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**STUDIES ON GROWTH PERFORMANCE AND
METHANE EMISSIONS IN GOATS FED TREE
FOLIAGES**

**MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES
ANIMAL HUSBANDRY**

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COMMITMENT

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. .

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Phonevilay Silivong

Dedication

To

My wife Souksadar Vongyalud
My son Phetsamone Silivong

My families

And

My country

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Contents

Dedication.....	6
Acknowledgment.....	7
Contents.....	8
Abstract.....	11
Paper 1.....	11
Paper 2.....	11
Paper 3.....	11
Paper 4.....	12
Abbreviations.....	13
Contents of the thesis.....	14
Introduction.....	15
Farming systems in Lao PDR.....	15
The role of goats in Lao PDR.....	15
Tree foliages as basal diets for goats.....	16
Climate change, greenhouse gas emissions.....	16
Hypothesis.....	16
Objectives.....	16
Review of literature.....	17
Livestock production systems in Laos.....	17
Potential for goat improvement.....	17
Sources of feed for goat production in Laos.....	18
Tree foliage as feed for goats.....	19
Livestock and climate change.....	20
Conclusions.....	21
References.....	21
Effect of sulphur and calcium nitrate on methane production by goats fed a basal diet of molasses supplemented with Mimosa (<i>Mimosa pigra</i>) foliage.....	25
Introduction.....	25
Material and methods.....	26
Location and duration.....	26
Animal and housing.....	26
Experimental treatments and design.....	27

Measurements	28
Chemical analyses.....	28
Statistical analysis.....	29
Results and discussion	29
Conclusions.....	32
Acknowledgments	32
References.....	33
Effect of supplements of potassium nitrate or urea as sources of NPN on methane production in an <i>in vitro</i> system using molasses and Paper mulberry or Muntingia foliages as the substrate ...	34
Introduction.....	34
Hypotheses.....	35
Materials and methods	35
Location and duration	35
Treatments and experimental design	35
Preparation of substrate and the <i>in vitro</i> system	36
Data collection and measurements.....	37
Chemical analyses.....	37
Statistical analysis.....	38
Results and discussion	38
Chemical composition	38
Gas production and concentration of methane.....	38
Conclusions.....	41
Acknowledgements.....	41
References.....	41
Feed intake, digestibility and N balance of goats fed Paper mulberry (<i>Broussonetia papyrifera</i>) or Muntingia (<i>Muntingia calabura</i>) foliages supplemented with NPN from potassium nitrate or urea.....	43
Introduction.....	43
Material and methods	44
Location and duration	44
Treatments and experimental and design.....	45
Animals and housing.....	45
Feeding and management	46
Measurements	46
Chemical analyses.....	47

Statistical analyses	47
Results and discussion	47
Conclusions.....	49
Acknowledgements.....	49
References.....	50
Effect of potassium nitrate and urea on growth performance of goats fed a basal diet of Paper mulberry (<i>Broussonetia papyrifera</i>) or Muntingia (<i>Muntingia calabura</i>) foliages.....	52
Introduction.....	52
Materials and method	53
Location and duration	53
Treatments and experimental design	54
Individual treatments were:.....	54
Feeding system.....	54
Animals and management.....	54
Measurements	55
Chemical analyses.....	55
Statistical analyses	55
Results and discussion	55
Chemical composition	55
Feed intake, growth rate and feed conversion	55
Rumen ammonia and pH	58
Conclusions.....	58
Acknowledgements.....	59
References.....	59

Abstract

Paper 1

Four weaned crossbred goats (Bach thao x local female), with an initial body weight of 10.5 ± 2.5 kg and 4-5 months of age, were assigned to a 2*2 factorial design in a 4*4 Latin square to compare the effect on rumen methane emissions, digestibility and nitrogen balance on a basal diet of molasses and Mimosa (*Mimosa pigra*) foliage, supplemented with NPN from calcium nitrate or urea, and sulphur (0.8%) from sodium sulphate.

Supplementing the basal diet with calcium nitrate led to a reduction in the methane/carbon dioxide ratio in the eructed breath of the goats compared with control animals supplemented with urea. The addition of sodium sulphate to the diet also reduced the methane/carbon dioxide ratio, with the two supplements having additive effects. Added sulphate increased both digestibility of crude protein and N retention. These criteria were not affected by the NPN source.

Key words: Digestibility, intake, NPN, N retention, sodium sulphate, urea

Paper 2

The aim of this study was to evaluate the effect of potassium nitrate or urea on methane production from Paper mulberry and Muntingia foliages in an *in vitro* incubation system with molasses as the energy source. The incubation was for 24 h with measurements of total gas and percent methane at intervals of 6, 12, 18 and 24 hours and determination of residual unfermented substrate at the end of each interval.

Gas production, percent methane in the gas and methane produced per unit DM solubilized, at each incubation interval, were higher with urea than with potassium nitrate as NPN source. The fermentability of the substrate was higher for Paper mulberry than for Muntingia as the forage source but there were no differences between the two foliages in methane produced per unit substrate DM solubilised.

Keywords: Climate change, fermentation, greenhouse gases, incubation

Paper 3

Four weaned female goats, with an initial body weight of 10 ± 0.5 kg and 4-5 months of age, were assigned to 4 treatments on a 2*2 factorial design in a 4*4 Latin square. The treatments were: foliages of Paper mulberry or Muntingia and potassium nitrate or urea as the source of NPN.

DM intake was higher for Paper mulberry than Muntingia but was not affected by NPN source. Coefficients of apparent digestibility of OM and crude protein, and N retention, were higher for Paper mulberry than Muntingia but were not affected by NPN source. N retention as percent of N digested did not differ between the two foliages, implying that digestibility of the crude protein, rather than its biological value, was the reason for the superiority of the Paper mulberry as a feed for goats.

Key words: Biological value, N retention, protein, trees.

Paper 4

The objective of the present study was to compare the effect of potassium nitrate or urea on the growth of goats given a basal diet of Paper mulberry or Muntingia foliages supplemented with molasses. The experiment was arranged as a 2*2 factorial with sixteen local goats (initial average body live-weight of 13.8 kg and 5-6 months of age) housed in individual cages to give 4 replicates of each treatment. The factors were: iso-nitrogenous source of NPN (6.0% potassium nitrate or 1.83% urea) and source of foliage (Paper mulberry or Muntingia) given at 3% of LW (DM basis). Diluted molasses (Brix value 40) was provided ad libitum.

Feed intake was reduced but rate of live weight gain and DM feed conversion were improved when potassium nitrate replaced urea as the NPN source. DM feed intake was the same but live weight gain was higher with Paper mulberry rather than Muntingia.

Key words: Feed intake, feed conversion, NPN

Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ADG	Average Daily Gain
CRD	Completely Randomized Design
CP	Crude Protein
CO ₂	Carbon Dioxide
DM	Dry Matter
FCR	Feed Conversion Ratio
GDP	Gross Domestic Product
KNO ₃	Potassium Nitrate
LWG	Live Weight Gain
MEKARN	Mekong Basin Animal Research Network
MG	Muntingia
N	Nitrogen
NPN	Non Protein Nitrogen
NH ₃	Ammonia
OM	Organic Matter
pH	Potential of Hydrogen Ion
PM	Paper mulberry
Prob/P	Probability
RCBD	Randomised Complete Block Design
SEM	Standard Error of the Mean
Sida/SAREC	Swedish International Development Agency-Department for Research Cooperation
U	Urea

Contents of the thesis

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

1. **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*. Retrieved January 28, 2012, from <http://www.lrrd.org/lrrd23/3/sili23058.htm>
2. **Silivong P,** Effect of supplements of potassium nitrate or urea as sources of NPN on methane production in an *in vitro* system using molasses and Paper mulberry or Muntingia foliages as the substrate
3. **Silivong P,** Feed intake, digestibility and N balance of goats fed Paper mulberry (*Broussonetia papyrifera*) or Muntingia (*Muntingia calabura*) foliages supplemented with NPN from potassium nitrate or urea
4. **Silivong P,** Effect of urea and potassium nitrate on growth performance of goats fed a basal diet of Paper mulberry (*Broussonetia papyrifera*) or Muntingia (*Muntingia calabura*) foliages

Introduction

Farming systems in Lao PDR

Laos is located in the central part of the Indochinese Peninsula. It is an inland state surrounded by China, Vietnam, Cambodia, Thailand and Myanmar. The land area is about 236,800 km² of which 80% is occupied by mountains and hilly regions. The agricultural land is limited to around 4% of total. The population is approximately 6.5 million with an annual growth rate of 2.3% in 2006. There are three main ethnic categories of people: The Lao Loum constitute 68%, the Lao Theung 22% and the Lao Soung 9%. Laos is one the least densely populated countries in Asia, with 23.3 people per km².

Agriculture generates about half the GDP, and rice and livestock products account for about 4% and more than 30% of the total agricultural production, respectively (STEA 2003). The forests of Lao PDR have very high biodiversity, containing at least 10,000 species of animals, reptiles, amphibians, birds, fish and vascular plants. Laos ranks as one of the biologically richest countries in the region. Approximately 1.5% of the total land area has been set aside for National Biodiversity Conservation level (Linkham et al no date). Approximately 80% of the population is engaged in subsistence farming, rather than commercial agriculture. Farming systems in Laos are mainly rice-based at subsistence level, where livestock production is a supplementary and complementary component of the systems. The lowland areas are predominantly occupied by Lao Lum, who cultivate paddy rice as their main occupation. The farm size averages about 1.9 ha per household with 2.24 tonnes of rice yield or an average of 344 kg of paddy rice per person per year. Apart from crop production, farmers also rear livestock, such as goats, cattle, buffaloes, pigs and poultry. They are reared mainly for monetary security and are an integral part of smallholder farming systems. Over 95% of the livestock is owned by smallholders. They are extremely important for the livelihood of smallholders as a sole means of accumulating assets, earning cash income, and providing draft power and manure for crops.

The role of goats in Lao PDR

Goats in Laos are of the local breed, with small body size, similar to the Katjang breed of goat, which is common throughout Southeast Asia. They reach a mature weight of about 40 kg and are raised for meat. The first kidding is usually at 12-18 months of age with a single kid. The does generally give birth twice a year, with a high incidence of twins after the first parturition. Goats are found more frequently in the upland areas than in lowlands, with the largest concentrations in Oudomxay, Luangprabang, Huaphan and Savannaket provinces. Goats traditionally are allowed to graze freely all year around in small groups in forests, follow cropland, natural grassland and communal land.

Goats play an important role in food production system in almost all developing countries. Their great popularity can be explained by their good adaptation to many different climates and the many uses for which they can be kept. Goats are of high importance to people because of the many functions they provide: they serve as a bank account which can be drawn upon when cash money is needed. Furthermore, goats provide milk and meat which are high-grade foods for

people and contain high quality protein to balance diets based on cereal grains. Goats are much more resistant than cattle; they are small animals and cost less per animal. Each farmer usually owns a number of goats, and goat keeping therefore touches on many people's lives.

Tree foliages as basal diets for goats

The use of foliages from trees and shrubs in animal nutrition has focused the attention of many researchers, due to the fact that these feed resources are locally available, perennial sources of feeds (Singh 1995; Leng 1997), rich in protein and particularly appropriate for small ruminants (Van Eys et al 1986; Robertson 1988; Chen et al 1992; Norton 1994; Kaitho 1997). In this connection, jackfruit (*Artocarpus heterophyllus*), mulberry (*Morus alba*) and cassava (*Manihot esculenta*) are multipurpose plants that could be successfully used in goat production in Laos conditions of animal husbandry, due to the fact that their agronomy is well known in practice by the farmers and, on some occasions, have been given to ruminants for feeding purposes (Theng Kouch et al 2003)

Climate change, greenhouse gas emissions

In a recent review Leng (2007) postulated that nitrate could replace carbon dioxide as an electron acceptor in the rumen with the generation of ammonia instead of methane. In this reaction, nitrate is reduced to nitrite and then to ammonia, resulting in lower methane gas emission. Therefore, it was hypothesized that a nitrate salt could potentially replace urea as a source of non-protein nitrogen because, as with urea, it would provide a fermentable nitrogen source for microbial protein synthesis; it possesses a higher-affinity than carbon dioxide to accept hydrogen, resulting in lower methane production.

Leng (2008) concluded that the presence of nitrate salts in the rumen will act as a competitive sink for the hydrogen produced by fermentation of carbohydrate such that it is converted to ammonia rather than methane.

Leng and Preston (2010) reviewed the research of Van Zijderveld et al (2010) and concluded that: "the CH₄ suppressing effects of nitrate and sulphate were independent and additive".

Hypothesis

It is hypothesized that:

- (i) there will be differences in methane emissions from goats fed different sources of tree foliage
- (ii) on all foliages, potassium nitrate will reduce production of methane as compared with feeding of urea as sources of NPN
- (iii) digestibility, N retention and growth rates of goats will be higher when the NPN source in the diet is potassium nitrate rather than urea

Objectives

The objectives of this study were to measure the methane production, digestibility and growth performance of goats fed basal diets of Paper mulberry and Muntingia foliages supplemented with molasses and nitrate salts or urea.

Review of literature

Livestock production systems in Laos

Agriculture is the mainstay of the economy in Laos. For the rural smallholders, who contribute most of the country's agriculture output, livestock keeping is often a vital source of cash income, a means to accumulate assets and a provider of inputs to crop production. Cattle, buffaloes, pigs, chickens and goats are the most important livestock species in the country. Demand for meat is increasing and there is a growing potential for exporting livestock and their products to neighbouring countries. There is great potential to enable a dynamic development of the livestock and fisheries sub-sector. Relatively extensive land areas throughout the country are well suited to pasture and fodder production. An estimated 7 to 8 million hectares of grazing land and associated water resource are under-utilized, comprising natural grassland, forest, barren lands, inland water resource and reservoirs, of which about two thirds are located in the uplands. It is estimated that in the future ruminant production in upland areas will increase and supply the demand of the lowland and urban areas, as intensification and mechanization in the lowland decrease land availability for grazing.

Livestock in Laos offers today one of the most promising opportunities for Lao farmers and foreign investors to commercialize, with high value products. According to the economic growth in the region, domestic and regional demand for livestock products is expected to increase.

Market demand for rural consumption and export of goat meat is strong, the price per kg of live weight presently being about US\$ 1.7 to 2.0 and goat meat is more expensive than cattle and buffalo meat, which sells at US\$ 1.3 to 1.5 per kg. Recently, according to survey data, the price of live goats traded in Luangprabang province in April 2011 had increased to US\$ 3 per kg live animal. This is one reason for the relatively high rate of increase in the goat population over the last 20 years (Stur et al 2002). The government has adopted a livestock development plan to strengthen and promote animal production and enhance national food security.

Potential for goat improvement

Goat production is important on smallholder farms in developing countries, in SE Asia to provide meat, milk and manure in integrated crop and livestock farming and is one source of income for farmers. However, like cattle and buffalo production, goat production in developing countries in SE Asia are faced with several constraints like a shortage of feed supply because of seasonality, low quality of feeds available. There is also the problem of emissions of greenhouse gases from enteric fermentation.

To make sure that goat production in developing countries in SE Asia will be efficient, sustainable and environmental friendly, more efficient utilization of crop residues and tree/shrub foliages and appropriate feeding methods ensuring efficient rumen functions are needed.

Recently, the use of nitrate salts as feed supplements has been shown to be entirely feasible as a means of providing fermentable nitrogen and simultaneously reducing enteric methane emission from ruminant livestock.

A principal characteristic of the goat is its dietary selectivity, which enables it to survive in apparently harsh environments. Some researchers have pointed out that the goat is more susceptible to parasites than sheep and that its resistance to the humid tropical climate is not as good as is the case for cattle or sheep (Dunn 1978; Baxendell 1984; Restrepo and Preston 1989).

The conventional feeding system in goats in Lao PDR is based mainly on the use of natural grasses. However, in the dry season forage is in short supply as natural pasture becomes dried and improved grasses cannot grow. Therefore, it is important to find an alternative feeding system because purchased supplements are too expensive for poor farmers. On the other hand, there are many trees and shrubs available. Preston and Leng (1987, web version 2009) and Leng (1997) have emphasized that in tropical countries one of the most appropriate ways to improve feed supplies for ruminants is through utilization of tree and shrub foliages.

The great challenge is to make the goats become environmentally friendly through the changing of the free range farming system to stall-feeding systems. Farmers tend to restrict their herds in order to avoid excessive damage to crops, for which the owner is held responsible. In recent years according to Phengsavanh and Ledin (2003), goat management practices have been changing, and vary from site to site depending mostly on land availability, labor and community regulations. Goats are reared only for meat and they reach a mature weight of about 40 kg in 2-3 years under local conditions. First kidding is at 12-18 months of age, usually a single kid at the first litter and twin kids later.

Goats are browsers and highly selective, and they can withstand the harsh conditions such as seasonal shortages of feed which means they are well adapted to the local environment. According to their small body size, they need low capital investment, and need smaller areas to graze than cattle. Local goat breeds have high reproductive rate with a great genetic variability. However, little attention has been given to feeding, management and health so small inputs will probably give good results.

The great potential in the development of goat production in Laos is the high market demand for goat meat for local consumption and export. This is an incentive to intensify goat production as improved nutrition will lead to faster growth rates which will result in carcasses with higher content of meat.

Improved nutrition requires increasing the energy density of the diet, ensuring efficient rumen function and providing a complimentary source of bypass protein (Preston and Leng 2009).

Sources of feed for goat production in Laos

In the traditional feeding systems in tropical countries, the native grasses, legumes and some foliages are the main feed resources for ruminants. Foliages from trees and shrubs are important feeds for grazing and browsing animals and often contain appreciable amounts of nutrients that are deficient in other feed resources (Komwihangilo et al 2001).

Goats have a habit of selecting their feed carefully when eating and are considered to be agile feeders (Dumont et al 1995; Ngwa et al 2000). According to Steele (1996) goats are continuously searching for feed and are more satisfied when they have a whole range of different plants

available including trees, shrubs and grasses. The anatomical characteristics of goat, small mouths and split upper lips, enable them to select even very small parts of a plant. Goats are characterized as generalized feeders since they adapt their choice according to what is available. However, goats are also considered to be very fastidious and even when having a very large selection to choose between they will only consume the most nutritious feed available (Van Soest 1982; Fajemisin et al 1996).

Animal feeding behavior has been the object of numerous studies and there are a number of explanatory theories regarding the principles of herbage selection by grazing animals (Dumont et al 1995). Knowledge of feeding behavior is of fundamental importance in management of pastures, especially with regard to the determination of opportune feeding strategies and the type and quantity of supplements to distribute (Claps et al 1997).

Tree foliage as feed for goats

There is a wide range of trees and shrubs, the foliage of which is suitable for feeding to goats (Daovy et al 2008). *Muntingia calabura* (Jamaica cherry is its common name) is widely grown in Lao PDR as a shade tree around household. It has recently been studied as a possible source of tree foliage for ruminants (Nguyen Xuan Ba and Le Duc Ngoan 2003). Nevertheless, very little is known about the nutritive value of foliage from *Muntingia* for ruminant species. *Muntingia* has mostly been used as a shade tree and sources of fruit. On the other hand, *Muntingia* stems are primarily used for firewood. It ignites quickly, and produces an intensely hot flame with little smoke. It is also used for beautification and shading purposes. It has also been considered for use as paper pulp.

Regarding the agronomy characteristics of *Muntingia*, there is information about the botanical characteristics. *Muntingia* is a tree belonging to the Elaeocarpaceae family. It is a small fast-growing evergreen tree with a dense, spreading crown and drooping branches. It reaches 8 to 13 m in height with a trunk 8.5 to 20 cm in diameter. It bears a cherry-like fruit in 1.5 to 2 years after planting as seed. The leaves are evergreen with a large canopy, alternate, lanceolate or oblong, long-pointed at the apex, oblique at the base; 5 to 12.5 cm long, dark-green and minutely hairy on the upper surface, gray- or brown-hairy on the underside; and irregularly toothed. The flowers, borne singly or in 2's or 3's in the leaf axils, are 1.25-2 cm wide with 5 green sepals and 5 white petals and many prominent yellow stamens. They last only one day, the petals falling in the afternoon. The abundant fruits are round, 3/8 to 1/2 in (1-1.25 cm) wide, with red or sometimes yellow, smooth, thin, tender skin and light-brown, soft, juicy pulp, with very sweet, musky, somewhat fig-like flavor, filled with exceedingly minute, yellowish seeds, too fine to be noticed in eating (Morton 1987).

Muntingia can grow everywhere (sandy land, humid areas, and high land areas) and it is well adapted in the dry season. The fresh biomass yield has been reported to be from 40 to 50 tonnes/ha/year (Nguyen Xuan Ba and Le Duc Ngoan 2003). However, there appears to be little information about the use of *Muntingia* in integrated farming systems. However, it was shown recently to be palatable to goats, supporting intakes of more than 26.9 g of DM/kg LW according to Pok Samkol (2003). Even higher intakes (>40 g DM/kg LW) were reported by Tran Trung Tuan (2008) when *Muntingia* was fed as the sole diet to growing goats.

Paper mulberry (*Broussonetia papyrifera*), a member of the Moraceae family, is a tree that is common in the northern part of Laos growing to 15 metres tall. The leaves are variable shape (even on the same branch), and have a crude protein content of 20% in DM according to Sangkhom and Preston et al (2009). The leaves could be a potential feed resource for goats. Presently the bark of Paper mulberry is used in the handicraft industry to make paper and envelopes. The leaves would be a by-product from this process. Some farmers said the leaves can be used to feed to pigs. It is reported that the twigs and young leaves are consumed by deer (http://en.wikipedia.org/wiki/Paper_Mulberry), so it should be palatable to goats also.

Livestock and climate change

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, goats, 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 is 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% 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 US\$ 50 million by 2020 (Millar and Photakoun 2008).

Since the start of the industrial era in 1750, human activities have contributed to climate change by the liberation of gases, mainly carbon dioxide and methane, causing global warming through the greenhouse effect (IPCC 2007). Livestock contributes about 9% of total carbon dioxide emissions, but 37% of the methane, and 65% of the nitrous oxide (Steinfeld et al 2006). There is therefore a great incentive to reduce methane emissions from livestock.

According to Smith et al (2007) agriculture produces 10-12% of total global anthropogenic greenhouse gas emissions, contributing 50% of all anthropogenic methane (CH₄). Ruminant livestock animals are a major source of total anthropogenic emissions producing an estimated 80 million tonnes of CH₄ annually accounting for 33% of anthropogenic emissions of CH₄ (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).

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). 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). Trinh Phuc Hao et al (2009) showed that nitrate could be safely fed as the major source of fermentable N in a diet of rice straw, provided the animals (goats) were adapted to the diet over a period of 2 weeks.

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. However, there appear to be no reports in goats showing that the reduction in methane is accompanied by more efficient production of meat or milk, which should theoretically happen.

Conclusions

- There is much potential for the use of tree foliages to intensify goat production in Laos
- There are few reports on methane emissions in goats fed forages
- Nitrate salts have potential to reduce the emissions of methane in ruminant live stock.

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Effect of sulphur and calcium nitrate on methane production by goats fed a basal diet of molasses supplemented with *Mimosa (Mimosa pigra)* foliage

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Abstract

Four weaned crossbred goats (Bach thao x local female), with an initial body weight of 10.5 ± 2.5 kg and 4-5 months of age, were assigned to a 2*2 factorial design in a 4*4 Latin square to compare the effect on rumen methane emissions, digestibility and nitrogen balance on a basal diet of molasses and *Mimosa (Mimosa pigra)* foliage, supplemented with NPN from calcium nitrate or urea, and sulphur (0.8%) from sodium sulphate.

Supplementing the basal diet with calcium nitrate led to a reduction in the methane/carbon dioxide ratio in the eructed breath of the goats compared with control animals supplemented with urea. The addition of sodium sulphate to the diet also reduced the methane/carbon dioxide ratio, with the two supplements having additive effects. Added sulphate increased both digestibility of crude protein and N retention. These criteria were not affected by the NPN source.

Key words: Digestibility, intake, NPN, N retention, sodium sulphate, urea

Introduction

Livestock contribute some 18% of greenhouse gases according to Steinfeld et al (2006). Enteric methane from fermentative digestion is the main source of these emissions. There is an urgent need to develop ways of reducing methane emissions from ruminants in order to meet future targets for mitigating global warming. From a survey of the relevant literature, Leng (2008) concluded that the presence of nitrate salts in the rumen will act as a competitive sink for the hydrogen produced by fermentation of carbohydrate such that it is converted to ammonia rather than methane. Trinh Phuc Hao et al (2009) showed that nitrate could be safely fed as the major source of fermentable N provided the animals (goats) were adapted to the diet over a period of 2 weeks. In their experiment, N retention was the same with nitrate as with urea as the source of fermentable N. Results of recent research in Australia (Nolan et al 2010) showed that the production of methane in the rumen gas of sheep fed oat hay was reduced by 25% by feeding potassium nitrate instead of urea as the nitrogen source.

Leng (2008) proposed that the conditions favouring the action of nitrate in reducing methane production were: a highly fermentable source of energy, a protein source which would mainly escape the rumen fermentation (eg: bypass protein; see Preston and Leng 2009) and an additional source of sulphur. Leng and Preston (2010) reviewed the research of Van Zijderveld et al (2010)

and concluded that: “the CH₄ suppressing effects of nitrate and sulphate were independent and additive”.

Molasses is a source of highly fermentable carbohydrate (contains >50% soluble sugars) and is very low in crude protein (<0.5% in DM). When mixed with urea and supplemented with cassava foliage it supported high growth rates in fattening cattle (Ffoulkes and Preston 1978).

Mimosa pigra is an invasive weed of the genus *Mimosa* in the family Fabaceae. This plant is considered to be one of the worst environmental weeds of the Mekong River basin (Storrs et al 2001). In Tram Chim National Park in Dongthap Province in the Mekong delta, there is growing concern over the rapid growth of the *Mimosa pigra* plant, that has taken over more than one seventh of the 7,600 ha of the park (Tran Triet et al 2007; Viet Nam-VNS). However, recent research (Nguyen Thi Thu Hong et al 2008) has shown that *Mimosa* foliage is an excellent feed for goats, supporting growth rates when given as the sole feed of almost 100 g/day. It was hypothesized that the presence of condensed tannins would confer bypass properties on the protein in the *Mimosa* and that this could explain its high nutritive value.

The objective of the present study was to compare the effect on rumen methane emissions, digestibility and nitrogen balance of goats given a basal diet of molasses and *Mimosa* foliage, supplemented with NPN from calcium nitrate or urea and additional sulphur from sodium sulphate.

It was hypothesized that the calcium nitrate would reduce methane emissions and that there would be a synergistic effect of giving additional sulphur.

Material and methods

Location and duration

The experiment was conducted in the experimental farm of An Giang University in Vietnam, from September through October 2010.

Animal and housing

Four crossbred weaned goats (Bach Thao x local female) with initial body weight of 10.5 ± 2.5 kg and 4-5 months of age were used (Photo 1). There were housed individually in metabolism cages made from bamboo (dimensions of width 0.8 m, length 1 m and height 0.8 m) and designed to collect separately feces and urine.



Photo 1. Goats confined in the metabolism cages

Experimental treatments and design

Four treatments were arranged in a 2*2 factorial design in a 4*4 Latin square with 10 days per period: 5 days for adaptation and 5 days for collection of feed refusals, feces and urine. The factors were:

- Source of NPN: Calcium nitrate or urea
- Supplementary sulphur: 0 or 0.8% S in the diet DM as sodium sulphate.

Individual treatments were:

- U: Urea as source of NPN (2% of diet DM)
- US: Same as Urea but with 0.8% added sulphur as sodium sulphate
- CN: N from calcium nitrate (3.8% of diet DM)
- CNS: Same as CN but with 0.8% added sulphur as sodium sulphate

The basal diet was molasses and Mimosa foliage both offered to appetite. The molasses was diluted to 20 Brix (20% DM) before dissolving in it the urea or nitrate and sodium sulphate. The diluted molasses was offered 3 times daily (7.00 am, 12.30 am and 5.00 pm).

Feeding and management

The Mimosa was collected daily from natural stands in the University campus (Photo 2) and was hung in bunches above the feed trough. Molasses was fed in a plastic bucket. Feeds offered and residues were weighed every morning.



Photo 2. *Mimosa pigra*

Measurements

Live weight was recorded in the morning before feeding at the beginning and end of each period. Feeds offered and refusals were collected daily during the 5 days of the collection period. Urine was collected in buckets with 20 ml of a solution of sulphuric acid (10% sulphuric acid concentrate + 90% distilled water). Feces were collected daily and stored at -18°C and, at the end of each period, sub-samples were mixed together and ground with a coffee grinder. Ratio of methane and carbon dioxide was measured at the end of the 2nd and 4th period, 2h after feeding in the morning.

Chemical analyses

The sub-samples of feed offered and refused and of feces were analysed for DM, N and ash according to AOAC (1990) methods. A sample of rumen fluid was taken by stomach tube on the last day of each period 2h after feeding in the morning. The pH was measured immediately with a glass electrode and digital pH meter. A drop of concentrated sulphuric acid was then added to preserve the samples prior to analysis for ammonia by steam distillation (Nguyen Van Lai and Ly 1997). Ratio of methane and carbon dioxide was measured by Gasmeter (Photo 3).



Photo 3. Goats were confined in a closed space for the measurement of the eructed gases with the Gasmeter

Statistical analysis

The data were analyzed by the General Linear Model option in the ANOVA program of the Minitab Software (version13.2). Sources of variation in the model were: treatments, periods, animals and error.

Results and discussion

Crude protein in Mimosa foliage was high contrasting with the very low values in molasses (Table 1).

Table 1. Chemical composition of dietary ingredients (% in DM, except DM which is on fresh basis)

	DM	N*6.25	Ash
Molasses	66.7	4.2	4.6
Mimosa	32.2	24.8	5.8

Intake of mimosa foliage was not affected by sources of NPN or sulphur (Table 2). However, molasses intake was lower when sulphate was fed and when urea rather than calcium nitrate was the source of NPN. Sulphur dioxide is added to sugar cane juice during the extraction of sucrose from sugar cane and ends up as sulphate in the molasses at a final concentration equivalent to about 0.4% S in the molasses. It is possible that the goats reduced their intake of molasses when sulphate was added, due to the increased bitter taste when supplementary sulphate was also given. There is no explanation for the higher intake of molasses when urea was fed unless the goats could detect the taste of the calcium nitrate. Total DM intake was not affected by sulphate supplementation but was higher when urea rather than nitrate was the NPN source.

Table 2. Mean values of feed intake by goats fed molasses and urea (U) or calcium nitrate (CN) with (S) or without (NS) sodium sulphate

	NS	S	Pro	CN	U	Pro	SEM
DM intake, g/day							
Mimosa	269	281	0.35	279	271	0.528	8.71
Molasses	124	99	0.044	76	147	0.001	8.36
Urea	4.9	4.9	0.964	0.0	9.8		
Calcium nitrate	13.2	13.7	0.474	26.9	0.0		
Sodium sulphate	0.0	17.3		8.6	8.7	0.924	0.38
Total	411	416	0.742	390	436	0.004	10.9
DM intake g/kg LW	33.8	34.8	0.36	32.4	36.2	0.001	0.79

Apparent coefficients of digestibility of DM and OM did not differ among treatments (Table 3). In contrast, coefficients for crude protein were higher with supplementary sulphur and for nitrate compared with urea. Daily N retention, and N retention as percent of N intake and of N digested, were higher with added sulphur but were not affected by the NPN source.

Table 3. Mean values of apparent digestibility and N balance by goats fed molasses and urea (U) or calcium nitrate (CN) with (S) or without (NS) sodium sulphate

	NS	S	Pro	CN	U	Pro	SEM
<i>Apparent digestibility, %</i>							
DM	67.6	69.8	0.104	69.6	67.8	0.190	0.93
OM	70.6	72.5	0.105	72.0	71.1	0.430	0.85
N*6.25	59.8	67.5	0.005	67.9	59.4	0.002	1.87
<i>N balance, g/day</i>							
Intake	11.7	11.9	0.690	11.3	12.3	0.032	0.32
Feces	4.6	3.7	0.001	3.4	4.8	0.001	0.14
Urine	2.7	2.2	0.143	2.5	2.4	0.613	0.21
<i>N retention</i>							
g/day	4.5	6.0	0.002	5.4	5.1	0.570	0.33
% of N intake	37.7	49.2	0.001	46.1	40.8	0.082	2.12
% of N digested	61.4	73.1	0.001	66.6	67.9	0.710	2.49

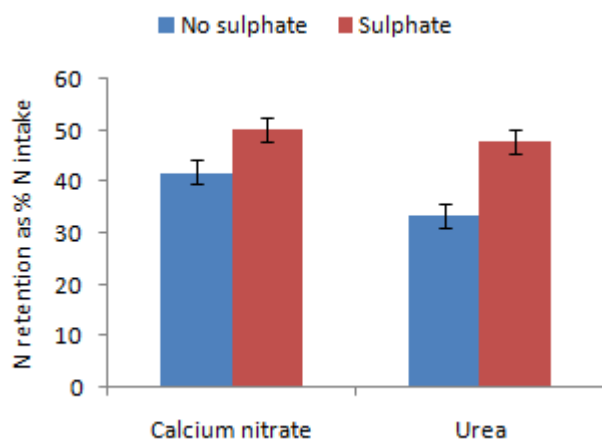


Figure 1. Effect of sodium sulphate on N retention as percent of N intake in goats fed molasses supplemented with Mimosa with calcium nitrate or urea as source of NPN.

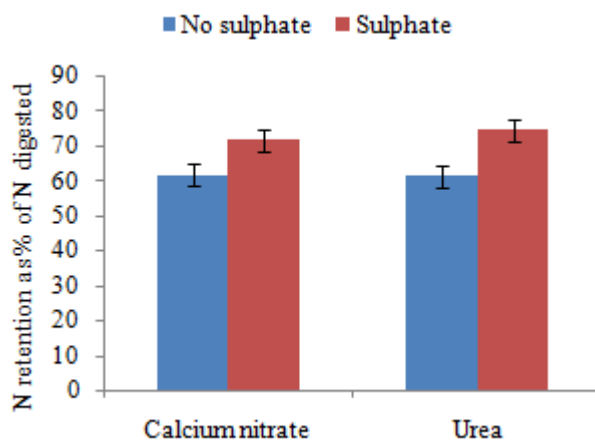


Figure 2. Effect of sodium sulphate on N retention as percent of N digested in goats fed molasses supplemented with Mimosa with calcium nitrate or urea as source of NPN.

There was no difference among treatments in rumen pH and NH_3 , but ratio of methane to carbon dioxide in eructed air was decreased by supplementation with sulphate and by replacing urea with calcium nitrate (Table 4 and Figures 3 and 4).

Table 4. Mean values of rumen traits and CH_4/CO_2

	NS	S	Pro	CN	U	Pro	SEM
Rumen pH	6.30	6.29	0.976	6.26	6.32	0.832	0.20
NH_3 (ml/kg)	251.00	239.00	0.491	33.73	36.61	0.779	7.10
CH_4/CO_2	0.0290	0.0254	0.001	0.0245	0.0299	0.001	0.01

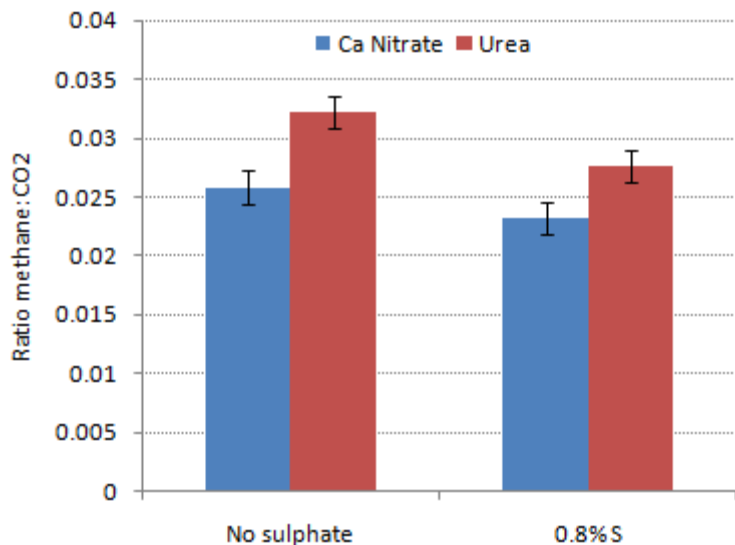


Figure 3. Effect of NPN source on ratio of methane to carbon dioxide in eructed gas from goats fed molasses and Mimosa foliage with or without added sodium sulphate

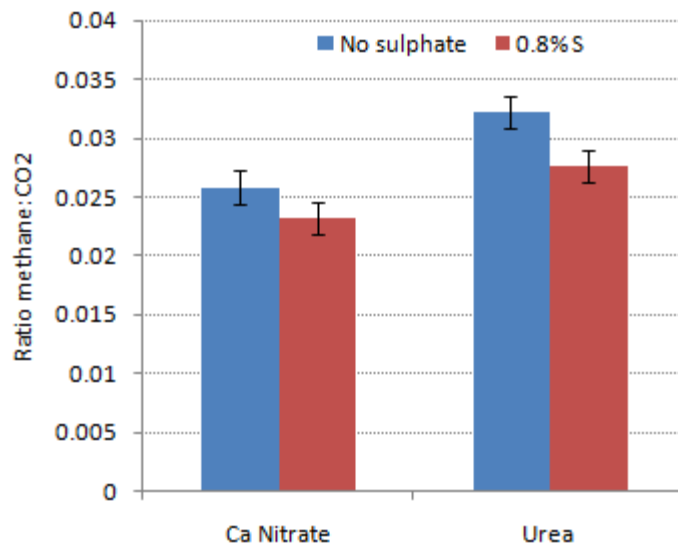


Figure 4. Effect of added sulphate on ratio of methane to carbon dioxide in eructed gas from goats fed molasses and Mimosa foliage with calcium nitrate or urea as source of NPN.

These results agree with the findings of Van Zijderveld et al (2010) who measured methane and carbon dioxide production (in respiration chambers) by sheep fed maize silage supplemented with urea, nitrate and sulphate or nitrate plus sulphate.

Leng and Preston (2010) showed that the observed reduction in methane production in the experiment of Van Zijderveld et al (2010) could be predicted from the change in the ratios of methane and carbon dioxide (Table 5). Applying this same approach to the methane/carbon dioxide ratios recorded in the present experiment showed degrees of reduction of methane broadly similar to those observed by Van Zijderveld et al (2010) (Table 6 and Figure 5) with additive effects for nitrate and sulphate.

Table 5. The relation between the reduction in methane production as calculated from the ratio of methane to carbon dioxide and as measured in calorimeters from repeated gas sampling over 24 hours.

Treatment	Methane /carbon dioxide ratio	Calculated reduction of methane, %	Measured reduction in methane,%
Control	0.058	0	0
+Nitrate	0.049	13	15.5
+Sulphate	0.042	32	27.5
+Nitrate+Sulphate	0.030	47	48.3

Table 6. Mean values for ratio of CH₄/CO₂ in goats fed molasses and Mimosa foliage, with calculated reduction of methane compared with data reported by Zijderveld et al (2010)

	Urea	Sulphate	Nitrate	Sulphate + Nitrate
CH ₄ /CO ₂	0.032	0.028	0.026	0.023
Reduction in CH ₄ , %				
This experiment	0	14.2	23.2	34.9
Zijderveld et al	0	16	32	47

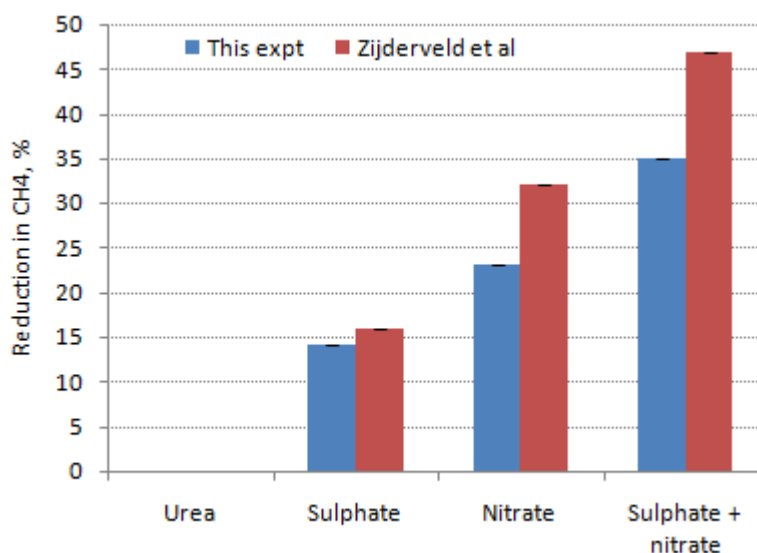


Figure 5. Effect of added sulphate and nitrate and sulphate plus nitrate on percent reduction in methane in the present experiment and that reported by Zijderveld et al (2010).

Conclusions

- The diet of molasses and Mimosa foliage supplemented with calcium nitrate led to a reduction in the methane/carbon dioxide ratio in the eructed breath of goats when compared with control animals supplemented with urea. Sulphur as sodium sulphate at 0.8% level in the diet also reduced the methane/carbon dioxide ratio. While supplementary sulphate increased both digestibility of crude protein and N retention, these parameters were similar when using different source of NPN.

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Effect of supplements of potassium nitrate or urea as sources of NPN on methane production in an *in vitro* system using molasses and Paper mulberry or Muntingia foliages as the substrate

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Abstract

The aim of this study was to evaluate the effect of potassium nitrate or urea on methane production from Paper mulberry and Muntingia foliages in an *in vitro* incubation system with molasses as the energy source. The incubation was for 24 h with measurements of total gas and percent methane at intervals of 6, 12, 18 and 24 hours and determination of residual unfermented substrate at the end of each interval.

Gas production, percent methane in the gas and methane produced per unit DM solubilized, at each incubation interval, were higher with urea than with potassium nitrate as NPN source. The fermentability of the substrate was higher for Paper mulberry than for Muntingia as the forage source but there were no differences between the two foliages in methane produced per unit substrate DM solubilised.

Keywords: Climate change, fermentation, greenhouse gases, incubation

Introduction

In developing improved systems for feeding live stock, account must also be taken of the impacts on the environment. It is estimated that live stock presently account for some 18% of total greenhouses gases which contribute to global warming (Steinfeld et al 2006). Enteric methane from fermentative rumen digestion is the main source of these emissions. There is an urgent need to develop ways of reducing methane emissions from ruminants in order to meet future targets for mitigating global warming. From a survey of the relevant literature, Leng (2008) concluded that the presence of nitrate salts in the rumen will act as a competitive sink for the hydrogen produced by fermentation of carbohydrate such that it is converted to ammonia rather than methane. Recent research has confirmed that nitrate reduces methane production in goats fed sugar cane (Nguyen Ngoc Anh et al 2010) and rice straw (Sophea and Preston 2011) as basal diets.

When urea is fed to cattle it often is recommended to dissolve the urea in molasses as one way to avoid possible toxicity problems with the urea (Preston and Leng 1987, web version 2009). The same idea should be applied to feeding of nitrate which is potentially more toxic than urea (Leng and Preston 2010). Combining foliage from cassava with molasses/urea was shown to support

high growth rates in cattle (Ffoulkes and Preston 1978). However, there are no reports of this kind of feeding system being used for goats.

Muntingia calabura belonging to the family Elaeocarpaceae grows everywhere (sandy land, humid areas, and high land area) and is well adapted to the dry season in Lao PDR. The farmers use it as shade tree around the homestead, and along the roads. It is a tall tree with a large canopy of leaves but it is not normally fed to animals (Nguyen Xuan Ba et al 2003). However, it was shown recently to be palatable to goats supporting intakes of more than 40 g of DM/kg LW DM with a DM digestibility of 68% (Tran Trung Tuan 2008).

Paper mulberry (*Broussonetia papyrifera*) of the family Moraceae is a tree that is common in the northern area of Lao growing to 15 meters tall. The leaves are variable shape (even on the same branch) (http://en.wikipedia.org/wiki/Paper_Mulberry), the leaves of Paper mulberry have crude protein content of 20% in DM according to Sangkhom and Preston (2009). The leaves could be a potential feed resource for goats. Presently the bark of Paper mulberry is used in the handicraft industry to make paper and envelopes. The leaves would be a by-product from this process. Some farmers indicate the leaves can be used to feed to pigs. It is reported that the twigs and young leaves are consumed by deer, so it should be palatable to goats (http://en.wikipedia.org/wiki/Paper_Mulberry).

The purpose of the present study was to use a simple *in vitro* method to determine methane production from a substrate based on molasses as energy supplemented with protein from leaves of Paper mulberry and *Muntingia* and using potassium nitrate and urea as sources of non-protein nitrogen.

Hypotheses

The hypotheses to be tested were:

- Methane production in an *in vitro* system will be reduced when potassium nitrate replaces urea as NPN in substrates of foliage of Paper mulberry or *Muntingia*

Materials and methods

Location and duration

The experiment was carried out at the Animal Science laboratory of the Faculty of Agriculture and Forest Resources, Souphanouvong University, Luang Prabang province, Lao PDR. The experiment was conducted from May to June 2011.

Treatments and experimental design

The experimental design was a 2*2 factorial arrangement of 4 treatments with four replications of each treatment.

The factors were:

Source of NPN:

Urea (U) or potassium nitrate (KN)

Source of foliage:

Paper mulberry (PM) or Muntingia (MG)

Individual treatments were:

- UMG: Urea + Muntingia
- UPM: Urea + Paper mulberry
- KNMG: Potassium nitrate + Muntingia
- KNPM: Potassium nitrate + Paper mulberry

Preparation of substrate and the *in vitro* system

A simple *in vitro* system was used (Photo 1) with recycled plastic bottles as flasks for the incubation and gas collection. The leaves from Paper mulberry and Muntingia foliages were chopped into small piece (2-3mm) and dried in oven at 60°C for 24 h then ground with a coffee grinder, and mixed with the molasses and either potassium nitrate or urea (according to the proportions shown in Table 1). The mixtures (12 g DM) were put in the incubator bottle with 960 ml of buffer solution (Table 2) and 240 ml of rumen fluid from a buffalo. The rumen fluid was taken at 3.00-4.00 am from the slaughter house from a buffalo immediately after the animal was killed. A representative sample of the rumen contents (including feed residues) was put in a vacuum flask and stored until 5.00 am the following morning when the contents were filtered through a layer of cloth before being added to the incubation bottle. The remaining air in the flask was flushed out with carbon dioxide. The incubation flask was connected by a plastic tube to a second flask (a calibrated recycled water bottle with the bottom removed) suspended in water so as to measure the gas production by water displacement. The bottles were incubated at 38°C in a water bath for 24 hours.

Table 1. Composition of diets (% DM basis)

	MG-U	PM-U	MG-KN	PM-KN
Molasses	68.17	68.17	64.0	64.0
Muntingia	30.00		30.0	
Paper mulberry		30.00		30.0
Urea	1.83	1.83		
Potassium nitrate			6.0	6.0
Total	100	100	100	100

Table 2. Ingredients of the buffer solution (g/liter)

CaCl ₂	NaHPO ₄ .12H ₂ O	NaCl	KCl	MgSO ₄ .7H ₂ O	NaHCO ₃	Cysteine
0.04	9.30	0.47	0.57	0.12	9.80	0.25

Source: Tilly and Terry (1963)



Photo 1. The *in vitro* fermentation system using recycled water bottles and water displacement to measure gas production

Data collection and measurements

Incubations were carried out for 6, 12, 18 and 24 h. At the end of each incubation, the methane concentration in the gas was measured with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK; Photo 2). Residual DM in the incubation bottle was determined by filtering the incubation residues through cloth to estimate DM loss during incubation (Photo 3).



Photo 2. Measurement of percentage of methane in the gas



Photo 3. The substrate residue filtered through cloth

Chemical analyses

Samples of Paper mulberry, *Muntingia* foliage and residual substrate were analysed for DM, ash and N according to methods outlined in Ly and Nguyen Van Lai (1997). The residual DM in the incubation bottle was determined by filtering through cloth and drying (70°C for 24 h).

Statistical analysis

The data from the experiment 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, foliage source, interaction NPN*foliages and error.

Results and discussion

Chemical composition

The DM content of Paper mulberry was lower and ash and crude protein higher than in Muntingia (Table 3). The solubility of the protein was relatively low in the leaves of both foliages and similar to levels reported for cassava leaves (Sangkhom et al 2012).

Table 3. The chemical composition of feed (% in DM, except DM which is on fresh basis)

	DM	N*6.25	Ash	Protein solubility, %
Molasses	80.4	5.4	10.5	
Muntingia leaves	44.9	16.6	4.6	12.5
Muntingia stem	37.3	6.8	4.2	
Paper mulberry leaves	29.0	26.7	11.9	19.4
Paper mulberry stem	17.0	15.8	12.4	

Gas production and concentration of methane

Gas production, percent methane in the gas and methane produced per unit substrate solubilized increased with length of incubation (Table 4; Figures 1-4). All of these criteria were lower when the NPN source was potassium nitrate rather than urea but there were no differences between the sources of the leave (Figure 5). The percent of substrate solubilized decreased with incubation time with the rate of decrease apparently greater for nitrate than for urea (Figure 2).

Table 4. Mean value for gas production, percentage of methane in the gas, methane production (ml), DM solubilized and methane production per DM solubilized according to source of NPN and origin of the leaves (Paper mulberry PM; Muntingia MG)

	PM	MG	Prob.	KN	Urea	Prob.	SEM
0-6 hours							
Gas production, ml	990	1079	0.662	812	1257	0.044	139
Methane, %	10.3	9.3	0.005	8.5	11.0	<0.001	0.2
Digested, %	73.1	68.2	0.006	72.6	68.7	0.025	1.05
Methane, ml/g DM solubilised	12.0	13.5	0.573	8.5	16.9	0.005	1.74
0-12 hours							
Gas production, ml	1368	1054	0.166	1011	1411	0.084	150
Methane, %	11.3	10.3	0.233	9.2	12.4	0.001	0.54
Digested, %	79.1	66.9	<0.001	74.7	71.3	0.158	1.60
Methane, ml/g DM solubilised	17.3	15.5	0.637	11.3	21.5	0.019	2.65
0-18 hours							
Gas production, ml	1324	873	0.136	751	1446	0.030	200
Methane, %	12.0	10.8	0.436	9.3	13.5	0.020	1.13
Digested, %	74.2	58.0	<0.001	62.4	69.8	0.049	2.38
Methane, ml/g DM solubilised	19.8	15.3	0.399	10.7	24.4	0.020	3.63
0-24 hours							
Gas production, ml	1551	1366	0.472	1159	1758	0.034	177
Methane, %	13.1	12.5	0.505	10.9	14.8	0.001	0.64
Digested, %	68.6	57.8	0.003	60.2	66.2	0.061	2.03
Methane, ml/g DM solubilised	25.9	26.5	0.918	18.8	33.7	0.023	4.04

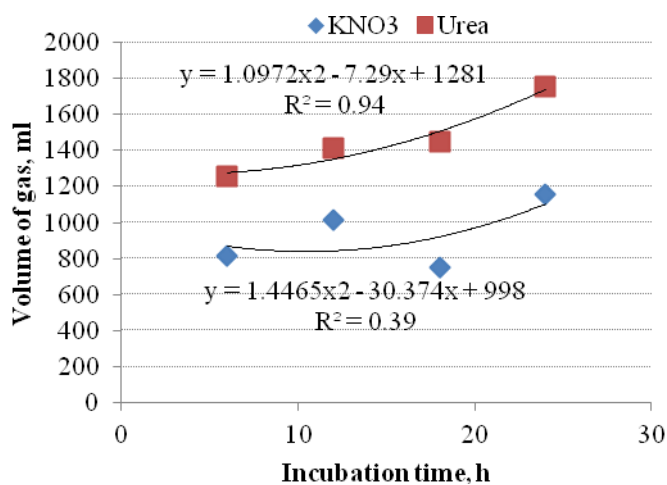


Figure 1. Effect of incubation time on gas production

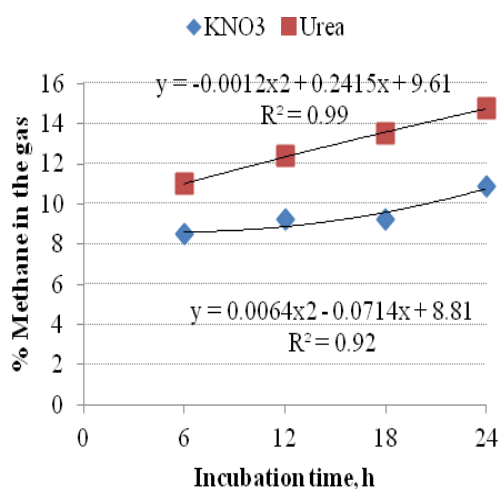


Figure 2. Effect of incubation time on methane content in the gas

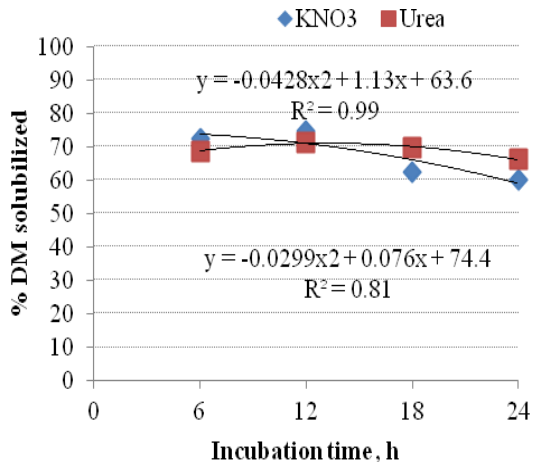


Figure 3. Effect of NPN source and incubation time on percent substrate solubilised

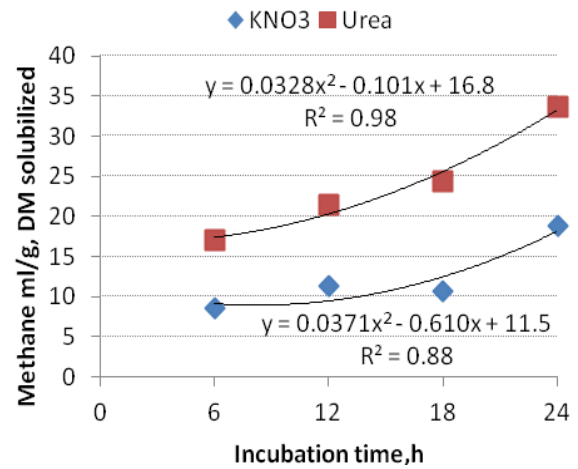


Figure 4. Effect of NPN source and incubation time on methane production per unit substrate solubilised.

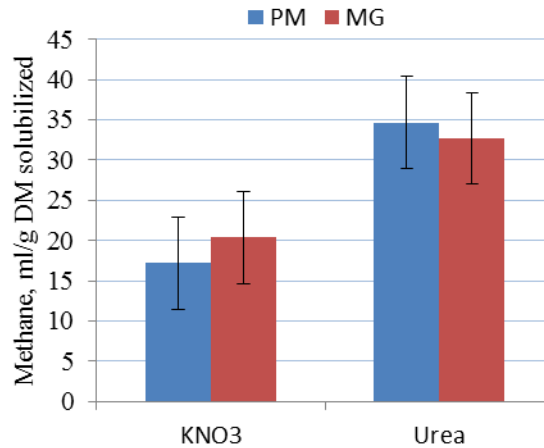


Figure 5. Effect of NPN source on methane production per unit substrate solubilized from Paper mulberry (PM) and Muntingia (MG) leaves.

The reduction in methane production per unit substrate solubilized, with nitrate compared with urea, are similar to those reported by Outhen et al (2011), Inthapanya et al (2011), Binh Phuong et al (2011) and Thanh et al (2011) who used a similar *in vitro* system but with different substrates. The fact that there were no differences between the leaves from Muntingia and from Paper mulberry suggests that the two sources of leaves had similar characteristics in terms of content, or absence, of anti-nutritional compounds such as cyanogenic glucosides, tannins or saponins, compounds which are known to affect methanogenesis in *in vitro* incubations (Cuzin and Labat 1992; Thanh et al 2011; Inthapanya et al 2012).

The increase in methane production with length of incubation is also in agreement with other reports from similar *in vitro* systems (Outhen et al 201; Sangkhom et al 2011; Binh Phuong et al 2011 and Thanh et al 2011). However, the slight reduction in substrate solubilized with length of incubation is in contrast with the earlier findings in the same *in vitro* system (Outhen et al 2011; Sangkhom et al 2011; Binh Phuong et al 2011 and Thanh et al 2011). We have no explanation for this effect.

Conclusions

- Increased time of incubation increased gas production, methane concentration in the gas and methane produced per unit substrate solubilized. Gas production, methane concentration in the gas and methane produced per unit substrate solubilized were reduced when urea was replaced by potassium nitrate.
- Methane production from Paper mulberry and Muntingia diets was similar.

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Feed intake, digestibility and N balance of goats fed Paper mulberry (*Broussonetia papyrifera*) or Muntingia (*Muntingia calabura*) foliages supplemented with NPN from potassium nitrate or urea

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Abstract

Four weaned female goats, with an initial body weight of 10 ± 0.5 kg and 4-5 months of age, were assigned to 4 treatments on a 2*2 factorial design in a 4*4 Latin square. The treatments were: foliages of Paper mulberry or Muntingia and potassium nitrate or urea as the source of NPN.

DM intake was higher for Paper mulberry than Muntingia but was not affected by NPN source. Coefficients of apparent digestibility of OM and crude protein, and N retention, were higher for Paper mulberry than Muntingia but were not affected by NPN source. N retention as percent of N digested did not differ between the two foliages, implying that digestibility of the crude protein, rather than its biological value, was the reason for the superiority of the Paper mulberry as a feed for goats.

Key words: Biological value, N retention, protein, trees.

Introduction

Livestock production plays an important role for the livelihood of farmers in rural areas in developing countries and this is also the case in Lao PDR. The main part of the cash income and the protein consumption in the households comes from livestock, and livestock can also provide organic fertilizer for crop production. Farmers in the lowland and upland areas keep many animal species such as cattle, buffaloes, goats, pigs and poultry (Phengsavanh 2003; Xaypha 2005). Ruminants provide a large part of the cash income for smallholder farmers and can also be used for draught power. The production of meat and milk from ruminants is generally based on traditional management methods using free range grazing systems.

Goats produce of meat, milk, skins and fiber for sale or family consumption. They have an ability to survive on low quality feeds or in difficult conditions on relatively small amounts of feed and they also have a higher reproduction rate compared to cattle (Steel 1996), with 1.4 to 1.9 litter per year and 1.7 to 1.9 kids per litter (Xaypha 2005). Goat production can be found everywhere in Laos at present, but the number of animals per household is small and the breeds

used are local breeds with small body size, low growth rates and poor productivity (Phimpachanhvongsod 2001).

According to Simbaya (2002) and Phengsavanh (2003) the major feed resources for the ruminants in Laos are native grasses, legumes and fodder tree leaves that are available around the farms and in the forest. These feeds generally have a high content of fiber and low content of protein, minerals and vitamins. Many researchers have found that foliages from fruit trees and other trees, legumes and some crops are important protein sources, with high intake and digestibility and offering them as feeds results in good growth performance in goats (Daovy et al 2009; Phengvichith and Preston 2011; Kounnavongsa et al 2010). In almost all provinces in Lao PDR, the main constraints to ruminant production are diseases and lack of feed, especially in the dry season (ADB 2001; Phengsavanh 2003; Phimpachanhvongsod et al 2004; Xaypha 2005).

Paper mulberry (*Broussonetia papyrifera*) is a tree that is common in the northern area of Laos growing to 15 meters (tall). The leaves of Paper mulberry have crude protein content of 19.7% in DM according to Sangkhom and Preston et al (2009). The leaves could be a potential feed resource for goats. Presently the bark of Paper mulberry is used in the handicraft industry to make paper and envelopes. The leaves would be a by-product from this process. Some farmers said the leaves can be used to feed to pigs. It is reported that the twigs and young leaves are consumed by deer (http://en.wikipedia.org/wiki/Paper_Mulberry), so it should be palatable to goats also.

Muntingia calabura belonging to the family Elaeocarpaceae grows everywhere (sandy land, humid areas, and high land area) and is well adapted to the dry season in Lao PDR. The farmers use it as shade tree around the homestead, and along the roadside. It is a tall tree with a large canopy of leaves but it is not normally fed to animals (Nguyen Xuan Ba et al 2003). Very little is known about the nutritive value of foliage from *Muntingia* for ruminant species. Pok Samkol (2003) reported that the foliage was palatable to goats; and that DM intake was higher when the foliage was offered hanging in the feed trough (33.5 g/kg LW) compared with giving the leaves alone (26.9 g/kg LW).

In an *in vitro* study (Phonevilay Silivong et al 2012), it was shown that gas production and methane produced per unit substrate solubilized was the same when leaves from Paper mulberry or *Muntingia* were the major sources of protein, and that with both foliages supplementation with potassium nitrate reduced methane production compared with urea as NPN source.

The hypothesis to be tested was:

- Feed intake, digestibility and N balance of goats fed Paper mulberry (*Broussonetia papyrifera*) or *Muntingia* (*Muntingia calabura*) foliages will be increased when the foliages are supplemented with potassium nitrate rather than urea

Material and methods

Location and duration

The experiment was carried out at the Animal Science farm of Souphanouvong University, which is located about 7.5 km from Luang Prabang district, Laos at an altitude of 1.000 m above sea level. The experiment was conducted from April to June 2011.

Treatments and experimental and design

Four growing goats were used to compare four treatments arranged in a 2*2 factorial design within a 4*4 Latin square with 10 days per period: 5 days for adaptation and 5 days for collection of feed refusals, feces and urine.

The factors were:

Source of NPN: Urea (U) or potassium nitrate (KN)

Source of foliage: Paper mulberry (PM) or Muntingia (MG)

Individual treatments (Table 1) were:

- MG-U: Urea (1.83% of diet DM) + Muntingia
- PM-U: Urea (1.83% of diet DM) + Paper mulberry
- MG-KN: Potassium nitrate (6% of diet DM) + Muntingia
- KN-PM: Potassium nitrate (6% of diet DM) + Paper mulberry

Table 1. The layout of the experiment

Period	Goat 1	Goat 2	Goat 3	Goat 4
1	MG-U	PM-U	MG-KN	KNPM
2	KNPM	MG-U	PM-U	MG-KN
3	MG-KN	KNPM	MG-U	PM-U
4	PM-U	MG-KN	KNPM	MG-U

Animals and housing

Four female weaned goats (local breed) with initial live-weight of 10 ± 0.5 kg and 4-5 months of age were used. These animals were purchased from farmers around the Luang Prabang district. They were housed individually in metabolism cages made from bamboo (dimensions of width 0.8 m, length 0.9 m and height 0.9 m) and designed to collect separately feces and urine (Photo 1). The goats were vaccinated against Pasteurellosis and foot and mouth disease and treated with Ivermectin (1ml/33 kg live weight) to control internal and external parasites. They were gradually introduced to the cages and diets over 7 days before beginning the experiment.



Photo 1. Goats confined in the metabolism cages

Feeding and management

Molasses diluted to a Brix (content of sugars) of 40 was used as the carrier for the NPN sources. The diluted molasses was fed in a plastic bucket and hung above the feed trough and was given ad libitum. The *Muntingia* and Paper mulberry foliage (Photo 2 and Photo 3) were collected daily from natural stands in the University campus and were fed at 3% of live weight on DM basis. They were hung in bunches above the feed trough. Feeds offered and residues were weighed every morning.



Photo 2. *Muntingia Calabura*



Photo 3. Paper mulberry

Measurements

Live weight was recorded in the morning before feeding at the beginning and at the end of each period. Feeds offered and refusals were collected daily during the 5 days of the collection period. Urine was collected in buckets with 20 ml of a solution of sulphuric acid (10% sulphuric acid concentrate + 90% distilled water). Feces were collected daily and stored at -18°C and at the end of each period, sub-samples were mixed together and ground with a coffee grinder.

Chemical analyses

The sub-samples of feed offered, refused and of feces were analysed for DM, N and ash according to AOAC (1990) methods. Urine was analysed for nitrogen by AOAC (1990) procedure

Statistical analyses

The data were analyzed by the General Linear Model option in the ANOVA program of the Minitab (2000). Sources of variation in the model were: goats, treatments, periods, NPN source, foliage source and error.

Results and discussion

Leaves and stems were higher in crude protein and lower in DM in Paper mulberry than in Muntingia (Table 2). Compared to other published data, the DM and crude protein of Muntingia in the leaves and stem were higher than the value (33.3% and 34.8% DM and 13% and 5% CP in DM) reported by Sitone et al (2011). DM and crude protein levels of Paper mulberry leaves and stems were higher than the values (28.4% and 17.7% for DM and 16% and 8.4% for CP) reported by Sangkhom and Preston et al (2009).

Table 2. Chemical composition of dietary ingredients (% in DM, except DM which is on fresh basis)

	DM	N*6.25	Ash
Molasses	80.3	5.4	10.5
Muntingia leaves	44.8	16.6	4.6
Muntingia stem	37.3	6.8	4.2
Paper mulberry leaves	29.1	26.7	11.9
Paper mulberry stem	17.1	15.8	12.5

Intakes of Paper mulberry and Muntingia foliages were not affected by NPN as potassium nitrate or urea. Total DM intake as g/kg LW was higher when nitrate rather than urea was the NPN source (Table 3). The foliages represented 60-70% of the total DM intake (Figure 1).

Table 3. Mean values of feed intake by goats fed Paper mulberry (PM) or Muntingia (MG) supplemented with potassium nitrate (KN) or urea (U)

	PM	MG	Pro	KN	U	Pro	SEM
DM intake, g/day							
Molasses	97.2	82.4	0.124	89.2	90.4	0.90	6.7
Paper mulberry	214.0	0.0	<0.001	108.0	105.0	0.72	6.08
Muntingia	0.0	234.0	<0.001	121.0	114.0	0.41	6.07
Potassium nitrate	8.8	9.2	0.66	18.0	0.0		
Urea	2.8	2.9	0.80	0.0	5.7		
Total	322.0	329.0	0.68	336.0	315.0	0.18	11.21

DM intake g/kg LW 31.3 30.8 0.70 32.4 29.7 0.053 0.97

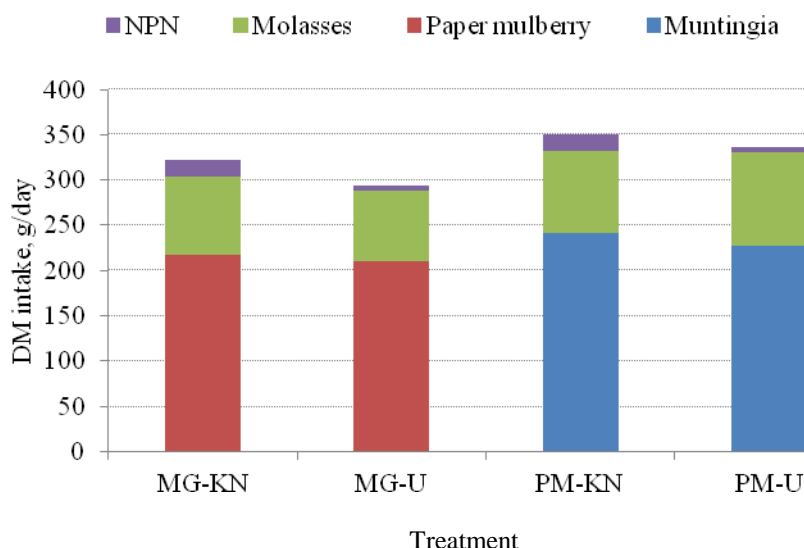


Figure 1. DM intake of goats fed Paper mulberry (PM) or Muntingia (MG) supplemented with potassium nitrate (KN) or urea (U).

Apparent coefficients of digestibility of OM and crude protein were higher for Paper mulberry than Muntingia, but there was no difference in DM digestibility (Table 4). Digestibility coefficients did not differ between the two sources of NPN. Daily N retention and N retention as percent of N intake were higher for Paper mulberry than Muntingia but there was no difference for N retained as percent of N digested (Table 4). Daily N retention and N retention as percent of N intake did not differ between sources of NPN but N retention as percent of N digested was higher for potassium nitrate than urea.

Table 4. Mean values of apparent digestibility and N balance by goats fed Paper mulberry (PM) or Muntingia (MG) supplemented with molasses and potassium nitrate (KN) or urea (U)

	PM	MG	Pro	KN	U	Pro	SEM
<i>Apparent digestibility, %</i>							
DM	75.7	75.0	0.742	73.8	76.9	0.151	1.51
OM	81.4	77.6	0.029	78.3	80.7	0.18	0.02
N*6.25	73.6	58.7	<0.001	65.1	67.1	0.309	1.38
<i>N balance, g/day</i>							
Intake	11.8	9.4	<0.001	10.4	10.7	0.308	0.22
Feces	2.1	3.2	<0.001	2.8	2.5	0.291	0.16
Urine	3.1	1.9	<0.001	2.1	3.0	<0.001	0.17
<i>N retention</i>							
g/day	6.6	4.3	<0.001	5.6	5.2	0.239	0.21
% of N intake	56.2	46.2	0.001	53.3	49.1	0.148	2.05
% of N digested	68.1	69.2	0.673	73.0	64.3	0.001	1.80

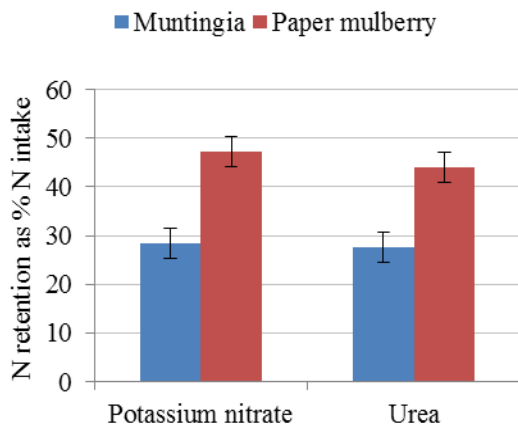


Figure 2. Effect of potassium nitrate or urea on N retention as percent of N intake by goats fed Paper mulberry or Muntingia supplemented with potassium nitrate or urea.

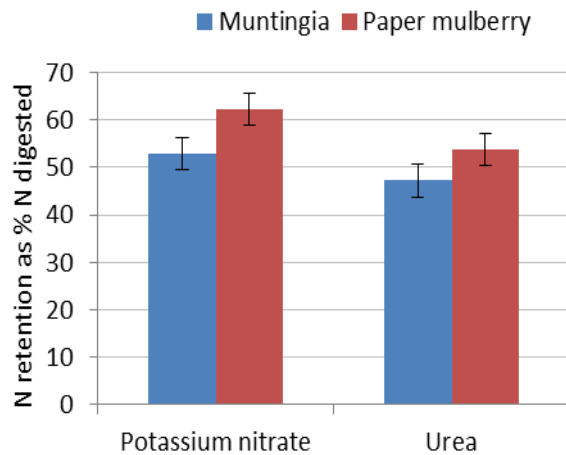


Figure 3. Effect of potassium nitrate or urea on N retention as percent of N digested by goats fed Paper mulberry or Muntingia supplemented with potassium nitrate or urea.

The higher ash content of the Paper mulberry compared with Muntingia would seem to explain the lack of difference between the foliages in apparent DM digestibility and the higher value for the former when the criterion was the apparent digestibility of the OM. The differences between the two foliages in daily N retention and N retention as percent of N intake, in favour of Paper mulberry, appears to be the result of the higher crude protein digestibility in this foliage. When N retention was expressed as percent of N digested there were no differences between the two foliages, implying that the biological value of the protein was the same in both.

Conclusions

- DM intake as g/kg LW, and coefficients of apparent digestibility of OM and crude protein of goats fed Paper mulberry were higher than those of goats fed Muntingia but were not affected by NPN source
- Daily N retention and N retention as percent of N intake of goats fed Paper mulberry were higher than these of goats fed Muntingia; however, N retention as percent of N digested between the two foliages was similar. It seems that Paper mulberry has overall superior nutritive value because of higher OM digestibility and higher crude protein content.

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Effect of potassium nitrate and urea on growth performance of goats fed a basal diet of Paper mulberry (*Broussonetia papyrifera*) or Muntingia (*Muntingia calabura*) foliages

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Abstract

The objective of the present study was to compare the effect of potassium nitrate or urea on the growth of goats given a basal diet of Paper mulberry or Muntingia foliages supplemented with molasses. The experiment was arranged as a 2*2 factorial with sixteen local goats (initial average body live-weight of 13.8 kg and 5-6 months of age) housed in individual cages to give 4 replicates of each treatment. The factors were: iso-nitrogenous source of NPN (6.0% potassium nitrate or 1.83% urea) and source of foliage (Paper mulberry or Muntingia) given at 3% of LW (DM basis). Diluted molasses (Brix value 40) was provided ad libitum.

Feed intake was reduced but rate of live weight gain and DM feed conversion were improved when potassium nitrate replaced urea as the NPN source. DM feed intake was the same but live weight gain was higher with Paper mulberry rather than Muntingia.

Key words: Feed intake, feed conversion, NPN

Introduction

Livestock are an important component of smallholder farming systems in Laos with sales which account for more than 50% of the family cash income. Livestock provide great benefits to farmers such as high nutritive value food, generating income and manure which is very important for maintaining soil fertility. They also provide draught power for transportation and crop production. They serve as financial assets so livestock offer an alternative for storing their savings or accumulated capital as a "living savings account". Keeping livestock is considered as an alternative form of insurance; they also enhance family status because the value of livestock can be an indicator of social importance. Over 95% of all livestock in Lao PDR is produced by smallholders.

The goat population in Laos is estimated to be about 268,900 heads (DLF 2007). Farmers tend to restrict their herds in order to avoid excessive damage to crops, for which the owner is held responsible. The great challenge is to make the goats become environmentally friendly through the changing of the free range farming system to stall feeding system.

A principal characteristic of the goat is its dietary selectivity, which enables it to survive in apparently harsh environments.

The conventional feeding system in goats in Lao PDR is based mainly on the use of natural grasses. However, in the dry season is difficult for forage production, as natural pasture becomes dried and improved grasses cannot grow. Therefore, it is important to find out an alternative for feeding system because purchased supplements are so expensive for poor farmers. On the other hand, there are many trees and shrubs available. Preston and Leng (1987, web version 2009) and Leng (1997) have emphasized that in tropical countries one of the most appropriate ways to improve feed supplies for ruminants is through utilization of tree and shrub foliages.

Paper mulberry (*Broussonetia papyrifera*) of the family Moraceae is a tree that is common in the northern area of Laos growing to 15 meters tall. The leaves of paper mulberry were reported to have a crude protein content 19.7% in DM according to Sangkhom and Preston et al (2009).

Muntingia (*Muntingia calabura*) belonging to the family Elaeocarpaceae grows everywhere (sandy land, humid areas, and high land area) and is well adapted to the dry season in Lao PDR. The farmers use it as shade tree around the homestead, and along the roads. It is a tall tree with a large canopy of leaves but it is not normally fed to animals (Nguyen Xuan Ba et al 2003). However, it was shown recently to be palatable to goats supporting intakes of more than 40 g of DM/kg DM with a DM digestibility of 68% (Tran Trung Tuan 2008).

In a previous study (Phonevilay et al 2011) it was shown that supplementing a foliage diet fed to goats with a nitrate salt led to reduced emissions of methane in the eructed gases, compared with feeding urea as the NPN source. An *in vitro* study (Phonevilay et al 2012), also showed that methane production was reduced by incorporating nitrate rather than urea in the substrate was based on leaf meals from Paper mulberry and Muntingia.

When these two foliages were compared in a digestibility and N balance experiment (Phonevilay et al 2012), the Paper mulberry was found to support higher feed intake and N retention than the Muntingia, probably because of the higher crude protein digestibility in the Paper mulberry.

The aim of the present study was therefore to determine if the apparently superior nutritive value of Paper mulberry over Muntingia would be reflected in better growth rates when potassium nitrate rather than urea was added to the diets as a source of NPN.

Materials and method

Location and duration

A feeding trial with growing goats was carried out at the Animal Science farm of Souphanouvong University, which is located about 7.5 km from Luang Prabang district, Laos at an altitude of 1000 m above sea level. The experiment was conducted from July to November 2011.

Treatments and experimental design

The experiment was arranged as a 2*2 factorial in a Randomized Block Design (RBD) with 4 treatments and 4 replicates of each treatment.

The treatments were:

- Source of NPN: Urea (U) or potassium nitrate (KN)
- Source of foliage: Muntingia (MG) or Paper mulberry (PM)

Individual treatments were:

- U-MG: Urea + Muntingia
- U-PM: Urea + Paper mulberry
- KN-MG: Potassium nitrate + Muntingia
- KN-PM: Potassium nitrate + Paper mulberry

Feeding system

Molasses diluted to 40 Brix was fed as a carrier for the NPN and was given ad libitum. The Paper mulberry and Muntingia foliages were collected daily from natural stands in the University campus and offered at 3% of LW (DM basis). They were hung in bunches above the feed trough. Fresh feeds were offered twice per day at 07:00 h and 16:00 h.

Animals and management

Sixteen local weaned goats with initial average body weight of 13.8 kg and 5-6 months of age were used. They included 4 males (non-castrated) and 12 females. These animals were purchased from the farmers around Luangprabang district. They were housed individually in cages made from bamboo (dimensions of width 0.8 m, length 0.9 m and height 0.9 m) (Photo 1). They were vaccinated against Pasteurellosis and foot and mouth disease and treated with Ivermectin (1 ml/33 kg LW) to control parasites. They were adapted to the pens and the feeds for 14 days before starting the experiment. The experiment lasted 114 days, including the adaptation period.



Photo 1. Goats confined in the cages

Measurements

Live weight was recorded in the morning before feeding at the beginning and at the end of the experiment and at intervals of 10 days during the experiment. Quantities of feed offered and residues were recorded daily. Every 10 days samples were taken for analysis of DM and N. Samples of Paper mulberry and Muntingia foliages offered and residues were separated into stem and leaves (containing attached petioles). Representative samples of each component were analyzed for DM, N and ash. Samples of rumen fluid were taken at the last day of the experiment, using a stomach tube. pH was measured on the fresh rumen fluid. A drop of concentrated sulphuric acid was added prior to determination of ammonia by steam distillation.

Chemical analyses

The sub-samples of feed offered and refused were analysed for DM, N and ash according to AOAC (1990) methods. pH was measured by digital pH meter. Samples of rumen fluid were analysed for ammonia by steam distillation (Nguyen Van Lai and Ly 1997).

Statistical analyses

The data were analyzed by the GLM option of the ANOVA program in the Minitab (2000) software. In the model the sources of variation were NPN source, foliage source, interaction NPN*foliage and error.

Results and discussion

Chemical composition

Leaves and stems were higher in crude protein and lower in DM in Paper mulberry than in Muntingia (Table 1).

Table 1. The chemical composition of the feeds (% in DM, except DM which is on fresh basis)

	DM	N*6.25	Ash	N solubility
Molasses	80.4	5.4	10.5	-
Muntingia leaves	41.0	16.6	5.9	12.5
Muntingia stem	36.8	6.8	4.9	-
Paper mulberry leaves	27.3	19.4	11.6	19.4
Paper mulberry stem	18.1	15.8	13.9	-

Feed intake, growth rate and feed conversion

The foliages accounted for some 75% of the dietary DM intake (Figure 1). DM feed intake was higher on Paper mulberry than on Muntingia and higher for urea than for nitrate (Table 3; Figures 2 and 3). Daily live weight gain was higher in goats fed Paper mulberry than those fed Muntingia; and higher for goats supplemented with nitrate compared with those given urea

(Table 4; Figures 4 and 5). DM feed conversion did not differ between foliages but tended to be better for nitrate compared with urea (Figures 6 and 7).

Table 2. Mean values of feed intake by goats fed Paper mulberry (PM) or Muntingia (MG) supplemented with molasses and potassium nitrate (KN) or urea (U)

	PM	MG	Pro	KN	U	Pro	SEM
DM intake, g/day							
Molasses	85.0	102	<0.001	52.8	134	<0.001	1.98
Paper mulberry	370	0.0	<0.001	192	177	<0.001	2.77
Muntingia	0.0	332	<0.001	172	159	<0.001	2.31
Potassium nitrate	14.3	13.4	<0.001	27.7	0.0		
Urea	4.1	4.0	0.78	0.0	8.1		
Total	473	451	<0.001	445	479	<0.001	4.56
DM intake g/kg LW	30.8	30.2	0.01	28.6	32.4	<0.001	0.18

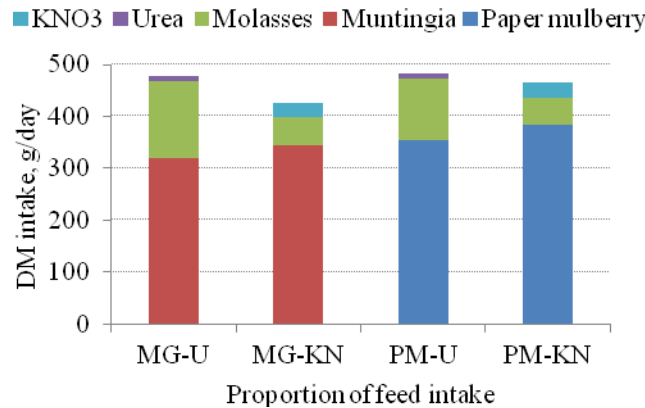


Figure 1. DM intake of goats fed Paper mulberry (PM) or Muntingia (MG) supplemented with molasses and potassium nitrate (KN) or urea (U).

Table 3. Feed consumption and live weight changes of growing goat during the experiment

	PM	MG	Pro	KN	U	Pro	SEM
Live weight, kg							
Initial	12.6	12.6	0.961	12.7	12.5	0.927	1.23
Final	16.5	15.5	0.614	16.5	15.5	0.631	1.37
Daily gain, g/day	41.4	25.6	0.012	40.0	27.1	0.032	3.79
DM intake, g	473	451	0.687	445	479	0.535	37.25
DM intake/LW, g/kg	31.4	30.7	0.009	29.1	33.0	<0.001	0.19
DM feed conversion	14.4	19.6	0.267	13.7	20.4	0.16	3.15

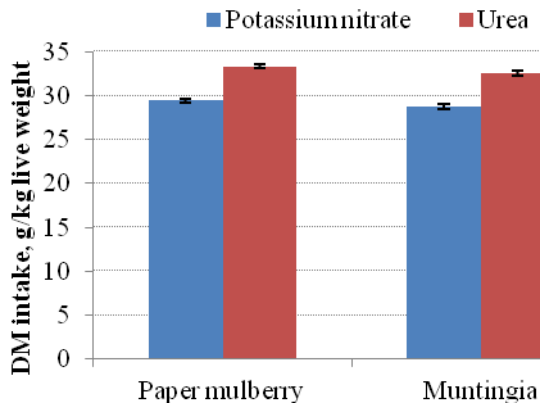


Figure 2. Effect of potassium nitrate compared with urea on DM intake of goats fed either Paper mulberry or Muntingia foliages.

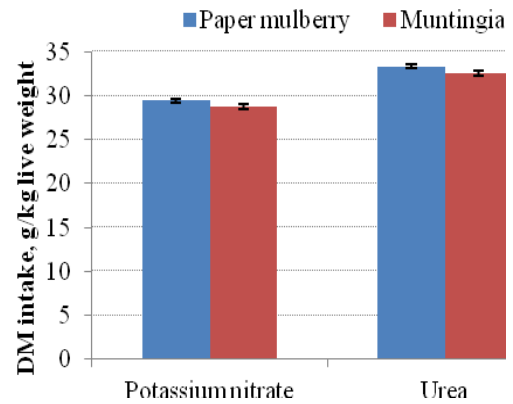


Figure 3. Effect of foliage source on DM intake of goats fed either potassium nitrate or urea as source of NPN.

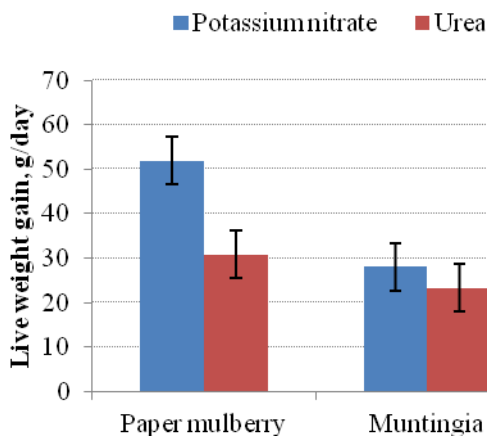


Figure 4. Effect of potassium nitrate compared with urea on live weight gain of goats fed either Paper mulberry or Muntingia foliages.

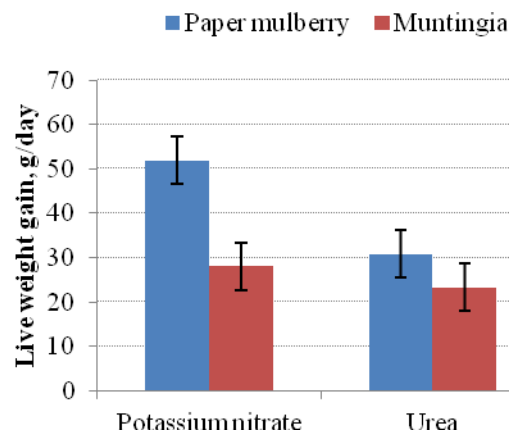


Figure 5. Effect of foliage source on live weight gain of goats fed either potassium nitrate or urea as source of NPN.

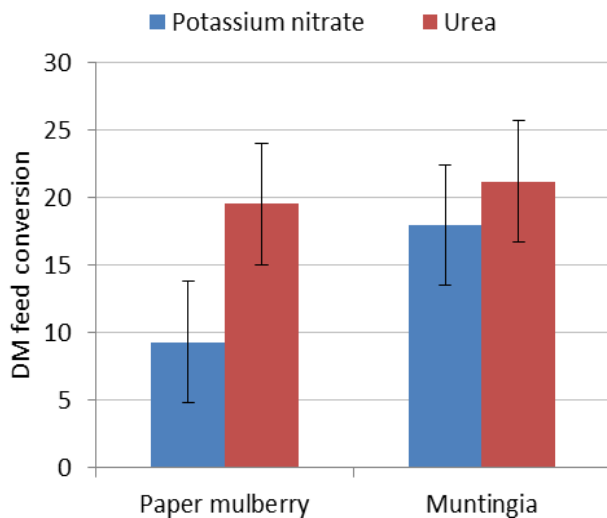


Figure 6. Effect of potassium nitrate compared with urea on DM feed conversion of goats fed either Paper mulberry or Muntingia foliages.

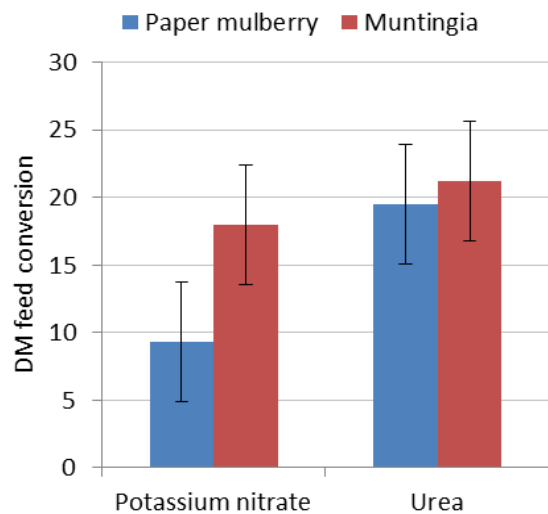


Figure 7. Effect of foliage source on DM feed conversion of goats of goats fed either potassium nitrate or urea as source of NPN.

On theoretical grounds dietary modifications that lead to reduced. The improved growth performance of the goats given potassium nitrate rather than urea as NPN source.

Rumen ammonia and pH

The values of rumen pH were similar and there were no difference among treatments. Rumen ammonia was higher for goats fed Paper mulberry compared with Muntingia and tended to be higher ($P=0.130$) with urea as NPN source compared with potassium nitrate. The higher values on Paper mulberry compared with Muntingia reflect the higher solubility of the crude protein in the former.

Table 4. Mean value of rumen pH and ammonia

Variables	PM	MG	Pro	KN	U	Pro	SEM
Rumen pH	7.16	7.29	0.097	7.17	7.29	0.130	0.05
NH ₃ , ml/litter	611	369	0.005	441	539	0.19	50.1

Conclusions

- Goats fed the tree foliages Paper mulberry and Muntingia grew faster and tended to have better DM feed conversion when they were supplemented with potassium nitrate rather than urea.
- The higher rumen ammonia levels in goats fed Paper mulberry rather than Muntingia probably reflected the greater solubility of the crude protein in the Paper mulberry.

Acknowledgements

This research is part of the requirements of the senior author for the MSc degree in Animal Production "Specialized in Response to Climate Change and Depletion of Non-renewable Resources" of Cantho University. The authors acknowledge support for this research from the MEKARN project financed by Sida and thank the staff of the Department of Animal Science, Faculty of Agriculture and Forest Resources, Souphanouvong University for providing the facilities to carry out the research.

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