MINISTRY OF EDUCATION AND TRAINING
CAN THO UNIVERSITY
School year: 2010-2012

SANGKHOM INTHAPANYA

MITIGATION OF METHANE PRODUCTION FROM RUMINANTS; EFFECT OF NITRATE AND UREA ON METHANE PRODUCTION IN AN *IN VITRO* SYSTEM AND ON GROWTH PERFORMANCE AND METHANE EMISSIONS IN GROWING CATTLE

MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES
ANIMAL HUSBANDRY

Code: 60 – 62 - 40

Scientific supervisors:

1- Associate professor Dr. Duong Nguyen Khang

2-Professor Dr. Thomas Reg Preston

An approval of the scientific evaluation committee

The thesis with the title: **"***Mitigation of methane production from ruminants; effect of nitrate and urea on methane production in an in vitro system and on growth performance and methane emissions in growing cattle***"** implemented by Mr Sangkhom Inthapanya was approved by the Scientific evaluation Committee at the Can Tho University.

Committee member **Secretary**

Opponent number 1 **Opponent number 2**

Can Tho City, March, 2012

Chair person

Curriculum vitae

I. Bio-data

Full name: Sangkhom Inthapanya Gender: Male

Date of birth: October, 02,1984

Place of birth *(ward/commune, district, city/province, country):* Naxagveuy village, Luang prabang district and Luang prabang province, Lao PDR

Father's birthplace: Xayabouly province Nationality: Lao

Position *(before participating MSc program):* Deputy of Animal Science Department

Workplace (*current or before participating MSc program):* Animal Science Department, Faculty of Agriculture and Forest Resource, Souphanouvong University, Lao PDR

Residence addresses *(house number, district, ward/district, and city/province):* 06/92, Naxangveuy village, Luang prabang district and Luang prabang province, Lao PDR

Tel: (856) 020 23981411 Fax: (856) 071 254932-34

E-mail: inthapanyasangkhom@yahoo.com; inthapanyasangkhom@gmail.com

II. Educational background

1. Undergraduate education

Type of training (*Permanent or impermanent)*: Permanent

Institution awarding degree: Faculty of Agriculture, National University of Laos

Major: Animal Science

Year earned: 2007

Country: Laos

Institution awarding degree: Faculty of Agriculture, National University of Laos

Country: Laos

Name of the thesis: The study of chemical composition and nutritive value of animal feed resource at Xieng Khuang province in northern of Lao PDR

Name of supervisor: 1. Mr. Viengsakoun Napasirth
2. Mr. Bounlerth Sivilay

2. Master of Sciences

Type of training (*Permanent or un-permanent)*: Permanent

Institution awarding degree: Faculty of Agriculture and Apply & Biology, Cantho University

Major: Animal Production

Year earned: 2012

Country: Vietnam

Institution awarding degree: Faculty of Agriculture and Apply & Biology, Cantho University

Country: Vietnam

Name of the thesis: Mitigation of methane production from ruminants; effect of nitrate and urea on methane production in an in vitro system and on growth performance and methane emissions in growing cattle

Name of supervisor: 1. Associate professor Dr. Doung Nguyen Khang
2. Professor Dr. Thomas Reg Preston

3. Foreign languages

1. English	Level of proficiency (IELTS):	Good (Overall score: 4.5)
2. Mother tongue' name	Level of proficiency:	Excellent

4. BSc Degree awarding

- Major: Feed resources analysis

- Degree number: 0232/NOUL.2007, date/month/year: 02 July, 2007 at National University of Laos, Country: Laos

Cantho City, *March, 2012*

Signature

Sangkhom Inthapanya

Comments

I assure that this thesis is a scientific work which was implemented by myself. All the figures and results presented in the thesis are true and not published in any previous theses.

Author

Sangkhom Inthapanya

Acknowledgement

The research in this thesis was conducted at laboratory and farm of Animal Science, Faculty of Agriculture and Forest Resource, Souphanouvong University, Luang prabang province, Laos with supported from Mekong Basin Animal Research Network (MEKARN) project and Norwegian Programme for Development, Research and Education (NUFU) project for funding this theses research and the scholarship for the MSc degree.

I would like to express my gratitude to:

Mekong Basin Animal Research Network (MEKARN) project and The Norwegian Programme for Development, Research and Education (NUFU) project, for the financial support of this study and research.

The Faculty of Agriculture and Forest Resource, Souphanouovng University, Laos and Department of Animal Science, Agriculture and Apply Biology, Cantho University, Vietnam.

Associate professor Dr. Duong Nguyen Khang, Head of the Department of Animal Physiology and Biochemistry, Nong Lam University, my supervisor, for all your support, advice and explanations throughout the research, also for your reading and correcting my thesis papers.

Professor Dr. Thomas Reg Preston and Professor Dr. Ron Leng, my teacher and adviser, for all your valuable guidance and support during the study

I would also like to extend sincere thanks to Professor Dr. Jan Erik Lindberg, International Coordinator MEKARN project; Professor Dr. Jan Berg, International Coordinator NUFU project; Dr Vanthong Phengvichith, National Agriculture and Forestry Researcher Institute (NAFRI), Associate professor Dr. Ngo Van Man, MEKARN regional coordinator and Mr. Nguyen Van Cong, MEKARN program officer and Mr. Nguyen Dzung, NUFU project assistant for their facilitation, help and support to the whole course. Associate professor Dr. Nguyen Van Thu, Head of the Department of Animal Science, Faculty of Agriculture and Apply Biology, Cantho University, Vietnam, Professors, Lecturers and assistant lectures in MEKARN courses, for giving me care and useful knowledge.

Warm thanks are extended to my father, Mr. Vanhkham Inthapanya for his great help and support. My mother, Ms. Chanhty Keovilay for her assistance and encouragement, my young sisters Sengsouphab Inthapanya and Sengsouliya Inthapanya for their supported and encouragement; to my wife, Ms. Vilanout Silaphet and my son Seviengkek Inthapanya for their love.

I would like to warmly thank Mr. Sengsouly Phongphanith and Aloun Ounalom, my analysis assistants and my colleagues in the Department of Animal Science, Faculty of Agriculture and Forest Resource, Souphanouvong University. All of my friends in the MSc course 2010-2012 from the three countries: Laos, Vietnam and Cambodia, for sharing the culture, friendship and creating a warm atmosphere throughout the time of the course.

I would like to thank all the people who contributed to this study.

Contents

Abstract

A series of experiments was carried out to determine the effect of nitrate and urea as fermentable nitrogen sources on methane production in an *in vitro* system and on growth performance and methane emissions in cattle fed lime-treated rice straw supplemented with fresh cassava foliage.

Paper 1:

An *in vitro* incubation system was used to evaluate the following treatments in a completely randomized 2*2 factorial arrangement with 4 replications; Cassava leaf meal plus urea (CLM-U), Cassava leaf meal plus calcium nitrate (CLM-CaN), *Mimosa pigra* leaf meal plus urea (MLM-U) and Mimosa leaf meal plus calcium nitrate (MLM-CaN). The basal substrate was cassava root meal.

Gas production did not differ between calcium nitrate and urea but was higher for mimosa than for cassava leaf meal after 48 hours of fermentation. The percentage of methane in the gas was lower for calcium nitrate than for urea at all incubation times but the degree of difference decreased with the length of the incubation. After 9 h of fermentation, nitrate reduced methane production by 53 and 48%, compared with urea on the mimosa and cassava leaf meal supplements. There were no consistent differences between the cassava and mimosa leaf meals in the methane content of the gas. Methane production increased, and the effect of the nitrate decreased, with fermentation time the trend being similar for both sources of leaf meal. The proportion of the substrate DM that was fermented in 48 h did not differ between sources of NPN nor between the two leaf meals. Overall, the production of methane per unit of substrate fermented was decreased by 32% when calcium nitrate replaced by urea as the NPN source. In vitro systems of feed evaluation should carefully select incubation times to represent more closely the period dominated by primary fermentation.

Paper 2:

The aim of this study was to evaluate the effect of combining alkali treatment of rice straw with non-protein nitrogen (NPN) from nitrate in reducing methane production in an *in vitro* incubation system. The treatments in a split-plot 2*6 arrangement with four replications were: alkali treated straw with NaOH (0; 1; 2; 3; 4%) plus lime ($Ca(OH)_2$) (4; 3; 2; 1; 0%) or untreated straw, and as NPN source potassium nitrate or urea. All treatments had fresh cassava leaf as protein source. The quantity of substrate was 12 g to which were added 240 ml rumen fluids (from slaughtered buffalo) and 960 ml of buffer solution. The incubation was for 24 h with measurements of total gas production, methane percentage at intervals of 4, 8, 12, 16 and 24 hours and determination of residual unfermented substrate at the end.

The proportion of substrate fermented after 24 h was increased by NaOH-lime treatment and tended to increase as lime replaced NaOH. There was no consistent effect of alkali treatment on methane percentage in the gas nor on methane production per unit substrate. Total gas production and methane percentage increased with incubation time and the gas production and the percentage of methane in the gas were reduced when nitrate replaced urea at all fermentation stages. After 24 h, the methane production per unit of fermented substrate was less when nitrate

replaced urea. It is concluded that lime can replace NaOH as a means of increasing the fermentability of rice straw and that methane production is decreased with potassium nitrate instead of urea as NPN source.

Paper 3:

Two experiments were carried out to study effects of processing of cassava leaves on the solubility of the protein and on methane production when they were incubated with cassava root meal in an in vitro incubation with urea or potassium nitrate as source of NPN.

Experiment 1: The treatments in a 2*4 factorial arrangement in a randomized block design were: leaves or petioles of cassava, and form of processing (fresh, ensiled, sun-dried and oven-dried). Protein solubility was decreased by ensiling and with the severity of drying.

Experiment 2: The treatments in a 2*4 factorial arrangement in a randomized block design were: form of processing cassava leaves (fresh, ensiled, sun-dried and oven-dried) and source of NPN (urea or potassium nitrate). An *in vitro* incubation system was used to determine the effects of the treatments on methane production and substrate fermentation. The quantity of substrate was 12 g DM to which was added 240 ml rumen fluid (from slaughtered buffalo) and 960 ml of buffer solution. The incubation was for 12 and 24 h with measurements of gas production, percent methane, substrate fermented and methane produced per unit substrate fermented.

Protein solubility in leaves and petioles decreased in the order: fresh, ensiled, sun-dried and oven-dried. Protein solubility in petioles was lower than in leaves. The gas production and methane percentage in the gas were increased with incubation time. The percentage of methane in the gas, and methane per unit substrate fermented were reduced when nitrate replaced urea as the NPN source and were lower for fresh and ensiled cassava leaves than for dried leaves at both 12 and 24 h of incubation. Methane produced per unit of fermented DM was inversely related to protein solubility.

Paper 4:

Sixteen male local "Yellow" cattle with initial weight 63-100 kg were fed lime-treated rice straw and fresh cassava foliage in a randomized complete block design (RCBD) with to two treatments: potassium nitrate or urea as NPN source. The NPN sources were dissolved in 100 g molasses diluted with 500 ml of water. The experiment lasted 120 days at the end of which concentrations of methane and carbon dioxide were determined in eructed gas mixed with air in a closed chamber in which the animals were kept for 5 minutes prior to measurement of the gases so as to ensure equilibration of the eructed gases with the air in the chamber.

Daily live weight gain and DM feed conversion were improved by supplementation with nitrate rather than urea. There was no difference between treatments in DM intake. Feed intake as g DM/kg live weight and growth rate were linearly and positively related to initial live weight. The ratio of methane to carbon dioxide in the mixed eructed gas and air was decreased by feeding nitrate with an overall 27% reduction in methane emission, for animals fed nitrate compared with those fed urea. This is the first research to report better growth rates and better

feed conversion ratios when nitrate replaces urea in a low quality diet. This result may be related to the pattern of feeding of the straw /molasses nitrate diet which was given every 6 hours.

Key words: *Alkali treatment, calcium nitrate, climate change, feed conversion, gas production, greenhouse gases, hydrogen cyanide, live weight gain, nitrogen solubility, potassium nitrate, rumen ammonia. tannins, urea*

Abbreviations

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CSF	Cassava foliage
CF	Crude fibre
CH_4	Methane
$CaNO_3$	Calcium nitrate
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
FCR	Feed conversion ratio
FMD	Foot and mouth disease
HCN	Hydrogen cyanide
KNO_3	Potassium nitrate
Lime	Calcium hydroxide
LW	Live weight
Mekarn	Mekong basin animal research network
N	Nitrogen
NDF	Neutral detergent fibre
NH_3	Ammonia
NOH	Sodium hydroxide
NPN	None protein nitrogen
NUFU	Norwegian Programme for Development, Research and Education
OM	Organic matter
pH	Power of/potential Hydrogen
Prob/P	Probability
RCBD	Randomized complete block design
RS	Rice straw
SE Asia	South East Asia
SEM	Standard error of the mean
Sida-SAREC	Swedish international development cooperation agency Department for research cooperation
U	Urea

Contents of thesis

This thesis is based on the following papers, which are referred to by the numbers 1, 2, 3 and 4.

1. **Sangkhom Inthapanya, Preston T R and Leng R A 2011:** Mitigating methane production from ruminants; effect of calcium nitrate as modifier of the fermentation in an *in vitro* incubation using cassava root as the energy source and leaves of cassava or *Mimosa pigra* as source of protein. *Livestock Research for Rural Development. Volume 23, Article #21.* Retrieved January 21, 2011, from http://www.lrrd.org/lrrd23/2/sang23021.htm
2. **Sangkhom Inthapanya, Duong Nguyen Khang, Leng R A and Preston T R 2011:** Effect of potassium nitrate as modifier of the fermentation in an *in vitro* incubation using as substrate NaOH and/or lime treated straw supplemented with fresh cassava leaves. *Livestock Research for Rural Development. Volume 23, Article #204.* Retrieved January 18, 2012, from http://www.lrrd.org/lrrd23/10/sang23204.htm
3. **Sangkhom Inthapanya, Preston T R, Duong Nguyen Khang and Leng R A 2012** Effect of processing of cassava leaves on protein solubility and methane production in an *in vitro* incubation using cassava root as source of energy. Livestock Research for Rural Development. Volume 24, Article #036. Retrieved, from http://www.lrrd.org/lrrd24/2/sang24036.htm
4. **Sangkhom Inthapanya, Preston T R, Duong Nguyen Khang and Leng R A 2012:** Effect of potassium nitrate and urea as fermentable nitrogen sources on growth performance and methane emissions in local "Yellow" cattle fed lime ($Ca(OH)_2$) treated rice straw supplemented with fresh cassava foliage. Livestock Research for Rural Development. Volume 24, Article #034. Retrieved, from http://www.lrrd.org/lrrd24/2/sang24027.htm

Introduction

In SE Asia, most farmers raise livestock for their main source of income and draft power. According to FAO (2005) over 75% of the population in Lao PDR relies on agriculture as the primary source of income. Cattle and buffaloes are important on smallholder farms in developing countries to provide meat, milk, traction power and manure in integrated crop and livestock farming (Preston and Leng 2009). In Lao PDR the populations of cattle, buffalo and sheep-goats have increased significantly from year 2002 to 2007 at the rates of 1.9, 0.61 and 9.46 % per annum, respectively (Anon 2007). However, a number of impediments and constraints have been shown to affect livestock productivity and efficiency. The main feed resources for the ruminants in Lao PDR are native grasses, legumes and tree leaves that are available in the natural grassland and forests (Phonepaseuth Phengsavanh and Ledin 2003). The availability of these feed resources is seasonally limited and both feed availability and quality are low, especially in the cropping season.

Preston and Leng (2009) and Leng (1997) have emphasized that the most appropriate ways to improve feed resources for ruminants are through efficient utilization of crop residues and tree/shrub foliage. However, to optimize performance correct feeding methods need to be applied ensuring that rumen function is efficient and secondly ensuring efficient assimilation of nutrients by providing a source of bypass nutrients (Preston and Leng 2009).

Rice straw is the most abundant crop residue in Asia, particularly in Lao PDR. It is the main feed in the dry season when natural grasses are in short supply to animals. Rice straw is characterized by high fiber level (39-53 % ADF) and nutrient deficiencies, especially protein (2 to 4% crude protein), vitamins, minerals and soluble carbohydrates. The straw itself is 98% covered with silica which has to be disrupted (McAllister et al 1994). Thus rice straw has low digestibility for ruminants in the range 41- 59 % (Napasirth et al 2005; Susuki et al 2004; Bui Van Chinh et al 2001; Tran Quoc Viet et al 2001).

There are two ways to improve the feeding value of rice straw: (i) by delignification treatments which also disrupt the silca covering, which may be physical, chemical or biological (Sundstol 1984; Doyle et al 1986); and (ii) supplementation with limiting nutrients in the rumen and essential amino acids (bypass protein) in the animal (Preston and Leng 2009). Treatment with sodium hydroxide was used in early trials on delignification of straw, but ammoniating the straw with urea has been the most widely used method (Chenost and Kayouli 1997). The partial replacement of urea by lime was reported by Nguyen Xuan Trach et al (2001) and Le Thi Thuy et al (2005) to be equally effective and more economical then ammonization methods.

Leng (2008) concluded that the inclusion of nitrate salts in feed supplements appeared to be entirely feasible as a means of providing fermentable nitrogen and simultaneously reducing enteric methane emissions from ruminant livestock. Whilst there is a risk of nitrite toxicity, nitrate reduction to ammonia in the rumen should theoretically improve microbial growth efficiency and retain the energy that is otherwise lost in methane.

The possibility of nitrate as an alternative hydrogen sink to carbon dioxide has been downplayed because of the possible toxic effects of nitrite, which is formed as an intermediate during the reduction of nitrate to ammonia (Lewis 1951). However, several reports have recently examined the potential of nitrate as a methane-lowering feed additive, and it has been shown to lower methanogenesis consistently (Leng and Preston 2010).

Recent researche in Vietnam (Nguyen Ngoc Anh et al 2010) and Cambodia (Iv Sophea and Preston 2010) have confirmed that the long term feeding of nitrate salts supported the same growth in goats as when urea was the NPN source, but brought about 30% reduction in production of methane. Much higher (50%) responses were reported in sheep in the Netherlands (Van Zijderveld et al 2010a) and in dairy cattle in Brazil (Van Zijderveld et al 2010b). However, there appear to be no reports showing that the reduction in methane is accompanied by more efficient production of meat or milk, which should theoretically happen.

It has been postulated (Leng 2008) that nitrate salts will be most effective as an NPN source when the diet is low in other sources of rumen-fermentable nitrogen and that the additional protein needed by the animal, over and above that produced by rumen microbes should be provided in the form of bypass protein. Fresh cassava foliage has been shown to be an effective source of bypass protein in diets where the dietary N was mostly in the form of urea (Ffoulkes and Preston 1978). Cassava is widely grown in all tropical countries and the fresh foliage / leaf meal has been shown to support increased growth rates in cattle fed on rice straw (Keo Sath et al 2008; Tham et al 2008). Recently, the foliage of *Mimosa pigra* has been shown to support high growth rates when fed as the sole diet to goats (Thu Hong et al 2008). The authors postulated that this could be explained by the high content of condensed tannins conferring "rumen escape' qualities on the protein. Mimosa is considered to be an invasive weed in many tropical countries (Tran Triet et al 2007); however, if a positive use could be found for the plant as an animal feed supplement it could become a useful plant rather than an environmental menace.

Hypotheses

The hypotheses to be tested were:

Paper I: Giving calcium nitrate rather than urea will reduce the methane production in *an in vitro* system using cassava root meal, with mimosa or cassava leaf meals as the source of protein.

Paper II: Lime ($Ca[OH]_2$) could replace NaOH to treat rice straw to improve the digestibility in an in *vitro* incubation and providing the NPN source as potassium nitrate rather than urea will reduce the methane production irrespective of the method of treating the straw.

Paper III a: Different ways of processing cassava leaves and petioles (fresh, ensiled, sun-dried, oven-dried) will affect solubility of the protein.

Paper III b: Different ways of processing cassava leaves (fresh, ensiled, sun-dried and oven-dried) will affect CH_4 production in an *in vitro* incubation with cassava root meal as energy source and urea or K-nitrate as source of NPN.

Paper IV: Growing cattle fed lime-treated rice straw and fresh cassava foliage will have better growth performance and produce less methane when supplemented with potassium nitrate rather than urea.

Literature review

Livestock production systems in Lao PDR

Lao People's Democratic Republic (Lao PDR) is a rural country with an estimated 6.3 million people in 2010. About 73 percent of its population lives in rural areas. Livelihoods of a large majority of these people are based on a combination of rice-based agriculture, the collection of forest products and livestock production (FAO 2010). Agriculture is changing and many Lao farmers are moving from subsistence farming, where they produce food for their family's self-sufficiency, to commercial farming in order to produce commodities for the market (NAFES 2006). Nowadays many Lao products are competitive in international markets including rice, coffee, maize, sugarcane, green tea, plantation timber, and non-timber forest products (MAF 2007). However, livestock is a particularly important activity for upland households and farmers as they receive more than 50 percent of their income by selling animals (Wilson 2007). Livestock is important for farmer's livelihood security because when crop production fails they can sell animals and buy rice (Ingxay et al 2009). In general, livestock are most used as a source of cash income by farmers who live in remote areas without road access to markets. They can walk livestock to markets but are unable to transport perishable crop products (Stur et al 2002). So far, the Lao government has given high priority in its rural development strategy to improving livestock production systems (ACIAR 2004).

Livestock production in Lao PDR contributes around 15% to national GDP and 33% of agricultural GDP (Government of Lao PDR (GOL); Millar and Photakoun 2008), and is thus of crucial importance to the economy (FAO launches 2009). This production includes livestock such as buffalo, cattle, pigs, poultry and insects. There is potential to improve and develop livestock production, because firstly smallholders already have local knowledge and experiences on livestock management and production which have been transferred from generation to generation, there is natural grassland and other areas that suitable for ruminant production and the most important is that the government of Laos has developed a strategy for diversifying its agricultural economy, particularly the livestock sector (Ingxay et al 2009).

In 2009, livestock production in Laos increased 4.4 percent and net livestock production, at 240,500 tonnes, now exceeds domestic consumption - indicating that Laos has the ability to export livestock to neighboring countries (Northern farmers fatten 2009). The government has 2006-2010 plans to export between 100,000 and 120,000 tonnes of livestock per year; however, exports in 2008 reached only 98,000 tonnes (More livestock 2009). About 75% of cattle and buffalo produced are consumed domestically, and the remaining 25% are exported to neighboring countries. Lao PDR exports about 100,000 head per year to Thailand (Millar and Photakoun 2008). In general, farmers like to keep the poorest animals for consumption and sell the good quality animals to the capital Vientiane, Thailand and Vietnam (Stur et al 2002). The National Growth and Poverty Eradication Strategy identifies targets of an average meat supply of 60 kg/capita/year and increased exports to value of $50 million by 2020 (Millar and Photakoun 2008). The Lao government has declared the goal of becoming a regional exporter of cattle and buffalo (Michael 2009).

The Department of Livestock and Fisheries (DLF) has planned to ensure cattle destined for export weigh at least 180-250 kg. Upland areas will be encouraged to breed cows because local weather conditions are suitable for livestock raising (More livestock 2009). Cattle are one type of livestock which is important for smallholder farms. Rural farmers mainly grow

crops as the primary commodity and they keep cattle for draft power and for manure as fertilizer for their crops. The numbers of buffaloes and cattle are increasing in Lao PDR, as shown in Table 1, despite a concurrent increase in the numbers exported to neighboring countries, principally China and Thailand (FAO 2011).

Table 1. Livestock Numbers ('000 head) by Province, 2007-2009

	Buffaloes			Cattle			Pigs		
	2007	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**
Phongsali	36	37	39	38	38	39	169	176	181
Luang Namtha	23	23	22	26	27	27	66	70	75
Oudomxai	37	36	39	34	36	39	103	105	123
Bokeo	23	24	28	30	30	33	50	55	69
Luang Prabang	58	58	60	55	57	59	162	179	198
Huaphanh	66	67	69	53	54	55	301	357	392
Xayabouri	53	53	55	68	70	71	138	166	185
North	**296**	**298**	**312**	**304**	**312**	**323**	**999**	**1108**	**1223**
Vientiane Municipality	16	18	19	71	83	84	52	55	62
Xienkhuang	46	53	51	71	73	76	76	79	82
Vientiane	69	72	71	122	133	133	94	97	100
Borikhamxai	44	44	45	54	55	56	58	60	62
Khammuane	74	75	73	65	66	69	61	62	69
Savannakhet	285	285	285	391	391	392	244	249	256
Centre	**534**	**547**	**544**	**774**	**901**	**810**	**595**	**602**	**631**
Saravane	99	112	121	112	120	123	328	374	367
Sekong	28	28	30	23	25	27	123	129	135
Champassak	119	123	124	120	125	129	137	148	170
Attapeu	47	47	47	14	15	17	24	26	29
South	**293**	**310**	**322**	**269**	**285**	**296**	**612**	**650**	**701**
Lao PDR	**1123**	**1155**	**1178**	**1347**	**1398**	**1429**	**2186**	**2360**	**2555**

Source: *MAF Agricultural Statistics Yearbook (2009).*

The majority of animals in Lao PDR are native breeds managed in a free range system in grassland and forest areas. The main purpose is meat production for consumption, traditional ceremonies and selling in the market. Besides that, farmers like to raise cattle as draught power, for transportation and manure (Ministry of agriculture and forestry 2003). However, cattle usually lose body weight in the dry season, when natural grasses are less available. There are opportunities to make better use of rice straw which normally is burned after harvest (Vongsamphanh et al 2004).

Utilization of feed resources for ruminant production

Livestock can utilize agricultural crop residues and farm by-products that are abundantly available particularly for large ruminants - buffalos or cattle which are able to use nutrients arising from poor quality forage or cellulosic biomass (Preston and Leng 2009).

Rice straw has been used as ruminant feed in traditional livestock farming communities in Asia. According to NAFRI, NAFES and NUOL (2005) rice straw is the most common roughage in Lao PDR, as it is available as the byproduct from crop cultivation. It is used to meet part of the nutrient requirements of ruminants in the rice-producing areas during the cropping season and in dry or drought periods. However, the animals fed only rice straw do not obtain enough nutrients to maintain high production levels due to the low nutritive value

of this highly lignified material. The high level of lignification and solidification, the slow and limited ruminal degradation of the carbohydrates and the low content of nitrogen are the main deficiencies of rice straw, affecting its value as feed for ruminants (Van Soest 2006).

Possible strategies to improve rice straw utilization

Basically, the key to improving the use of crop residues for ruminants is to overcome their inherent barriers to rumen microbial fermentation. In the case of rice straw, the important factors that restrict bacterial degradation in the rumen are its high levels of lignification and silidification, and its low content of nitrogen, vitamins and minerals. To improve the feeding value of rice straw, the straw can be treated with different means and methods and other required nutrients can be supplied to the ration of the animal. Strategies to improve the utilization of rice straw may use alkaline, acidic or oxidative agents. Among these, alkali agents have been most widely investigated and practically accepted for application on farms. Basically, these alkali agents can be absorbed into the cell wall and chemically break down the ester bonds between lignin and hemicellulose and cellulose, and physically make the structural fibers swollen (Chenost and Kayouli 1997; Lam et al 2001). These processes enable the rumen microorganisms to attack more easily the structural carbohydrates, enhancing degradability and palatability of the rice straw (Prasad et al 1998; Shen et al 1999; Selim et al 2004).

The most commonly used alkaline agents are sodium hydroxide (NaOH), ammonia (NH_3), Lime ($Ca[OH]_2$) and urea. Chemical treatments appear to be the most practical method for use on-farm, as no expensive machinery is required, the chemicals are relatively cheap and the procedures to use them are relatively simple. However, the chemicals themselves are not harmless and safety precautions are needed for their use.

NaOH treatment

Several NaOH treatment methods to improve the use of crop residues for ruminant feeding have been developed as reviewed by Jackson (1977), Berger et al (1994) and Arieli (1997). The principal advantages of the different NaOH treatment methods are increased degradability and palatability of treated straw, compared to untreated straw (Chaudhry and Miller 1996; Vadiveloo 2000). However, NaOH is not widely available as a resource for small-scale farmers and may be too expensive to use. In addition, the application of NaOH can be a cause of environmental pollution, resulting in a high content of sodium in the environment (Sundstol and Coxworth 1984).

Urea treatment

Rice straw can also be treated with urea, which releases ammonia after dissolving in water. For practical use by farmers, urea is safer than using anhydrous or aqueous ammonia and also provides a source of nitrogen (crude protein) in which straw is deficient (Schiere and Ibrahim 1989). Since urea is a solid chemical, it is also easy to handle and transport (Sundstol and Coxworth 1984) and urea can be obtained easily in many developing countries. In addition, urea is considerably cheaper than NaOH or NH_3. Vadiveloo (2003) reported that rice varieties with a low degradability responded better to urea treatments than higher quality straw, increasing the in vitro dry matter degradability from 45 to 55-62%. Urea treatment may therefore be most suitable for small-scale farmers to improve the quality of straws, particularly varieties showing a low degradability. In the past, numerous investigations involving urea treatment of rice straw, with or without additional supplementation, were

performed not only in the laboratory (Reddy 1996; Shen et al 1998; 1999; Vadiveloo 2003) but also in field trials (Prasad et al, 1998; Vu et al, 1999; Akter et al 2004; Pradhan et al 1997). Sirohi and Rai (1995) demonstrated that a combination of 3% urea plus 4% lime at 50% moisture for 3 weeks incubation time was the most effective treatment for improving degradability of rice straw. Using urea is regarded as a practical and available method in livestock production, especially in developing countries, as it is relatively cheap, adds nitrogen to the ration and is relatively safe to work with.

Lime treatment

Lime ($CaO/Ca(OH)_2$) is a weak alkali agent with a low solubility in water. It has been reported that lime can be used to improve the utilization of straw and also can be used to supplement the ration with calcium, which has been found to be in a negative balance in cattle fed only rice straw (Hadjipanayiotou 1984; Pradhan et al 1997; Chaudhry 1998). Soaking and ensiling are two methods of treating straw with lime. Although lime treatments increase the degradability of straw, the dry matter intake often decreases, due to a reduced acceptability of the treated feed by animals. Pradhan et al (1997) reported that ensiling rice straw with 4 or 6% $Ca(OH)_2$ showed a higher IVDMD than using 4 or 6% urea. However, mould growth was noticed in the $Ca(OH)_2$ treated straw. It was suggested that a combination of lime and urea would give better results than urea or lime alone. This combination has the advantage of an increased degradability and an increased content of both calcium and nitrogen. Additive effects of lime and the other alkali agents have been demonstrated (Saadulah et al 1981; Hadjipanayiotou 1984). The use of lime may be safer and more cost effective to use than NaOH.

Alkali treatment of straw will increase is digestibility but supplementation is also needed to ensure there is an adequate balance of nutrients available to the animal. Therefore, as described by Preston and Leng (2009), when considering way to optimize the utilization of feed resources for ruminants it is necessary to apply basic concepts that include:

- Ensuring optimum conditions for microbial growth in the rumen to make this part of the digestive system of the animal as efficient as possible by providing macro and micro minerals and sources of ammonia
- Providing a protein meal that is relative slowly degraded by microbial action such that a proportion of the dietary protein enters the intestine for enzymic digestion
- Modifying the rumen microbial ecosystem (eg: defaunation by oil drenching) to minimize protozoa that prey on bacteria and reduce protein flow to the intestine.

For this purpose there is a need to supply readily fermentable nitrogen and minerals as well the "unknown" factors often associated with green feeds (Preston and Len 2009). However, even when the needs of rumen micro-organisms are met, basal diets such as rice straw must also be supplemented with sources of bypass or escape protein (Preston and Leng 2009) in order to meet the requirements for production. In this respect cassava foliage has proved to be a valuable supplement in diets that otherwise supply only rumen nutrients (Ffoulkes and Preston 1978). Several authors showed that on diets of rice straw fed to growing cattle, rates of live weight gain were increased by supplements of fresh cassava foliage (Seng Mom et al 2001), cassava leaf meal (Ho Thanh Tham 2008) and sun-dried cassava hay (Keo Sath et al 2008).

Impact of global greenhouse gases

Greenhouse gas (GHG) emissions have become an increasingly important topic worldwide due to their effects on global warming and climate change. Since the industrial revolution in the 1750's, there has been a global increase in atmospheric concentrations of GHGs. Evidence that the global temperature is increasing (IPCC 2007) has resulted in an international effort to reduce anthropogenic GHG emissions to the atmosphere.

Sources of GHG emissions include fossil fuel use, enteric fermentation from livestock and manure management, rice agriculture, biomass burning and waste management. The remaining emissions come from natural sources including wetland, gas hydrates, permafrost, termites, oceans, freshwater bodies, non-wetland soils, volcanoes and wildfires (IPCC 2007, Sejian et al 2011). The largest growth in GHG emissions has come from energy supply and transport and industry, while emissions from residential and commercial building and agriculture sectors have been growing at a slower rate.

According to Smith et al (2007) agriculture produces 10-12% of total global anthropogenic greenhouse gas emissions, contributing 50% of all anthropogenic methane (CH_4). Ruminant livestock animals are a major source of total anthropogenic emissions producing an estimated 80 million tonnes of CH_4 annually accounting for 33% of anthropogenic emissions of CH_4 (Beauchemin et al 2008). There is therefore an urgent need to develop ways of reducing methane production from ruminants which are major contributors to global warming (CONAM 2001).

Ruminants and the environment

Methane formation in the rumen is important for the ruminant animal because it removes hydrogen (H_2) arising from the fermentation of feed that would otherwise accumulate and have adverse effects for rumen function (Moss et al 2000). The methanogens in the rumen maintain low H_2 concentrations, which allow the primary fermentation of feed to proceed (Buddle et al 2011). The net effect of CH_4 formation is that four moles of H_2 are removed for each mole of CO_2 reduced to CH_4 (Table 2). The free energy change (ΔG) associated with CH_4 production phosphorylates adenosine di-phosphate (ADP) to form adenosine tri-phosphate (ATP), which provides energy for maintenance and growth of the methanogenic archaea (Russell and Wallace 1997).

Table 2. Key reactions in the rumen and free energy (ΔG) change that is available for doing work

Reaction	Formula	(ΔG) (KJ/M)
Glucose to acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_4O_2 + 4H_2 + 2CO_2$	- 142.4
Glucose to propionate	$C_6H_{12}O_6 + 2H_2 \rightarrow 2C_2H_6O_2 + 2H_2O$	- 303.9
Glucose to butyrate	$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2H_2 + 2CO_2$	- 233.1
Glucose to lactate	$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$	- 116.8
Lactate to propionate	$C_3H_5O_3 + H_2 \rightarrow C_3H_5O_2 + H_2O$	- 93.6
Methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	- 134.9
Acetogensis*	$2CO_2 + 4H_2 \rightarrow C_2H_4O_2 + 2H_2O$	- 72.2
ATP generation	$ADP + P_i \rightarrow ATP + H_2O$	- 10.4

** not demonstrated in the rumen*

H_2O: water; H_2: hydrogen; CO_2: carbon dioxide; CH_4: methane; P_i: high energy phosphate; ADP: adenosine di-phosphate; ATP: adenosine tri-phosphate; M: mole

Source: *Kohn and Boston (2000)*

Methane production from ruminant digestion not only contributes to the global greenhouse effect (Rossi et al 2001), but it also represents a substantial waste of feed energy (Waghorn et al 2007). As a percentage of gross energy (GE) consumed by ruminants, 2 to 12%, is lost as CH_4. This variation is associated with factors including diet quality. Losses from ruminants grazing temperate forages are typically 5 to 7% of GE intake (GHI) (Lassey et al 1997, Pinares-Patino et al 2003d). In general, CH_4 emissions are closely related to the digestible OM intake (DOMI). About 55 to 65% of digestion occurs in the rumen (Moss et al 2000, Waghorn et al 2007), but the rumen does not account for all of the CH_4 produced by the animal. It has been estimated that 10 to 30% of OM digestion occurs in the hindgut (Moss et al 2000) and while its contribution may be relatively small, the hindgut does produce and contribute to overall CH_4 emissions (Ellis et al 2008).

Increased demand for global supply of animal products will drive an increase in livestock populations, resulting in higher total emissions of CO_2, CH_4 and N_2O and result in greater use of N fertilizers. This is a concern for the environment and as a consequence research in pastoral situations is now re-focused on increased use of legume forages in pastures to overcome some environmental issues. According to (Waghorn et al 2002), sheep fed fresh forages such as white clover *(Trifolium repens)*, lotus major *(Lotus pedunculatus)* and other legumes (Waghorn et al 2002) had much lower CH_4 yields. The lower CH_4 yields from sheep fed alternative forage species present an opportunity for GHG mitigation whilst increasing animal productivity.

Methane emissions from ruminants

Methane from ruminants is produced when feed macromolecules are fermented by microorganisms in the gastro-intestinal tract (GIT). The catabolism yields volatile fatty acids (VFAs), CO_2, ammonia (NH_3), H_2 and heat. Volatile fatty acid and NH_3 are absorbed via the rumen wall, where CO_2 is both absorbed and eructed (Preston and Leng 1987). Methane production is the last step of the fermentation process and is carried out by methanogenic archaea (methanogens), which in the rumen predominately utilize H_2 as an energy source to reduce CO_2 to CH_4. According to Moate et al (1997) the methane produced by methanogens accounts for about 25% of ruminal gases and it is absorbed and eructed with CO_2. Cattle produce about 150 to 420 L of CH_4 per day (107 to 300 g CH_4/day) and sheep about 25 to 55 L per day (18 to 39 g CH_4/day), depending on intake (Czerkawski 1969, Holter and Young 1992, McAllister et al 1996).

Methanogens comprise a range of species and populate the rumen at 10^8 to 10^{10} cell/litre of rumen fluid (Stewart, 1991, Kumar et al 2009). Although H_2 and CO_2 are preferred substrates, formate, acetate, methanol and mono-, di and tri-methylamine can also be utilized as substrate for CH_4 formation (Wolin et al 1997). Cleavage of methyl groups from compounds such as pectin, methylamines and methylated suphides, can also serve as precursors for CH_4 formation; as well as breakdown products of methylated amino compounds and methionine (Ellis et al 2008). Short chain alcohols can also serve as electron donor for CO_2 reduction; where secondary alcohols are oxidized to ketones and primary alcohols are reduced to carboxylic acids (Widdel 1986, Zellner and Winter 1987).

Methanogens are unique because they have a high affinity for very low H_2 concentrations (Stewart 1991). They are consistently more competitive for H_2 compared to other H_2 utilizing microbes (i.e. sulphate reducing bacteria and acetogenic bacteria) because they use pathways with a more negative change in free energy (ΔG) (Janssen 2010). For example, an alternative H_2 utilizing pathway is the reduction of CO_2 to acetate (acetogenesis), which is

thermodynamically less favorable (ΔG= - 72.2 KJ) than the reduction of CO_2 to CH_4 (ΔG= - 134.9KJ) (Kohn and Boston 2000). A negative ΔG enables a reaction to produced, so in conditions where species are using fermentation pathways with a large negative ΔG, they will dominate the microbial community (Janssen 2010).

Digestion and fermentation in the rumen

Forage digestion, especially of plant cell walls, arises from a symbiotic association between the host ruminant and gut microflora (bacteria, archaea, protozoa and fungi) (Akin 1993). The majority of digestion takes place in the reticulo-rumen (termed rumen), which is the primary source of CH_4 and the remainder in the lower gut (mainly caecum and colon). In general, 55 to 65% of the apparent OM digestion takes place in the rumen, about 20 to 30% in the small intestine and 5 to 15% in the large intestine (Waghorn et al 2007).

The rumen functions as a large anaerobic fermentation vat buffered with bicarbonate from saliva to maintain the pH between 5.6 and 6.8 and temperature is about 39°C (Hungate 1966, Kolver and De Veth 2002). A pH between 6.0 and 6.8 provides an ideal environment for the microflora and enzymes from rumen microbes responsible for fermentation of the feed (Leng 1984, Fisher et al 1995). As well as mechanisms involved in feed retention (e.g. chewing and particle size reduction), the rumen system allows ingested feed to be retained for an extended period. This enables extensive digestion by microbial enzymes, and is assisted by cell rupture and breakdown through mastication and rumination (Leng 1984). Rumen contractions move and mix the contents ensuring contact between microorganisms and particles and facilitate eructation of gases (Leng 1984).

During the fermentation process, energy is conserved in the form of ATP and utilized for the maintenance and growth of the microbial population (France and Dijkstra 2005). Dietary carbohydrates such as cellulose, hemicellulose, pectin, starch and soluble sugar are degraded to hexoses and pentose before being fermented to VFAs via pyruvate (France and Dijkstra 2005). The products of fermentation are primarily acetate, propionate and butyrate, and NH_3 from proteolysis, with CO_2 and H_2 (Janssen 2010). Acetyl-Co A is an intermediate in the formation of both acetate and butyrate from pyruvate, while propionate formation occurs mostly via the succinate pathway from pyruvate.

In addition to production of microbial biomass, small concentrations of formate, ethanol, lactate and succinate are produced during fermentation. Proteins are hydrolyzed to ammonia and peptide; each amino acid is then delaminated to CH_3 and a fatty acid (Wallace et al 1997). Dietary lipids are hydrolyzed by bacterial lipases into glycerol and their constituent long-chain fatty acids (LCFAs). The complex fermentation system results in varying amounts of H_2 formation and consequently CH_4 (Janssen 2010).

The proportions of VFAs produced from digestion are important for ruminant production because they differ in their end uses and in the efficiency of energy capture. The ratio of glucogenic (propionate) to non-glucogenic (acetate and butyrate) VFAs will affect the energetic efficiency and composition of the products (milk and meat) from the ruminant (Bannink and Tamminga 2005). When host energy needs are met, surplus acetate and butyrate must be stored as fat (Waghorn et al 2007). Propionate is more versatile and may be converted to glucose or to glycogen for storage, as well as to fatty acids (McDonald et al 2002). Propionate production in the rumen represents a 7% increase in the efficiency of energy capture from hexoses relative to acetate (Beever 1993) and results in a net utilization of H_2, while production of acetate and butyrate yield H_2 (Figure 1).

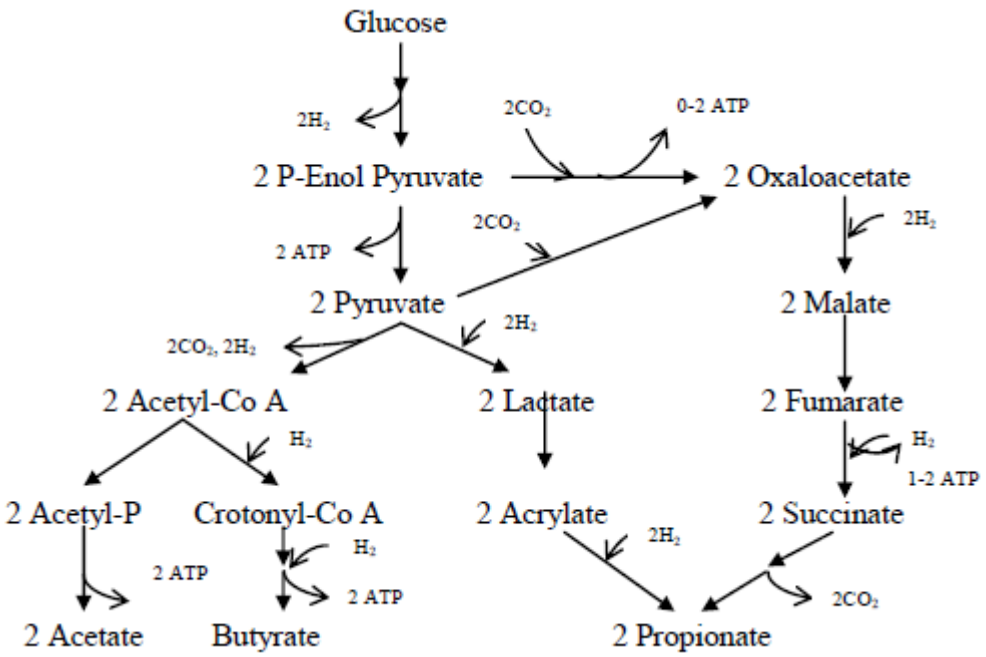

Figure 1. Fermentation pathways in the rumen. ***Source:*** *Ungerfeld and Kohn (2006)*

Hydrogen and methanogenesis

Anaerobic fermentation reactions are controlled by reduced cofactors (nicotinamide adenosine dinucleotide, NADH; nicotinamide adenosine dinucleotide phosphate, NADPH; and flavin adenosine dinucleotide, FADH), which are oxidized (NAD^+, $NADP^+$, FAD^+) by the donation of electrons to hydrogen ions (H^+) to form H_2 (Hino and Asanuma 2003; Martin et al 2010; Kittelmann and Janssen 2011). These cofactors are required for energy generation (as ATP) for microbial growth, and most of the H_2 produced in the rumen is used for methanogensis (Janssen 2010).

The total pool of H_2 in the rumen is small and the concentration of dissolved H_2 is 0.1 to 50 µM. The rate of CH_4 formation is determined by the rate at which H_2 enters the dissolved pool, because the partial pressure of H_2 drives methanogenesis (Janssen 2010). Methanogens maintain a low H_2 partial pressure in the rumen, which prevents oxidation of NADH to form products such as ethanol or lactate (Miller 1995), with the release of H_2. If H_2 accumulates, the oxidation of NADH is inhibited because it is thermodynamically unfavorable and because of feedback inhibition. Microbial growth, forage digestion and the associated production of acetate, butyrate and propionate is inhibited when NAD^+, $NADP^+$, and FAD^+ accumulate (Joblin 1999) and this will stop fermentation.

Requirement of non-protein nitrogen source to reduce methane emissions

Enteric CH_4 is produced under anaerobic conditions in the rumen, by methanogenic *Archaea* that gain energy by reducing CO_2 with H_2 to form CH_4. Leng (2008) proposed that nitrate could potentially replace urea in low protein diets to provide a source of rumen ammonia and at the same time provide a hydrogen sink to reduce enteric methane production. The use of nitrate as a source of rumen fermentable nitrogen had previously been discouraged, due to the possible toxic effects of nitrite that under some circumstances is formed as an intermediate during the reduction of nitrate to ammonia in the rumen (Leng and Preston 2010). Nitrate is

not toxic to ruminants but absorbed nitrite binds haemoglobin forming methaemoglobinaemia which lowers the oxygen carrying capacity of blood. Nitrite accumulates in rumen fluid when nitrate is suddenly introduced by an intra-ruminal injection (Lewis 1951). Trinh Phuc Hao et al (2009) showed that nitrate could be safely fed as the major source of fermentable N to goats provided the animals were adapted to the diet over a period of 2 weeks.

It has been suggested that the use of nitrate as a source of fermentable nitrogen for rumen microbial synthesis will be facilitated if there is no competing source of ammonia in the diet, such as a readily fermentable protein. The suggestion is that nitrate reduction to ammonia could be suppressed by end product feed-back inhibition from high ammonia levels in rumen fluid (Leng 2008).

Potential of cassava foliage as sources of bypass protein for ruminants

Many developing countries are faced with the challenge of rapidly increasing populations without depleting the natural resource base. With the increasing use of cereal grains for human food, cassava *(Manihot esculenta, Crantz)* has been identified as a possible replacement for grain in the diet of animals, if properly supplemented with protein, minerals and in some cases, essential vitamins. The crop has multiple advantages including good yield, tolerance of poor soils, drought resistance and can be left in the ground, thus permitting staggered harvests. Cassava production in Laos is yearly mainly for root production by small-holder farmers in upland areas. The crop's starchy roots are mainly sold for use as animal feed and industrial processing but also serve as a secondary staple food.

Cassava foliage

Cassava is currently the third most important crop in Laos, after rice and maize. It is widely grown throughout the country by upland farmers but in small areas using local varieties and with very few inputs (CIAT 2001). Roots or tubers can be harvested between 7 and 18 months, depending on variety. The potential use of cassava foliage as an animal feed has recently been studied and described by several authors. Phuc et al (2001) investigated the use of cassava foliage as a feed for pigs and poultry. Van et al (2001) and Khang (2004) described the foliage of cassava as a protein source for small ruminants and cattle. The tubers are rich in energy and the foliage or leaves have high protein content (Preston 2001), and can be used for animal feeding. A high edible biomass yield has been reported, ranging from 3.8 to 7.9 tones DM/ha (Tung et al 2001; Van et al 2001; Hong et al 2003; Wanapat 2003; Khang 2004; Vongsamphanh 2004; Thang 2005; Wanapat 2008). Protein content in cassava leaves ranges between 160 g to 250 g/kg dry matter (DM) and with almost 85% of the crude protein (CP) fraction as true protein (Ranvindran 1993).

Feeding fresh cassava foliage to cattle and goats did not show any effect of toxicity from HCN or tannin, when the cassava was managed as semi perennial forage with repeated harvests at 50-80 day interval under fertilization (Seng Mom et al 2001; Seng Sokerya and Rodriguez 2001; Theng Kouch et al 2003). In fattening cattle, Ffoulkes and Preston (1978) reported that the fresh foliage could be used as the sole source of protein and fibre for supplementing a liquid diet of molasses-urea, supporting growth rates of more than 800 g/day in fattening cattle. Similarly, Seng Mom et al (2001) reported that when fresh cassava foliage was given to local "Yellow" cattle fed rice straw and rumen supplement, the daily weight gain increased from 210 to 302 g/day while Le Huu Khoung and Doung Nguyen Khang (2005) reported that increasing levels of fresh cassava foliage increased total DM intake and rate of live weight gain.

Up to the present time, the potential of cassava foliage as a protein source in ruminant feeds has not yet been fully exploited. The main reason for this is probably the content of hydrogen cyanide (HCN), which may affect animal health. Different kinds of processing like sun drying or ensiling, will reduce the cyanide content of cassava foliage to a level which is safe for cattle (Khieu Borin et al 2005; Phengvichith and Ledin 2007). Cassava leaves are known to contain variable levels of condensed tannins (0.26% in DM, Wanapat et al, 2000; 3.2% in DM, Netpana et al 2001; 3.2% in DM, Bui Phan Thu Hang and Ledin 2005). Condensed tannins at moderate levels are known to have positive effects on the nutritive value of the feed by forming insoluble complexes with dietary protein, resulting in "escape" of the protein from the rumen fermentation (Barry and McNabb 1999). A five-fold increase in N retention of goats fed ammoniated rice straw (by ensiling with urea) to intake of fresh cassava leaves at 1% of live weight (DM basis) was reported by Ho Quang Do et al (2002).

The negative impact is caused by the HCN and the tannins in cassava foliage. When there is high tannin content in forage the tannins are probably combined with protein to form indigestible complexes, which result in low feed intake and performance (Barry and McNabb 1999; Man and Wiktorsson 2001). The HCN is an anti-nutritional factor in cassava foliage when fed to animals, as reported in many studies (Poonam and Hahn 1984; Dufour 1988; Ravindran 1993; Wanapat 2008). In principle, cyanogen is hydrolysed by enzymes to release HCN, which can take place in the rumen by microbial activity. Then the HCN is absorbed rapidly to the blood and detoxified in the liver by the enzyme rhodanese, which converts CN to thiocyanate (SCN) which is excreted in the urine (Kumar 1992). However, excess cyanide ions inhibit cytochrome oxidase and consequently stop ATP formation and tissues suffer energy deprivation and death quickly follows.

The presence and role of condensed tannins in cassava leaves was discussed by Reed et al (1982). Tannins in cassava leaves have been shown to increase with maturity (Gomez and Valdivieso 1984; Ravindran and Ravindran 1988) and also to vary between cultivars (Padamaja 1989). It was reported that tannin content in fresh cassava leaves varied from 30 to 50 g/kg DM (Ravindran 1993) and from 32.6 to 43 mg/kg in sun-dried cassava leaves (Wanapat 2003; Netpana et al 2001). Free tannin contents of cassava leaves were markedly lowered by drying (Padmaja 1989). Similar effects were also found by Khang (2004) and Borin (2005), who reported that sun drying and ensiling reduced tannin contents in cassava foliage, although ensiling after sun wilting was more effective. Tannin content was reduced from 3.51% in fresh cassava foliage to 2.42% and 2.74% after drying and ensiling, respectively. HCN and tannin contents of cassava leaves processed in different ways are summarized in Table 3.

Table 3. HCN and tannins contents of cassava leaves processed in different ways

	HCN (mg/kg DM)	Reference	CT (g/kg DM)	Reference
FCF	840	Man (2001)	40	Chataprasarn (2005)
	983	Khang (2004)	35.1	Khang (2004)
CH	120	Ho Bunyeth (2005)	31	Wanapat et al (2000)
	128	Dung (2003)	23	Dung (2003)
PCF	341	Khang (2004)	24.2	Khang (2004)
ECF	408	Khang (2004)	27.4	Khang (2004)
	292	Man (2001)	45	Man (2001)
CLM	-		31	Khang (2004)

CT= Condensed tannins; FCF= Fresh cassava foliage; CH= Cassava hay; PCF= Pelleted cassava foliage; ECF= Ensiled cassava foliage; CLM= Cassava leaf meal

Numerous studies have shown the potential of the tannin content in cassava leaves to play an anthelminthic role for the control of nematode parasites in ruminants (Seng Sokerya and Preston 2003; Neptpana et al 2001; Le Huu Khoung and Doung Nguyen Khang 2005). In an experiment with urea-treated rice straw fed to Sindhi x yellow cattle plus 0.72 kg DM/day of Napier grass and 0.26 kg DM/day of cassava root meal per 100 kg LW, provision of 100 g CP/day of fresh cassava foliage per 100 kg LW increased LW gain with an indication of reduced levels of nematode eggs in the faeces (Khuong and Khang 2005).

Mimosa foliage

Mimosa pigra has been shown to support high growth rates when fed as the sole diet to goats (Thu Hong et al 2008). The authors postulated that this could be explained by the high content of condensed tannins conferring "rumen escape' qualities on the protein. Mimosa is considered to be an invasive weed in many tropical countries (Tran Triet et al 2007); however, if a positive use could be found for the plant as an animal feed supplement it could become a useful plant rather than an environmental menace.

Potential of condensed tannins in foliage to reduce methane production

Improving diet quality can both improve animal performance and reduce CH_4 production, but also improve efficiency by reducing CH_4 emissions per unit of animal product. Recent studies have shown that plant secondary metabolites (PSM) at low concentrations could be used to manipulate rumen fermentation favorably. At appropriate dose level, saponins or saponin-containing plants have been shown to suppress protozoal population, increase bacteria and fungi population, increase propionate production, and yield and efficiency of microbial protein synthesis as well as to decrease methanogenesis hence improve performance in ruminants (Diaz et al 1994; Makkar et al 1995; Hess et al 2003). Tannins especially condensed tannins (CT) also decrease methane production and increase efficiency of microbial protein synthesis as reported by (Makkar et al 1995). Condensed tannins (CT) were generally found to have a higher value in mature cassava leaves, but levels were lower in cassava hay harvested at a young stage (Wanapat 2001).

Condensed tannins (CT) have been shown to reduce CH_4 production by 13 to 16% (DMI basis) (Carulla et al 2005; Grainger et al 2009; Waghorn et al 2002; Woodward et al 2004), mainly through a direct toxic effect on methanogens. However, high condensed tannins (CT) concentrations (>55 g CT/kg DM) may reduce voluntary feed intake and digestibility (Beauchemin et al 2008; Grainger et al 2009; Min et al 2003). Plant saponins also hold potential to reduce CH_4, with some saponin sources more effective than others, with CH_4 suppression attributed to their anti-protozoal properties (Beauchemin et al 2008).

Conclusions

- Crop residues especially rice straw are important feed resources for ruminants in SE Asia. Their feeding value can be improved by simple methods involving the use of readily available chemicals such as urea and lime.
- Ruminants are a major source in agriculture of anthropogenic methane emissions through enteric CH_4 produced under anaerobic conditions in the rumen, by methanogenic *Archaea* that gain energy by reducing CO_2 with H_2 to form CH_4. Use of nitrate salts as a source of non-protein nitrogen in ruminant diets can act as a

hydrogen sink to reduce enteric methane production and also provide a source of rumen ammonia for micro-organisms.

- Foliage of cassava and mimosa can be potentially valuable supplements for ruminant animals, as the presence of condensed tannins confers "rumen escape' qualities on the protein. Tannin-rich plants can also have potential to reduce methane production.

References

ACIAR 2004 Research that works for developing countries and Australia. Project ID: AH/2004/046: Forage legumes for supplementing village pigs in Lao PDR Retrieved 1 September 2008 from http://www.aciar.gov.au/project/AH/2004/046

Akin DE 1993 Perspectives of cell wall biodegradation-session synopsis. In forage cell wall structure and digestibility (eds. H Jung, D Buxton, R Hatfield and J Ralph), pp. 73-82, American society of agronomy, Inc; Crop science society of America, Inc; Soil science society of America, Inc; Madison, Wisconsin, USA

Akter Y, Akbar M A, Shahjalal M and Ahmed, T. U 2004 Effect of urea molasses multi-nutrient blocks supplementation of dairy cows fed rice straw and green grasses on milk yield, composition, live weight gain of cows and calves and feed intake. Pak. J. Biol. Sci. 7(9):1523-1525

Anon 2007 The conclusion of animal raising system, ministry of Agriculture and forestry, Veterinary and fishery in 2007 and plan in 2008.

Arieli, A 1997 Whole cottonseed in dairy cattle feeding: a review. Anim. Feed Sci. Technol. 72:97-110

Bannink A and Tamminga S 2005 Rumen function. In Quantitative Aspects of ruminant digestion and metabolism (eds. J Dijkstra, J Forbes and J France), pp. 263-288, CABI Publishing, Cambridge, USA

Barry T T N and McNabb W W C 1999 The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. British Journal of Nutrition 81(4), 263-272.

Beauchemin K A, Kreuzer M, O'Mara F and McAllister T A 2008 Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture 48, 21-27. doi: 10.1071/EA07199

Beever DE 1993 Rumen function. In Quantitative Aspect of rumen digestion and Metabolism (eds. J Forbes and J France), pp. 87-215, CABI International, Wallingford, UK

Berger L L, Fahey G C, Bourquin L D and Tilgeyer E C 1994 Modification of forage quality after harvest. In: Forage Quality, Evaluation, and Utilisation (Ed. G. C. Fahey). American Society of Agronomy, Inc, Madison, USA. pp. 922-966

Borin K 2005 Cassava foliage for monogastric animals - Forage yield, digestion, influence on gut development and nutritive value. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala

Buddle BM, Denis M, Attwood GT, Altermann EH, Janssen PH, Ronimus RS, Pinarespatino CS, Muetzel S and Wedlock DN 2011 Strategies to reduce methane emissions from farmed ruminants grazing on pasture. The Veterrinary Journal 188, 11-17

Bui Phan Thu Hang and Ledin Inger 2005 Utilization of Melastoma (*Melastoma affine,* D. Don) foliage as a forage for growing goats with cassava *(Manihot Esculenta,* Crantz) hay supplementation. Proceedings International Workshop on Small Ruminant Production and Development in South East Asia (Editor: Inger Ledin), Hanoi, Vietnam, 2-4 March 2005. http://www.mekarn.org/procsr/hangctu.pdf

Bui Van Chinh, Le Viet Ly, Nguyen Huu Tao, Nguyen Van Hai and Tran Bich Ngoc 2001 Effects of drying, ensiling or urea-treatment on the use of sugarcane leaves as ruminant feed: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 12-19.

Carulla JE, Kreuzer M, Machmüller A and Hess H D 2005 Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research 56, 961-970

Chantaprasarn B 2005 Study on effects of different harvest intervals on cassava foliage (cassava hay) and root yield and effects of sunflower oil supplementation in cassava hay based-diets for lactating dairy cows. MSc thesis in the programme "Tropical Livestock Systems". SLU, Dept of Animal nutrition and management, Uppsala, Sweden

Chaudhry A S 1998 Nutrient composition, digestion and rumen fermentation in sheep of wheat straw treated with calcium oxide, sodium hydroxide and alkaline hydrogen peroxide. Anim. Feed Sci. Technol. 74:315-328

Chaudhry A S and Miller E L 1996 The effect of sodium hydroxide and alkaline hydrogen peroxide on chemical composition of wheat straw and voluntary intake, growth and digesta kinetics in store lambs. Anim. Feed Sci. Technol. 60:69-86

Chenost M and Kayouli C 1997 Roughage Utilization in Warm Climates. FAO Animal and Health Paper 135. Rome. http://www.fao.org/docrep/003/w4988e/w4988e00.htm

CIAT 2001 Solution that gross frontiers. Cassava production system. http://www.Ciat.cgiar.org/asia/cassava.htm

CONAM (Consejo Nacional del Ambiente) 2001 Communication Nacinal del Peru a la Convencion de las Nacions Unida sobre Cambio Climatico. Primera Communication. Consejo Nacional del Ambiente, Lima, Peru 118p

Czerkawski JW 1969 Methane production in ruminants and its significance. World review of nutrition and dietetics 11, 240-282

Diaz A, Avendan O M and Escobar A 1994 Evaluation of Sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters. Livestock Research and Rural Development. pp. 1–10

Doyle P T, Devendra C and Pearce G R 1986 Rice straw as a feed for ruminants. International Development program of Australian Universities and colleges. Canberra. Australia. 117 P.

Dufour D 1988 Cyanide content of cassava (Manihot esculenta , Euphorbiaceae) cultivars used by Tukanoan Indians in Northwest Amazonia. Economic Botany 42(2), 255-266

Dung N T 2003 Evaluation of cassava intercropping systems and cassava hay as a feed for growing goats. MSc thesis in the programme "Tropical Livestock Systems". SLU, Dept of Animal nutrition and management, Uppsala, Sweden

Ellis JL, Dijkstra J, Kebreab E, Bannink A, Odongo NE, McBride BW and France J 2008 Aspects of rumen microbiology central to mechanistic modeling of methane production in cattle. Journal of Agricultural Science 146, 213-233

FAO 2005 FAOSTAT data. Food and Agriculture Organization, Rome http://faostat.external.fao.org/

FAO 2010 National gender profile of Agricultural households. Report based on the Lao Expenditure and Consumption Surveys, National Agricultural Census and the National Population Census, pp 1

FAO 2011 Special report http://www.wfp.org/foodsecurity/reports/CFSAM

FAO launches project to boost animal production 2009 The Vientiane Times. Retrieved 25 September 2009, from www.LaoFAB.org

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. http://www.utafoundation.org/TAP/ TAP33/331.pdf

Fisher DS, Burns JC and Moore JE 1995 The nutritive evaluation of forage. In forages volume1: An introduction to Grassland Agriculture (eds. R Barnes, D Miller and C Nelson), pp. 105-116, Iowa State University Press, Iowa, USA

France J and Dijkstra J 2005 Volatile fatty acid production. In Quantitative Aspects of ruminant digestion and metabolism (eds. J Dijkstra, J Forbes and J France), pp. 157-175, CABI Publishing, Cambridge, USA

Gomez G and Valdivieso M 1984 Cassava for animal feeding: Effect of variety and plant age on production of leave and roots. Animal Feed Science and Technology 11: 49-55

Grainger C, Clarke T, Auldist M J, Beauchemin K A, McGinn SM, Waghorn GC and Eckard RJ 2009 Mitigation of greenhouse gas emissions from dairy cows fed pasture and grain through supplementation with *Acacia mearnsii* tannins Canadian Journal of Animal Science (in press)

Hadjipanayiotou M 1984 Effect of level and type of alkali on the digestibility in vitro of ensiled, chopped barley straw. Agric. Wastes 10:187-194

Hess H D, Kreuzer M, Diaz TE, Lascano CE, Carulla JE, Solvia CR and Machmuller A 2003 Saponin rich tropical fruita affect fermentation and methanogenesis in faunated and defaunated rumen fluid, J. Biol. pp.79-94

Hino T and Asanuma N 2003 Suppression of ruminal methanogenesis by decresing the substrates available to methanogenic bacteria. Nutrition abstracts and reviews series B: Livestock feeds and feeding 73, 1R-8R

Ho Bunyeth 2005 Cassava foliage as supplement for goats fed paragrass *(Brachiaria mutica)* in full confinement, or with grazing in semi-confinement. MSc thesis in the programme "Tropical Livestock Systems" SLU, Dept of Animal nutrition and management, Uppsala, Sweden

Ho Quang Do, Vo Van Son, Bui Phan Thu Hang, Vuong Chan Tri and Preston T R 2002 Effect of supplementation of ammoniated rice straw with cassava leaves or grass on intake, digestibility and N retention by goats. Livestock Research for Rural Development. Volume 14 (3) http://www.lrrd.org/lrrd14/3/do143b.htm

Holter JB and Young AJ 1992 Nutrition, feeding and calves: methane prediction in dry and lactating Holstein calves. Journal of dairy science 75, 2165-2175

Hong NTT, Wanapat M, Wachirapakorn C, Pakdee P and Rowlinson P 2003 Effects of timing of initial cutting and subsequent cutting on yields and chemical compositions of cassava hay and its supplementation on lactating dairy cows. Asian-Australasian Journal of Animal Sciences 16(12), 1763-1769

Hungate RE 1966 The rumen and its microbes. Academic Press, New York

Ingxay P, Vongsouvanna K, and Vongtilath S 2009 Food Security through Livestock (Cattle and Buffalo) Production: Case study at Phonxay District, Luang Phabang Province, Lao PDR. Draft Report for the Agriculture and Forestry Policy Research Centre, Vientiane, LaoPDR: NAFES

IPCC 2007 Climate change 2007: The Physical Science Basis. Cambridge University Press, United Kingdom and New York, USA

Iv Sophea and Preston T R 2010 Effect of different ratios of supplementary sodium nitrate replacing urea on growth rates and methane production in goats fed sugar palm-soaked rice straw and mimosa foliage. http://www.mekarn.org/workshops/pakse/abstracts/sophea.htm

Jackson M G 1977 Review article: The alkali treatment of straw. Anim. Feed Sci. Technol. 2:105-130

Janssen PH 2010 Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Animal feed science and technology 160, 1-22

Joblin KN 1999 Ruminal acetates and their potential to lower ruminant methane emissions. Australian Journal of Agriculture Research 50, 1307-1313

Kang DN 2004 Cassava foliage as protein source for cattle in Vietnam. Agraria Doctoral thesis. Dept. of animal Nutrition and Management, SLU, Uppsala, Sweden

Keo Sath, Borin K and Preston T R 2008 Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org./lrrd20/supplement/sath2.htm

Khieu Borin, Lindberg JE and Ogle RB 2005 Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. Animal Science 80(3), 319-324

Khuong L H and Khang D N 2005 Effect of fresh cassava foliage on growth and feacal nematoda egg counts in Sindhi x yellow cattle fed urea-treated rice straw basal diet. Workshop-seminar "Making better use of local feed resources" (Editors: T. R. Preston and B. Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005

Kittelmann S and Janssen PH 2011 Characterization of rumen ciliate community composition in domestic sheep, deer and cattle, feeding on varying diets, by means of PCR-DGGE and clone liv=braries. FEMS microbiology ecosystem 75, 468-481

Kohn RA and Boston RC 2000 The role of thermodynamics in controlling rumen metabolism. In modeling nutrient utilization in farm animals (eds. JP McNamara, J France and DE Beever), pp. 11-24, CAB International

Kolver ES and de Veth MJ 2002 Prediction of the ruminal pH from pasture-based diets. Journal of Dairy Science 84, 1255-1266

Kumar R 1992 Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. In: Speedy, A. & Pugliese, P. (Eds.) Proceedings of Legume Trees and Other Fodder Trees as Protein Source for Livestock, 14-18 October. Kuala Lumpur, Malaysia. : FAO, Rome. pp. 145-160

Kumar S, Puniya AK, Puniya M, Dagar SS, Sirohi SK, Singh K and Griffith GW 2009 Factors affecting rumen methanogens and methane mitigation strategies. World Journal of Microbiology and Biotechnology 25, 1557-1566

Lam TBT, Kadoya K and Iiyama K 2001 Bonding of hydroxycinnamic acids to lignin: ferulic and p-coumaric acids are predominantly linked at the benzyl position of lignin, not the b-position, in grass cell walls. Phytochem. 57:987-992

Lassey KR, Ulyatt MJ, Martin RJ, Walker CF and Shelton ID 1997 Methane emissions measured directly from grazing livestock in New Zealand. Atmospheric Environment 31, 2905-2914

Le Huu Khoung and Doung Nguyen Khang 2005 Effect of fresh cassava foliage on growth and faecal nematode egg counts in Sindhi x Yellow cattle fed urea treated rice straw. In: Regional Seminar Workshop on Livestock Based Sustainable Farming Systems in the Lower Mekong Basin. Cantho University Vietnam 23-25, May 2005 (Eds, Reg Preston and Brian Ogle). pp 125-131

Le Thi Thuy Hang, Ngo Van Man and Wiktorsson H 2005 Effects of urea and lime treatment of fresh rice straw on storage stability, and hygienic and nutritional quality. http://www.mekarn.or/msc2003-05/theses05/thuyh_p1.pdf

Leng R A 1997 Tree foliage in ruminant nutrition. FAO, Animals Production and Health Paper. 139 http://www.fao.org/docrep/003/w7448e/w7448e00.htm

Leng R A 2008 The potential of feeding nitrate to reduce enteric methane production in ruminants. Report to Department of Climate Change, Commonwealth Government, Canberra. Available at http://www.penambulbooks.com/Downloads/Leng-Final%20Modified%20% 2017-9- 2008. pdf [Verified 17 July 2010]

Leng R A and Preston T R 2010 Further considerations of the potential of nitrate as a high affinity electron acceptor to lower enteric methane production in ruminants. Livestock Research for Rural Development. Volume 22, Article #221. http://www.lrrd.org/lrrd22/ 12/leng22221.htm

Leng RA 1984 Microbial interactions in the rumen. In ruminant physiology concepts and consequences, proceeding of a symposium held at the University of Western Australia, pp. 161-173

Lewis D 1951 The metabolism of nitrate and nitrite in the sheep; the reduction of nitrate in the rumen of the sheep. Biochem. J.48:175–180

MAF 2007 The State of Food and Agriculture in Lao PDR. Report to the 34th Session of the FAO Conference, Rome, Italy 17-24 November. Retrieved 10 July 2009 from http://www. maf. gov.la/index.php?option=com content&task= view&id=130&Itemid=9

Makkar HPS, Blu¨mmel M and Becker K 1995 In vitro effects of and interactions between tannins and saponins and fate of saponins tannins in the rumen, Journal of the Science of Food and Agriculture. pp. 481–493

Man N V 2001 Better use of local forages for dairy cattle in Vietnam: Improving grasses, rice straw and protein rich forages. Doctoral thesis Swedish University of Agricultural Sciences, Uppsala

Man NV and 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

Martin C, Morgavi DP and Doreau M 2010 Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351-365

McAllister T A, Bae H D, Jones G A and Cheng K J 1994. Microbial attachment and feed digestion in the rumen. Journal of Animal Science 72, 3004–3018

McAllister T, Okine E, Mathison G and Cheng K-J 1996 Dietary, environmental and microbiological aspect of methane production in ruminants. Canadian Journal of Animal Science 76, 231-243

McDonald P, Edwards RA, Greenhalgh JD and Morgan CA 2002 Animal nutrition. Pearson education limited, Harlow, England

Millar, J and Photakoun, V 2008 Livestock development and poverty alleviation: revolution or evolution for upland livelihoods in Lao PDR? International Journal of Agricultural Sustainability 6(1), 89-102

Miller TL 1995 Ecology of methane production and hydrogen sinks in the rumen. In ruminant physiology: Digestion, Metabolism, Growth and Reproduction (ed. W Engelhardt, S Leoonhard-Marek, G Breves and D Giesecke), pp. 317-331, Ferdinand Enke Verlag, Stuttgart

Min BR, Barry TN, Attwood GT and McNabb WC 2003 The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 106, 3–19. doi: 10.1016/S0377-8401(03)00041-5

Ministry of agriculture and forestry 2003 What should we do without veterinarians. Department of Animal Husbandry and Fishery. Faculty of Agriculture and Forestry, National University of Laos

Moate PJ, Clark T, Davis LH and Laby RH 1997 Rumen gases and bloat in grazing dairy cows. Journal of Agriculture Science 129, 459-469

More livestock needed for export, food security 2009 The Vientiane Times. Retrieved 4 June 2009, from www.LaoFAB.org

Moss A, Jouany J and Newbold J 2000 Methane production by ruminants: its contribution to global warming. Animal Research 49, 231-253

NAFES 2006 Extension for Everyone: why Laos needs an inclusive approach to agricultural extension. Vientiane, Lao PDR: National Agriculture and Forestry Extension Service, Retrieved 25 October 2007, from http://www.laoex.org/manuals.htm

NAFRI, NAFES and NUOL 2005 Smallholder Livestock Systems and Upland Development. Improving Livelihoods in the Uplands of the Lao PDR

Naparsirth, V, Sommart, K and Polsen, S 2005 Effects of energy feed source in concentrate on voluntary Feed intake, Ruminal Fermentation, Digestibility and Rate of Passage in Beef cattle, Khon Kaen University Research Journals, pp. 23-35.

Netpana N, Wanapat M, Poungchompu O and Toburan W 2001 Effect of condensed tannins in cassava hay on fecal parasitic egg counts in swamp buffaloes and cattle. In: Proceedings International Workshop on Current Research and Development on Use of Cassava as Animal Feed. T R Preston, B Ogle and M Wanapat (Ed) http://www.mekarn.org/procKK/netp.htm

Nguyen Ngoc Anh, Khuc Thi Hue, Duong Nguyen Khang and Preston T R 2010 Effect of calcium nitrate as NPN source on growth performance and methane emissions of goats fed sugar cane supplemented with cassava foliage. http://www.mekarn.org/workshops/pakse/abstracts/anh grrc.htm

Nguyen Xuan Trach, Mo M and Cu Zuan Dan 2001 Treatment and supplementation of rice straw for ruminant feeding: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 178-204.

Northern farmers fatten up on livestock breeding 2009 The Vientiane Times. Retrieved 25 July 2009, from www.LaoFAB.org

Padamaja G 1989 Evaluation techniques to reduce assayable tannin and cyanic in cassava leaves Journal of Agriculture and Food chemistry. 37: 712-716

Phengvichith V and Ledin I 2007 Effects of supplementing gamba grass(Andropogon gayanus) with cassava (Manihot esculenta Crantz) hay and cassava root chips on feed intake, digestibility and growth in goats. Asian-Australasian Journal of Animal Sciences 20(5), 725-732.

Phonepaseuth Phengsavanh and Ledin Inger 2003 Effect of Stylo 184 (Stylosanthes guianensis CIAT 184) and Gamba grass (Andropogon gayanus cv. Kent) in diets for growing goats; Livestock Research for Rural Development 15 (10). http://www.lrrd.org/lrrd15/10/seut1510.htm

Phuc BH, Ogle B, Lindberg JE 2001 Nutritive value of cassava leaves for monogastric animals. In: Preston, T., R, Ogle, B., Wanapat, M. (Eds.), International workshop on Current research and development on use of cassava as animal feed. July 23-24, 2001. SIDA SARAC project. Khon Kaen University, Thailand. Pp. 23-24

Pinares-Patino CS, Ulyatt MJ, Waghorn GC, Lassey KR, Barry TN, Holmes CW and Johnson DE 2003d Methane emissions by alpaca and sheep fed on lucerne hay or grazed on pastures of perennial ryegrass/white clover or bridsfoot trefoil. Journal of Agriculture Science 140, 215-226

Poonam VR and Hahn SK 1984 An automated enzymic assay for determining the cyanide content of cassava (Manihot esculenta Crantz) and cassava products. Journal of the Science of Food and Agriculture 35(4), 426-436

Pradhan R, Tobioka H and Tasaki I 1997 Effect of moisture content and different levels of additives on chemical composition and in vitro dry matter digestibility of rice straw. Anim. Feed Sci. Technol. 68:273-284.

Prasad RDD, Reddy MR and Reddy GVN 1998 Effect of feeding baled and stacked urea treated rice straw on the performance of crossbred cows. Anim. Feed Sci. Technol. 73:347-352

Preston T R 2001 Potential of cassava in integrated farming systems. . International Workshop Current Research and Development on Use of Cassava as Animal Feed, Khon Karn, Thailand July 23-24, 2001. http://www.mekarn.org/procKK/pres.htm

Preston T R and Leng R A 1987 Matching Livestock Systems to Available Resources in the Tropics and Subtropics. Penambul Books Australia. Web version http://www.utafoundation.org/ P&L/preston&leng.htm

Preston T R and Leng R A 2009 Matching ruminant production systems with available resources in the tropics and subtropics. Penambul Book Ltd Armidale, NSW, Australia. http:// www. utafoundation.org/P&L/preston&Leng.htm

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

Ravindran, V 1993 Cassava leaves as animal feed: potential and limitations. Journal of the Science of Food and Agriculture 61(2), 141-150

Reddy DV 1996 Evaluation of rice straw-poultry droppings based rations supplemented with graded levels of rice bran in fistulated buffaloes. Anim. Feed Sci. Technol. 58:227-237

Reed J D, McDowell E R, Van Soest J P and Horvath J P 1982 Condensed Tannin: A factor limiting the use ofcassava forage. Journal of Science food and Agriculture. pp 213-220

Rossi F, Vecchia P and Masoero F 2001 Estimate of methane production from rumen fermentation. Nutrient Cycling in Agroecosystems 60, 89-92

Russell JB and Wallace RJ 1997 Energy yield and energy consuming reactions. In the rumen microbial ecosystem (eds. P Hobson and C Stewart), pp. 246-282, Blackie Academic & Professional, London

Saadulah M, Haque M and Dolberg F 1981 Treatment of rice straw with lime. Trop. Anim. Prod. 6:116-120

Schiere J B and Ibrahim MNM 1989 Feeding of urea-ammonia treated rice straw: A compilation of miscellaneous reports produced by the Straw Utilization Project (Sri Lanka). Pudoc, Wageningen.

Sejian V, Lal R, Lakritz J and Ezeji T 2011 Measurement and prediction of entric methane emissions. International Journal of Biometeorology 55, 1-16

Selim ASM, Pan J, Takano T, Suzuki T, Koike S, Kobayashi Y and Tanaka K 2004 Effect of ammonia treatment on physical strength of rice straw, distribution of straw particles and particle-associated bacteria in sheep rumen. Anim. Feed Sci. Technol. 115:117-128

Seng Mom, Preston T R, Leng R A and Meulen Uter 2001 Response of young cattle fed rice straw to supplementation with cassava foliage and a single drench of cooking oil. Livestock Research for Rural Development (13) 4: http://www.cipav.org.co/lrrd/lrrd13/4/seng134.htm

Seng Sokerya and Preston T R 2003 Effect of grass or cassava foliage on growth and nematode parasite infestation in goat fed low or high protein diets in confinement. MSc. Thesis, MEKARN-SLU

Seng Sokerya and Rodriguez L 2001 Foliage from cassava, Flemingia macropfylla and bananas compared with grasses as forage source for goats: effects on growth rate and intestinal nematodes. Livestock Research for Rural Development 13(2) http://www.cipav.org.co/lrrd/ lrrd13/2/soke132.htm

Shen HS, Ni DB and Sundstel F 1998 Studies on untreated and urea-treated rice straw from three cultivation seasons: 1. Physical and chemical measurements in straw and straw fractions. Anim. Feed Sci. Technol. 73:243-261

Shen HS, Sundstel F, Eng ER and Eik LO 1999 Studies on untreated and urea-treated rice straw from three cultivation seasons: 3. Histological investigations by light and scanning electron microscopy. Anim. Feed Sci. Technol. 80:151-159

Sirohi SK and Rai SN 1995 Associative effect of lime plus urea treatment of paddy straw on chemical composition and in vitro digestibility. Indian J. Anim. Sci. 65:1346-1351

Smith P, Martino D, Cai Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C, Scholes B and Sirotenko O 2007 Agriculture. In: Climate Change 2007:

Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds B. Metz, O.R. Davidson, P.R. Bosch, R. Dave & L.A. Meyer. Cambridge University Press, Cambridge: United Kingdom and New York, NY, USA

Stewart CS 1991 The rumen bacteria. In rumen microbial metabolism and ruminant digestion (ed. J Jouany), pp. 15-26, Institute National De La Recherche Agronomique, Paris

Stur W, Gray D, and Bastin G 2002 Review of the Livestock Sector in the Lao People's Democratic Republic. Prepared for the Asian Development Bank. International Livestock Research Institute, Manila, Philippines. Retrieved 29 October 2007, from http://www.ciat.cgiar.org/asia/pdf/adb_livestock_review.pdf

Sundstol F 1984 Ammonia treatment of straw: method for treatment and feeding experience in Norway. Animal feed science and technology. 60:65 -74 S Sp

Sundstol F and Coxworth EM 1984 Ammonia treatment. In: Straw and Other Fibrous By-products as Feed (Ed. F. Sundstol and E. Owen). Developments in Animal Veterinary Sciences, 14. Elsevier, Amsterdam, pp. 196-247

Susuki, T, Phawphaisal, I, Odai, M, Kawamoto, H, Ponraagdee, W, Pholsen, P, Narmsileee, R, Indramanee, S and Oshio, S 2004 Utilization of mixed silage prepared with sugarcane brewer's gain and rice straw. New dimesions and challenges for sustainable livestock farming volume III. Proceeding of the 11th Animal Science Congress The Asian-Australasian of Animal Production Societies. pp. 346-348.

Tham H T, Man N V and Preston T R 2008 Estimates of protein fractions of various heat-treated feeds in ruminant production. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org/lrrd20/supplement/tham2.htm

Thang CM 2005 Effects of intergrated production of cassava and Phaseolus calcaratus legume and use as a protein source for growing cattle (MSc. thesis). Diss.: Swedish University of Agricultural Science, Uppsala, Sweden

Theng Kouch 2003 Studies on utilization of tree and shrubs as sole feedstuff by growing goats; foliages preferences and nutrient utilization. MSc Thesis, MEKARN-SLU

Thu Hong N T, Quac V A, Kim Chung T T, Hiet B V, Mong N T and Huu P T 2008 Mimosa pigra for growing goats in the Mekong Delta of Vietnam. Livestock Research for Rural Development. Volume 20, Article #208. http://www.lrrd.org/lrrd20/12/hong20208.htm

Tran Quoc Viet, Dao Duc Kien, Le Viet Ly and Orskov, E. R 2001 Dried Rice straw-chicken litter and urea-treated rice straw as main fodder resources for local cattle in dry season: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 12-19.

Tran Triet, Le Cong Kiet, Nguyen Thi Lan Thi and Pham Quoc Dan 2007 The invasion by *Mimosa pigra* of wetlands of the Mekong Delta, Vietnam. [on-line]. Available from: http://www.ento.csiro.au/weeds/pdf/mimosa_symposium/07Trietetal.pdf

Trinh Phuc Hao, Ho Quang Do, Preston T R and Leng R A 2009 Nitrate as a fermentable nitrogen supplement for goats fed forage based diets low in true protein. *Livestock Research for Rural Development. Volume 21, Article #10* http://www.lrrd.org/lrrd21/1/trin21010.htm

Tung CM, Liang JB, Tan SL, Ong HK and Jelan ZA 2001 Foliage productivity and growth persistency of three local cassava varieties. Asian-Australasian Journal of Animal Sciences 14, 1253-1259

Ungerfeld EM and Kohn RA 2006 The role of thermodynamics in the control of ruminal fermentation. In ruminant physiology digestion, Metabolism and Impact of nutrition on Gene expression, Immunology and Stress (eds. K Sejrsen, T Hvelplund and MO Nielsen), pp. 55-86, Wageningen academic publisher, the Netherlands

Vadiveloo J 2000 Nutritional properties of the leaf and stem of rice straw. Anim. Feed Sci. Technol. 83:57-65

Vadiveloo J 2003 The effect of agronomic improvement and urea treatment on the nutritional value of Malaysian rice straw varieties. Anim. Feed Sci. Technol. 108:33-146

Van DTT, Thanh MNT, Binh DV 2001 Cassava as small ruminant feed in the hilly and mountainous area of Bavi district. In: Preston, T., R, Ogle, B., Wanapat, M. (Eds.), International workshop on Current research and development on use of cassava as animal feed. July 23-24, 2001. SIDA SARAC project. Khon Kaen University, Thailand. Pp. 23-24

Van Soest PJ 2006 Review: rice straw, the role of silica and treatments to improve quality. Animal Feed Science. Technology 130:137-171

Van Zijderveld S M, Dijkstra J, Gerrits W J J, Newbold J R and Perdok H B 2010b Dietary nitrate persistently reduces enteric methane production in lactating dairy cows. In Greenhouse gases and animal agriculture conference. October 3-8, 2010 Banff, Canada, T119, page 127 http://www.ggaa2010.org/pdfs/Proceedings_Abstracts.pdf

Van Zijderveld S M, Gerrits WJ J, Apajalahti J A, Newbold J R, Dijkstra J, Leng R A and Perdok H B 2010a Nitrate and sulfate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science. Volume 93, 5856-5866 http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS0022030210006387.pdf

Vongsumphanh P and Wanapat M 2004 Comparisons of cassava hay yield and chemicals composition of local and introduced varieties and effect of levels of cassava hay supplementation in native beef cattle feed on rice straw. Livestock Research Center, National Agriculture and forestry Research institute, Ministry of Agriculture and forestry, Vientiane, Lao PDR. Department of Animal Science, Faculty of Agriculture, Khon Kean University

Vu DD, Cuong LX, Dung CA and Hai PM 1999 Use of urea-molasses-multinutrient block and urea-treated rice straw for improving dairy cattle productivity in Vietnam. Prev. Vet. Med. 38:187-193

Waghorn GC, Burke JL and Kolver ES 2007 Principles of feeding value. In pasture and supplements for grazing animal (eds. P Rattray, I Brookes and A Nicol), pp. 35-60, New Zealand Society of animal production, Hamilton, New Zealand

Waghorn GC, Tavendale MH and Woodfield DR 2002 Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grassland Association 64, 167–171

Wallace RJ, Onodera R and Cotta MA 1997 Metabolism of nitrogen-containing compounds. In the rumen microbial ecosystem (eds. P Hoson and C Stewart), pp. 283-328, Blackie academic and professional, London

Wanapat M 2001 Role of cassava hay as animal feed in the tropics. In: International Workshop Current Research and Development on Use of Cassava as Animal Feed, Khon Karn, Thailand July 23-24, 2001. http://www.mekarn.org/procKK/wana3.htm

Wanapat M 2003 Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. Asian-Australasian Journal of Animal Sciences 16(3), 463-472

Wanapat M 2008 Potential uses of local feed resources for ruminants. Tropical Animal Health and Production 41(7), 1035-1049.

Wanapat M, Pimpa O, Sripuek W, Puramongkol T, Petlum A, Boontao U, Wachirapakorn C and Sommart K 2000 Cassava hay: an important on-farm feed for ruminants. In: Proceedings of international workshop on "Tannins in livestock and human nutrition" (Ed. J. D. Brooker), Adelaide, Australia, May 31-June 2, 1999. ACIAR proceeding No. 92, 171pp

Widdel F 1986 Growth of methanogenic bacteria in pure culture with 2-propanol and other alcohols as hydrogen donors. Applied and Environmental Microbiology 51, 1056-1062

Wilson RT 2007 Status and prospects for livestock production in Lao People's Democratic Republic [Electronic Version]. Tropical Animal and Health Production 39(6), 443-452

Wolin MJ, Miller TL and Stewart CS 1997 Microbe-microbe interactions. In the rumen microbial ecosystem (eds. P Hobson and C Stewart), pp. 469-491, Blackie academic and professional, London

Woodward SL, Waghorn GC and Laboyrie P 2004 Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduced methane emissions from dairy cows. Proceedings of the New Zealand Society of Animal Production 64, 160–164

Zellner G and Winter J 1987 Secondary alcohols as hydrogen donors for CO_2-reduction by methanogens. FEMS microbiology letters 44, 323-328

Mitigating methane production from ruminants; effect of calcium nitrate as modifier of the fermentation in an *in vitro* incubation using cassava root as the energy source and leaves of cassava or Mimosa *pigra* as source of protein

Sangkhom Inthapanya, T R Preston* and R A Leng**

Souphanouvong University, Lao PDR
inthapanyasangkhom@yahoo.com
** Finca Ecologica, TOSOLY, UTA (Colombia)*
AA#48, Socorro, Santander, Colombia
*** University of New England, Armidale NSW, Australia*

Abstract

An *in vitro* incubation system was used to evaluate the following treatments in a completely randomized 2*2 factorial arrangement with 4 replications; Cassava leaf meal plus urea (CLM-U), Cassava leaf meal plus calcium nitrate (CLM-CaN), *Mimosa pigra* leaf meal plus urea (MLM-U) and Mimosa leaf meal plus calcium nitrate (MLM-CaN). The basal substrate was cassava root meal.

Gas production did not differ between calcium nitrate and urea but was higher for mimosa than for cassava leaf meal after 48 hours of fermentation. The percentage of methane in the gas was lower for calcium nitrate than for urea at all incubation times but the degree of difference decreased with the length of the incubation. After 9 h of fermentation, nitrate reduced methane production by 53 and 48%, compared with urea on the mimosa and cassava leaf meal supplements. There were no consistent differences between the cassava and mimosa leaf meals in the methane content of the gas. Methane production increased, and the effect of the nitrate decreased, with fermentation time the trend being similar for both sources of leaf meal. The proportion of the substrate DM that was fermented in 48 h did not differ between sources of NPN nor between the two leaf meals. Overall, the production of methane per unit of substrate fermented was decreased by 32% when calcium nitrate replaced by urea as the NPN source. In vitro systems of feed evaluation should carefully select incubation times to represent more closely the period dominated by primary fermentation.

Key words: *Climate change, gas production, Greenhouse gases, tannins, urea*

Introduction

According to Smith et al (2007) agriculture produces 10-12% of total global anthropogenic greenhouse gas emissions, contributing 50% of all anthropogenic methane (CH_4). Ruminant livestock animals are a major source of total anthropogenic emissions producing an estimated 80 million tonnes of CH_4 annually accounting for 33% of anthropogenic emissions of CH_4 (Beauchemin et al 2008). There is therefore an urgent need to develop ways of reducing methane production from ruminants which are major contributors to global warming (CONAM 2001).

Enteric CH_4 is produced under anaerobic conditions in the rumen by methanogenic *Archaea* that gain energy by reducing CO_2 with H_2 to form CH_4. Leng (2008) proposed that nitrate could potentially replace urea in low protein diets to provide a source of rumen ammonia and at the same time provide a hydrogen sink to reduce enteric methane production. The use of nitrate as a source of rumen fermentable nitrogen had previously been discouraged, due to the possible toxic effects of nitrite that under some circumstances is formed as an intermediate during the reduction of nitrate to ammonia in the rumen (Leng and Preston 2010). Nitrate is not toxic to ruminants but absorbed nitrite binds haemoglobin forming methaemoglobin which lowers the oxygen carrying capacity of blood. Nitrite accumulates in rumen fluid when nitrate is suddenly introduced by an intra-ruminal injection (Lewis 1951). Trinh Phuc Hao et al (2009) showed that nitrate could be safely fed as the major source of fermentable N to goats provided the animals were adapted to the diet over a period of 2 weeks.

It has been suggested that the use of nitrate as a source of fermentable nitrogen for rumen microbial synthesis will be facilitated if there is no competing source of ammonia in the diet, such as a readily fermentable protein. The suggestion is that nitrate reduction to ammonia could be suppressed by end product feed-back inhibition from high ammonia levels in rumen fluid (Leng 2008). This requires that any supplementary protein should be mainly in the form of 'bypass' protein that will escape the rumen fermentation. When low protein feeds are the major component of the diet such bypass protein will have a stimulatory effect on feed intake and production (Preston and Leng 1987). The protein in cassava (*Manihot esculenta*, Crant) leaves is considered to be a good source of bypass protein (Ffoulkes and Preston 1978; Wanapat et al 1997; Keo Sath et al 2008). It is widely cultivated in all tropical counties and is thus logical forage to provide the additional protein required in diets high in non-protein nitrogen.

Recently, the foliage of *Mimosa pigra* has been shown to support high growth rates when fed as the sole diet to goats (Thu Hong et al 2008). The authors postulated that this could be explained by the high content of condensed tannins conferring "rumen escape' qualities on the protein. Mimosa is considered to be an invasive weed in many tropical countries (Tran Triet et al 2007). However, if a positive use could be found for the plant as an animal feed supplement it could become a useful plant rather than an environmental menace.

In vitro fermentation procedures to evaluate the nutritive value of feeds were first promoted by Tilly and Terry (1963) and were subsequently developed by Ørskov et al (1980) and Menke and Steingass (1988) among others. An *in vitro* method was used recently by Khan and Chaudhry (2009) to evaluate the effect of spices as potential modifiers of methane production in diets for ruminants.

The purpose of the present study was to develop and use a simple *in vitro* method to screen the potential methane production from a diet based on cassava root as the energy source supplemented with protein from mimosa or cassava leaves, using calcium nitrate and urea as sources of non-protein nitrogen.

Hypothesis

Giving calcium nitrate rather than urea will reduce the methane production in a diet based on cassava root meal with mimosa or cassava leaf meals as the source of protein.

Materials and Methods

Location

The experiment was conducted in the laboratory of the Faculty of Agriculture, An Giang University, Vietnam, from August to October 2010.

Treatments and experiment design

An *in vitro* incubation system was used to evaluate the following treatments in a completely randomized 2*2 factorial arrangement with 4 replications

- Cassava leaf meal plus urea (CLM-U)
- Cassava leaf meal plus calcium nitrate (CLM-CaN)
- Mimosa leaf meal plus urea (MLM-U)
- Mimosa leaf meal plus calcium nitrate (MLM-CaN)

The basal substrate was cassava root meal (CRM).

Table 1. Ingredients in the substrate, g

	Urea		**CaN**	
	CLM	**MLM**	**CLM**	**MLM**
Cassava root meal	8.76	8.76	8.34	8.34
Cassava leaf meal	3.00		3.00	
Mimosa leaf meal		3.00		3.00
Urea	0.24	0.24		
$Ca(NO_3)_2.4H_20$			0.66	0.66
	12.0	**12.0**	**12.0**	**12.0**

The in vitro system

Recycled water bottles (capacity 1500 ml) were used for the fermentation and collection of the gas (Photo 1). A hole was made in the lid of each of the bottles, which were inter-connected with a plastic tube (id 4 mm). The bottle receiving the gas had the bottom removed and was suspended in a larger bottle (5 litre capacity) partially filled with water, so as to collect the gas by water displacement. The bottle that was suspended in water was calibrated at 50 ml intervals to indicate the volume of gas.

Photo 1. The *in vitro* system

Photo 2. Using "plasticine" to seal the junction between the gas inlet tube and the bottle used to measure gas volume by water displacement

Preparation of diets and rumen fluid

The components of the substrate (cassava root and mimosa leaves or cassava leaves) were chopped into small pieces and dried in an oven at 105 °C for 24 h prior to being milled in a coffee grinder. They were then mixed with the source of NPN (calcium nitrate or urea). A representative sample of the mixtures (12 g DM) (Table 1) was put in the fermentation bottle to which was added 0.96 litres of buffer solution (Table 2) and 240 ml of rumen fluid (obtained from a newly slaughtered buffalo in the town abattoir), prior to displacing the air with carbon dioxide. Each junction of the connecting tube with the bottles was covered by "plasticine" (modelling clay) to ensure a gas-tight seal (Photo 2). The bottles with substrate were then incubated at 38°C in a water bath for 48h.

Table 2. Ingredients of the buffer solution

Ingredients	**$CaCl_2$**	**$NaHPO_4.12H_2O$**	**NaCl**	**KCl**	**$MgSO_4.7H_2O$**	**$NaHCO_3$**	**Cysteine**
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

***Source**: Tilly and Terry 1963*

Data collection and measurements

Gas production was estimated by water displacement and the percentage of methane was measured (by infra-red sensor, Crowcom Instruments Ltd, UK; Photo 3), after 9, 18 and 48h of fermentation. At the end of the incubation the total gas volume and total methane production were calculated. Residual insoluble DM from the substrate was determined by filtration through cloth (Photo 3) and drying the residues at 100°C for 24 h.

Photo 3. Measurement of methane with the Crowcom meter

Photo 4. The substrate residue filtered through cloth

Statistical analysis

The data from each treatment were analyzed by the General Linear Model (GLM) option in the ANOVA program of the Minitab Software (version13.2) (Minitab 2000).

Sources of variation in the model were: substrate, NPN source, interaction substrate*NPN source and error.

Results and Discussion

Gas production did not differ between fermentation supported by either calcium nitrate or urea but was higher for the substrate mixes containing mimosa foliage than for cassava leaf meal after 48 hours of fermentation (Table 3). The percentage of methane in the gas was

lower for calcium nitrate than for urea over all incubation times but the difference decreased with increasing time of the incubation (Figure 1). There were no consistent differences in methane production between the flasks containing cassava and mimosa leaf meals. The proportion of the insoluble DM that was degraded in 48 h did not differ between sources of NPN nor between the two leaf meals. Overall, the production of methane per unit of substrate fermented was decreased by 32% when calcium nitrate replaced urea as the NPN source.

Table 3. Mean values for gas production, percentage of methane in the gas, substrate fermented and methane production per substrate fermented according to source of protein and NPN

	Protein source			NPN source			
	CLM	**MLM**	**P**	**CaN**	**Urea**	**P**	**SEM**
Gas production, ml							
0-9h	769	863	0.20	694	938	0.04	48.6
10-18h	725	763	0.53	688	738	0.39	34.3
19-48h	413	375	0.030	575	463	0.17	35.9
Total	1906	2094	0.020	1894	2106	0.35	55.8
Methane, %							
0-9h	17.0	16.3	0.20	11.1	22.1	0.001	0.39
10-18h	25.4	23.4	0.001	21.8	27.0	0.001	0.30
19-48h	43.3	36.4	0.003	38.1	41.5	0.091	1.30
DM fermented after 48h, %	63.8	64.8	0.73	62.6	66.0	0.26	2.03
Methane, ml/g DM fermented	65.8	66.5	0.87	57.2	75.1	0.002	3.11

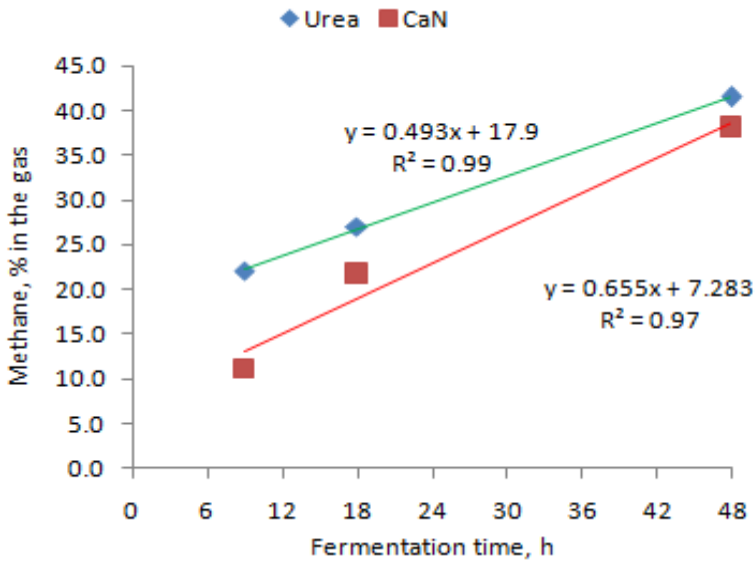

Figure 1. Effect of fermentation time on methane content of the gas

The increase in the methane content of the gas with increasing time of incubation may reflect the likely order of use of fermentation substrates. Initially any soluble sugars, starches and proteins will be degraded by the microbial consortium with a much slower hydrolysis of structural carbohydrates such as cellulose and hemicelluloses and insoluble protein, all of which are also fermented to VFA. As the more digestible components of the substrate are solubilized and converted into VFA there will be a change in the microbial population to those organisms that utilize VFA as an energy source (similar to the reactions that occur in a biodigestor) with the production of methane and carbon dioxide gas. In the rumen the short

turnover time of rumen contents ensures that most of the digestible energy is contained in the VFA and microbial biomass, and methane production is limited. However, as the fermentation moves into a secondary fermentation considerable amount of the VFA energy are released as methane and microbes lyse to provide substrate for other organisms to grow. In both primary and secondary fermentation, nitrate can act as a high affinity electron acceptor but the residual nitrate after 9 hours may primarily limit the effectiveness in reducing methane. The longer incubation times may also be sufficient to allow denitrification to nitrogen of any non-protein nitrogen, also diluting the methane percentage, whilst increasing gas production. It is also likely that sulphur compounds will act as high affinity electron acceptors when nitrate is fully utilized with time of incubation. The sulphur content of the substrate was unlikely to be higher than 0.4% of the dry matter so that the hydrogen sulphide produced would be small. However, the smell of hydrogen sulphide is likely to indicate the time for termination of any experiment since it indicates the exhaustion of nitrate which is a potent inhibitor of sulphur reducing bacteria (Bracht and Kung 1997). The result after 9 h of incubation, for which the reduction in methane was 50%, is probably more indicative of the *in vivo* situation. The per cent reduction in methane production by replacing urea by a nitrate salt is in agreement with recently published studies with sheep (Nolan et al 2009; Van Zijderveld et al 2010a, b), goats (Iv Sophea et al 2010; Nguyen Ngoc Anh et al 2010; Ngoc Huyen Le Thi et 2010) and cattle (Do Thi Thanh Van et al 2010) and *in vitro* (Guo et al 2009).

The in vitro system used in this study was simple to install and operate, and is especially relevant for use in developing countries where conventional laboratory glassware is not always available. However, future experiments with this system should be restricted to incubation times not exceeding 12 hours to make the results more applicable to the *in vivo* situation in ruminant livestock.

Conclusions

- After 9 h of fermentation, nitrate reduced methane production by 53 and 48%, compared with urea on the mimosa and cassava leaf meal supplements, respectively.
- Methane production increased, and the effect of the nitrate decreased, with fermentation time the trend being similar for both sources of leaf meal.
- In vitro systems of feed evaluation should carefully select incubation times to represent more closely the period dominated by primary fermentation.

Acknowledgements

This research was done by the senior author with support from NUFU as part of the requirements for the MSc degree in Animal Production "Specialized in Response to Climate Change and Depletion of Non-renewable Resources" of Cantho University, Vietnam. The authors acknowledge support for this research from the MEKARN project financed by Sida. Special thanks to Mrs Le Thi Thuy Hang and Mr Ho Xuan Nghip who provided valuable help in the laboratory

References

Beauchemin K A, Kreuzer M, O'Mara F and McAllister T A 2008 Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture 48, 21-27.

Bracht J P and Kung L Jr 1997 Inhibition of sulphide production *in vitro* ruminal fermentations. Abstracts from the 24th Biennial Conference on Rumen Function. Chicago, IL. 24:20

CONAM (Consejo Nacional del Ambiente) 2001 Communication Nacinal del Peru a la Convencion de las Nacions Unida sobre Cambio Climatico. Primera Communication. Consejo Nacional del Ambiente, Lima, Peru 118p.

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. Tropical Animal Production Volume 3, Number 3, pp 186-192 http://www.utafoundation.org/TAP/TAP33/3_3_1.pdf

Guo WS, Schaefer, D M Guo, XX, Ren LP, Meng QX 2009 Use of nitrate-nitrogen as a sole dietary nitrogen source to inhibit ruminant methanogenesis and improve microbial nitrogen synthesis *In vitro, A*sian-Australian Journal of Animal Science **22**, 542-549

Iv Sophea, Khieu Borin, Leng R A and Preston T R 2010 Effect of different levels of supplementary potassium nitrate replacing urea on growth rates and methane production in goats fed sugar palm-soaked rice straw and mimosa foliage MSc Thesis, MEKARN-SLU http://www.mekarn.org/MSC2008-10/theses/sophea.htm

Khan M M H and Chaudhry A S 2009 Effect of spice supplementation on in vitro methane production using ground wheat as a substrate. Advances in Animal Biosciences: Proceedings of the British Society of Animal science.

Leng R A 2008 The potential of feeding nitrate to reduce enteric methane production in ruminants. A Report to the Departmernt of Climate Change Commonwealth Government of Australia. ACT Canberra Australia For paper and PPT presentation see http://www.penambulbooks.com/

Leng R A and Preston T R 2010 Further considerations of the potential of nitrate as a high affinity electron acceptor to lower enteric methane production in ruminants. Livestock Research for Rural Development. Volume 22, Article #221. http://www.lrrd.org/lrrd22/12/leng22221.htm

Lewis D 1951 The metabolism of nitrate and nitrite by the sheep. 1. Reduction of nitrate in the rumen of the sheep. Biochemistry Journal 48: 175-179

Menke K H and Steingass H 1988 Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development. Volume 28. Pp 7-55

Nolan J V, Hegarty R S, Hegarty J, Godwin I R and Woodgate R 2010 Effects of dietary nitrate on rumen fermentation, methane production and digesta kinetics in sheep. Animal Production Science 50 (8) 801–806

Ngoc Huyen Le Thi, Do H Q, Preston T R and Leng R A 2010 Nitrate as fermentable nitrogen supplement to reduce rumen methane production. Livestock Research for Rural Development. Volume 22, Article #146. http://www.lrrd.org/lrrd22/8/huye22146.htm

Nguyen Ngoc Anh, Khuc Thi Hue, Duong Nguyen Khang and Preston T R 2010 Effect of calcium nitrate as NPN source on growth performance and methane emissions of goats fed sugar cane supplemented with cassava foliage. MEKARN Conference on Livestock production, climate change and resource depletion (Editors: Reg Preston and Brian Ogle). Pakse, Laos, November 7-9 2010 http://www.mekarn.org/workshops/pakse/abstracts/_anh-grrc.htm

Ørskov E R, DeB Hovell F D and Mould F 1980 The use of the nylon bag technique for the evaluation of feedstuffs. Tropical Animal Production Volume 5, Number 3. http://www.utafoundation.org/TAP/TAP53/53_1.pdf

Sath K, Borin K and Preston T R 2008 Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org/lrrd20/supplement/sath2.htm

Smith P, Martino D, Cai,Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C, Scholes B and Sirotenko O 2007 Agriculture. In: Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds B. Metz, O.R. Davidson, P.R. Bosch, R. Dave & L.A. Meyer. Cambridge University Press, Cambridge: United Kingdom and New York, NY, USA.

Thu Hong N T, Quac V A, Kim Chung T T, Hiet B V, Mong N T and Huu P T 2008 Mimosa pigra for growing goats in the Mekong Delta of Vietnam. Livestock Research for Rural Development. Volume 20, Article #208. http://www.lrrd.org/lrrd20/12/hong20208.htm

Tilley J M A and Terry R A 1963 A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18 : 104.

Tran Triet, Le Cong Kiet, Nguyen Thi Lan Thi and Pham Quoc Dan 2007 The invasion by *Mimosa pigra* of wetlands of the Mekong Delta, Vietnam. [on-line]. Available from: http://www.ento.csiro.au/weeds/pdf/mimosa_symposium/07Trietetal.pdf

Trinh Phuc Hao, Ho Quang Do, Preston T R and Leng R A 2009 Nitrate as a fermentable nitrogen supplement for goats fed forage based diets low in true protein. *Livestock Research for Rural Development. Volume 21, Article #10* http://www.lrrd.org/lrrd21/1/trin21010.htm

Van Zijderveld S.M, Gerrits W.J J, Apajalahti J A, Newbold J R, Dijkstra J, Leng R A and Perdok H B 2010a Nitrate and sulfate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science. Volume 93, 5856-5866 http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS0022030210006387.pdf

Van Zijderveld S M, Dijkstra J, Gerrits W J J, Newbold J R and Perdok H B 2010b Dietary nitrate persistently reduces enteric methane production in lactating dairy cows. In Greenhouse gases and animal agriculture conference. October 3-8, 2010 Banff, Canada, T119, page 127 http://www.ggaa2010.org/pdfs/Proceedings_Abstracts.pdf

Wanapat M, Pimpa O, Petlum A and Boontao U 1997 Cassava hay: A new strategic feed for ruminants during the dry season. *Livestock Research for Rural Development. Volume 9 (2), Article #18* http://www.lrrd.org/lrrd9/2/metha92.htm

Effect of potassium nitrate as modifier of the fermentation in an *in vitro* incubation using NaOH and/or lime treated straw supplemented with fresh cassava leaves as substrate

Sangkhom Inthapanya, Duong Nguyen Khang*, T R Prestonand R A Leng*****

Souphanouvong University, Lao PDR
inthapanyasangkhom@yahoo.com
[*]Department of Animal Physiology and Biochemistry, Nong Lam University, Ho Chi Minh City, Vietnam
***Finca Ecológica TOSOLY, AA#48, Socorro, Colombia*
****University of New England, Armidale NSW, Australia*

Abstract

The aim of this study was to evaluate the effect of combining alkali treatment of rice straw with non-protein nitrogen (NPN) from nitrate in reducing methane production in an *in vitro* incubation system. The treatments in a split-plot 2*6 arrangement with four replications were: alkali treated straw with NaOH (0; 1; 2; 3; 4%) plus lime ($Ca(OH)_2$) (4; 3; 2; 1; 0%) or untreated straw, and as NPN source potassium nitrate or urea. All treatments had fresh cassava leaf as protein source. The quantity of substrate was 12 g to which were added 240 ml rumen fluids (from slaughtered buffalo) and 960 ml of buffer solution. The incubation was for 24 h with measurements of total gas production, methane percentage at intervals of 4, 8, 12, 16 and 24 hours and determination of residual unfermented substrate at the end.

The proportion of substrate fermented after 24 h was increased by NaOH-lime treatment and tended to increase as lime replaced NaOH. There was no consistent effect of alkali treatment on methane percentage in the gas nor on methane production per unit substrate. Total gas production and methane percentage increased with incubation time and the gas production and the percentage of methane in the gas were reduced when nitrate replaced urea at all fermentation stages. After 24 h, the methane production per unit of fermented substrate was less when nitrate replaced urea. It is concluded that lime can replace NaOH as a means of increasing the fermentability of rice straw and that methane production is decreased with potassium nitrate instead of urea as NPN source.

Keywords: *Alkali, climate change, gas production, greenhouse gases, urea*

Introduction

The greenhouse gases (GHG) emissions from the agriculture sector account for about 25.5% of total global radiative forcing and over 60% of anthropogenic sources (FAO 2009). Animal husbandry accounts for 18% of GHG emissions. Emission of methane (CH_4) is responsible for nearly as much radiative forcing as all other non-CO_2 GHG gases combined (Beauchemin and McGinn 2005). While atmospheric concentrations of GHGs have risen by about 39%

since pre-industrial era, CH_4 concentration has more than doubled during this period (WHO 2009). Reducing GHG emissions from agriculture, especially from livestock, should therefore be a top priority since it could curb global warming fairly rapidly (Sejian et al 2010).

Ruminants, such as cattle, buffalo, sheep and goats, are the major contributors of total methane agricultural emissions (Leng 1993; Lassey 2007; Chhabra et al 2009). In ruminants, the H_2 produced in rumen fermentation is normally removed by the reduction of CO_2 to methane. However, nitrate has a higher affinity for H_2 than CO_2 and is first used to reduce of NO_3 to NO_2 and then NO_2 to NH_3, thereby reducing methane production from CO_2. Renewed recognition that nitrate supplements in ruminants compete successfully for H_2 and thus decrease methane production is a promising development (Leng 2008).

The use of nitrate as a source of rumen fermentable nitrogen had previously been discouraged due to the possible formation of toxic nitrite under some circumstances during nitrate reduction of nitrate to ammonia (Leng and Preston 2010). However, recent research has shown that if the adaptation to nitrate is done gradually, toxicity is not a problem (Hao Trinh Phuc et al 2009).

The key to improving the use of crop residues for ruminants is to overcome the inherent barriers to rumen microbial fermentation, which in rice straw is the lignification of the cellulose and hemicellulose cell wall components, and to provide supplements of fermentable nitrogen, vitamins and minerals. Among the strategies to improve the straw fermentation, alkaline substances have been the most widely investigated and accepted for practical on-farm application (Chenost and Kayouli, 1997). The most commonly used alkaline agents are sodium hydroxide (NaOH), ammonia (NH_3), lime ($Ca[OH]_2$) and urea. Chemical treatments appear to be the most practical for on-farm use since inexpensive machinery is required, chemicals are relatively cheap and the procedures are relatively simple. Hao Trinh Phuc et al. (2009) showed that in goat production studies given a low-protein rice straw diet, similar improvements in growth rate and N retention were obtained irrespective of whether the animals were supplemented with nitrate or urea. In a similar cattle study, Le Thi Ngoc Huyen et al (2010) compared sodium nitrate and urea as iso-nitrogenous sources of supplementary N to NaOH-treated rice straw, molasses and cottonseed meal. They found that methane production was reduced by nitrate supplementation while feed intake, digestibility and growth rate did not differ between treatments.

The protein in cassava (*Manihot esculenta*, Crant) leaves is considered to be a good source of bypass protein (Ffoulkes and Preston 1978; Wanapat et al 1997; Sath et al 2008). It is widely cultivated in all tropical counties and is thus logical forage to provide the additional protein required in diets high in non-protein nitrogen.

The purpose of the present study was to determine if lime ($Ca[OH]_2$) could replace sodium hydroxide as an alkaline source to improve straw digestibility, and how this might affect methane production in an *in vitro* incubation, in which the NPN source was either potassium nitrate or urea.

Hypothesis

The hypotheses to be tested were:

- Lime ($Ca[OH]_2$) could replace NaOH to treat rice straw to improve the digestibility in an in vitro incubation.
- Providing the NPN source as potassium nitrate rather than urea will reduce the methane production irrespective of the method of treating the straw.

Materials and methods

Location and duration

An in vitro incubation was conducted in the laboratory of the Faculty of Agriculture and Forest resources, Souphanouvong University, Luang Prabang province, Lao PDR, from May to June 2011.

Treatments and experimental design

The experimental design was arranged as a split-plot 2*6 factorial arrangement with four replications of the following treatments:

Source of NPN:

- Urea
- Potassium nitrate

Alkali treatment:

- NaOH 0% plus $Ca(OH)_2$ 4%
- NaOH 1% plus $Ca(OH)_2$ 3%
- NaOH 2% plus $Ca(OH)_2$ 2%
- NaOH 3% plus $Ca(OH)_2$ 1%
- NaOH 4% plus $Ca(OH)_2$ 0%
- Untreated

The main plots (one run with 4 flasks) were the NPN sources; the split plots were the alkali treatments.

Table 1. Ingredients in the substrate (g)

	Urea	**KNO_3**
Rice straw	8.00	7.55
Fresh cassava leaf	3.73	3.73
Urea	0.22	
KNO_3		0.72
	12.0	**12.0**

The in vitro system

The equipment and procedure was that used by Sangkhom Inthapanya et al (2011) (Photos 1 and 2).

Photo 1. The in vitro system

Photo 2. Gas production after fermentation

Experimental procedure

The rice straw was chopped into small pieces of around 1-2 cm of length, then ground (1mm sieve). A sample of 500 g was taken to treat with combinations of NaOH (% in straw DM: 0, 1, 2, 3, 4) and lime (% in straw DM: 4, 3, 2, 1, 0). The alkalis were dissolved / suspended in water (50% solution) and applied to the straw, which was then stored in plastic bags at room temperature for 14 days. The treated straw was mixed with 30% (DM basis) of fresh cassava leaf before the incubation, and with either potassium nitrate (6% of substrate DM) or urea (2% of substrate DM) as source of NPN.

Representative samples of the substrates (12 g DM which included 8 g straw, 3.73g fresh cassava leaf, 0.72 g potassium-nitrate or 0.22 g urea) were put in the incubation bottle to which were added 0.96 litters of buffer solution (Table 2) and 240 ml of rumen fluid (obtained from a recently slaughtered buffalo at the village abattoir), prior to filling each bottle with carbon dioxide. The bottles were incubated at 38 ^{0}C in a water bath for 4, 8, 12, 16 and 24 h.

Table 2. Ingredients of the buffer solution

Ingredients	**$CaCl_2$**	**$NaHPO_4.12H_2O$**	**NaCl**	**KCl**	**$MgSO_4.7H_2O$**	**$NaHCO_3$**	**Cysteine**
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

***Source**: Tilly and Terry (1963)*

Data collection and measurements

The gas volume and the percentage of methane in the gas were recorded at 4, 8, 12, 16 and 24 h with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK, photo 3). At 24 h the residual DM in the incubation bottle was determined by filtering through cloth and drying (100 ^{0}C for 24 h) the residue (Photo 4).

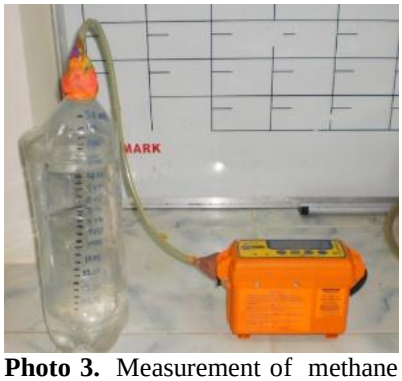

Photo 3. Measurement of methane with the Crowcom meter

Photo 4. The substrate residue filtered through cloth

Chemical analyses

Samples of straw and fresh cassava leaf were analysed for DM, ash and N according to methods outlined in Ly and Nguyen Van Lai (1997).

Statistical analysis

The data were analyzed by the General Linear Model (GLM) option in the ANOVA program of the Minitab (2000) Software. Sources of variation in the model were: NPN source, alkali treatment, interaction NPN*alkali and error.

Results and discussion

Chemical composition

The DM content of rice straw treated with lime or NaOH was lower than in untreated straw (Table 3). Straw treated with lime had higher ash content than straw treated with NaOH. The relatively low solubility of the N in the fresh cassava leaf was indicative of potential bypass protein characteristics.

Table 3. Mean values for dry matter (DM), ash, crude protein and N solubility of substrate ingredients

	Rice straw (RS)	RS*-NaOH	RS-Lime	Fresh cassava leaf
DM, %	94.9	90.8	90.3	33.4
Ash in DM, %	15.1	15.2	16.4	5.88
Crude protein in DM, %	4.91	5.33	5.78	25.2
Solubility of N, %	12.2	13.3	13.4	32.5

Effects of alkali treatment and NPN sources on total gas and methane production

The proportion of substrate fermented after 24 h was increased by NaOH-lime treatment and tended to increase as lime replaced NaOH (Table 4; Figures 1 and 2). The total gas production and percentage of methane in the gas increased with time of incubation (Figures 3 and 4). The gas production and the percentage of methane in the gas were reduced when nitrate replaced urea all stages of the fermentation. After 24 h, the percentage methane in the gas and the methane production per unit of fermented substrate was less when nitrate replaced urea as the source of NPN (Figures 5 and 6). There was no consistent effect of alkali treatment of the straw on methane percentage in the gas nor on methane production per unit substrate fermented (Figures 5 and 6).

Table 4. Mean value for gas production, percentage of methane in the gas, substrate solubilized, methane production per substrate solubilized according to source of alkaline or no alkaline and NPN

		Alkaline						NPN source		
	Untreated	**N0L4**	**N1L3**	**N2L2**	**N3L1**	**N4L0**	**Prob**	**Urea**	**KNO_3**	**Prob**	**SEM**
0-4 hours											
Gas production, ml	207	234	218	234	251	244	<0.001	246	216	<0.001	3.81
Methane, %	6.38	7.13	6.75	6.63	7.13	7.63	<0.001	7.63	6.25	<0.001	0.11
Methane, ml	13.3	16.8	14.8	15.6	17.9	18.8	<0.001	18.6	13.5	<0.001	0.39
0-8 hours											
Gas production, ml	230	236	225	225	245	239	0.255	268	198	<0.001	3.69
Methane, %	8.06	8.63	8.13	8.25	7.63	8.75	0.004	9.13	7.35	<0.001	0.11
Methane, ml	18.9	20.8	18.4	18.9	18.9	21.4	0.078	24.5	14.6	<0.001	0.47
0-12 hours											
Gas production, ml	261	308	276	270	274	298	0.123	357	205	<0.001	8.01
Methane, %	11.4	13.1	12.5	11.1	12.0	11.0	<0.001	14.9	8.75	<0.001	0.17
Methane, ml	31.5	45.7	37.7	31.5	34.8	34.6	0.007	54.0	17.8	<0.001	1.62
0-16 hours											
Gas production, ml	283	341	301	286	300	315	0.003	394	215	<0.001	6.17
Methane, %	13.2	14.6	14.6	13.8	14.4	13.9	0.025	17.9	10.2	<0.001	0.22
Methane, ml	39.4	55.8	47.8	42.6	45.8	47.4	0.001	71.0	21.9	<0.001	1.53
0-24 hours											
Gas production, ml	310	411	390	356	355	385	<0.001	458	278	<0.001	6.00
Methane, %	14.8	16.1	17.4	15.5	16.0	16.4	<0.001	19.3	12.8	<0.001	0.19
Methane, ml	47.9	68.7	73.3	57.5	59.3	66.2	<0.001	88.7	35.6	<0.001	1.30
Total gas production, ml	1291	1530	1410	1371	1424	1480	<0.001	1723	1112	<0.001	17.9
Total methane, ml	151	208	192	166	177	188	<0.001	257	104	<0.001	3.85
Overall methane, %	11.2	12.8	12.8	11.6	11.9	12.1	<0.001	14.9	9.30	<0.001	0.11
DM solubilized after 24 h, %	21.4	29.8	28.4	27.4	27.9	26.6	<0.001	28.5	25.4	<0.001	0.33
Methane, ml/g DM solubilized	18.8	19.6	21.7	17.8	18.1	21.2	0.002	26.5	12.6	<0.001	0.42

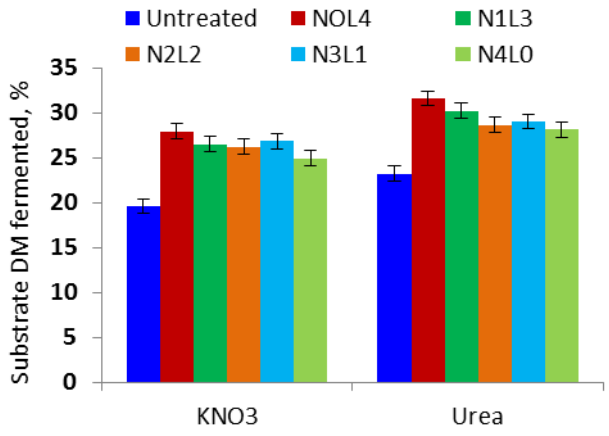

Figure 1. Effect on percentage of substrate fermented of combinations of lime (L) and NaOH (N)

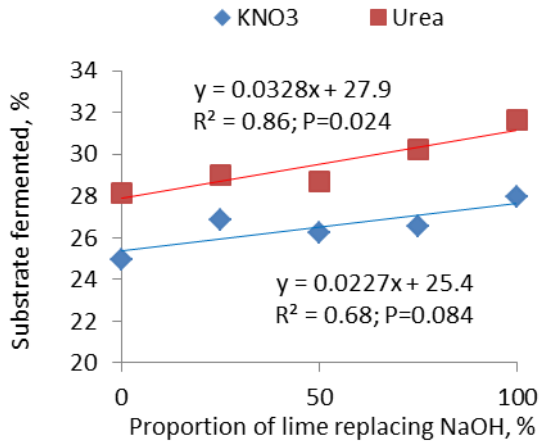

Figure 2. Effect on percentage of substrate fermented of replacing NaOH with lime treatment

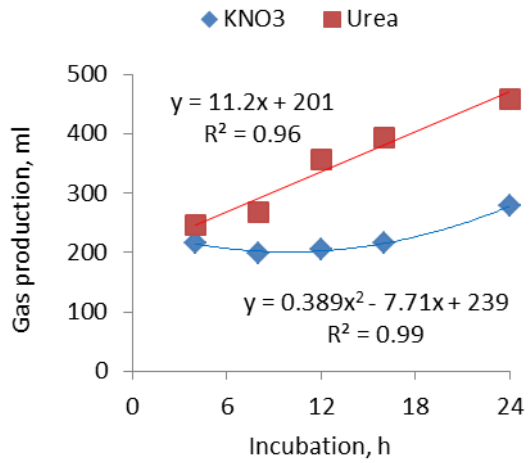

Figure 3. Effect of incubation time on gas production with KNO_3 or urea as NON source

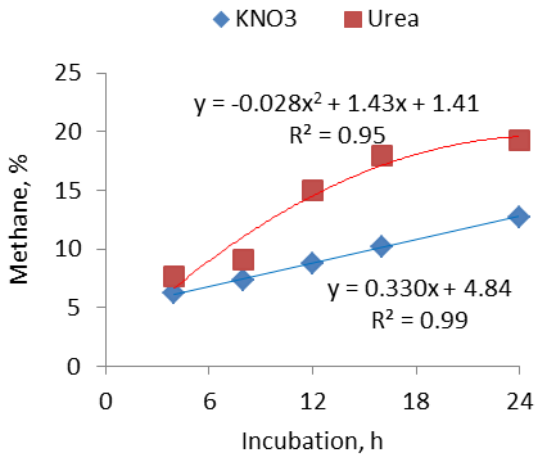

Figure 4. Effect of incubation time on methane content of the gas with KNO_3 or urea added to the substrate

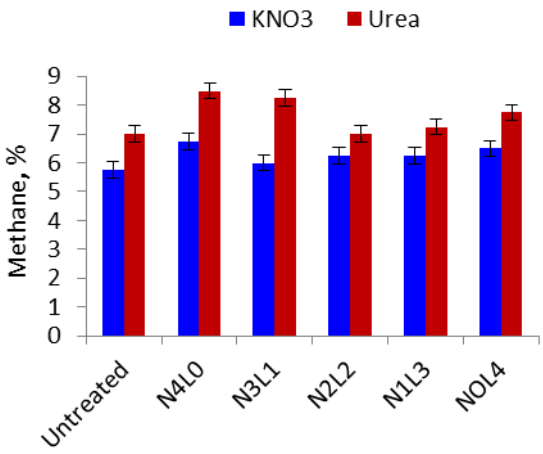

Figure 5. Effect of alkali treatment and proportion of lime (L) and NaOH (N) in the alkali medium on percentage methane in the gas

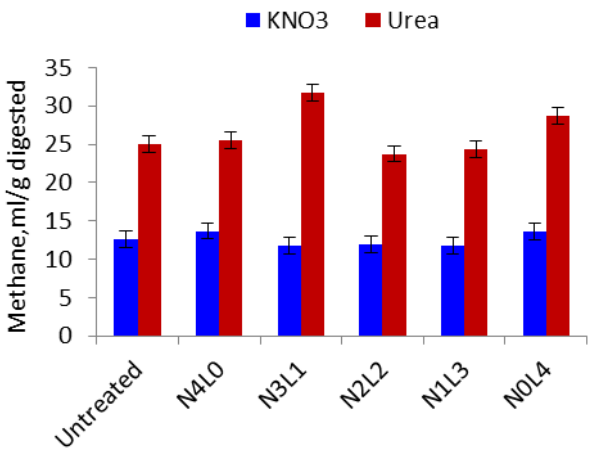

Figure 6. Effect of alkali treatment and proportion of lime (L) and NaOH (N) in the alkali medium on methane produced per unit substrate fermented

Conclusions

- The total gas production and percentage of methane in the gas increased with incubation time.
- The gas production and the percentage of methane in the gas were reduced when nitrate replaced urea as the NPN source at all stages of the fermentation.
- After 24 h, the methane production per unit of fermented substrate was less when nitrate replaced urea as NPN source.
- The proportion of substrate fermented after 24 h was increased by NaOH-lime treatment and tended to increase as lime replaced NaOH.
- There was no consistent effect of alkali treatment on methane percentage in the gas nor on methane production per unit substrate.

Acknowledgements

This research was done by the senior author with support from NUFU as part of the requirements for the MSc degree in Animal Production "Specialized in Response to Climate Change and Depletion of Non-renewable Resources". The authors acknowledge support for this research from the MEKARN project financed by Sida. Special thanks to Mr Nouyang and Mr Jator who provided valuable help in the laboratory. Thanks also to the staff of Department of Animal Science laboratory, Faculty of Agriculture and Forest Resource, Souphanouvong University for providing the facilities to carry out this research.

References

Beauchemin K A and McGinn S M 2005 Methane emissions from feedlot cattle fed barley or corn diets. Journal of Animal Science 83: 653-661.

Chenost M and Kayouli C 1997 Roughage Utilization in Warm Climates. FAO Animal Production and Health Paper 135 Rome.

Chhabra A, Manjunath K R, Panigrahy S and Parihar J S 2009 Spatial pattern of methane emissions from Indian livestock. Current Science 96(5): 683-689.

FAO 2009 Submission to UNFCCC AWG LCA, Enabling Agriculture to Contribute to Climate Change. Retrieved from: http://unfccc.int/resource/ docs/2008/smsn/igo/036.pdf

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. Tropical Animal Production Volume 3, Number 3, pp 186-192 http://www.utafoundation.org/TAP/TAP33/3_3_1.pdf

Hao Trinh Phuc, Do Ho Quang, Preston T R and Leng R A 2009 Nitrate as a fermentable nitrogen supplement for goats fed forage based diets low in true protein. Livestock Research in Rural Development 21(1). Available at http://www.lrrd.org/lrrd21/1/trin21010.htm

Lassey K R 2007 Livestock methane emission: From the individual grazing animal through national inventories to the global methane cycle. Agriculture Meteorology, 142: 120-132.

Le Thi Ngoc Huyen, Ho Quang Do, Preston T R and Leng RA 2010 Nitrate as fermentable nitrogen supplement to reduce rumen methane production. *Livestock Research for Rural Development* 22(8). Available at http://www.lrrd.org/lrrd22/8/huye22146.htm

Le Thuy Binh Phuong, Preston T R and Leng R A 2011 Mitigating methane production from ruminants; effect of supplementary sulphate and nitrate on methane production in an *in vitro* incubation using sugar cane stalk and cassava leaf meal as substrate. *Livestock Research for Rural Development. Volume 23, Article #22.* Retrieved, from http://www.lrrd.org/lrrd23/2/phuo23022.htm

Leng R A and Preston T R 2010 Further considerations of the potential of nitrate as a high affinity electron acceptor to lower enteric methane production in ruminants. Livestock Research for Rural Development. Volume 22, Article #221. http://www.lrrd.org/lrrd22/12/leng22221.htm

Leng R A 2008 Report to Department of Climate Change, Commonwealth Government, Canberra. Available at http://www.penambulbooks.com/Downloads/Leng-Final%20 Modified% 20%2017-9-2008.pdf

Leng R A 1993 The impact of livestock development on environmental change. FAO Corporate Documentary Repository, pp: 1-14.

Ly J and Nguyen Van Lai 1997 Laboratory manual. http://www.mekarn.org/labman/ Default.htm

Minitab 2000 Minitab Software Release 13.2

Phommasack Outhen, Preston T R and Leng R A 2011 Effect of supplementation with urea or calcium nitrate and cassava leaf meal or fresh cassava leaf in an *in vitro* fermentation using a basal substrate of sugar cane stalk. *Livestock Research for Rural Development. Volume 23, Article #023.* http://www.lrrd.org/lrrd23/2/ outh 23023.htm

Sangkhom Inthapanya, Preston T R and Leng R A 2011 Mitigating methane production from ruminants; effect of calcium nitrate as modifier of the fermentation in an in vitro incubation using cassava root as the energy source and leaves of cassava or *Mimosa pigra* as source of protein. *Livestock Research for Rural Development. Volume 23, Article #21.* http://www.lrrd.org/lrrd23/2/sang23021.htm

Sath K, Borin K and Preston T R 2008 Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org/ lrrd20/supplement/sath2.htm

Sejian V, R Lal, Lakritz J and Ezeji T 2010 Measurement and prediction of enteric methane emission. International Journal . Biometeorology DOI: 10.1007/s00484-010-0356-7.

Silivong P, Preston T R and Leng R A 2011 Effect of sulphur and calcium nitrate on methane production by goats fed a basal diet of molasses supplemented with Mimosa (Mimosa pigra) foliage. Livestock Research for Rural Development. Volume 23, Article #58. http://www.lrrd.org/lrrd23/3/sili23058.htm

Thanh V D, Preston T R and Leng R A 2011 Effect on methane production of supplementing a basal substrate of molasses and cassava leaf meal with mangosteen peel (Garcinia mangostana) and urea or nitrate in an in vitro incubation. Livestock Research for Rural Development. Volume 23, Article #98. http://www.lrrd.org/lrrd23/4/ than23098.htm

Tilley J M A and Terry R A 1963 A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18: 104.

Wanapat M, Pimpa O, Petlum A and Boontao U 1997 Cassava hay: A new strategic feed for ruminants during the dry season. . *Livestock Research for Rural Development. Volume 9 (2), Article #18* http://www.lrrd.org/lrrd9/2/metha92.htm

WHO 2009 Department of health statistics and health information systems, World Health Statistics.

Effect of method of processing of cassava leaves on protein solubility and methane production in an *in vitro* incubation using cassava root as source of energy

Sangkhom Inthapanya, T R Preston*, Duong Nguyen Khang and R A Leng*****

Faculty of Agriculture and Forest Resource, Souphanouvong University, Lao PDR
inthapanyasangkhom@yahoo.com
**Finca Ecologica, TOSOLY, AA#48, Socorro, Santander, Colombia*
***Department of Animal Physiology and Biochemistry, Nong Lam University,*
Ho Chi Minh City, Vietnam
****University of New England, Armidale NSW, Australia*

Abstract

Two experiments were carried out to study effects of processing of cassava leaves on the solubility of the protein and on methane production when they were incubated with cassava root meal in an in vitro incubation with urea or potassium nitrate as source of NPN.

Experiment 1: The treatments in a 2*4 factorial arrangement in a randomized block design were: leaves or petioles of cassava, and form of processing (fresh, ensiled, sun-dried and oven-dried). Protein solubility was decreased by ensiling and with the severity of drying.

Experiment 2: The treatments in a 2*4 factorial arrangement in a randomized block design were: form of processing cassava leaves (fresh, ensiled, sun-dried and oven-dried) and source of NPN (urea or potassium nitrate). An *in vitro* incubation system was used to determine the effects of the treatments on methane production and substrate fermentation. The quantity of substrate was 12 g DM to which was added 240 ml rumen fluid (from slaughtered buffalo) and 960 ml of buffer solution. The incubation was for 12 and 24 h with measurements of gas production, percent methane, substrate fermented and methane produced per unit substrate fermented.

Protein solubility in leaves and petioles decreased in the order: fresh, ensiled, sun-dried and oven-dried. Protein solubility in petioles was lower than in leaves. The gas production and methane percentage in the gas were increased with incubation time. The percentage of methane in the gas, and methane per unit substrate fermented were reduced when nitrate replaced urea as the NPN source and were lower for fresh and ensiled cassava leaves than for dried leaves at both 12 and 24 h of incubation. Methane produced per unit of fermented DM was inversely related to protein solubility.

Key words: *Climate change, gas production, greenhouse gases, hydrogen cyanide*

Introduction

Cassava is currently the third most important crop in Laos, after rice and maize. It is widely grown throughout the country by upland farmers but in small areas using local varieties and with very few inputs (CIAT 2001). The potential use of cassava foliage as an animal feed has been studied and described by several authors. Phuc et al (2001) investigated the use of cassava foliage as a feed for pigs and poultry. Ffoulkes and Preston (1978) reported the feeding of fresh cassava foliage as the only source of roughage and protein in diets for cattle based on molasses-urea. Van et al (2001) and Khang (2004) described the use of foliage of cassava as a protein source for small ruminants and cattle. The potential of cassava foliage as a protein source in ruminant feeds has not been fully exploited, probably because of the risk of toxicity resulting from the content of precursors of hydrogen cyanide (Wanapat 2001). However, it is known that the capacity to liberate HCN from cassava foliage is reduced by processing such as sun drying or ensiling (Khieu Borin et al 2005; Phengvichith and Ledin 2007).The role of cyanide as inhibitor of methanogenesis in sludge fermentation has been discussed by Gijzen et al (2000), Eikmanns and Thauer (1984), Smith et al (1985) and Cuzin and Labat (1992).

Cassava leaves are known to contain variable levels of condensed tannins; about 3% in DM according to Netpana et al (2001) and Bui Phan Thu Hang and Ledin (2005). Condensed tannins at moderate levels are known to have positive effects on the nutritive value of the feed by forming insoluble complexes with dietary protein, resulting in "escape" of the protein from the rumen fermentation (Barry and McNabb 1999). Numerous studies have also shown the potential of the tannin content in cassava leaves to play an anthelminthic role for the control of nematode parasites in ruminants (Seng Sokerya and Preston 2003; Neptpana et al, 2001; Khoung and Khang 2005). Condensed tannins (CT) are also reported to decrease methane production and increase the efficiency of microbial protein synthesis (Makkar et al 1995). Reductions of CH_4 production by 13 to 16% were reported by Carulla et al (2005), Waghorn et al (2002), Grainger et al (2009) and Woodward et al (2004), apparently through a direct toxic effect on methanogens.

In a recent study, Ho Quang Do et al (2010, unpublished data) showed major effects of protein solubility on methane production in an *in vitro* incubation. Methane production was reduced by almost 50% when the protein source was fish meal (protein solubility 16.6%) compared with groundnut (protein solubility 76.2%) when sodium nitrate was the source of NPN and was 25% less when urea was the source of NPN.

The purpose of the present study was to determine the possible relationship between the solubility of the protein in cassava foliage and how this might affect methane production in an *in vitro* incubation, in which the NPN source was either potassium nitrate or urea.

Hypothesis

- It was hypothesized that the solubility of the protein in cassava leaves would be reduced by drying and that this would result in a reduced production of methane in an in vitro incubation with cassava root meal as energy source and potassium nitrate or urea as source of NPN.

Materials and methods

Location and duration

The experiment was conducted in the laboratory of the Department of Animal Science, Faculty of Agriculture and Forest Resources, Souphanouvong University, Luang Prabang province, Lao PDR, from September to October 2011.

Experiment 1: Protein solubility of leaves and stems of cassava foliage

Treatments and experimental design

The experimental design was arranged as a random block design with a 2*4 factorial arrangement of the following treatments:

Source of material:

- Leaves
- Petioles

Processing:

- Fresh
- Ensiled
- Sun-dried
- Oven-dried

Experimental procedure

Cassava foliage was collected from the Souphanouvong University campus. The samples were collected in the morning and immediately put into sealed plastic bags to avoid moisture loss.

Leaves and petioles were separated and chopped into small pieces around 0.5-1.0 cm of the length and then ground through a 1mm sieve. Part of the material was sun-dried for 24 h. Another part was dried in the oven for 24 h at 80°C. For the ensiling treatment, the material was put in sealed plastic bags, and the air removed prior to storage at room temperature for 14 days.

Protein solubility was measured by weighing 3 g of samples (DM basis), followed by liquidizing and shaking in 100 ml of M NaCl for 3 h. The suspension was then filtered through Whatman No. 4 filter paper and washed 3 times with distilled water. All the filtrate was then transferred to a kjeldahl flask for digestion, distillation and titration according to AOAC (1990). Protein solubility was calculated as the N content of the filtrate as a percentage of the N in the original sample.

Photo 1. The shaking

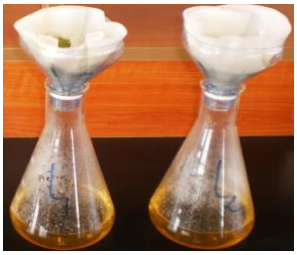

Photo 2. The filtering

Photo 3. The digestion

Photo 4. The distillation

Statistical analysis

Data were analyzed by the General Linear Model option in the ANOVA program of the Minitab software (Minitab 2000). Sources of variation in the model were: plant part, processing and interaction plant part*process and error.

Experiment 2: Effect of processing of cassava leaves on methane production in an* in vitro *incubation with cassava root meal as energy substrate and potassium nitrate or urea as source of NPN

Treatments and experimental design

The experiment was arranged as a 2*4 factorial of the following treatments in a random block design:

Source of NPN:

- Urea
- Potassium nitrate

Processing of cassava leaves:

- Fresh
- Ensiled
- Sun-dried
- Oven-dried

The basal substrate was cassava root meal.

Table 1. Ingredients in the substrate, g

	FCL-U	SCL-U	OCL-U	ECL-U	FCL-KN	SCL-KN	OCL-KN	ECL-KN
Cassava root meal	8.76	8.76	8.76	8.76	8.28	8.28	8.28	8.28
Fresh cassava leaf	3.00				3.00			
Ensiled cassava leaf				3.00				3.00
Sun-dried cassava leaf		3.00				3.00		
Oven-dried cassava leaf			3.00				3.00	
Urea	0.24	0.24	0.24	0.24				
KNO_3					0.72	0.72	0.72	0.72
Total	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0

The in vitro system

The equipment and procedure was that used by Sangkhom Inthapanya et al (2011) (Photos 5 and 6).

Photo 5. The in vitro system

Photo 6. Gas production after fermentation

Experimental procedure

The leaves of cassava were chopped into small pieces of around 0.5-1.0 cm of length, then ground (1mm sieve). They were mixed with cassava root meal and either potassium nitrate or urea (Table 1) prior to adding 0.96 liters of buffer solution (Table 2) and 240 ml of rumen fluid (obtained from a newly slaughtered buffalo in the village abattoir), and put in the incubation bottle which was then gassed with carbon dioxide. The bottles were incubated at 38 ^{0}C in a water bath for 12 and 24 h.

Table 2. Ingredients of the buffer solution

Ingredients	**$CaCl_2$**	**$NaHPO_4.12H_2O$**	**NaCl**	**KCl**	**$MgSO_4.7H_2O$**	**$NaHCO_3$**	**Cysteine**
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

***Source**: Tilly and Terry (1963)*

Data collection and measurements

The gas volume and the percentage of methane in the gas (Crowcon infra-red analyser; Crowcon Instruments Ltd, UK; Photo 3) were recorded for the separate incubations after 12 and 24 h. At the end of each incubation the residual DM in the incubation bottle was determined by filtering through cloth and drying (100°C for 24 h) the residue (Photo 4).

Statistical analysis

The data were analyzed by the General Linear Model (GLM) option in the ANOVA program of the Minitab (2000) Software. Sources of variation in the model were: NPN source; leaves of cassava, interaction NPN*leaves and error.

Results and discussion

Chemical composition

On all processing treatments, the petioles had lower content of DM and of crude protein in the DM (Table 3).

Table 3. Dry matter (DM) and crude protein (CP) in the diets

	DM, %	CP in DM, %
Fresh cassava		
Leaves	31.8	23.4
Petioles	25.8	16.8
Ensiled cassava		
Leaves	28.2	21.2
Petioles	17.6	15.9
Sun-dried cassava		
Leaves	66.7	21.9
Petioles	54.1	15.6
Oven-dried cassava		
Leaves	92.6	21.6
Petioles	85.2	16.0

Protein solubility

The protein solubility decreased in the order: fresh, ensiled, sun-dried and oven-dried processing (Table 4; Figure 1). Petioles had lower values than leaves.

Table 4. Mean values of protein solubility of cassava leaves and petioles subjected to different forms of processing

	Fresh	Ensiled	Sun-dried	Oven-dried	SEM	Prob.
Leaves	33.1	31.6	29.9	29.4	1.26	<0.001
Petioles	25.2	21.1	22.0	21.0	1.26	<0.001

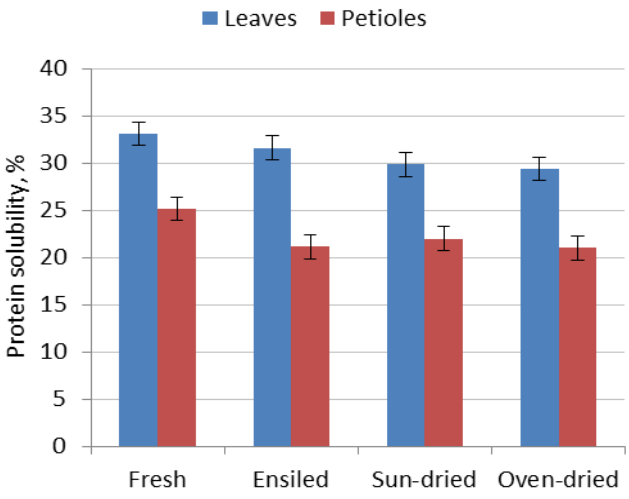

Figure 1. Protein solubility in leaves and petioles of cassava

Methane production

Gas production, per cent methane on the gas, substrate fermented and methane produced per unit substrate fermented were higher for 24 than 12 h incubation and higher for nitrate than for urea (Figures 2-9).

Gas production was lower for fresh leaves than for ensiled or dried leaves when urea was the NPN source, but with no differences for processing with nitrate as NPN. Per cent methane in the gas was lower for fresh and ensiled leaves compared with dried leaves at 12 h with a similar but less marked tendency for the 24 h incubation. The proportion of substrate fermented was higher for fresh and ensiled leaves compared with dried leaves for both nitrate and urea supplements and for both times of incubation. Methane produced per unit substrate fermented was lower for fresh and ensiled leaves than for dried leaves at both12 and 24 h incubation, the differences being more marked for the shorted incubation time.

Table 5. Mean values of gas production, methane percentage in the gas, substrate solubilized and methane per substrate solubilzed according to process of cassava leaves and NPN source

	FCL	ECL	SCL	OCL	Prob.	KNO_3	Urea	Prob.	SEM#
0-12 hours									
Gas production, ml	744	938	1013	988	<0.001	669	1172	<0.001	28/20
Methane, %	12	11	14	15	<0.001	12	14	<0.001	0.46/0.32
Methane, ml	91.6	109	140	152	<0.001	78.2	168	<0.001	5.0/3.5
Digested, %	49.7	51.6	45.1	45.1	<0.001	39.9	55.8	<0.001	0.90/0.64
Methane, ml/g substrate fermented	15.7	17.7	25.9	28.1	<0.001	17.7	26.0	<0.001	1.1/0.77
0-24 hours									
Gas production, ml	1075	1188	1138	1163	0.326	847	1434	<0.001	43/31
Methane, %	16	16	17	18	<0.001	15	18	<0.001	0.36/0.25
Methane, ml	170	194	193	213	<0.001	127	258	<0.001	7.8/5.5
Digested, %	56.7	59.1	54.4	50.5	<0.001	46.7	63.7	<0.001	1.8/1.3
Methane, ml/g substrate fermented	25.5	27.4	30.3	35.6	<0.001	24.8	34.5	<0.001	1.2/0.88

FCL: Fresh leaves; ECL: Ensiled leaves; SCL: Sun-dried leaves; OCL: Oven-dried leaves
#SEM for process/NPN

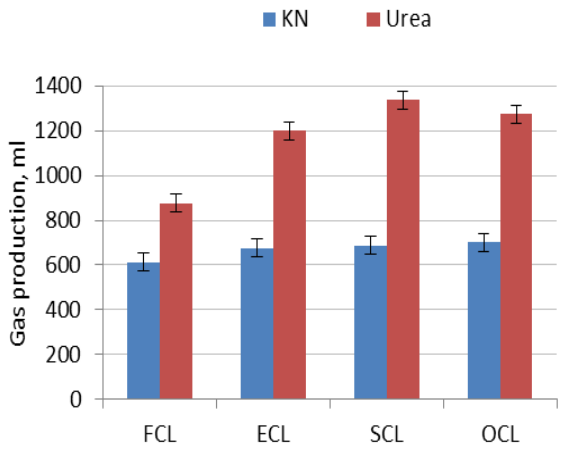

Figure 2. Effect of NPN source on gas production for 12 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

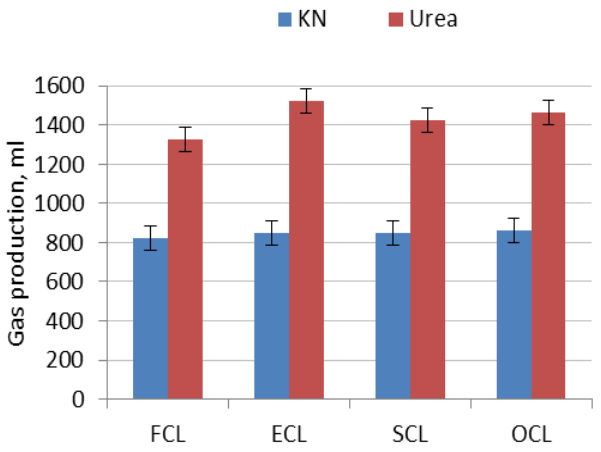

Figure 3. Effect of NPN source on gas production for 24 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

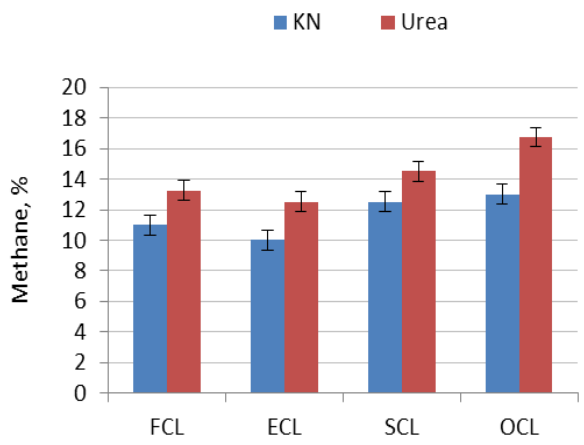

Figure 4. Effect of NPN source on methane per cent in the gas for 12 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

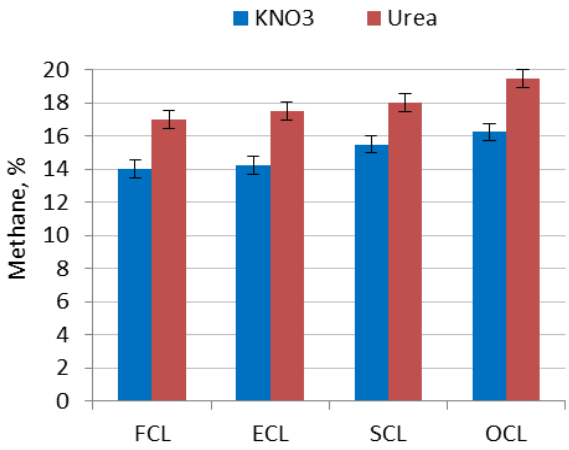

Figure 5. Effect of NPN source on methane per cent in the gas for 24 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

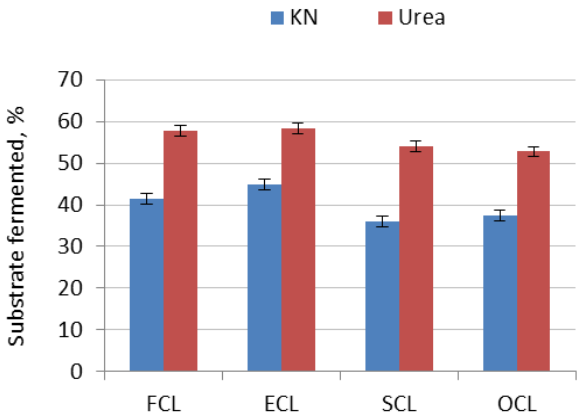

Figure 6. Effect of NPN source on substrate fermented for 12 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

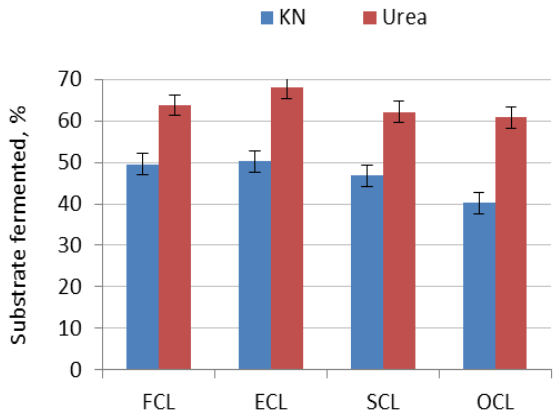

Figure 7. Effect of NPN source on substrate fermented for 24 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

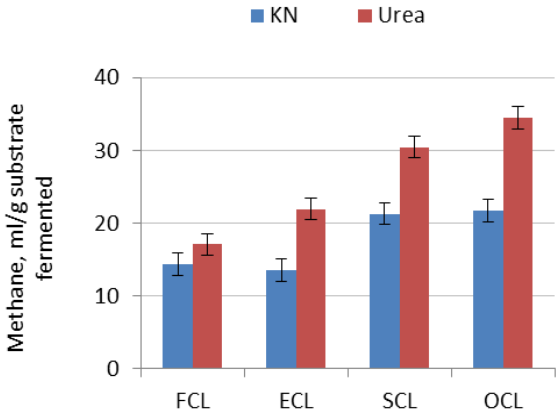

Figure 8. Effect of NPN source on methane per unit substrate fermented for 12 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

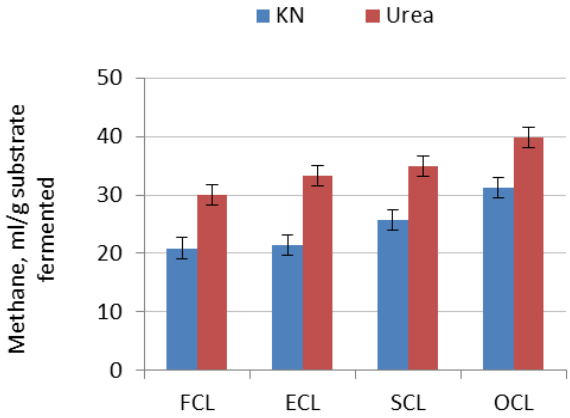

Figure 9. Effect of NPN source on methane per unit substrate fermented for 24 h incubation time with fresh, ensiled, sun-dried and oven-dried cassava leaves

The lower methane production per unit of dry matter digested in substrates with fresh cassava leaves compared with dried leaves indicates that treatments that increase cyanide concentrations (eg: in fresh cassava leaves) decrease the amount of methane produced, which may be explained if cyanide inhibits the metabolism of acetate to methane and carbon dioxide as happens in sludge type fermentations (Gijzen et al 2000; Eikmanns and Thauer 1984; Smith et al 1985; Cuzin and Labat 1992).

The original hypothesis that decreasing protein solubility would reduce methane production was disproved as the contrary effect was observed (Figure 10). It would appear that the effects of drying on cyanide release potential were more important than the slight differences in protein solubility brought about by drying.

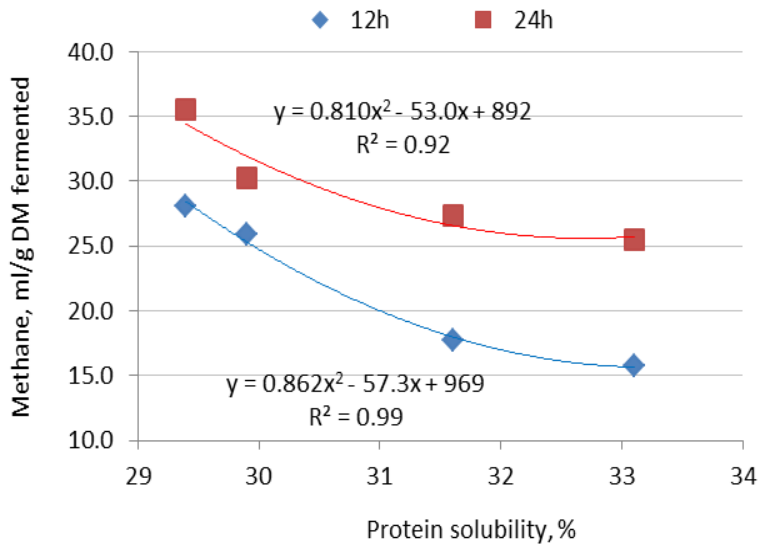

Figure 10. Apparent relationship between protein solubility in cassava leaves and production of methane per unit substrate fermented

Conclusions

- Protein solubility in leaves and petioles decreased in the order: fresh, ensiled, sun-dried and oven-dried
- Protein solubility in petioles lower than in leaves
- The percentage of methane in the gas and quantity of methane per unit of fermented substrate was lower with fresh cassava leaves when compared with dried cassava leaves in an *in vitro* incubation with cassava root meal as energy source
- The gas production, percentage of methane in the gas and methane produced per unit substrate fermented, were reduced when nitrate replaced urea as the NPN source in *the in vitro* incubation

Acknowledgements

The author expresses gratitude to the MEKARN project, supported by Sida, for financial support for this research. Special thanks are given to Mr Sengsouly Phongphanith who provided valuable help in the laboratory. I also acknowledge the Souphanouvong University,

Faculty of Agriculture and Forest Resources, Department of Animal Science, for providing infrastructure support.

References

AOAC 1990 Official methods of analysis 15th ed. AOAC, Washington, D.C

Barry T T N and McNabb W W C 1999 The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. British Journal of Nutrition 81(4), 263-272.

Bui Phan Thu Hang and Ledin Inger 2005 Utilization of Melastoma (*Melastoma affine*, D. Don) foliage as a forage for growing goats with cassava *(Manihot Esculenta*, Crantz) hay supplementation. Proceedings International Workshop on Small Ruminant Production and Development in South East Asia (Editor: Inger Ledin), Hanoi, Vietnam, 2-4 March 2005. http://www.mekarn.org/procsr/hangctu.pdf

Carulla J E, Kreuzer M, Machmüller A and Hess H D 2005 Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research 56, 961-970.

CIAT 2001 Solutions that cross frontiers. Cassava production system. http://www.Ciat.cgiar.org/asia/cassava.htm

Diaz A, Avendan O M and Escobar A 199 Evaluation of *Sapindus saponaria* as a defaunating agent and its effects on different ruminal digestion parameters. Livestock Research for Rural Development. Animal Production. Volume 5 (2) http://www.lrrd.org/lrrd5/2/cefe.htm

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. Tropical Animal Production. http://www.utafoundation.org/TAP/TAP33/ 331.pdf

Gijzen H J, Bernal E and Ferrer H 2000 Cyanide toxicity and cyanide degradation in anaerobic wastewater treatment. Water Research. Volume. 34, No. 9, pp. 2447-2454

Grainger C, Clarke T, Auldist M J, Beauchemin K A, McGinn S M and Waghorn G C 2009 Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen). excretion from grazing dairy cows, Canadian Journal of Animal Science, 89, 241–251

Hess H D, Kreuzer M, Diaz T E, Lascano C E, Carulla J E, Solvia C R and Machmuller A 2003 Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid, Journal Animal Feed Science and Technology. Volume 109, pp 79-94.

IPCC 2001 *Climate Change 2001: The Scientific Basis*, Technical Summary by Workgroup I of the Intergovernmental Panel on Climatic Change, Cambridge, UK, and Cambridge University Press.

Khang D N 2004 Cassava foliage as protein source for cattle in Vietnam. Agraria Doctoral thesis. Dept. of animal Nutrition and Management, SLU, Uppsala, Sweden.

Khieu Borin, Lindberg J E and 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.

Khuong L H and Khang D N 2005 Effect of fresh cassava foliage on growth and feacal nematoda egg counts in Sindhi x yellow cattle fed urea-treated rice straw basal diet. Workshop-seminar "Making better use of local feed resources" (Editors: T. R. Preston and B. Ogle) MEKARN-CTU, Cantho, 23-25 May, 2005.

Le Thuy Binh Phuong, Preston T R and Leng R A 2011 Mitigating methane production from ruminants; effect of supplementary sulphate and nitrate on methane production in an *in vitro* incubation using sugar cane stalk and cassava leaf meal as substrate. *Livestock Research for Rural Development. Volume 23, Article #22.* Retrieved , from http://www.lrrd.org/lrrd23/2/phuo23022.htm

Ly J and Nguyen Van Lai 1997 Laboratory manual. http://www.mekarn.org/labman/Default.htm

Makkar H P S, Blu¨mmel M and Becker K 1995 In vitro effects of and interactions between tannins and saponins and fate of saponins tannins in the rumen, Journal of the Science of Food and Agriculture. pp. 481–493.

Minitab 2000 Minitab Software Release 13

Netpana N, Wanapat M, Poungchompu O and Toburan W 2001 Effect of condensed tannins in cassava hay on fecal parasitic egg counts in swamp buffaloes and cattle. In: Proceedings International Workshop on Current Research and Development on Use of Cassava as Animal Feed. T R Preston, B Ogle and M Wanapat (Ed) http://www.mekarn.org/procKK/netp.htm

Phengvichith V and Ledin I 2007 Effects of supplementing gamba grass(Andropogon gayanus) with cassava (Manihot esculenta Crantz) hay and cassava root chips on feed intake, digestibility and growth in goats. Asian-Australasian Journal of Animal Sciences 20(5), 725-732.

Phommasack Outhen, T R Preston T R and Leng R A 2011 Effect of supplementation with urea or calcium nitrate and cassava leaf meal or fresh cassava leaf in an *in vitro* fermentation using a basal substrate of sugar cane stalk. *Livestock Research for Rural Development. Volume 23, Article #023.* http://www.lrrd.org/lrrd23/2/ outh 23023.htm

Phuc B H, Ogle B, Lindberg J E 2001 Nutritive value of cassava leaves for monogastric animals. In: Preston T R, Ogle B and Wanapat M (Editors.), International workshop on Current research and development on use of cassava as animal feed. July 23-24, 2001.. Khon Kaen University, Thailand. Pp. 23-24. http://www.mekarn.org/procKK/phuc.htm

Preston T R 2001 Potential of cassava in integrated farming systems. . International Workshop Current Research and Development on Use of Cassava as Animal Feed, Khon Kaen University, Thailand July 23-24, 2001. http://www.mekarn.org/procKK/pres.htm

Sangkhom Inthapanya, Preston T R and Leng R A 2011 Mitigating methane production from ruminants; effect of calcium nitrate as modifier of the fermentation in an in vitro incubation using cassava root as the energy source and leaves of cassava or *Mimosa pigra* as source of protein. *Livestock Research for Rural Development. Volume 23, Article #21.* http://www.lrrd.org/lrrd23/2/sang23021.htm

Seng Sokerya and Preston T R 2003 Effect of grass or cassava foliage on growth and nematode parasite infestation in goats fed low or high protein diets in confinement. MSc. Thesis, MEKARN-SLU

Tilley J M A and Terry R A 1963 A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18: 104.

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

Van D T T, Thanh M N T and Binh D V 2001 Cassava as small ruminant feed in the hilly and mountainous area of Bavi district. In: Preston T R, Ogle B and Wanapat M (Eds.), International workshop on Current research and development on use of cassava as animal feed. July 23-24, 2001. SIDA SARAC project. Khon Kaen University, Thailand. Pp. 23-24. http://www.mekarn.org/procKK/van.htm

Waghorn, G.C, Tavendale, M.H and Woodfield, D.R 2002 Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grassland Association 64, 167–171.

Wanapat M 2001 Role of cassava hay as animal feed in the tropics. In: International Workshop Current Research and Development on Use of Cassava as Animal Feed, Khon Kaen University, Thailand July 23-24, 2001. http://www.mekarn.org/procKK/wana3.htm

Woodward S L, Waghorn G C and Laboyrie P 2004 Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduced methane emissions from dairy cows. Proceedings of the New Zealand Society of Animal Production 64, 160–164.

Effect of potassium nitrate and urea as fermentable nitrogen sources on growth performance and methane emissions in local "Yellow" cattle fed lime ($Ca(OH)_2$) treated rice straw supplemented with fresh cassava foliage

Sangkhom Inthapanya, T R Preston*, Duong Nguyen Khang and R A Leng*****

Souphanouvong University, Lao PDR
inthapanyasangkhom@yahoo.com
**Finca Ecológica TOSOLY, AA 48 Socorro, Colombia*
**Department of Animal Physiology and Biochemistry, Nong Lam University, Ho Chi Minh City, Vietnam
****University of New England, Armidale NSW, Australia*

Abstract

Sixteen male local "Yellow" cattle with initial weight 63-100 kg were fed lime-treated rice straw and fresh cassava foliage in a randomized complete block design (RCBD) with to two treatments: potassium nitrate or urea as NPN source. The NPN sources were dissolved in 100 g molasses diluted with 500 ml of water. The experiment lasted 120 days at the end of which concentrations of methane and carbon dioxide were determined in eructed gas mixed with aif in a closed chamber in which the animals were kept for 5 minutes prior to measurement of the gases so as to ensure equilibration of the eructed gases with the air in the chamber

Daily live weight gain and DM feed conversion were improved by supplementation with nitrate rather than urea. There was no difference between treatments in DM intake. Feed intake as g DM/kg live weight and growth rate were linearly and positively related to initial live weight. The ratio of methane to carbon dioxide in the mixed eructed gas and air was decreased by feeding nitrate with an overall 27% reduction in methane emission, for animals fed nitrate compared with those fed urea.

This is the first research to report better growth rates and better feed conversion ratios when nitrate replaces urea in a low quality diet. This result may be related to the pattern of feeding of the straw /molasses nitrate diet which was given every 6 hours.

Key words: *Climate change, feed conversion, greenhouse gases, live weight gain, rumen ammonia*

Introduction

In SE Asia, most farmers raise livestock for their main source of income and draft power. According to FAO (2005) over 75% of the populations in Lao PDR rely on agriculture as their primary source of income. Cattle and buffaloes are important on smallholder farms in developing countries to provide meat, milk, traction power and manure

in integrated crop and livestock farming (Preston and Leng 2009). In Lao PDR the populations of cattle, buffalo and sheep-goats have increased significantly from year 2002 to 2007 at the rates of 1.9, 0.61 and 9.46 % per annum, respectively (Anon 2007). However, a number of impediments and constraints have been shown to affect livestock productivity and efficiency. The main feed resources for the ruminants in Lao PDR are native grasses, legumes and tree leaves that are available in the natural grassland and forests (Phonepaseuth Phengsavanh and Ledin 2003). The availability of these feed resources is seasonally limited and both feed availability and quality are low, especially in the cropping season.

Preston and Leng (2009) and Leng (1997) have emphasized that the most appropriate ways to improve feed resources for ruminants are through efficient utilization of crop residues and tree/shrub foliages. However, to optimize performance correct feeding methods need to be applied ensuring that rumen function is efficient and secondly ensuring efficient assimilation of nutrients by providing a source of bypass nutrients (Preston and Leng 2009).

Rice straw is the most abundant crop residue in Asia, particularly in Lao PDR. It is the main feed in the dry season when natural grasses are in short supply to animals. Rice straw is characterized by high fiber level (39-53 % ADF) and nutrient deficiencies, especially protein (2 to 4% crude protein), vitamins, minerals and soluble carbohydrates. The straw itself is 98% covered with silica which has to be disrupted (McAllister et al 1994). Thus rice straw has low digestibility for ruminants in the range 41- 59 % (Napasirth et al 2005; Susuki et al 2004; Bui Van Chinh et al 2001; Tran Quoc Viet et al 2001).

There are two ways to improve the feeding value of rice straw: (i) by delignification treatments which also disrupt the silca covering, which may be physical, chemical or biological (Sundstol 1984; Doyle et al 1986); and (ii) supplementation with limiting nutrients in the rumen and essential amino acids (bypass protein) in the animal (Preston and Leng 2009). Treatment with sodium hydroxide was used in early trials on delignification of straw but, ammoniating the straw with urea has been the most widely used method (Chenost and Kayouli 1997). The partial replacement of urea by lime was reported by Nguyen Xuan Trach et al (2001) and Le Thi Thuy et al (2005) to be equally effective and more economical then ammoniation methods.

Leng (2008) concluded that the inclusion of nitrate salts in feed supplements appeared to be entirely feasible as a means of providing fermentable nitrogen and simultaneously reducing enteric methane emissions from ruminant livestock. Whilst there is a risk of nitrite toxicity, nitrate reduction to ammonia in the rumen should theoretically improve microbial growth efficiency and retain the energy that is lost in methane in the nutrients absorbed.

The possibility of nitrate as an alternative hydrogen sink to carbon dioxide has been downplayed because of the possible toxic effects of nitrite, which is formed as an intermediate during the reduction of nitrate to ammonia (Lewis 1951). However, several reports have recently examined the potential of nitrate as a methane-lowering feed additive, and it has been shown to lower methanogenesis consistently (Leng and Preston 2010).

Recent researches in Vietnam (Nguyen Ngoc Anh et al 2010) and Cambodia (Iv Sophea and Preston 2010) have confirmed that the long term feeding of nitrate salts supported the same growth in goats as when urea was the NPN source, but brought about 30% reduction in production of methane. Much higher (50%) responses were reported in sheep in the Netherlands (Van Zijderveld et al 2010a) and in dairy cattle in Brazil (Van Zijderveld et al 2010b). However, there appear to be no reports showing that the reduction in methane is

accompanied by more efficient production of meat or milk, which should theoretically happen.

It has been postulated (Leng 2008) that nitrate salts will be most effective as an NPN source when the diet is low in other sources of rumen-fermentable nitrogen and that the additional protein needed by the animal, over and above that produced by rumen microbes should be provided in the form of bypass protein. Fresh cassava foliage has been shown to be an effective source of bypass protein in diets where the dietary N was mostly in the form of urea (Ffoulkes and Preston 1978). Cassava is widely grown in all tropical countries and has been shown to support increased growth rates in cattle fed on rice straw (Keo Sath et al 2008; Tham et al 2008).

In order to avoid the use of both urea and sodium hydroxide for straw treatment we showed in an earlier paper (Sangkhom et al 2011) that hydrated lime ($Ca[OH]_2$) could effectively replace sodium hydroxide with similar improvements in the digestibility of the straw. The objective of the studies reported in this paper was to investigate the use of a combination of lime-treated straw and fresh cassava foliage as the basal diet for fattening the local "Yellow" breed of cattle, with associated mitigation of methane emissions by incorporation in the diet of potassium nitrate.

Materials and methods

Location and duration

The experiment was conducted in the farm of Souphanouvong University, 7 km from Luang Prabang city, Luang Prabang province, Lao PDR. This experiment was conducted for 4 months, from July to October 2011 excluding adaptation and organizing period.in

Treatments and experimental design

Sixteen local male “Yellow” cattle (Photo 1) were assigned to 8 blocks according to live weight and within blocks, in a randomized complete block design (RCBD), to two treatments, which were:

- Urea at 1.83% of diet DM
- Potassium nitrate at 6% of diet DM

The basal diet was lime-treated rice straw fed ad libitum, fresh cassava foliage at 300 g/kg diet (DM basis) and 100 g of molasses/day diluted with 500 ml of water as the carrier for the urea and nitrate salts

Animals and housing

The cattle had an initial weight in the range of 63 to 100 kg. They were confined in separate pens (Photo 2). Vaccination was done against epidemic diseases and drenching against internal parasites before the commencement of the experiment.

Photo 1. Local "Yellow" cattle

Photo 2. The cattle kept in individual pens

Feeding and management

The cattle were gradually introduced to the NPN sources over a period of two weeks. The rice straw is offered at 120% of recorded intake the previous week. It was bought from farmers in the area. The straw was treated with lime ($Ca(OH)_2$) at 4% of DM, dissolved/suspended in water (50% solution), and stored in sealed plastic bags for 14 days before feeding (Photo 3). The NPN sources was dissolved in the diluted molasses and sprayed over the straw prior to each new feed of straw, which was four times per day. Cassava foliage was harvested daily from farmer areas. It was offered two times a day, at 7.00 am and 4.30 pm and put in to individual wood feeders. Water was freely available.

Photo 3. Storage of lime-treated rice straw

Photo 4. Cassava at the time of harvesting the foliage

Data collection and measurements

Feeds offered were weighed before giving them to the cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measure the feed intake. The live weights of the cattle were taken at the beginning, every 2 weeks and at the end of the experiment, using an electronic balance. Samples of rumen fluid were taken by a stomach tube; two hours post feeding in the morning at the end of the experiment for determining rumen ammonia and pH. At the end of the experiment, a sample of mixed eructed and respired gas from each animal was analysed for methane: carbon dioxide ratio using the Gasmet equipment (GASMET 4030; Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland), based on the approach suggested by Madsen et al (2008). The cattle were held for 5 minutes in wooden crates covered with polyethylene film before taking the measurements, so that the gases emitted from the animal could equilibrate with the air in the

box (Photo 5). Samples of air in the animal house were also analyzed for the methane: carbon dioxide ratio.

Photo 5. Wooden crates enclosed in plastic used to house the cattle during the 5 minute period of adaptation/measurement using the GASMET infra-red analyser.

Chemical analysis

Samples of feeds offered and residues were collected every 14 days to determine dry matter (DM), ash, crude protein (CP) following the procedure of Ly and Nguyen Van Lai (1997). Rumen pH was measured immediately after taking rumen fluid from the animal with a digital pH meter. Rumen ammonia was measured by steam distillation and titration with 0.1 N H_2SO_4. Protein solubility was determined by shaking 3 g sample with 100 ml 1M NaCl for 3 hours, filtering through Whatman No.4 filter paper and determining nitrogen in the filtrate.

Statistical analysis

The data were analyzed by the general linear model option of the ANOVA program in the Minitab (2000) software (version 13.31). In the model the sources of variation were blocks, source of NPN, straw treatment, the interaction NPN*straw and error. Live weight gains were calculated from the linear regression of live weight (Y) on days in the experiment (X).

Results and Discussion

Chemical composition of the feeds

The leaves of the fresh cassava had higher DM, CP, Ash, OM and nitrogen solubility than fine stems (Table 1). The DM and crude protein contents of the lime-treated rice straw were lower than was reported by Sangkhom et al (2011) but the solubility of the nitrogen was higher. Two thirds (65.4%) of the fresh cassava foliage was in the form of leaves.

Table 1. Chemical composition of the feeds

	----As per cent in DM----				
	%DM	**CP**	**Ash**	**OM**	**Nitrogen solubility**
Treated-straw	83.3	5.35	11.3	88.7	13.9
Fresh cassava					
Leaves	31.2	23.4	7.54	92.5	35.8
Stem	25.5	17.4	7.28	92.7	29.6
Molasses	73.9	4.95	6.32	93.7	

Feed intake

Intake of rice straw, of total DM and of crude protein was similar on both nitrate and urea treatments (Table 2).

Table 2. Mean values of feed intake for local "Yellow" cattle fed lime-treated rice straw supplemented with fresh cassava foliage

Item	**Urea**	**K-nitrate**	**SEM**	**Prob.**
DM intake, g/day				
Rice straw	2355	2322	93	0.87
Cassava foliage	814	830	18	0.60
Molasses	74	72		
Urea	62			
K-nitrate		210		
Total	3303	3434	109	0.41
DM intake, g/ kg LW	36.2	36.8	1.6	0.67
N*6.25 intake, g/day				
Rice straw	127	126		
Fresh cassava foliage	178	181		
Molasses	3.65	3.65		
Urea	169			
K-nitrate		159		
Total	477	469	4.139	0.19
CP (N*6.25) in DM, %	14.4	13.7	0.026	<0.001

Growth rate

The growth rate was higher for cattle fed potassium nitrate compared with urea (Table 3, Figures 1 and 2). The animals used were of village origin and were immature with a rough condition score of 2 out of a scale of 5. Overall growth rates were lower than reported for Yellow cattle in Cambodia fed similar diets (250 g/day, Seng Mom et al 2001; 243 g/day, Sophal et al 2010). However, initial weights were lower for the "Yellow" cattle in our experiment (83 kg) compared with those used in Cambodia (range of 100 to 127 kg). The data in Figures 5 and 6 show clearly that both relative DM feed intake (as g/kg live weight) and daily live weight gain were increased linearly with increasing initial live weight. Thus cattle that had an initial live weight of 100 kg (as in the Cambodia data) had a growth rate of 205 g/day which is closer to the range of growth rates (243-250 g/day) reported for Yellow cattle in Cambodia (Seng Mom et al 2001; Sophal et al 2010).

DM feed conversion followed the same response pattern as for growth rate with better conversion for the potassium nitrate treatment compared with urea (Table 3; Figure 5Mc). The range of values were similar to those (16 to 26) reported for *Bos indicus* cattle fed similar

diets (Ongol in the case of Keo Sath et al 2010; and Red Sindhi crosses in the case of Tham et al 2010). Seng Mom et al (2001) and Sophal et al (2010) reported a range of DM feed conversion of 13 to 17, better than in the present experiment and probably a reflection of the higher growth rates.

Table 3. Mean values for live weight and conversion ratio in local "Yellow" cattle fed lime-treated straw supplemented with fresh cassava foliage

	Urea	K-nitrate	SEM	Prob.
Live weight, kg				
Initial	82.6	82.5		
Final	95.6	99.0	1.09	0.049
Daily gain, g/day	131	168	10.0	0.032
DM intake, g/day	3303	3434	353	0.41
DM feed conversion	24.9	21.1	0.93	0.02

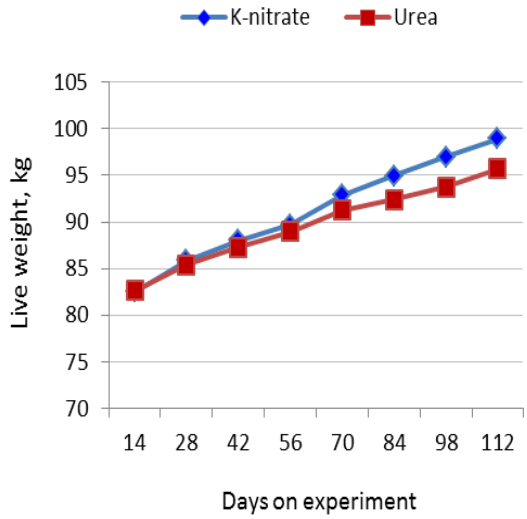

Figure 1. Growth curves of local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea as NPN source

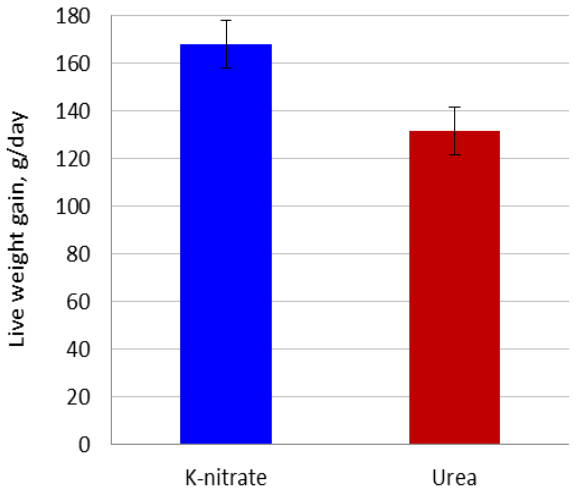

Figure 2. Live weight gain of local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea as NPN source

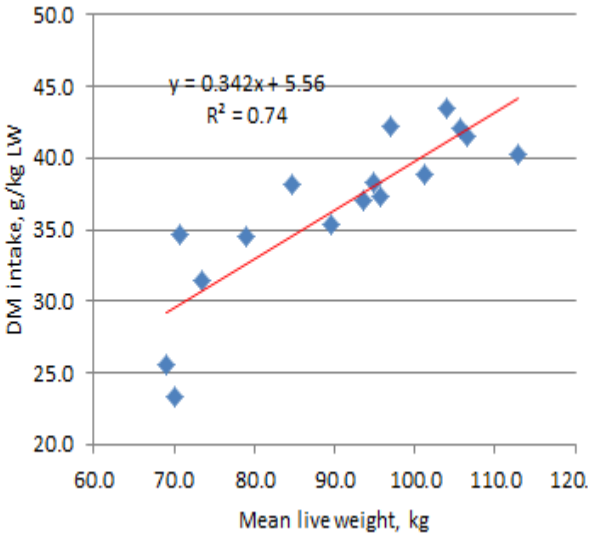

Figure 3. Effect of initial live weight on feed DM intake (as g/kg live weight) by local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea as NPN source

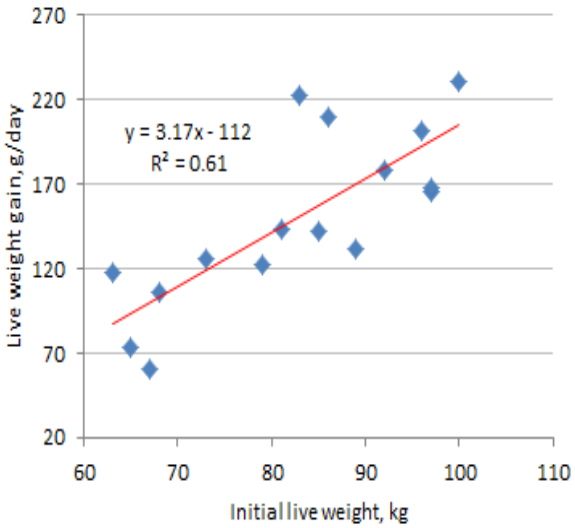

Figure 4. Effect of initial live weight on daily live weight of local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea as NPN source

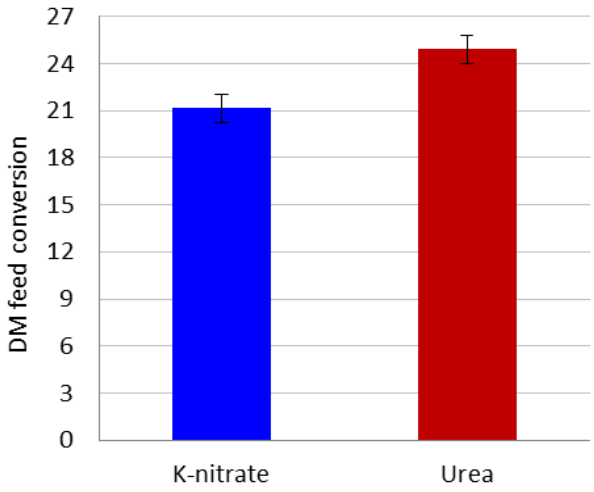

Figure 5. DM feed conversion of local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea as NPN source

Rumen parameters and ratio of methane and carbon dioxide

There was no difference among treatment in the rumen pH value, but the concentration of rumen ammonia was higher when urea rather than potassium nitrate was the NPN source (Table 5). The concentrations of methane to carbon dioxide in the mixed eructed gas and air were decreased by replacing urea with potassium nitrate (Table 4 and Figure 6). The reduction in methane mission due to nitrate was 27%. Similar beneficial effects of dietary nitrate to mitigate methane production in ruminants have been reported by Nolan et al (2010), Van Zijderveld et al (2010a; b), Nguyen Ngoc Anh et al (2010), Hulshof et al (2010) and Do Thi Thanh Van et al (2010).

Table 4. Mean values for rumen pH, ammonia, concentrations of methane and carbon dioxide in mixed eructed gases/surrounding air from local "Yellow" cattle fed lime-treated straw supplemented with fresh cassava foliage and either potassium nitrate or urea as the NPN source

	Urea	**K-nitrate**	**SEM**	**Prob.**
Rumen pH	8.12	8.10	0.046	0.696
NH_3, mg/litre	219	200	4.786	0.015
CO_2	1626	1945	86	
CH_4	109	107	5.1	
CO_2 (air)	430	430		
CH_4 (air)	2.13	2.13		
CO_2 (corrected)	1410	1679	268	
CH_4 (corrected)	107	104	16.9	
CH_4/CO_2	0.0679	0.0498	0.0048	0.02

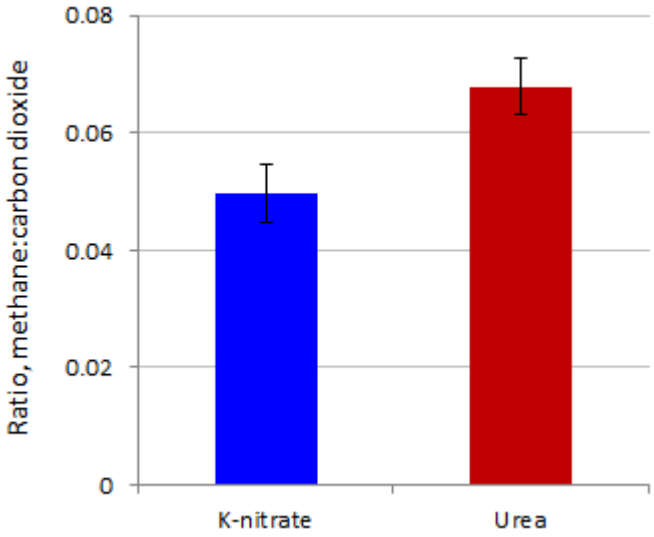

Figure 6. Ratio of methane to carbon dioxide in expired breath of local "Yellow" cattle fed lime-treated rice straw supplemented with cassava foliage and K-nitrate or urea

Despite the expected increased efficiency in using feed energy from incorporation of nitrate salts in ruminant diets, there appear to be no reports in the literature of improved growth and feed conversion from feeding of nitrate salts. It is tempting to hypothesize that the response observed in the present experiment reflected the result of a feeding system planned to optimize rumen function with supplementation of bypass protein, whereas in other reported feeding trials concentrate supplements, rich in maize and soybean meal, or based on maize silage, were the basis of the diets. Such feeding systems improve animal performance but may compromise the efficiency of the ruminant microbial mode of digestion. A response to increased microbial growth efficiency would only be discernible where it improved the N balance of the animal. In much of the high quality feeding systems protein is not limiting and often wastefully metabolized as amino acids in the animal with excretion of urea at an energy cost.

Conclusions

- Local “Yellow “cattle fed lime-treated rice straw and fresh cassava foliage had better growth rate and feed conversion when potassium nitrate replaced urea as the source of NPN.
- The ratio of methane to carbon dioxide in the mixed eructed gas and air was lower for cattle fed potassium nitrate than for those fed urea, the reduction in methane emission being of the order of 27%.

Acknowledgements

This research was done by the senior author with support from NUFU as part of the requirements for the MSc degree in Animal Production "Specialized in Response to Climate Change and Depletion of Non-renewable Resources". The authors acknowledge support for this research from the MEKARN project financed by Sida. Special thanks to Mr Thong vanh Bountum; Mr Keomany and Mr Bounlung Silivong who provided valuable help in the farm. We also thank the staff of Department of Animal Science, Faculty of Agriculture and Forest Resources, Souphanouvong University for providing the facilities to carry out this research.

References

Anon 2007 The conclusion of animal raising system, ministry of Agriculture and forestry, Veterinary and fishery in 2007 and plan in 2008.

Bui Van Chinh, Le Viet Ly, Nguyen Huu Tao, Nguyen Van Hai and Tran Bich Ngoc 2001 Effects of drying, ensiling or urea-treatment on the use of sugarcane leaves as ruminant feed: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 12-19.

Chenost M and Kayouli C 1997 Roughage Utilization in Warm Climates. FAO Animal and Health Paper 135. Rome. http://www.fao.org/docrep/003/w4988e/w4988e00.htm

Do Thi Thanh Van, Duong Nguyen Khang and Preston T R 2010 Methane emission in yellow cattle fed diets based on NaOH treated rice straw, cassava root and leaf meal, supplemented with sodium nitrate or urea as sources of non-protein nitrogen. http://www.mekarn.org/workshops/pakse/abstracts/van.NIAS.htm

Doyle P T, Devendra C and Pearce G R 1986 Rice straw as a feed for ruminants. International Development program of Australian Universities and colleges. Canberra. Australia. 117 P

FAO 2005 FAOSTAT data. Food and Agriculture Organization, Rome http://faostat.external.fao.org/

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. http://www.utafoundation.org/TAP/ TAP33/331.pdf

Hulshof R B A A, Berndt J J, Demarchi A, Gerrits W J J and Perdok H.B 2010 Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based

diets. Greenhouse Gas Conference Banff Canada , page 81 http://www.ggaa2010.org/pdfs/Proceedings_Abstracts.pdf

Iv Sophea and Preston T R 2010 Effect of different ratios of supplementary sodium nitrate replacing urea on growth rates and methane production in goats fed sugar palm-soaked rice straw and mimosa foliage. http://www.mekarn.org/workshops/pakse/abstracts/sophea.htm

Keo Sath, Borin K and Preston T R 2008 Effect of levels of sun-dried cassava foliage on growth performance of cattle fed rice straw. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org./lrrd20/supplement/sath2.htm

Le Thi Thuy Hang, Ngo Van Man and Wiktorsson H 2005 Effects of urea and lime treatment of fresh rice straw on storage stability, and hygienic and nutritional quality. http://www.mekarn.or/msc2003-05/theses05/thuyh_p1.pdf

Leng R A 1997 Tree foliage in ruminant nutrition. FAO, Animals Production and Health Paper. 139 http://www.fao.org/docrep/003/w7448e/w7448e00.htm

Leng R A 2008 The potential of feeding nitrate to reduce enteric methane production in ruminants. Report to Department of Climate Change, Commonwealth Government, Canberra. Available at http://www.penambulbooks. com/Downloads/Leng-Final%20Modified%20% 2017-9-2008. pdf [Verified 17 July 2010]

Leng R A 2010 Further considerations of the potential of nitrate as a high affinity electron acceptor to lower enteric methane production in ruminants. *Livestock Research for Rural Development. Volume 22, Article #221* . Retrieved December 2, 2010, from http://www.lrrd.org/lrrd22/12/leng22221.htm

Leng R A and Preston T R 2010 Further considerations of the potential of nitrate as a high affinity electron acceptor to lower enteric methane production in ruminants. Livestock Research for Rural Development. Volume 22, Article #221. http://www.lrrd.org/lrrd22/12/leng22221.htm

Lewis D 1951 The metabolism of nitrate and nitrite in the sheep; the reduction of nitrate in the rumen of the sheep. Biochem. J.48:175–180.

Ly J and Nguyen Van Lai 1997 Laboratory manual. http://www.mekarn.org/labman/Default.htm

McAllister T A, Bae H D, Jones G A and Cheng K J 1994. Microbial attachment and feed digestion in the rumen. Journal of Animal Science 72, 3004–3018

Minitab 2000 Minitab Software Release 13.

Naparsirth, V, Sommart, K and Polsen, S 2005 Effects of energy feed source in concentrate on voluntary Feed intake, Ruminal Fermentation, Digestibility and Rate of Passage in Beef cattle, Khon Kaen University Research Journals, pp. 23-35.

Nguyen Ngoc Anh, Khuc Thi Hue, Duong Nguyen Khang and Preston T R 2010 Effect of calcium nitrate as NPN source on growth performance and methane emissions of goats fed

sugar cane supplemented with cassava foliage. http://www.mekarn.org/workshops/pakse/abstracts/anh grrc.htm

Nguyen Xuan Trach, Mo M and Cu Zuan Dan 2001 Treatment and supplementation of rice straw for ruminant feeding: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 178-204.

Nolan J V, Hegarty J, Godwin I R and Woodgate R 2010 Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. *ASAP special issue (Volume 50, issue 5-6): Livestock production in a changing environment*

Phonepaseuth Phengsavanh and Ledin Inger 2003 Effect of Stylo 184 (Stylosanthes guianensis CIAT 184) and Gamba grass (Andropogon gayanus cv. Kent) in diets for growing goats; Livestock Research for Rural Development 15 (10). http://www.lrrd.org/lrrd15/10/seut1510.htm

Preston T R and Leng R A 2009 Matching Livestock Systems to Available Resources in the Tropics and Subtropics. Penambul Books Australia. Web versionhttp://www.utafoundation.org/P&L/preston&leng.htm

Sangkhom I, Khang D N, Leng R A and Preston T R 2011 Effect of potassium nitrate as modifier of the fermentation in an *in vitro* incubation using as substrate NaOH and/or lime treated straw supplemented with fresh cassava leaves. Livestock Research for Rural Development. Volume 23, Article #204. Retrieved November 1, 2011, from http://www.lrrd.org/lrrd23/10/sang23204.htm

Seng Mom, Preston T R, Leng R A and Meulen U ter 2001 Response of young cattle fed rice straw to supplementation with cassava foliage and a single drench of cooking oil Livestock Research for Rural Development (13) 4: http://www lrrd org/lrrd13/4/seng134 htm

Sophal C, Borin K and Preston T R 2010 Effects of supplements of water hyacinth and cassava hay on the performance of local "Yellow" cattle fed a basal diet of rice straw. *Livestock Research for Rural Development. Volume 22, Article #166.* Retrieved November 1, 2011, from http://www.lrrd.org/lrrd22/9/soph22166.htm

Sundstol F 1984 Ammonia treatment of straw: method for treatment and feeding experience in Norway. Animal feed science and technology. 60:65 -74 S Sp.

Susuki T, Phawphaisal I, Odai M, Kawamoto H, Ponraagdee W, Pholsen P, Narmsileee R, Indramanee S and Oshio S 2004 Utilization of mixed silage prepared with sugarcane brewer's gain and rice straw. New dimesions and challenges for sustainable livestock farming volume III. Proceeding of the 11th Animal Science Congress The Asian-Australasian of Animal Production Societies. pp. 346-348.

Tham H T, Man N V and Preston T R 2008 Estimates of protein fractions of various heat-treated feeds in ruminant production. Livestock Research for Rural Development. Volume 20, supplement. http://www.lrrd.org/lrrd20/supplement/tham2.htm

Tran Quoc Viet, Dao Duc Kien, Le Viet Ly and Orskov, E. R 2001 Dried Rice straw-chicken litter and urea-treated rice straw as main fodder resources for local cattle in dry

season: Improved utilization of by-products for animal feed in Vietnam. Proceeding NUFU project conference 28-30 March, 2001. pp. 12-19.

Van Zijderveld S M, Gerrits WJ J, Apajalahti J A, Newbold J R, Dijkstra J, Leng R A and Perdok H B 2010a Nitrate and sulfate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science. Volume 93, 5856-5866 http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS0022030210006387.pdf

Van Zijderveld S M, Dijkstra J, Gerrits W J J, Newbold J R and Perdok H B 2010b Dietary nitrate persistently reduces enteric methane production in lactating dairy cows. In Greenhouse gases and animal agriculture conference. October 3-8, 2010 Banff, Canada, T119, page 127 http://www.ggaa2010.org/pdfs/Proceedings_Abstracts.pdf

General conclusions

The research in this thesis was conducted in the laboratory of the Faculty of Agriculture, An Giang University, Vietnam (Experiment 1) and in the laboratory and farm of the Animal Science, Faculty of Agriculture and Forest Resource, Souphanouvong University, Laos (Experiment 2; 3 and 4). All the research was supported by the Mekong Basin Animal Research Network (MEKARN) project. The Norwegian Programme for Development, Research and Education (NUFU) funding the scholarship for the MSc degree at Cantho University.

The experiments were carried out to determine the effect of nitrate and urea as fermentable nitrogen sources on methane production in an *in vitro* system and on growth performance and methane emissions in cattle fed lime-treated rice straw supplemented with fresh cassava foliage.

The results of the experiments are summarized as follows:

- The gas production, and methane percentage in the gas, increased with incubation time and were reduced when nitrate replaced urea at all fermentation stages.
- The methane production per unit of fermented substrate was less when nitrate replaced urea as NPN source
- Treatment with alkali as lime ($Ca(OH)_2$ can replace NaOH as a means of increasing the fermentability of rice straw.
- Protein solubility in cassava petioles was lower than in leaves and decreased in the order: fresh, ensiled, sun-dried and oven-dried.
- The methane percentage in the gas, and methane per unit substrate fermented were reduced when nitrate replaced urea as the NPN source and were lower for fresh and ensiled cassava leaves than for dried leaves at all stages of fermentation time
- Local “Yellow “cattle fed lime-treated rice straw supplemented with fresh cassava foliage had better growth rate and feed conversion when potassium nitrate replaced urea as the source of NPN
- The ratio of methane to carbon dioxide in the mixed eructed gas and air was lower for cattle fed potassium nitrate than for those fed urea, the reduction in methane emission being of the order of 27%.