dietary Ca exceeds the requirement. In ruminant species, dietary P is required not only for the metabolism of the animal itself, but also for the microorganisms it hosts in the digestive tract. Phosphorous homeostasis is largely regulated by saliva recirculation and intestinal absorption and P is virtually not excreted with the urine (Mogodiniyai Kasmaei & Holtenius, 2012; Hill *et al.*, 2008). Apparent P absorption in cattle seldom exceeds 55%, but may be much lower when P is fed in excess of the requirement (Ekelund *et al.*, 2006). Both Na and K absorption occurs efficiently from the gastrointestinal tract of cattle and their homeostasis is mainly regulated by urinary excretion of the surplus of absorbed electrolytes. Magnesium is mainly absorbed from the rumen of ruminants. The true absorption of Mg appears to be around 20% over a wide range of forage-based diets and with varying Mg content (Schonewille *et al.*, 2008). However, high K intake can negatively interact with the absorption. The surplus of absorbed Mg is excreted with urine and the urinary excretion can be used as a marker of Mg status in cattle (Martens & Schweigel, 2000). The S requirements are normally covered with sulphur-containing amino acids which are part of the dietary protein. Sulphur is absorbed in the small intestine as sulphur-containing amino acids.

4 Summary of materials and methods

4.1 Experimental site

The study in Paper I was carried out Kampong Cham (south-east) and Seim Reap (north-west) province, representatives of the two different agro-ecological zones in Cambodia. These provinces have a high population density and most of the inhabitants depend on agriculture for their livelihood and a high density of cattle keeping.

All experiments in Papers II, III and IV were conducted at the research farm of the Centre for Livestock and Agriculture Development (CelAgrid), about 25 km south of Phnom Penh, Cambodia, from May 2009 to May 2011. The climate is tropical monsoonal, with two seasons; a dry season (November-April) and a rainy season (May-October). The temperature during the experimental period ranged from 24 to 35 ºC.

4.2 Experimental design, treatments and diets

Paper I: A total of 48 feed samples of rice bran, rice straw, para grass, cassava foliage, leucaena and water hyacinth were collected from two regions of Cambodia (each feed sample was collected from four different places within each region) for nutrient composition analysis with the focus on macro minerals. A total of 56 faeces and urine samples from 28 cows (14 in each region; one urine and one faeces sample from each cow) were collected from the same regions as feed sampling in order to identify potential mineral deficiencies.

Papers II and III: The study was arranged as a 4 x 4 double Latin square design, with four treatments and four periods for data collection. Each period consisted of 10 days for adaptation and five days for data collection. The four diets were rice straw *ad libitum* and para grass at 1% DM of BW as the basal diet and differed only the level of cassava foliage (0, 0.8, 1.6 and 2.4 g CP/kg BW). Para grass was collected from a monoculture grown in the area and stored for one day before feeding. Cassava foliage (including petioles and leaves) was collected after root harvesting and then sun-dried for 3-5 days until the leaves became crispy. The cassava foliage was stored in hay bags under a roof.

In Paper IV, an ensiling experiment and an animal performance experiment were conducted. In the ensiling trial, four levels (0(M_0), 2 (M_2), 4 (M_4) and 6 (M_6) %) of sugar palm syrup were tested as an additive when making para grass silage. The forages and syrup were thoroughly mixed by hand, compacted into plastic bags and sealed with rubber bands. There were 72 silo plastic bags of 1 kg fresh matter in total (4 treatments, 6 sampling times and 3 replicates per treatment). Samples were taken at day 0, 2, 5, 14, 90 and 180 after closure for measurement of DM, pH and NH_3-N concentration. A completely randomised design, with 3 experimental diets and 5 replicates per treatment, was used for measurement of digestibility and growth response in cattle. The first diet consisted of rice straw fed *ad libitum* and rumen supplement at 0.25% of BW as the control, which represented farmers' practice. In the other two diets, animals received rice straw *ad libitum* and sugar palm syrup supplied at ensiling, or at feeding mixed into para grass silage fed at a level of 1.3 kg DM/100 kg BW. A 3% addition of sugar palm syrup was used to ensile para grass, indicated as "feeding syrup at ensiling". The other silage was prepared without sugar palm syrup. Animals that received silage without sugar palm syrup were instead supplied with sugar palm syrup at 3% of fresh silage matter, indicated as "feeding syrup at feeding". After thorough mixing, the materials were stored anaerobically in cylindrical containers lined with double plastic bags sealed with rubber bands and were stored under roof for at least 10 weeks. Each container contained about 140 kg FM grass silage.

4.3 Animal management and measurements

A total of 23 cattle of the Local Yellow breed, aged of 18 months, were used in the experimental study. Prior to the experiment, they were treated for intestinal parasites and vaccinated against food-and-mouth disease. Eight male cattle with an average BW of 121 kg were kept in individual metabolic cages which were designed with an upper floor for collecting faeces and a lower floor allowing total collection of urine in Papers II and III. Fifteen female cattle with an average BW of 110 kg were kept in individual pens with roof and concrete floor for Paper IV. The cattle had free access to the experimental diets and water. The experimental diets were offered three times a day, at 08:00, 12:00 and 16:00.

The intake of the individual feeds by each animal was recorded daily based on the amount of feeds offered and refused in the morning of the next day. In the digestibility study, faecal and urinary excretion were determined for each day in each period (Papers II and III) or only faeces of individual animals were collected and recorded daily in the last 5 days of the experimental period (Paper IV). During the collection periods, all faeces excreted were collected every 4 hours and a sub-sample from these faeces samples was taken daily and stored in a freezer. Total collection of urine (Papers II and III) was performed in buckets containing 10 mL 10% sulphuric acid to preserve nitrogen and around 1% of the total urine excreted was sampled and stored in the freezer. The samples from each animal were pooled according to each 5-day collection period for Papers II and III and according to each treatment for Paper IV before analysis. Blood samples of each animal were taken from the jugular vein on the last day of each period and the plasma was harvested and frozen for Papers II and III. Rumen fluid samples of each animal were taken using a stomach tube 2 hours post-feeding on the last day and analysed immediately in Paper IV. During the trial, cattle were individually weighed in the morning before feeding at the start and end of each period in Papers II and III to measure daily weight gain. In Paper IV the cattle were weighed on two consecutive days at the start and end of the trial.

The indoor temperature of the experimental house was recorded three times per day, at 06:00, 12:00 and 18:00, during the trial.

4.4 Sample analysis

Dry matter (DM) was determined using microwave radiation according to Undersander *et al.* (1993). Crude protein (CP), calculated as N x 6.25, and ash were determined according to AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the procedure of Goering & Van Soest (1970). Plasma urea (Paper II) was analysed by means of a commercial enzymatic kit (Abbott Urea nitrogen B7D7F8 Abbott, Wiesbaden, Germany). Mineral concentrations (Ca, P, K, Na, Mg, S and Mn) in feeds, faeces and urine (Papers I, II and III) were determined by inductively coupled plasma optical emission spectroscopy (Spectroflame, Spectro Analytical Instruments GmbH, Kleve, Germany) using HNO_3 for extraction according to Balsberg-Påhlsson (1990). Blood plasma levels of Ca and Mg (Paper III) were analysed by the same method. In Paper IV, NH_3-N was determined according to AOAC (1990) and pH was determined using an electronic pH meter.

4.5 Statistical analysis

In all papers, the Minitab software version 16.1.1 (Minitab, 2010) was used. Data were analysed by analysis of variance (ANOVA) using the General Linear Model procedure of Minitab software. The data in Paper I were described by mean values and their standard deviation using descriptive statistics. Each roughage, faeces and urine excretion was analysed separately using ANOVA with a one factorial design.

Turkey's pair-wise comparison procedures test was used to determine differences between treatment means which showed significant differences at the probability level of $p<0.05$ in Papers II-IV.

5 Summary of results

5.1 Nutrient composition and macro mineral content of feeds

The CP, NDF and mineral concentration (g/kg DM) varied greatly among the six forages sampled, with CP ranging from 43 to 283 g/kg DM and NDF from 384 to 745 g/kg DM (Paper I). The nutrient composition of feedstuffs used during the experiments (Papers II-IV) is summarised in Table 1.

Table 1. *Nutritional value of feeding materials used in Papers II, III and IV*

Feedstuffs	Paper	DM g/kg	CP	OM	NDF	ADF
			g/kg DM			
Fresh para grass	II, III	194	146	879	691	459
Fresh para grass	IV	196	145	878	705	408
Silage* with 3% syrup	IV	313	135	880	712	435
Silage* without syrup	IV	307	139	884	722	460
Rice straw	II, III	895	35	866	745	503
Rice straw	IV	899	22	888	725	498
Cassava leaves	II, III	879	232	923	558	324
Cassava petioles	II, III	886	88	917	588	494
Rumen supplement	IV	668	503	855	159	136
Sugar palm syrup	IV	685	0.06	-	-	-

*Para grass silage

Cassava foliage, leucaena and water hyacinth had a high Ca content (13.5-17.9 g/kg DM), while rice bran had a low Ca concentration (0.5 g/kg DM). Rice bran had a high P concentration (7.8 g/kg DM), 10-fold higher than that in rice straw. The K content of all forages except rice bran was relatively high, ranging from 16.1 to 45.3 g/kg DM (Paper I). Cassava foliage had a high concentration of Ca and Mg compared with rice straw and para grass.

Interestingly, the Na content of para grass was approximately 10-fold higher than that of cassava leaves and petioles or of rice straw. Rice straw had a low P, Mg and S content, 0.5, 0.7 and 0.6 g/kg DM, respectively. Para grass had a high S and P concentration, 3.2 and 2.5 g/kg DM, respectively (Paper II).

5.2 Faeces and urine for assessing potential mineral deficiency in cows (Paper I)

The cattle sampled in both areas of Cambodia relied completely on free grazing on unproductive areas or after harvesting crop land. The faecal and urinary mineral excretion did not differ significantly between the two regions, except for K and S excretion in faeces. The concentration of all minerals in faecal DM was slightly higher in the TS region compared with the MK region. The concentration of Ca, P, Mg, K, S. Na, and Mn in faeces accounted for 5.5, 2.5, 2.1, 6.5, 1.7, 1.4 and 1.1 g/kg DM, respectively. Among the minerals, urinary excretion was lowest for P (0.001 g/L) and highest for K (10.9 g/L). The results of excretion analyses indicated that Ca, P, Na and Mg intake was insufficient in the cattle sampled in both regions.

5.3 Effect of cassava foliage on mineral uptake and excretion (Paper II)

The total intake of Ca, P, Mg, K, S and Mn was significantly increased with increasing intake of cassava foliage, but Na intake was not affected. The two highest levels of cassava foliage intake did not generate any difference in mineral intake, except for Ca and Mg intake. Animal fed no cassava foliage had significantly lower mineral intake than those supplemented with cassava foliage. There was a significant diet effect on Mg, S and Mn digestibility, but Ca, P, K and Na digestibility was not affected by treatment. Digestibility of Mg and Mn increased with increasing actual intake of cassava foliage, while S digestibility decreased.

Faecal excretion of Ca, Mg, S and Mn increased significantly with increasing cassava foliage intake, while P, K and Na excretion in faeces was not affected by treatment. The highest intake of cassava foliage generated the highest faecal mineral excretion. Mineral losses in urine were affected by diet only for P excretion. There was a significant effect of treatment on Ca, P and Mg retention, with the highest value observed for supplementation with 1.6 g CP/kg BW cassava foliage.

5.4 Effect of cassava foliage on feed intake, digestibility and N retention (Paper III)

Intake of DM, OM, NDF and ADF increased significantly with increasing intake of cassava foliage. The total DM intake per 100 kg BW ranged from 2.7 to 3.2 kg and the highest DM intake was related to high cassava foliage intake. There were no differences in intake of DM, NDF and ADF between two lowest and the two highest levels of cassava foliage intake.

Digestibility of DM, OM, NDF and ADF was significantly higher in the group fed no cassava foliage than in the other groups. There were no differences in digestibility between diets supplemented with different amounts of cassava foliage. Nitrogen retention increased from 16 to 28 g/day with the first level of cassava foliage inclusion, but levelled out at the two highest levels. Nitrogen excretion increased in both faeces and urine as a response to higher intake of cassava foliage.

5.5 Effect of cassava foliage on blood plasma

The plasma urea concentration increased with increasing level of cassava foliage intake (Paper III). The group fed no cassava foliage had significantly lower urea concentration in plasma than the group fed the highest level of cassava foliage, but no significance difference appeared between diets supplemented with different levels of cassava foliage. Moreover, there were no treatment effects on Ca and Mg concentration in blood plasma (Paper II).

5.6 Effect of applying syrup to para grass silage at feeding or ensiling time on intake and digestibility (Paper IV)

There was a significant effect of diet on daily DM intake. The total DM intake per 100 kg BW ranged from 2.2 to 2.6 kg and the animals fed the rice straw and rumen supplement diet had higher DM intake than the groups fed two different silages with straw. However, CP intake was significantly lower in animals fed the rice straw and rumen supplement diet than in those on both silage diets. There were no differences in apparent nutrient digestibility between treatments except for CP digestibility. The digestibility of CP was significantly lower in the group fed the rice straw and rumen supplement diet than in the groups fed diets with silage inclusion.

5.7 Effect of applying syrup on para grass silage on rumen pH and NH_3-N (Paper IV)

There was no significant treatment effect on rumen NH_3-N concentration between diets supplying syrup to silage at feeding or ensiling in comparison with rice straw with rumen supplement. The rumen pH concentration was slightly lower in both groups fed silage (pH 6.9) compared with the group fed rice straw and rumen supplement (pH 7.1).

5.8 Effect of sugar palm syrup on para grass silage (Paper IV)

There were clear differences among treatments in silage pH and NH_3-N levels. Increasing levels of sugar palm syrup (M_0 to M_6) markedly decreased pH and NH_3-N values in the para grass silages. The silages made with the two highest levels of syrup had the lowest pH values after 5-14 days (pH 4.6). After 14 days, the pH value of the syrup-treated silages increased slowly to pH 5.1-5.5 at day 180, possibly due to air entry. The pH in the untreated control silage was only slightly reduced (6.1) and the concentration of NH_3-N was high (>200 g NH_3-N/kg N). The NH_3-N concentration increased rapidly during the first 14 days, followed by a slower increase towards a peak at day 90. The addition of sugar palm syrup clearly improved the fermentation characteristics of the para grass silage, even if the pH and NH_3-N levels were generally high. Based on this experiment, an application of 2-3% sugar palm syrup on a FM basis can be recommended.

5.9 Growth performance of cattle

There were no signs or symptoms of disease or toxicity among the animals during the experiments. The daily weight gain ranged from 145 to 492 g (Papers II-IV). The daily weight increased significantly with increasing level of CP from cassava foliage in the diet (Papers II and III). However, at the two highest levels of cassava foliage offered (1.6 and 2.4 g CP/kg BW) no difference in daily weight gain was observed. The highest positive response of cattle fed rice straw and para grass with CP from cassava foliage was observed at an intake of 1.3 g CP/kg BW.

In Paper IV, there were significant effects of treatment on ADG and FCR. Cattle fed rice straw and rumen supplement gained 145 g per day. Combining rice straw with para grass silage gave rise to a gain of 224 g per day. The FCR was better for both diets with silage inclusion compared with the rice straw and rumen supplement diet. Applying syrup at feeding or ensiling to para grass silage did not give rise to any differences in ADG or FCR.

6 General discussion

6.1 Identifying mineral status of cattle based on feed and faecal or urinary excretion

Blood serum or blood plasma has been used as a determinant of the status of some minerals. However, the concentration of a mineral or its functional form in blood does not always adequately reflect the mineral status of an animal. For example, bone reserves of Ca and P can protect the animal against dietary deficiency of these minerals. Judson & McFarlane (1998) summarise the possibility of assessing the status of various minerals in livestock using a specific sample. The use of feed, urine and faeces for mineral assessment has been considered because of the ease of sample collection. Feed analyses provide useful supporting data for estimating the mineral element intake if representative samples of all feeds can be obtained. The kidney plays an important role in homeostatic control of Na and Mg utilisation in animals (Underwood & Suttle, 1999). Little Mg and Na is excreted when absorption fails to meet the nutritional requirements of the animal. Faecal P concentrations have been used as an indicator of livestock at risk of P deficiency (Ternouth, 1990).

Most naturally occurring mineral deficiencies in livestock are associated with specific regions, and are related to both soil mineral concentration and soil characteristics (McDowell, 1985), which affect the uptake of the mineral from forages. A number of minerals are essential for the animal and the diets should cover the requirements. In this thesis Ca, P, Mg, Na, K, S and Mn were investigated. The results in Paper I indicate that the grazing cattle sampled in two areas of Cambodia had insufficient Ca, P, Na and Mg intake during the study period. Many factors could contribute these observed deficiencies. For example, the mineral content varied between different forages, especially for Ca and P, as reported in Paper I. On the other hand, as the dry season progresses the amount of feed gradually decreases both qualitatively and quantitatively, including the mineral content, so grazing cattle would suffer nutrient deficiency. Supplementary feeds are not commonly used for cattle in Cambodia and the diet is generally based on rice straw with a low mineral content. However in some cases cattle are occasionally fed with rice bran (personal observation), which could match the P requirement. However, rice bran is primarily fed to other livestock, particularly pigs. McDowell (1996) concluded that Ca, P, Mg and Na are most likely to be lacking under grazing conditions for ruminants. In addition, P deficiency in cattle is usually associated with P-deficient soils and known P-deficient areas.

Mineral concentration varied among six feedstuffs studied in this thesis. The P concentration was high in rice bran, but it was low in rice straw and that in cassava foliage, leucaena leaves and para grass and water hyacinth was marginal. Gowda *et al.* (2004) reported from India that leguminous green fodders are excellent sources of Ca and Mg and moderate sources of P, while cereal by-products such as rice bran are excellent sources of P but relatively poor in Ca. Most cereal grains and their by-products usually contain less Ca than the critical value (Gowda *et al.*, 2004). The K concentration in all forages investigated in this thesis was relatively high and matched the requirement. This is in line with observations by Kumagai *et al.* (1996), who reported that K concentrations on pasture are high compared with cattle requirements. It has also been reported that K deficiency is uncommon in ruminants (Suttle, 2010). Forage mineral composition is dependent on many factors, including soil characteristics, plant species, stage of plant growth, climate conditions and fertilisation practices (McDowell, 1985). Sandy soils often allow minerals to leach more easily from the topsoil than heavier clay soils. Soil acidity also affects the available of soil minerals for uptake by roots and subsequent translocation to plant tissues (Brady, 1974). Greene *et al.* (1987) showed that actively growing plant tissues have much higher concentrations of P, Mg and K than non-actively growing plant tissues.

In Paper II (pen-fed growing cattle), it was concluded that P and S should be considered when feeding cassava foliage to cattle on a rice straw and para grass basal diet. The highest P and S intake accounted for 0.13% and 0.18 % of diet DM, respectively. These were lower levels than recommended by NRC (2000). Rumen microbes have P and S requirements a part from the animal's requirements, which must be met for optimum rumen microbial activity (Hugan, 1996). Deficiency of S would alter the balance of microbes in the rumen, since S metabolism is closely associated with N metabolism (Kandylis, 1984), and could thus affect the digestibility of forages. Supplementation of such diets with S may be necessary to provide an optimum dietary nitrogen to sulphur (N:S) ratio. The dietary N:S ratio required for the most efficient utilisation by rumen microorganisms is about 10-13.5:1 for sheep and about 13.5-15:1 for cattle (Kandylis, 1984). Furthermore, Onwuka *et al.* (1992) showed that S appeared to be vital for cyanide detoxification when feeding cassava foliage or other feed containing this substance. Therefore increased supplementation with S up to 4% DM in the ration can be beneficial for cows fed fresh or hay cassava foliage (Promkot & Wanapat, 2009). Papers I and II showed the same deficiency of P. This could be explained by different feeding compared with other studies. Grain was not fed to the cattle used in Papers I and II. Khalili *et al.* (1993) found that when farmers did not feed their cattle sufficient grain, the plasma P level of grazing cattle was lower than acceptable during the rainy season and faecal P was also low compared with that in the same cattle fed grain. However, bone P plays a role as a P reservoir for resorption when body requirements temporarily exceed dietary intake (Minson, 1990). In contrast to monogastrics, where P is lost primarily in the urine, P losses in ruminants occur largely in faeces (Underwood & Suttle, 1999). Urinary P in Paper II represented 0.4-0.6% of excrete P, while on both P depletion and repletion diets, urinary P as a percentage of total excreted P ranged from <1 to 4% (Bortolussi *et al.*, 1999; Bortolussi *et al.*, 1996). Holechek *et al.* (1985) pointed out that cattle faeces are easy to sample and obviously have some relationship to an animal's diet, since dietary P can be predicted from faecal P. A critical faecal P level below 0.2% DM in grazing cattle indicates forage P levels which are too low to prevent P deficiency symptoms (Moir, 1966).

Faecal excretion of minerals in non-lactating cows in Paper I was markedly lower than that in growing cattle in Paper II. Mineral deficiency in specific feeds and varying mineral concentration in forages could affect mineral intake in grazing cattle, which might explain the lower faecal excretion compared with Paper II. Using cassava foliage compensated for the low mineral content in rice straw and para grass in growing cattle in Paper II, which clearly showed that animals were free from mineral deficiency, except P. Cassava foliage has high Ca, so could compensate for the low Ca in rice straw and para grass. Para grass is high in Na and this could compensate for the low content in rice straw and cassava foliage. Khorasani *et al.* (1997) showed positive linear correlations between P, Mg and Ca intake and their faecal excretion. Paper II showed that the digestibility of K and Na were high (the lowest digestibility of Na was 80% and of K 86%). The high urinary K and Na excretion reflects the high capability for absorption of both these minerals. However, urinary Na was lower in Paper I than in Paper II and there were indications that the animals were Na-deficient. Similarly, faecal Ca and Mg concentrations in Paper I were about half those in Paper II. Low faecal mineral content could indicate a low mineral intake or low DM digestibility. Clinical signs of mineral deficiency were not observed in this thesis. However, the interpretation of such signs is difficult if more than one mineral is deficient; deficiency is associated with other disorders such as parasites and may lead to increased susceptibility of the animal to disease (Suttle, 2010).

6.2 Evaluation of cassava foliage as a protein and mineral source on apparent digestibility and mineral balance

Supplying tropical legumes could provide soluble carbohydrates, fermentable N and insoluble protein, as well as some minerals, for rumen and post-rumen digestion (Leng, 1997; Devendra, 1995). Legume forages contain more than twice as much Ca as grasses and are more than adequate to meet animal requirements (Greene, 2000). Feeding fresh cassava foliage as sole source of protein and fibre in diet of molasses containing 2.5% urea was reported to be feasible by Ffoulkes & Preston (1977a), with no advantage in providing soy bean meal. Under these conditions, the cassava intake contributed up to 40% of the total diet and cattle daily weight gain was almost 900 g. A mixture of cassava foliage with cane stalk improved total intake without affecting cane stalk intake or DM digestibility (Ffoulkes & Preston, 1977b). A similar response in N retention and apparent nutrient digestibility of growing cattle has been reported for a mixture of cassava foliage and stylosanthes foliage as the sole source (Thang *et al.*, 2009).

Increasing the level of sun-dried cassava foliage improved total DM intake and N intake of cattle fed a basal diet of rice straw and para grass, but slightly reduced apparent digestibility of DM, OM, NDF and ADF (Paper III). Rice straw intake markedly decreased as the level of cassava foliage intake increased. This confirms findings by Vanzant & Cochran (1994) that voluntary intake of tallgrass prairie hay decreases, whereas total DM intake increases, when the amount of alfalfa hay is increased. The highest intake of sun-dried cassava foliage in Paper III was 27% of the total diet, but the highest N retention was observed at the level of approximately 21% of the total diet (40 % of N intake from cassava foliage). The capability of cattle for intake of cassava foliage in Paper III was higher than reported by Khang & Wiktorsson (2006) and Sath *et al.* (2008a), who found the highest cassava intake to be approximately 20% of the total diet. Several factors might explain the difference in results, *e.g.* the breed of the animal, differences in basal diets, the proportion of cassava foliage in the diet and different varieties of cassava used in term of nutritional composition and level of anti-nutritional substances

(HCN and tannins). High levels of HCN and tannins in fresh cassava foliage result in a slightly reduced intake and adverse effects on growth rate (Hue *et al.*, 2010; Thang *et al.*, 2009; Khang & Wiktorsson, 2006). Different varieties and stages of maturity of cassava foliage and differences in nutrient composition can affect the HCN concentration; local cultivars have a low HCN compared with commercial cultivars (Gómez *et al.*, 1985). Borin *et al.* (2005) found that the HCN content in cassava foliage could be reduced by up to 63% and 78% by sun-drying and ensiling, respectively. However ruminants are capable of detoxifying HCN into less toxic compounds (Sousa *et al.*, 2003), so if cattle eat cassava foliage slowly or over a period of time they do not suffer from toxicity. The other concern when feeding cassava foliage to cattle, besides the HCN and tannins, is the high fibre/cell wall content, which is characterised by high NDF and ADF content. The cell wall fraction of forages usually increases with maturity. Faster and more complete digestion of the feed by microbes apparently reduces the fill of the feed in the rumen, and thus enables an increase in feed intake. Faverdin (1999) suggested that an improvement in feed digestibility and microbial activity can be observed by supplying sufficient CP that is degradable in the rumen. In studies by Phengvichith & Ledin (2007), an increasing level of cassava foliage up to 30% of expected DM intake (accounting for 24% of total DM intake) in goats fed Gamba grass improved apparent digestibility of DM, OM, CP, NDF and ADF. The apparent digestibility results in this thesis thus contradict those reported by Phengvichith & Ledin (2007), which could be explained by the difference in chemical composition of cassava foliage in the two studies. Cassava foliage was cut every 90 days and fertiliser was applied at cutting time before feeding to goats in the study by Phengvichith & Ledin (2007), which could have contributed to better nutritional profile. In this thesis, cassava foliage was collected at tubers harvesting (12-15 months). In addition, balancing carbohydrate and N availability in the diet will act as a mechanism for maximising the capture of ruminally degradable N and the efficiency of N utilisation by rumen microbes and the host animal (Hristov *et al.*, 2005). Therefore the two highest levels of cassava foliage in this thesis did not further improve N utilisation, possibly due to the limitation of available energy for rumen microbes.

The Ca and Mg intake increased with increasing level of sun-dried cassava foliage, but there was no effect on blood plasma of Ca and Mg from increasing the level of cassava foliage (Paper II). Similarly, Kamiya *et al.* (2005) found that plasma Ca in cows was not affected by dietary Ca level. The Ca concentration in cassava foliage was approximately 6-fold higher than that in para grass and rice straw. Cassava foliage and para grass had high Mg concentration, approximately 5-fold higher than rice straw. Thus growing cattle consuming para grass and rice straw already had sufficient Mg for their requirements. However, the Ca intake of cattle consuming para grass and rice straw was only about 0.29% of DM, *i.e.* lower than that recommended by NRC (2000). In this thesis cassava foliage inclusion at 12% of total intake gave Ca intake of 0.45% of DM, which was above the level recommended for cattle by NRC (2000). Thus using cassava foliage could compensate for low Ca and Mg levels in feeds such as rice straw and para grass. On the other hand, the P content was relatively low in cassava foliage and even at the highest level of supplementation of cassava foliage P deficiency was not avoided for growing cattle fed rice straw and para grass. The highest P intake in this thesis was 0.13% of DM, which was lower than the recommended level. In this thesis S intake was 0.15% of DM, which was considered to be satisfactory, since the intake should not be less than 0.10% of DM (McDowell, 1985). Cassava foliage is characterised by a marked deficiency of sulphur containing AA (*i.e.* methionine and cysteine) (Ravindran, 1993; Gómez & Valdivieso, 1985). Sulphur supplementation has been shown to be beneficial for rumen microorganisms when feeding cassava foliage in terms of fermentation and HCN detoxification (Promkot & Wanapat, 2009; Promkot *et al.*, 2007). The highest Ca, P and Mg retention found in this thesis was observed at a cassava intake of 21% of total DM in the diet, showing the same trend as the N retention. Mineral supplementation might be less important for cattle if energy and protein requirements are inadequate (McDowell, 1996).

6.3 Ensiling of para grass with sugar palm syrup

The fermentation quality of silage can be evaluated based on different fermentation products, for instance pH, lactic acid, propionic acid, butyric acid, level of NH_3-N and ethanol. In this thesis pH and NH_3-N were analysed. Forage crops for silage preparation should contain an adequate level of fermentative substrates in the form of WSC to stimulate LAB growth. Wilkinson *et al.* (1981) and Pettersson & Lindgren (1990) suggested a concentration of at least 20-25 g WSC/kg FM in order to achieve good silage fermentation in a temperate climate. If the WSC content is lower than 20 g/kg FM, the forages should be wilted or a silage additive used to reduce the risk of poor fermentation. In general tropical forages are lower in WSC than temperate forages, so the fermentation pathways also differ. Use of additives such as acetic acid rather than lactic acid has been suggested for tropical grass silage (McDonald *et al.*, 1991). The WSC concentration in Napiergrass (*Pennisetum* *purpureum* Schumach) ranges from 3.9 to 4.9 % DM and decreases with maturity (Yunus *et al.*, 2000).

In this thesis, the pH of para grass silage ensiled without sugar palm syrup was only slightly reduced (pH 6.1) and the concentration of NH_3-N was high. This could be explained by a shortage of fermentable substrates in para grass. However, addition of sugar palm syrup gave a more pronounced reduction in silage pH and reduced the production of NH_3-N. The main factor contributing to the quality of para grass silage was the supplementation with sugars from sugar palm syrup. Applying sugars in the form of molasses has been shown to improve the quality of silage (Henderson, 1993; McDonald *et al.*, 1991). The sugar palm syrup (75 brix) used in this experiment contained about 75% WSC. Thus when 3% of sugar palm syrup was added to fresh grass, about 23 g WSC/kg was added, which is within the range recommended above. The lowest pH in silage of all syrup application rates (M_2:4.9, M_4: 4.7, M_6: 4.6) was observed after 5-14 days and pH rose slightly by 0.5 pH-units until day 180. This might be explained by ingression of air and stimulation of undesirable microorganism growth, leading to increased pH (Lindgren *et al.*, 1985). Exposure to oxygen could not be completely avoided at silage making. Mould or yeast growth on the surface of silage is an indicator of the presence and distribution of oxygen in the silo. If forage DM content is too high at ensiling, it is more difficult to achieve adequate compaction.

Kung & Muck (2000) reported that the target levels of fermentation profiles which indicate well-fermented forages vary between different materials and are probably dependent on the DM content. For example, for grass silage with <70% moisture, an ammonia-N value of less than 120 g/kg N and a pH of 4.3-4.7 are suggested. The pH alone is an unreliable guide, because with increasing DM level, stable conditions may be achieved at much higher pH (McDonald *et al.*, 1991). Weissbach (1996) predicted that pH should be no higher than 0.0257*(% DM of silage) + 3.71. The pH in the para grass silages analysed in this thesis were higher than the critical pH predicted. Silage is assumed to be well fermented only if both ammonia-N and pH are below the suggested critical values. Nevertheless, the results presented in this thesis indicate that application of 2-3% (W/W) sugar palm syrup to para grass biomass can produce silage of acceptable quality. The confirms previous finding for tropical grass silages (*Digtitaria decumbens, Panicum maximum cv* Hamil*, Setaria sphacelata cv.* Kazugula) showing that addition of 2-3% molasses leads to satisfactory preservation (Tjandraatmadja *et al.*, 1994). Furthermore, Castle & Watson (1985) compared different proportions of molasses (1-3%) and formic acid (2.0 L/tonne) applied to a low DM ryegrass and concluded that the molasses treatment was as effective as the formic acid treatment. An &

Lindberg (2004) suggested that good quality silage of pre-wilted sweet potato leaves can be produced by adding 6% sugar cane molasses. However, it must be emphasised that successful preservation of silage is not only a question of adequate additive, but also of factors such as rapid filling and compaction of the silo, good air exclusion and adequate sealing to limit mould growth.

6.4 Growth response of cattle fed para grass silage and effect of adding syrup at feeding or ensiling

The use of silage might be a way to improve animal production in tropical regions because animals can be still fed when fresh forage supplies are inadequate. The Cambodian cattle studied in this thesis did not eat much of the para grass silage at the beginning of the experiment and the desired intake of para grass silage was obtained after about 10 days of adaptation. Spraying sugar palm syrup on the silage at feeding appear to stimulate silage intake (personal observation).

The total DM intake of both silage-based diets was slightly lower than that of rice straw with rumen supplement. This is in agreement with Touqir *et al.* (2007), who reported low total intake of silage-based diets of jambo grass silage and mott grass silage in comparison with fresh jumbo grass. Low DM intake with silage-based diets possibly occurs because of the presence of fermentation products (Thomas & Thomas, 1985). However, the pH in rumen fluid of the animals studied in this thesis must be regarded as high and thus it is less plausible that low rumen pH limited intake. The NDF content of para grass, about 70% of DM, was virtually similar to that of rice straw. It is reasonable to assume that the high fibre content of both rice straw and para grass was the main factor limiting feed intake. The DM, NDF and CP digestibility of para grass silage diets in this thesis was similar to that of jambo grass silage and mott grass silage fed to buffalo (Touqir *et al.*, 2007). It is interesting that the daily weight gain was higher on both para grass silage diets than on the rice straw diet. It is possible that the cattle benefited from the higher protein digestibility of the para grass diets. On the other hand, it is reasonable to assume that it was available energy in the form of fermentable carbohydrates rather than digestible protein that limited performance. However, the possibly could not be excluded that the difference in weight gain, at least to some extent, was an artefact due to difference in fill of the digestive tract and /or fluid balance.

Adding sugar palm syrup at feeding or ensiling to para grass silage did not significantly affect DM intake or any of the other parameters studied. Mouldy silages were not fed and it can be speculated that this masked potential unpalatable factors in the para grass silage that was not supplemented with syrup. The sugar supplied during ensiling was presumably fermented to acids, whereas the sugar supplied at feeding time might have enhanced the palatability of the untreated silage. Untreated para grass was subjectively judged as being of acceptable fermentation quality in this thesis. A similar ADG for beef steers fed silage alone or silage supplemented with molasses has been reported by Petit *et al.* (1994). Other studies show that molasses supplementation at feeding increases intake of silage and total DM (Murphy, 1999; Yan *et al.*, 1997).

7 General conclusions and implications

7.1 Conclusions

- Several of the local feeds used in Cambodia have a mineral content that does not cover the requirements of cattle.

- Cattle grazing in two different agro-ecological zones of Cambodia with a high density of cattle were deficient in Ca, P, Na and Mg.

- Combining forages of different mineral content could improve the mineral status of grazing cattle.

- Cassava foliage supplementation improved dry matter intake and nitrogen retention, but decreased the apparent digestibility of nutrients in cattle fed diets based on rice straw and para grass.

- Cassava foliage is a good Ca source which compensates for the low Ca content in rice straw and para grass, but P deficiency appears to be exaggerated in cattle with higher cassava intake.

- Para grass silage is an excellent nitrogen source that improves the performance of cattle on a rice straw-based diet.

- Acceptable fermentation quality of para grass silage can be achieved by adding at least 20 g sugar palm syrup/kg para grass fresh matter at ensiling.

7.2 Implications and future research

The findings in this thesis about the macro mineral content in different forages represent information which can be used to meet the mineral requirements of cattle. This could be done by combining forages with different mineral profiles. Rice bran proved to be good source of phosphorous and could be used as an alternative to commercial P-containing mineral supplements. The proportion of nitrogen from cassava foliage comprise up to 40% of diet DM. Farmers could use cassava foliage as a protein source to cattle fed rice straw and para grass silage-based diets and thus they should collect this forage after harvesting the root. However, supplementation with P and S might be required. Cassava is a poor S source and has a high content of HCN, and S is involved in the detoxification process. The feeds available for cattle in Cambodia are generally limited in protein. Therefore supplementing with para grass silage during the dry season would be beneficial. Para grass has a relatively high content of digestible protein and applying 2-3% sugar palm syrup to para grass forage at feeding or ensiling could be an option for Cambodian smallholders. However the fibre content of para grass is high, which might restrict rumen digestibility and, in turn, feed intake.

Research is needed both on-station and on-farm to investigate other potential protein forages and locally available sources which are not yet in use as feeds for cattle. Preservation of feeds is an interesting option during periods when they plant biomass is available in excess. The preserved feeds may be used in periods with food shortage. Preservation techniques should be easy to adopt by smallholders, with low investment costs. Future research also needs to focus on the possibility of reducing methane emissions from ruminants.

References

Allen, M.S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science* 83(7), 1598-1624.

Allen, M.S. & Mertens, D.R. (1988). Evaluating constraints on fiber digestion by rumen microbes. *Journal of Nutrition* 118(2), 261-270.

Ammerman, C.B., Chicco, C.F., Moore, J.E., Van Walleghem, P.A. & Arrington, L.R. (1971). Effect of dietary magnesium on voluntary feed intake and rumen fermentations. *Journal of Dairy Science* 54(9), 1288-1293.

An, L.V. & Lindberg, J.E. (2004). Ensiling of sweet potato leaves (*Ipomoea batata (*L.) Lam) and the nutritive value of sweet potato leaf silage for growing pigs. *Asian-Australasian Journal of Animal Sciences* 17(4), 497-503.

AOAC (1990). Official method of analysis. 15th edn, Association of Official Analytical Chemists. edition (K Helrick editor). Arlington pp 1230.

Arbabi, S. & Ghoorchi, T. (2008). The effect of different levels of molasses as silage additives on fermentation quality of foxtail millet (*Setaria italica*) silage. *Asian Journal of Animal Sciences* 2(2), 43-50.

Balsberg-Påhlsson, A.M. (1990). Pretreatment and preparation of extracts from fresh and decaying plain material. In: Guidance in chemical methods for plant-ecological studies. Meddelanden från Växtekologiska Avdelningen, Lund University, Lund, Sweden, pp. 18-19. (in Swedish).

Barry, T.N., Manley, T.R. & Duncan, S.J. (1986). The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. *British Journal of Nutrition* 55, 123-137.

Bertilsson, J. & Murphy, M. (2003). Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. *Grass and Forage Science* 58(3), 309-322.

Bolsen, K.K., Ashbell, G. & Weinberg, Z.G. (1996). Silage fermentation and silage additives - Review. *Asian-Australasian Journal of Animal Sciences* 9(5), 483-493.

Borin, K. (2007). Avian influenza and future backyard poultry farmers. In: Proceedings of the world poultry science association "Asian pacific federation working group on small-scale family poultry farming

symposium, Swissotel Le Concorde Hotel, Bangkok, Thailand, 5-6 March, 2007, 97-102.
Borin, K., Lindberg, J.E. & Ogle, R.B. (2005). Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. *Animal Science* 80(3), 319-324.
Bortolussi, G., Ternouth, J.H. & McMeniman, N.P. (1996). Dietary nitrogen and phosphorus depletion in cattle and their effects on liveweight gain, blood metabolite concentrations and phosphorus kinetics. *Journal of Agriculture Sciences* 126, 493-501.
Bortolussi, G., Ternouth, J.H. & McMeniman, N.P. (1999). Phosphorus repletion of cattle previously exposed to nitrogen and phosphorus defciencies. *Australian Journal of Agricultural Research* 50, 93-99.
Brady, N.C. (1974). The nature and properties of soils. 8th ed. Mac- Millan Publishing Co. NewYork.
Buxton, D.R. (1996). Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* 59(1-3), 37-49.
Castle, M.E. & Watson, J.N. (1985). Silage and milk production: studies with molasses and formic acid as additive for grass silage. *Grass and Forage Science* 40, 85-92.
Coenen, M. (2005). Exercise and stress: Impact on adaptive processes involving water and electrolytes. *Livestock Production Science* 92(2 SPEC. ISS.), 131-145.
Devendra, C. (1995). Composition and nutritive value of browse legumes. In D'Mello, J.P.F and Devendra, C. (Eds). Tropical legumes in animal nutrition CAB international. ISBN 0 85198 926 8
Devendra, C. & Sevilla, C.C. (2002). Availability and use of feed resources in crop– animal systems in Asia. *Journal of Agricultural Systems* 71, 59-73.
Dolberg, F. & Finlayson, P. (1995). Treated straw for beef production in China. *World Animal Review (Multilingual Edition)* 82(1), 14-24.
Dryden, G. (2008). Animal Nutrition Science. Cambridge University Press, Cambridge, pp 130-150.
Durand, M. & Komisarczuk, S. (1988). Influence of major minerals on rumen microbiota. *Journal of Nutrition* 118(2), 249-260.
Ekelund, A., Sporndly, R. & Holtenius, K. (2006). Influence of low phosphorus intake during early lactation on apparent digestibility of phosphorus and bone metabolism in dairy cows. *Livestock Science* 99(2-3), 227-236.
FAO (2005). Livestock information, sectors analysis and policy branch: Cambodia. *FAO: Rome, Italy.*
Faverdin, P. (1999). The effect of nutrients on feed intake in ruminants. *Proceedings of the Nutrition Society* 58, 523-531.
Ffoulkes, D. & Preston, T.R. (1977a). Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. *Tropical Animal Health and Production* 3(3), 186-192.

Ffoulkes, D. & Preston, T.R. (1977b). Effect on voluntary intake and digestibility of supplementing chopped sugar cane stalk with cane tops, banana leaves or cassava forage. *Tropical Animal Health and Production* 4(1), 37-41.

Goering, H. & Van Soest, P.J. (1970). Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture hand book No.379. United State Department of Agriculture, Washington D.C., USA.

Gómez, G. & Valdivieso, M. (1985). Cassava foliage: chemical composition, cyanide content and effect of drying on cyanide elimination. *Journal of the Science of Food and Agriculture* 36, 433-441.

Gómez, G., Valdivieso, M. & Noma, A.T. (1985). The influence of cultivar and plant age on the chemical composition of field-grown cassava leaves and roots. *Qualitas Plantarum Plant Foods for Human Nutrition* 35(2), 109-119.

Gowda, N.K.S., Ramana, J.V., Prasad, C.S. & Singh, K. (2004). Micronutrient content of certain tropical conventional and unconventional feed resources of southern India. *Tropical Animal Health and Production* 36, 77-94.

Greene, L.W. (2000). Designing mineral supplementation of forage programs for beef cattle. *Journal of Animal Science* 77, 1-9.

Greene, L.W., Pinchak, W.E. & K., H.R. (1987). Seasonal dynamics of minerals in forages at the Texas Experimental Ranch. *Journal of Range Management* 40, 502-506.

Groff, E.B. & Wu, Z. (2005). Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. *Journal of Dairy Science* 88(10), 3619-3632.

Hao, N.V. & Ledin, I. (2001). Performance of growing goats fed *Gliricidia maculata*. *Journal of Small Ruminant Research* 39, 113-119.

Heinritz, S.N., Martens, S.D., Avila, P. & Hoedtke, S. (2012). The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. *Animal Feed Science and Technology* 174(3-4), 201-210.

Henderson, N. (1993). Silage additives. *Journal of Animal Feed Science and Technology* 45(1), 35-56.

Hill, S.R., Knowlton, K.F., Kebreab, E., France, J. & Hanigan, M.D. (2008). A model of phosphorus digestion and metabolism in the lactating dairy cow. *Journal of Dairy Science* 91(5), 2021-2032.

Holechek, J.L., Galyean, M.L., Wallace, J.D. & Wofford, H. (1985). Evaluation of faecal indices for predicting phosphorus status of cattle. *Grass and Forage Science* 40, 489-492.

Hristov, A.N., Ropp, J.K., Grandeen, K.L., Abedi, S., Etter, R.P., Melgar, A. & Foley, A.E. (2005). Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *Journal of Animal Science* 83, 408-421.

Hue, K.T., Van, D.T.T. & Ledin, I. (2008). Effect of supplementing urea treated rice straw and molasses with different forage species on the performance of lambs. *Small Ruminant Research* 78(1-3), 134-143.

Hue, K.T., Van, D.T.T., Ledin, I., Spörndly, E. & Wredle, E. (2010). Effect of feeding fresh, wilted and sun-dried foliage from cassava (*Manihot esculenta Crantz*) on the performance of lambs and their intake of hydrogen cyanide. *Livestock Science* 131(2-3), 155-161.

Hugan, J. (1996). Nutritional needs of rumen microbes. In Bakrie, B., Hogan, J., Liang, J.B., Tareque, A.M.M. & Upadhyay, R.C. (Eds.) Ruminant nutrition and production in the tropics and subtropics. ACIAR Monograph No. 36. ISBN 1 86320 166 1.

Huyen, N.T., Wanapat, M. & Navanukraw, C. (2012). Effect of Mulberry leaf pellet (MUP) supplementation on rumen fermentation and nutrient digestibility in beef cattle fed on rice straw-based diets. *Journal of Animal Feed Science and Technology* 175, 8-15.

Judson, G.J. & McFarlane, J.D. (1998). Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Australian Journal of Experimental Agriculture* 38(7), 707-723.

Jung, H.G. & Allen, M.S. (1995). Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of Animal Science* 73(9), 2774-2790.

Kaitho, R.J., Umunna, N.N., Nsahlai, I.V., Tamminga, S. & Van Bruchem, J. (1998). Effect of feeding graded levels of *Leucaena leucocephala*, *Leucaena pallida*, *Sesbania sesban* and *Chamaecytisus palmensis* supplements to teff straw given to Ethiopian highland sheep. *Journal of Animal Feed Science and Technology* 72(3-4), 355-366.

Kamiya, Y., Kamiya, M., Tanaka, M. & Shioya, S. (2005). Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. *Animal science Journal* 76, 325-330.

Kandylis, K. (1984). The role of sulphur in ruminant nutrition. A review. *Journal of Livestock Production Science* 11, 611-624.

Kang, S., Wanapat, M., Pakdee, P., Pilajun, R. & Cherdthong, A. (2012). Effects of energy level and Leucaena leucocephala leaf meal as a protein source on rumen fermentation efficiency and digestibility in swamp buffalo. *Journal of Animal Feed Science and Technology* 174(3-4), 131-139.

Khalili, M., Lindgren, E. & Varvikko, T. (1993). A survey of mineral status of soils, feeds and cattle in the Selale Ethiopian highlands. I. macro elements. *Tropical Animal Health and Production* 25, 162-172.

Khang, D.N. & Wiktorsson, H. (2004). Effects 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(8), 751-762.

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

Khorasani, G.R., Janzen, R.A., McGill, W.B. & Kennelly, J.J. (1997). Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage or alfalfa silage. *Journal of Animal Science* 75, 239-248.

Kongmanila, D., Bertilsson, J., Ledin, I. & Wredle, E. (2012). Effect of feeding different levles of foliage from *Erythina variegata* on the performance of growing goats. *Tropical Animal Health and Production* 44, 1659-1665.
Kongmanila, D. & Ledin, I. (2009). Chemical composition of some tropical foliage species and their intake and digestibility by goats. *Asian-Australasian Journal of Animal Sciences* 22(6), 803-811.
Kronqvist, C., Emanuelson, U., Traven, M., Sporndly, R. & Holtenius, K. (2012). Relationship between incidence of milk fever and feeding of minerals during the last 3 weeks of gestation. *Animal* 6(8), 1316-1321.
Kumagai, H., Swasdiphanich, S., Prucsasri, P., Yimmongkol, S., Rengsirikul, B. & Thammageeratiwong, P. (1996). A study on the mineral status of beef and dairy catlle and buffalo in cental Thailand. *Asian-Australasian Journal of Animal Sciences* 9(5), 525-531.
Kung, L. & Muck, R.E. (2000). Fermentation profile guidelines, target levels. Dairyland Laboratories Inc. Arcadia, Wisconsin, USA. www.dairylandlabs.com/pages/interpretations/vfa_chart.php.
Leng, R.A. (1997). Tree foliage in ruminant nutrition. *FAO: Animal Production and Health Papers 139. Rome, Italy. ISBN 9251040869*.
Leng, R.A. (2004). Requirements for protein meals for ruminant meat production in developing countries. In FAO proceeding: Animal Production and Health. Proteine sources for the animal feed industry. Rome, Italy. ISBN 9251040120.
Lindgren, S., Pettersson, K., Kaspersson, A., Jonsson, A. & Lingvall, P. (1985). Microbial dynamics during aerobic deterioration of silages. *Journal of the Science of Food and Agriculture* 36(9), 765-774.
MAFF (2007). Ministry of Agriculture, Forestry and Fisheries: Annual Conference Report on Agriculture, Forestry and Fisheries of Cambodia: Direction for sectore development.
MAFF (2012). Ministry of Agriculture, Forestry and Fisheries: Statistic of livestock. http://www.stats.maff.gov.kh/km/index.php?page=stat&mode=cattle&option=com_content&Itemid=27.
Man, N.V. & Wiktorsson, H. (2001). Cassava tops ensiled with or without molasses as additive effects on quality, feed intake and digestibility by heifers. *Asian-Australasian Journal of Animal Sciences* 14(5), 624-630.
Man, N.V. & Wiktorsson, H. (2002). Effect of molasses on nutritional quality of cassava and gliricidia tops silage *Asian-Australasian Journal of Animal Sciences* 15(9), 1294-1299.
Martens, H. & Schweigel, M. (2000). Pathophysiology of grass tetany and other hypomagnesemias-Implications for clinical management. *Veterinary Clinics of North America-Food Animal Practice* 16(2), 339-368.
McDonald, P., Edwards, R.A., Greenhalgh, J.E.D. & Morgan, C.A. (2002). Animal Nutrition. 6th ed. Pearson Education Limited. ISBN 0 582 419069: Printed by Ashford Colour Press Ltd., Gosport.

McDonald, P., Henderson, A.R. & Heron, S.J.E. (1991). The Biochemistry of Silage. 2nd Edn. Chalcombe Publications, Marlow, Bucks, UK. ISBN: 0-948617-22-5.
McDowell, L.R. (1985). Nutrition of Grazing Ruminants in Warm Climates. Academic Press, New York.
McDowell, L.R. (1996). Feeding minerals to cattle on pasture. *Journal of Animal Feed Science and Technology* 60, 247-271.
McLeod, M.N. (1974). Plant tannins-their role in forage quality. *Nutrition Abstracts and Reviews* 44(11), 803-815.
Mendieta-Araica, B., Sporndly, R., Reyes-Sanchez, N. & Sporndly, E. (2011). Moringa (Moringa oleifera) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. *Livestock Science* 137(1-3), 10-17.
Merchen, N.R. & Bourquin, L.D. (1994). Processes of digestion of forage-based diets by ruminants. In: G.C. Fahey, Jr. et al. (Editors), Forage quality, evaluation, and utilization. American Society of Agronomy, Madison, WI, pp. 564-612.
Minitab (2010). Minitab Statistical Software, Release 16.1.1 for Window. (Minitab Inc. State College PA, USA)
Minson, D.J. (1990). Forage in ruminant nutrition. Academic Press, New York.
Mogodiniyai Kasmaei, K. & Holtenius, K. (2012). Phosphorus net absorption in dairy cows subjected to abomasal infusion of inorganic phosphorus - a pilot study. DOI: 10.1111/j.1439-0396.2012.01282.x. *Journal of Animal Physiology and Animal Nutrition*.
Moir, K.M. (1966). Diagnosis of phosphorus deficiency in grazing beef cattle. *Queensland J. Agric. Anim. Sci.* 23, 97-100.
Moloney, A.P., Almiladi, A.A., Drennan, M.J. & Caffrey, P.J. (1994). Rumen and blood variables in steers fed grass silage and rolled barley or sugar cane molasses-based supplements. *Journal of Animal Feed Science and Technology* 50, 37-54.
Mui, N.T., Ledin, I., Ud´en, P. & Binh, D.V. (2001). Effect of replacing a rice bran–soya bean concentrate with Jackfruit (*Artocarpus heterophyllus*) or Flemingia (*Flemingia macrophylla*) foliage on the performance of growing goats. *Livestock Production Science* 72, 253-262.
Murphy, J.J. (1999). The effects of increasing the proportion of molasses in the diet of milking dairy cows on milk production and composition. *Journal of Animal Feed Science and Technology* 78, 189-198.
NRC (2000). Nutrient Requirements of Beef Cattle. Seventh Revised Edition. NATIONAL ACADEMY PRESS Washington, D.C. 1996.
Onwuka, C.F.I. (1992). Tannin and saponin contetns of some tropical browse species fed to goats. *Tropical Agriculture* 69(2), 176-180.
Onwuka, C.F.I. (1999). Molasses block as supplementary feed resource for ruminants. *Archivos de Zootecnia* 48(181), 89-94.

Onwuka, C.F.I., Akinsoyinu, A.O. & Tewe, O.O. (1992). Role of sulphur in cyanide detoxification in ruminants. *Small Ruminant Research* 8, 277-284.

Oude Elferink, S.J.W.H., Driehuis, F., Gottschal, J.C. & Spoelstra, S.F. (2000). Silage fermentation processes and their manipulation. in the proceedings of the FAO electronic conference on tropical silage. Rome, Italy.

Petit, H.V., Veira, D.M. & Yu, Y. (1994). Growth and carcass characteristics of beef steers fed silage and different levels of energy with or without protein supplementation. *Journal of Animal Science* 72(12), 3221-3229.

Pettersson, K.L. & Lindgren, S. (1990). The influence of the carbohydrate fraction and additives on sillage quality. *Grass and Forage Science* 45(2), 223-233.

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

Preston, T.R. & Leng, R.A. (1987). Matching Ruminant Production Systems with Available Fed Resources in the Tropics and Sub-Tropics. *Penambul Book. Armidale, Australia. ISBN 0958829012*.

Promkot, C. & Wanapat, M. (2009). Effect of elemental sulfur supplementation on umen environment parameters and utilization efficiency of fresh cassava foliage and cassava hay in dairy cattle. *Asian-Australasian Journal of Animal Sciences* 22(10), 1366-1376.

Promkot, C., Wanapat, M., Wachirapakorn, C. & Navanukraw, C. (2007). Influence of sulfur on fresh cassava foliage and cassava hay incubated in rumen fluid of beef cattle. *Asian-Australasian Journal of Animal Sciences* 20(9), 1424-1432.

Ramírez-Pérez, A.H., Sauvant, D. & Meschy, F. (2009). Effect of phosphate solubility on phosphorus kinetics and ruminal fermentation activity in dairy goats. *Animal Feed Science and Technology* 149(3-4), 209-227.

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

Reed, J.D., McDowell, R.E., Van Soest, P.J. & Horvath, P.J. (1982). Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture* 33, 213-220.

Sansoucy, R. (1995). New developments in the manufacture and utilization of multinutrient blocks. *World Animal Review (Multilingual Edition)* 82(1), 78-83.

Saris, N.E.L., Mervaala, E., Karppanen, H., Khawaja, J.A. & Lewenstam, A. (2000). Magnesium: An update on physiological, clinical and analytical aspects. *Clinica Chimica Acta* 294(1-2), 1-26.

Sath, K., Borin, K. & Preston, T.R. (2008a). Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. *Livestock Research for Rural Development* 20(SUPPL.).

Sath, K., Borin, K. & Preston, T.R. (2008b). Survey on feed utilization for cattle production in Takeo province. *Livestock Research for Rural Development* 20(SUPPL.).

Schonewille, J.T., Everts, H., Jittakhot, S. & Beynen, A.C. (2008). Quantitative prediction of magnesium absorption in dairy cows. *Journal of Dairy Science* 91(1), 271-278.

Schroeder, B. & Breves, G. (2006). Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. *Animal Health Research Reviews* 7(1-2), 31-41.

Soliva, C.R., Kreuzer, M., Foidl, N., Foidl, G., Machm¨uller, A. & Hess, H.D. (2005). Feeding value of whole and extracted Moringa oleifera leaves for ruminants and their effects on ruminal fermentation in vitro. *Journal of Animal Feed Science and Technology* 118, 47-62.

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

Suttle, N.F. (2010). Mineral Nutrition of Livestock, 4th Edition.

Ternouth, J.H. (1990). Phosphorus and beef production in northern Australia. 3. Phosphorus in cattle-a review. *Tropical Grasslands* 24, 159-169.

Thang, C.M., Ledin, I. & Bertilsson, J. (2009). Effect of feeding cassava and/or Stylosanthes foliage on the performance of crossbred growing cattle. *Tropical Animal Health and Production* 42(1), 1-11.

Thomas, C. & Thomas, P.C. (1985). Factors affecting the nutritive value of grass silages. In: (Ed. W. Haresign and D. J. A. Cole). Recent Advances in Animal Nutrition. Butterworths, London, 223-256.

Thu, N.V. & Udén, P. (2001). Effect of urea-molasses cake supplementation of swamp buffaloes fed rice straw or grasses on rumen environment, feed degradation and intake. *Asian-Australasian Journal of Animal Sciences* 14(5), 631-639.

Tjandraatmadja, M., Norton, B.W. & Mac Rae, I.C. (1994). Ensilage characteristics of three tropical grasses as influenced by stage of growth and addition of molasses. *World Journal of Microbiology and Biotechnology* 10(1), 74-81.

Touqir, N.A., Khan, M.A., Sarwar, M., Mahr un, N., Ali, C.S., Lee, W.S., Lee, H.J. & Kim, H.S. (2007). Feeding value of jambo grass silage and mott grass silage for lactating Nili buffaloes. *Asian-Australasian Journal of Animal Sciences* 20(4), 523-528.

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

Underwood, E.J. & Suttle, N.F. (1999). The Mineral Nutrition of Livestock, 3rd edn, ISBN: 0851995578 (CAB International, New York).

Vanzant, E.S. & Cochran, R.C. (1994). Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality, Tallgrass-Prairie forage. *Journal of Animal Science* 72, 1059-1067.

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

Wanapat, M., Pimpa, O., Petlum, A. & Boontao, U. (1997). Cassava hay: A new strategic feed for ruminants during the dry season. *Livestock Research for Rural Development* 9(2), 57-61.

Wanapat, M., Sundstøl, F. & Garmo, T.H. (1985). A comparison of alkali treatment methods to improve the nutritive value of straw. I. Digestibility and metabolizability. *Animal Feed Science and Technology* 12(4), 295-309.

Weissbach, F. (1996). New developments in crop conservation. p: 11-25. In: Proc. 11th Intern. Silage Conf., Aberystwyth, Wales, UK. IGER, Aberystwyth, Wales, UK.

Wilkinson, J.M., Chapman, P.F., Wilkins, R.J. & R.F., W. (1981). Interrelationships between pattern of fermentation during ensilage and initial crop composition. p.631-634. In: Proc. 14th International Grassland Congress. Lexington, Kentucky, USA. Westview Press, Boulder, Colorado, USA.

Wilson, P. (1980). Effect of magnesium supplements on the digestion of forages and milk production of cows with hypomagnesemia. *Journal of Animal Production* 31, 153-160.

Yan, T., Roberts, D.J. & Higginbotten, J. (1997). The effects of feeding high concentration of molasses and supplementary with nitrogen and unprotected tallow on intake and performance of dairy cows. *Animal Science* 64, 17-24.

Yokota, H., Fujii, Y. & Ohshima, M. (1998). Nutritional quality of napier grass (*pennisetum purpureum schum*.) silage supplemented with molasses and rice bran by goats. *Asian-Australasian Journal of Animal Sciences* 11(6), 697-701.

Yunus, M., Ohba, N., Shimojo, M., Furuse, M. & Masuda, Y. (2000). Effects of adding urea and molasses on Napiergrass silage quality. *Asian-Australasian Journal of Animal Sciences* 13(11), 1542-1547.

Acknowledgements

The Swedish International Development Agency/Department for Research Cooperation with Developing Countries (Sida/SAREC), through the Mekong Basin Animal Research Network (MEKARN), is gratefully acknowledged for financing my study. I would like to appreciate my appreciation to the Centre for Livestock and Agriculture Development (CelAgrid) for granting me access to the experimental facilities at the farm and the Swedish University of Agricultural Sciences and the Department of Animal Nutrition and Management for providing a good academic environment and facilities for my work in Sweden.

There are a number of people who helped make this thesis work successful. I would like to express my deepest thanks to:

My main supervisor Professor Kjell Holtenius, for his great efforts in helping me, giving constructive advice and useful guidance during the study, particularly with respect to scientific editing of manuscripts and then publishing papers and writing this thesis. Dr. Thomas Pauly, my co-supervisor, for patiently guiding me through answering my questions, his valuable inputs and correcting manuscripts and thesis. Dr. Khieu Borin, my local supervisor, for his encouragement and allowing me to continue my studies.

I would like to express my special thanks to Professor Inger Ledin for her kindness in helping me on my first time in Sweden and spending time teaching me English. It was an unforgettable time in my life. My gratitude to Dr. Eva Wredle for her help with my accommodation during my stay in Sweden. Many thanks are also extended to former and present MEKARN committees for their smooth facilitation and support.

I would also like to express my gratitude to:

All professors, lecturers and assistant lecturers at SLU and staff at HUV for their help and sharing their great knowledge and experience, particularly during the periods I spent in Sweden.

All my former students, Sokun, Lida, Seala, Khen who helped me to conduct the experiments, particularly the digestibility study, and make para grass silage, and colleagues at CelAgrid, Phiny, Chhay Ty, Kerya, Samkol and Sina who always gave some comments during my work and helped in analysing samples.

All past and present PhD-students whom I met at HUV and other departments for your company and allowing me to be part of your group. My friends from Vietnam, Laos, China, Burkina Faso, Nicaragua and Sweden for their friendship, help and fun during my stay in Sweden. Special thanks to Daovy and Malavanh for sharing some food and many pleasant dinners together, particularly taking care of me when I was ill. Mr. Thida shared his great skills in the EndNote program with me. I would also like to thank the students at SLU who allowed me to join in volley ball games with them in the Ultuna gym, which gave me lot of fun and let me get to know many of them.

I would like to convey my sincere gratitude to my parents, my brothers and sisters for their endless encouragement and support during my studies. Last but not least to my wife, Much Chanmuny, for her love and endurance during my time away from home.