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

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EFFECT OF FOLIAGES RICH IN BYPASS PROTEIN ON APPARENT DIGESTTIBILITY, GROWTH AND METHANE EMISSION IN GOATS

MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES
ANIMAL HUSBANDRY

Code: 60 – 62 - 40

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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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ACKWOWLEDGEMENT

The research was conducted at livestock research center and laboratory and farm of Cattle Station Liverstock Research Center, Laos, with supported from Mekong Basin Animal Research Network (MEKARN) project and NUFU project for funding this thesis 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 Program for Development, Research and Education (NUFU) project, for the financial support of this study and research.

The Can Tho University of Agriculture and Apply Biology, Department of Animal Science, Vietnam.

Associate professor Dr. Vanthong pheangvichit, Head of Research Unit national Agriculture and Forestry research institute, 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, 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. Brain Ogle, International Coordinator, MEKARN project; Professor Dr. Jan Berg, International Coordinator, NUFU project; Associate professor Dr. Ngo Van Man, MEKARN regional coordinator 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, Can Tho University, Vietnam, Professors, Lecturers and assistant lectures in MEKARN courses, for giving me care and useful knowledge.

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

Dedication

To

My wife Khonsavanh Douangphachanh
My son Tulaphone and Xayphakone
My duagther Lindy

My families

And

My country

Effect of foliages rich in bypass protein on apparent digestibility, growth and methane emissions in goats

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Abstract

Two experiments were conducted to investigate the effect of foliage rich in bypass protein on apparent digestibility, growth and methane emission in goats.

Paper 1

The purpose of the present study was to determine if supplementation with foliage of Mimosa would improve the nutritive value for goats of *Muntingia calabura* and at the same time lead to a reduction in methane production. Four male weaned crossbred goats (Bachthao x local female) with an initial weight of 11.61 ± 0.3 kg were used in a 4 x 4 Latin Square design to compare replacement of *Muntingia calabura* foliage with *Mimosa pigra* at four levels: 0, 25, 50 and 75% (DM basis). The amounts offered were based on a feeding level of 40 g DM/kg live weight at the start of each period. The goats were fed with 50% of their daily ration at 08:00h and 50% at 14:00h. The feed offered for each goat, and residues from the previous day, were weighed every morning. Fresh water was always available. The ratio of leaves plus petioles and stems was determined on samples (1 kg of foliage) taken 2 times per day. 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.

Mimosa pigra had a higher content of protein and OM in leaves and stem, and a slightly higher leaf: stem ratio, than Muntingia. Most indices of nutritive value were improved when mimosa foliage replaced Muntingia in the diet of the goats. There was a positive linear relationship between the proportion of N derived from mimosa and DM intake , but not between CP content of the DM and DM intake. Increasing the rate of replacement of muntingia N by mimosa N had no effect on apparent digestibility of DM but resulted in a positive curvilinear increase in apparent digestibility of crude protein. There was a tendency (P=0.07) for apparent OM digestibility to increase with increasing proportion of Mimosa N in the diet. Nitrogen intake increased as Mimosa replaced Muntingia. This was due partly to a higher per cent of crude protein in Mimosa than in Muntingia and also to the increase in DM intake as Mimosa replaced Muntingia. There were no differences in daily excretion rates of N in feces and urine thus N retention increased markedly as the proportion of N from Mimosa increased. However, when the N balance was expressed on a per cent basis, it was apparent that the N in Mimosa was used more efficiently for tissue synthesis than the N in Muntingia. The mean values of ruminal pH appeared (P=0.078) to be slightly lower on the diets containing Mimosa as were the values for rumen ammonia (P=0.14). The per cent reduction in methane production was calculated on the basis that CO_2 production reflects energy utilization by the animal thus the ratio of methane to carbon dioxide in eructed gas is a measure of relative production of methane. The ratio of methane to carbon dioxide in the eructed breath of the goats was lowered by Mimosa at all levels with the greatest reduction (50%) on the 50:50 ratio of the foliages.

Key words: Back Thao, greenhouse gases, metabolism, methane:carbon dioxide ratio

Paper 2

Twenty male, native goats approximately 8 months of age with average live weight of 12 kg were fed fresh foliage of *Tithonia* diversifolia as the basal diet. They were allocated to a completely randomized 2x2 factorial design with five animals per treatment. The factors were with or without a source of fermentable carbohydrate (sugar cane); and with or without a source of bypass protein (*Gliricidia sepium* foliage). Measurements were made of growth rate over a 120 day feeding trial and of apparent digestibility over a 5-day period following the feeding trial.

In the feeding trial, DM intake and live weight gain were increased by supplementation with either sugar cane or Gliricidia but there was no further advantage for live weight gain in supplying both supplements at the same time. DM feed conversion tended to be improved by Gliricidia supplementation. As with live weight gain it appeared that either supplement improved DM feed conversion but the effects were not additive.

In the digestibility trial, total DM intake was increased by supplementation with either sugar cane or Gliricidia. Intake was lowest for goats fed fresh Tithonia foliage as the sole feed and highest when both sugar cane and Gliricidia were given together. Apparent digestibility of DM was increased by supplementation with sugar cane but not with Gliricidia. Apparent digestibility of crude protein was decreased by sugar cane supplementation and with a tendency to be increased by supplementation with Gliricidia.

Key words: Bypass protein, digestibility, fermentable carbohydrate,

Abbreviations

ADF	Acid detergent fiber
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CF	Crude fiber
CaN	Calcium nitrate
CP	Crude protein
DM	Dry matter
FCR	Feed conversion ratio
FMD	Foot and mouth disease
HCN	Hydrogen cyanide
LW	Live weight
Mekarn	Mekong basin animal research network
N	Nitrogen
NDF	Neutral detergent fiber
NH_3	Ammonia
OM	Organic matter
pH	potential Hydrogen
Prob/P	Probability
RCD	Randomized complete design
SEM	Standard error of the mean
U	Urea

Introduction

Most of the Lao population are farmers who cultivate rice integrated with livestock production and fisheries. Livestock production is an important component of smallholder farms in Lao PDR, with sales of the livestock accounting for more than 50 % of cash income in many upland and highland areas. Over 95 % of all livestock is produced by smallholders (ILRI 2002). Livestock is found on most farms in the Lao PDR, with 89 % of all farm households raising one or more livestock types. The main feed resources for ruminants are native grasses, legumes and tree leaves that are available in the natural grassland and forests (Phonepaseuth Phengsavanh and Ledin 2003). Availability of feed resources is very dependent on season and land utilization. The quantity and quality of available feed resources affects the performance and value of cattle. During periods of abundant feed supply, cattle are in good body condition. Utilization of crop residues and other forages and/or tree leaves is the most appropriate way for production of ruminants according to Preston and Leng (1987) and Leng (1997).

In recent years, the human population has increased rapidly and the demand for food, in particular livestock products, is expected to increase in all developing countries. Livestock a plays major role in the livelihoods of small-farmers in Southeast Asia and contributes to the regional and national economic development.

Goat production in Lao PDR is mainly in smallholder production systems. In the past in the central part of the country goats grazed freely in the forest and other areas near the production areas. This is not a case anymore due to the expansion of cropping areas thus goats are prohibited to roam freely in planting season when most feeds are available. Farmers are forced to keep goats in confinements either near their houses or on cropping land. This means that the animals cannot access most available feed resources. The amount of given feed is always small and it is believed that goats are infected with worms more seriously in such systems, so that they often loose weight during this time.

By introducing high quality fodder trees as feed, farmers will be helped to solve the problem and enable them to produce more goats for sale.

Tithonia diversifolia is a highly productive shrub that is widely distributed in all tropical latitudes. It is found in several of the upland regions in Lao PDR. Research in Vietnam showed that biomass yields exceed those from Guinea grass (Nguyen Van Sao et al 2010). These researchers reported annual yields of 25 tonnes/ha of DM and 6 tonnes/ha of crude protein. Of equal importance was the finding that soil fertility was improved after growing Tithonia. The leaves of Tithonia are readily consumed by goats in the fresh state (Pathoummalangsy and Preston 2008); however, growth rates when fed as the sole feed were poor (Preston T R , Unpublished data). Nguyen Van Sao et al (2010) showed that N retention was much lower in goats fed Tithonia as the sole feed compared with when it was supplemented with stylosanthes or Jackfruit foliages. Earlier work by Macheha and Rosales (2005) showed that the protein in Tithonia leaves was rapidly degraded in the rumen, which would explain the losses in the urine and the poor N retention and growth rates. The research by Pathoummalangsy and Preston (2008) confirmed the low N retention when Tithonia was the sole feed of goats. However, N retention was increased more than two-fold by supplementing the Tithonia with foliage from mulberry (*Morus alba*) or with a rapidly rumen fermentable carbohydrate such as cassava root chips.

Another approach to improving the nutritive value of Tithonia foliage could be through the use of supplements rich in condensed tannins (CT) since these compounds in low concentrations (20–45 g CT/kg DM) reduce rumen forage protein degradation due to reversible binding to the protein with resultant improvements in animal productivity.

Potential sources of CT are the foliages of *Gliricidia sepium* and *Mimosa pigra* which grow widely in Lao PDR. In South Vietnam, mimosa was found to support growth rates of over 90 g/day in goats and it was hypothesized that this was due to the content of CT which were in the range of 2 to 10% in the DM, depending on the stage of growth of the mimosa (Thu Hong et al 2008).

Hypotheses

The hypotheses to be tested were:

Paper 1

Supplementation with foliage of Mimosa would improve the nutritive value for goats of *Muntingia calabura* and at the same time lead to a reduction in methane production.

Paper 2

The protein in Tithonia leaves is very soluble and easily fermented in the rumen of goats, leading to losses of N in the urine. Combining tithonia foliage with a tannin-rich plant such as Gliricidia or Mimosa will facilitate the reaction of the tithonia protein with tannins to form complexes less available to rumen microbes, thus enhancing the “bypass” properties of the Tithonia protein. Sugar cane as a readily fermentable source of carbohydrates will also enhance utilization of Tithonia protein.

Literature review.

Characteristics and behavior of tropical goats

Goats have many advantages in smallholder livestock systems in the tropics and sub-tropics, as they are hardy and well adapted to a wide range of conditions. They can thrive and produce well in tropical or cold climates and in humid as well as dry regions. Breeding animals are relatively inexpensive and can therefore be kept by poor as well as by better off farmers. They can consume a wide variety of grasses, weeds and leaves and by preference browse woody plants and shrubs. Their small size makes them suitable for home production of meat, milk, skins, fibre and cheese (Leng 2010). As browsers, goats utilize terrain that is too rough for sheep and cattle.

Countering these benefits are two major problems facing any smallholder goat production system: (1) feeding management and (2) susceptibility to gastro-intestinal parasites. With their browsing habits and inquisitive nature, goat have often been blamed in many areas of the world for causing overgrazing, soil erosion, deforestation and destruction of crops and over- utilization of feed supplies intended for other grazing animals. In addition, goat are susceptible to severe effects on their growth and survival from infection with gastro intestinal parasites. Both of these limitations can be controlled through a combination of grazing management, housing at night and supplementary feeding with legume leaves.

Nutrition, feed preferences and feeding habits

An important characteristic of the feeding behavior of goats is that they can walk for long distances in search for feed, and this behavior assists them in meeting their nutrient requirements (Devendra and Coop 1982). When fresh forage cannot meet the nutrient needs, supplemental feeding may be necessary. Supplements should only be fed to the point where they support profitable levels of production. It is questionable as to whether goats can be fed profitably with concentrates, to the extent that other livestock can. The amount of pasture needed to support goats will vary considerably, depending upon the quality of the pasture and management system. Goats do well on improved pastures, as well as brushy, woody areas. They are natural browsers and if given the opportunity will choose browse and weeds over grasses (Schoenian 1999).

Goats are highly selective and have narrow mouth parts and cleft upper lips. This allows them to select more tender and nutritious parts of the vegetation.

Feed resources available for ruminant production in developing countries

Animals have crucial roles to play in human food production, either directly or indirectly. In developing countries, ruminants are primarily kept as a source of draft power, as an accessible source of funds when sold, for milk production (mostly as a byproduct, but in some cases large dairy herds have been established) and often they are important as an indication of wealth and standing. Generally they are only slaughtered for food when crops and other resources become scarce.

Whilst cattle have remained largely as draft animals in developing countries, more emphasis is now on them as a source of high quality food (protein) particularly for the middle class. People demand more animal protein in their diet as their standard of living increases. Over many years, milk and meat availability increased only through expanding numbers of animals and there was little increase in production per animal (Jackson 1981); this was largely owing to the secondary importance of meat or milk production to draft power. The main feed resources for large ruminants have remained crop residues, agro-industrial byproducts or pastures from infertile lands, which, in general, support only low levels of production (Leng 1997). Large ruminants are mainly found in cropping areas where land for pasture is scarce or non-existent, crop residues (straws and stoves) remain a single major resource used for feeding ruminants in developing countries. Another major resource is grassland that is either too difficult to cultivate or is of low inherent fertility. Pastures hand cut from roadsides, railway embankments, boundaries between crops and any other wastelands are also a major feed resource particularly in Asia. Another source of significant amounts of feeds is agro-industrial byproducts such as molasses, sugar cane tops, sisal waste and oilseed cakes or meals.

Cellulose is the greatest single feed resource for ruminants worldwide, with some 100 billion tons available worldwide annually. Cellulosic (fibrous) biomass is only usable as a major component of a diet by herbivores and ruminants in particular as it requires microbial fermentative activity for its digestion (Preston and Leng 2009).

The potential uses of tree foliages

Ligneous plants, which may be large/small trees or shrubs, are an important component of the cellulosic and high protein fodder resources available for use by livestock and wildlife alike. Foliage has been used as animal feed since Roman times and it appears to be the preferred forage of goats and some breeds of sheep as well as numerous species of deer, particularly on the arid savannahs. In more recent times, trees and shrubs have been introduced into cropping and grazing systems to provide green fodder high in protein to supplement the available low protein forage. These are grown in banks or hedges, between crops (alley farming) or as components of pastures and also as shade trees.

Tree foliage is being increasingly recognized as a potentially high quality feed resource for ruminants, particularly to supply crude protein. This is especially true in harsh and arid conditions where trees often provide more edible biomass than pasture and this biomass remains green and high in protein, even when pastures dry off and senesce. Because of their deep rooted nature, trees are able to tap water and nutrient resources deep in the soil profile. Many trees have micro-organisms associated with their root systems that allow them to mobilize soil bound mineral resources such as phosphorus and to fix N_2 from the atmosphere into organic compounds.

In some mountainous or arid areas, it has been found that grazing ruminants contribute 90% to rangeland production and use 40–50% of the total feed available (Le Hourou 1980). In the wet tropics of Latin and Central America, the Caribbean Islands. SE Asia. and Africa, fodder from trees and shrubs, especially from leguminous species, are being used widely as sources of dietary supplement for ruminants.

The role of *Tithonia divesiforlia* as livestock feed

Commonly known as Mexican or Wild sun flower, tithonia is a shrub belonging to the family Asteraceae. Tithonia originated from Mexico and is now widely distributed throughout the humid and sub humid tropics in Central and South America, Asia and Africa (Sonke 1997). It is common in indigenous fallow systems in Southeast Asia. Tithonia was probably introduced into Africa as an ornamental. It has been reported in Kenya (Niang et al 1996), Malawi (Ganunga et al 1998) and Nigeria (Ayeni et al 1997).

There were now several researches relevant to its use for livestock production. Mahecha and Rosales (2005) reported that the crude protein content in the forage of Tithonia was 24.2 % in DM basis and more than 40 % of protein was soluble. The leaves of Tithonia are readily consumed by goats in the fresh state (Pathoummalangsy and Preston 2008), however, growth rates when fed as the sole feed were poor (Preston T R , Unpublished data). Nguyen Van Sao et al (2010) showed that N retention was much lower in goats fed Tithonia as the sole feed compared with feeding of stylosanthes or Jackfruit foliages as supplements to the Tithonia. Earlier work by Macheha and Rosales (2005) showed that the protein in Tithonia leaves was rapidly degraded in the rumen, which would explain the losses in the urine and the poor N retention and growth rates. The research by Pathoummalangsy and Preston (2008) confirmed the low N retention when Tithonia was the sole feed of goats. However, N retention was increased more than two-fold by supplementing the Tithonia with foliage from mulberry (*Morus alba*) or with a rapidly rumen fermentable carbohydrate such as cassava root chips.

Another approach to improving the nutritive value of Tithonia foliage could be through the use of supplements rich in condensed tannins (CT) since these compounds in low concentrations (20–45 g CT/kg DM) reduce rumen forage protein degradation due to reversible binding to the protein with resultant improvements in animal productivity.

The role of *Mimosa pigra* and *Gliricidia sepium* supplements rich in bypass protein

Potential sources of CT are the foliages of *Gliricidia sepium* and *Mimosa pigra* which grow widely in Lao PDR. In South Vietnam, mimosa was found to support growth rates of over 90 g/day in goats and it was hypothesized that this was due to the content of CT which were in the range of 2 to 10% in the DM, depending on the stage of growth of the mimosa (Thu Hong et al 2008). There are several reports showing increases in live weight gain by supplementing forages with Gliricidia foliage: in cattle (Seijas et al 1994; Combellas et al 1996; Preston and Leng 2009); in goats (Abdulrazak et al 2006); and in sheep (Izaguirre et al 2011).

The role of Sugar cane as livestock feed

Sugar cane consists of three principal fractions, stalk, tops (or growing point) and green leaves. The chemical composition and digestibility, which differ between these fractions have been studied by several authors. Perez and Garcia (1975) found that whole sugar cane contained over 40% total sugars, 3.2% crude protein (CP) and 35% acid detergent fiber (ADF), all expressed in percent of dry matter (DM), and 11.3 MJ/kg DM. In the work reported by Mui et al (1996) The DM content was 28-29% in the leaves, 16-18% in the tops and 24-29% in the stalks. DM digestibility has been found to be between 29 and 72% for the stalk or stalk mixed with tops and leaves (Ferreiro and Preston 1977). While the digestibility of the fiber in sugar cane does not exceed 25%, in contrast to the grasses the overall digestibility does not decrease with maturity. There is rather a slight increase in digestibility, since accumulation of soluble cell content (sugars) more than offsets the decline in wall digestibility caused by lignifications of the structural cell wall carbohydrates (Preston 1977).

In the last decades much work has been done on sugar cane, concerning digestibility, metabolism and effect of supplementation with non-protein nitrogen, minerals or protein-rich foliages. The studies have generally focused on milk and meat production in the systems with dual-purpose cattle. There is, however very little known about the feeding of chopped whole sugar cane to growing goats.

When evaluating tropical forages feed intake is a very important component of the feeding value as a high feed consumption creates the necessary condition for an increase in production (McDonald et al., 1992) There are many factor effecting feed intake, among which different methods of processing have been examined to increase the feed intake. A decrease particle size by chopping or grinding generally result in higher intakes of roughages in small ruminants (Gherardi et al., 1992; Mc Sweeney and Kennedy, 1992). The methods of processing sugar cane for animal feeding have been based on the concept that digestibility and voluntary feed intake would be enhanced by eliminating the more lignified part to the sugar cane stems or by decreasing the length of the long fiber in the rind. Voluntary intake by cattle was increased by 38% when the stalk were chopped to 15 cm lengths and the rind removed, result in a DM intake of 18.7 g/kg body weight (Preston, 1997).

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Effect of a tannin-rich foliage (*Mimosa pigra*) on feed intake, digestibility, N retention and methane production in goats fed a basal diet of *Muntingia calabura*

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Abstract

Four male weaned crossbred goats (Bachthao x local female) with an initial weight of 11.6 ± 0.3 kg were used in a 4 x 4 Latin Square design to compare replacement of *Muntingia calabura* foliage with *Mimosa pigra* at four levels: 0, 25, 50 and 75% (DM basis).

Increasing the replacement of N in Muntingia by that in Mimosa led to increases in DM intake, apparent digestibility of crude protein and N retention. Methane production was reduced by replacing Muntingia N with Mimosa N with the greatest effect (42% reduction) with 72% of the diet N from Mimosa.

Key words: Back Thao, greenhouse gases, metabolism, methane:carbon dioxide ratio

Introduction

Muntingia (common name Jamaica cherry) (*Muntingia calabura*) belongs to the family Elaeocarpacae. According to Nguyen Xuan Ba and Le Duc Ngoan (2003) it can grow everywhere (sandy land, humid areas, and high land area) and is well adapted to the dry season. Traditionally, it is used as a shade tree around the homestead, and along the roadsides. It is a tall tree with a large canopy of leaves but it is not normally used as feed for animals. 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). Tran Trung Tuan (2009) hypothesized that the nutritive value for goats of Muntingia leaves and stems would be improved when supplemented with a rapidly rumen degradable forage (water spinach) and/or a source of leaves with known capacity to act as a source of bypass protein. However, contrary to expectations, N retention was high on all the diets and was not improved by either the Jackfruit or the water spinach.

Giant Mimosa (*Mimosa pigra*) has been described as one of the worst invasive weeds of the Mekong River basin (Photo 1). However, Nguyen Thi Thu Hong et al (2008) found that with suitable management (Photo 2) it produced a foliage of high nutritive value which when offered as the sole feed to goats supported growth rates in a grazing situation of 94 g/day and 61 g/day in confinement.

Photo 1. *Mimosa pigra* as an invasive weed in the Mekong delta (MWBP/RSCP 2006)

Photo 2. *Mimosa pigra* managed as a forage for goats (Nguyen Thi Thu Hong et al 2008)

Ruminant animals major contributors of greenhouse gases (Steinfeld 2006) which are considered to be the main causes of global warming (IPPC 2007). There is therefore a major incentive to find ways in which emissions of these gases can be reduced. Methane which is produced by enteric fermentation is the major GHG produced by ruminants. Recently, a number of authors have studied the effects of naturally occurring compounds in plants which might act to modify the rumen fermentation and specifically to reduce methane production. Puchala et al (2005) fed Angora goats a forage legume (*Lespedeza cuneata)* with a moderately high content of condensed tannins (17.9 g/kg DM) compared with a mixture of grasses (*Digitaria ischaemum* and *Festuca arundinacea*) with negligible tannin content (0.5 g/kg DM). Methane production was 10.7 g/kg digestible DM on the high tannin legume forage compared with 21.5 on the low tannin grass. Mimosa has been shown to contain from 4 to 8 % of condensed tannins in the DM of the leaves, the level increasing with stage of growth (Nguyen Thi Thu Hong et al 2008).

The purpose of the present study was to determine if supplementation with foliage of Mimosa would improve the nutritive value for goats of *Muntingia calabura* and at the same time lead to a reduction in methane production.

Materials and Methods

Location

The experiment was conducted at the experimental farm of the Faculty of Animal Science, An Giang University, Vietnam, from August to October 2010.

Treatments and experimental design

Four male weaned crossbred goats (Bachthao x local female) with an initial weight of 11.6 ± 0.3 kg and aged from 3 to 4 months were used to evaluate four experimental diets consisting of different ratios of Muntingian and Mimosa. The experimental design was a 4*4 Latin-square (Table 1), with periods of 10 days. The experimental diets were:

- MP0: Foliage of *Muntingia calabura* offered ad libitum
- MP25: A mixture of Muntingia foliage 75 % + 25% as *Mimosa pigra* foliage (DM basis)
- MP50: Muntingia 50% + 50% Mimosa (DM basis)
- MP75: Muntingia 25 % + 75% Mimosa (DM basis)

Table 1. Layout of the experiment

Period	Goat 1	Goat 2	Goat 3	Goat 4
1	MP 0	MP25	MP50	MP75
2	MP75	MP 0	MP2	MP50
3	MP50	MP75	MP 0	MP2
4	MP25	MP50	MP75	MP 0

Animals and housing

The goats were confined in metabolism cages made from bamboo, adapted for the separate collection of feed residues, feces and urine. They were gradually introduced to the cages and diets over 7 days before beginning the experiment.

Feeds and feeding system

The foliages of Muntingia and Mimosa were collected daily from natural stands in the University campus. The branches were hung separately in bunches above the feed trough. The amounts offered were based on a feeding level of 40 g DM/kg live weight at the start of each period. The goats were fed with 50% of their daily ration at 08:00h and 50% at 14:00h.The feed offered for each goat, and residues from the previous day, were weighed every morning. Fresh water was always available. The ratio of leaves plus petioles and stems was determined on samples (1 kg of foliage) taken 2 times per day.

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% concentrated sulphuric acid + 90% distilled water). Feces were collected daily and stored at -18ºC and, at the end of each period, sub-samples were mixed together and ground with a coffee grinder prior to analysis for DM and N. Ratio of methane and carbon dioxide in eructed gas was measured (Photo 3) at the end of the 2nd and 4th period, 2h after feeding in the morning, using a portable "Gasmet 4030" meter (Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland).

Chemical analyses

The sub-samples of the foliages offered to and refused by the goats, and of feces, were analyzed for DM content by micro-wave radiation (Undersander et al 1993) and for N and ash following standard procedures as outlined by AOAC (1990). Urine was analysed for N by AOAC (1990) procedures. 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).

Photo 3. Goats were confined in a closed space for the measurement of the eructed gases with the Gasmet 4030 equipment

Statistical analysis

The data were analyzed with the GLM option of the analysis of variance software of Minitab Version 13.31 (Minitab 2000). Sources of variation in the model were animals, periods, foliages and error.

Results and Discussion

Feed composition

Mimosa pigra had a higher content of protein and OM content in leaves and stem, and a slightly higher leaf: stem ratio, than Muntingia (Table 2).

Table 2. Feed characteristics of the foliage of Muntingia and Mimosa

	DM	**CP**	**OM**	**Proportion (fresh basis)**
	(%)	(% in DM)	(% in DM)	(%)
Muntingia				
Leaves	33.3	13.0	84.0	55.4

Stems	34.8	5.0	90.0	44.6
Mimosa				
Leaves	34.5	21.0	93.0	60.4
Stems	31.6	11.0	92.0	39.6

Feed intake and apparent digestibility

Most indices of nutritive value were improved when mimosa foliage replaced Muntingia in the diet of the goats (Tables 3 and 4).

Table 3 . Mean values for feed intake and live weight change of goats fed mixtures of Mimosa (MP) with Muntingia

	MP 0	MP 25	MP50	MP75	SEM	P
DM intake, g/day						
Muntingia	270	218	149	92		
Minmosa	0	119	230	303		
Total	270	337	379	395	15.0	0.001
CP, % of DM consumed	13.5	15.8	18.0	18.5	0.45	0.001
DM intake, g/kg LW	28.8	34.3	39.3	41.0	1.36	0.001
LW gain, g/day	54.0	58.5	81.5	68.5	3.73	0.001

There was a positive linear relationship between the proportion of N derived from mimosa and DM intake (Figure 1), but not between CP content of the DM and DM intake (Figure 2),

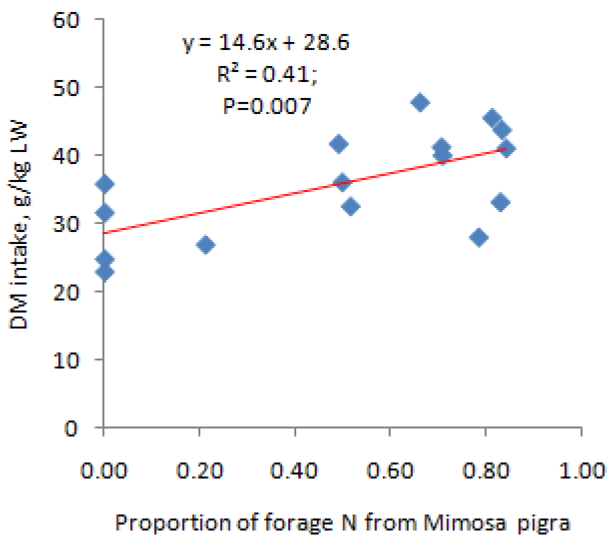


Figure 1. Effect of proportion of forage protein from Mimosa on DM intake in goats fed mixtures of Mimosa with Muntingia

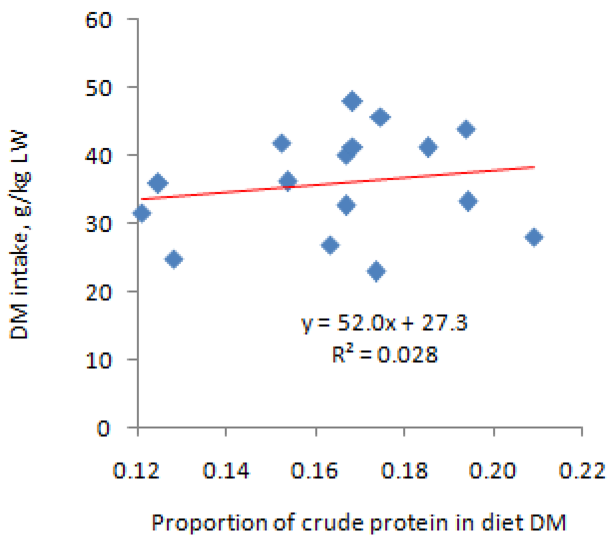
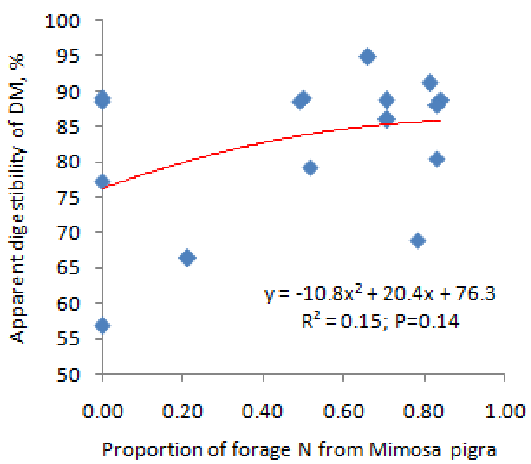


Figure 2. Effect of proportion of crude protein in diet DM on DM intake in goats fed mixtures of Mimosa with Muntingia

Increasing the rate of replacement of muntingia N by mimosa N had no effect on apparent digestibility of DM (Figure 3) but resulted in a positive curvilinear increase in apparent digestibility of crude protein (Figure 4). There was a tendency (P=0.07) for apparent OM digestibility to increase with increasing proportion of Mimosa N in the diet (Figure 5).

Table 4. Mean values for apparent digestibility of the diets.

	MP0	MP25	MP50	MP75	SEM	Prob.
Apparent digestibility. %						
DM	78.0	80.7	84.6	87.5	2.57	0.056
OM	64.0	71.2	73.7	76.2	1.66	0.001
N	69.3	78.0	73.5	62.7	2.13	0.001
N balance, g/day						
Intake	5.75	8.51	10.5	11.8	0.43	0.001
Feces	1.66	3.07	2.65	2.59	0.36	0.13
Urine	1.73	3.09	2.57	2.13	0.39	0.18
Retention	2.35	2.35	5.32	7.05	0.23	0.001
N retention						
As % of /N intake	38.1	26.2	50.3	59.4	4.2	0.006
As % of N digested	52.1	40.1	67.9	76.6	4.5	0.005



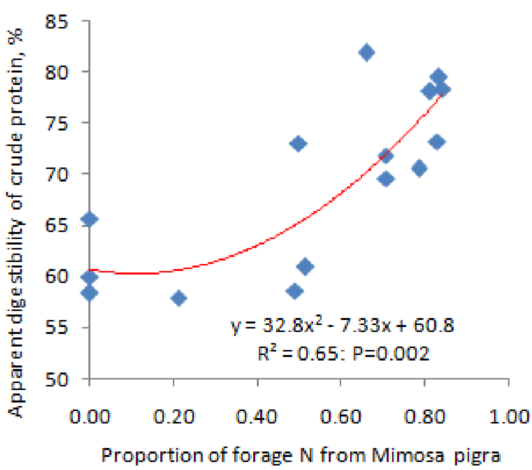


Figure 3. Relationship between apparent digestibility of DM and the proportion of forage N derived from Mimosa

Figure 4. Relationship between apparent digestibility of crude protein and the proportion of forage N derived from Mimosa

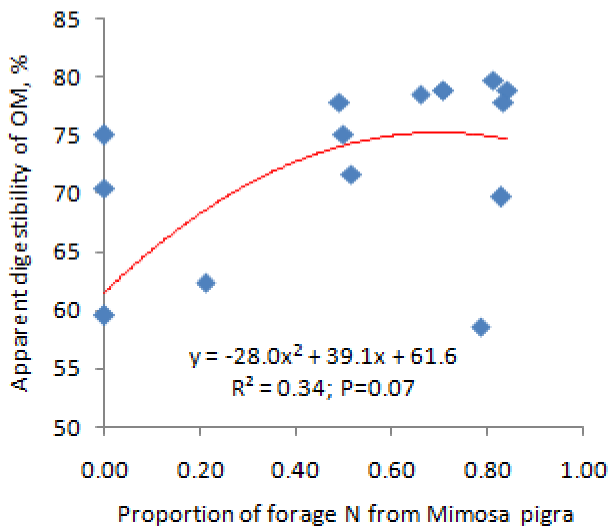


Figure 5. Relationship between apparent digestibility of organic matter and the proportion of forage N derived from Mimosa

Nitrogen balance

Nitrogen intake increased as Mimosa replaced Muntingia (Table 4). This was due partly to a higher per cent of crude protein in Mimosa than in Muntingia (Table 2) and also to the increase in DM intake as Mimosa replaced Muntingia. There were no differences in daily excretion rates of N in feces and urine (Table 4) thus N retention increased markedly as the proportion of N from Mimosa increased. (Figures 6 and 8). However, when the N balance was expressed on a per cent basis (Figure 7), it was apparent that the N in Mimosa was used more efficiently for tissue synthesis than the N in Muntingia.

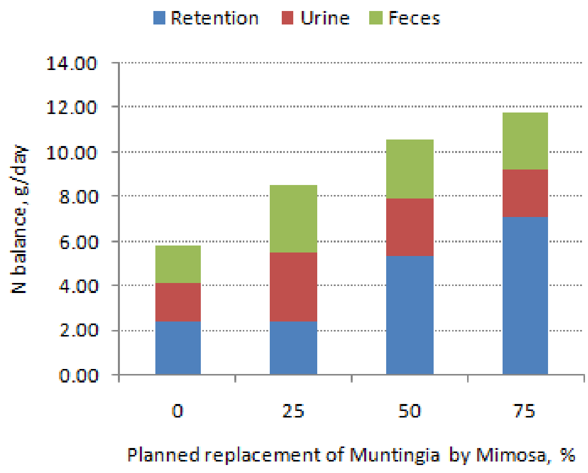


Figure 6. N balance according to the planned replacement of Muntingia by Mimosa

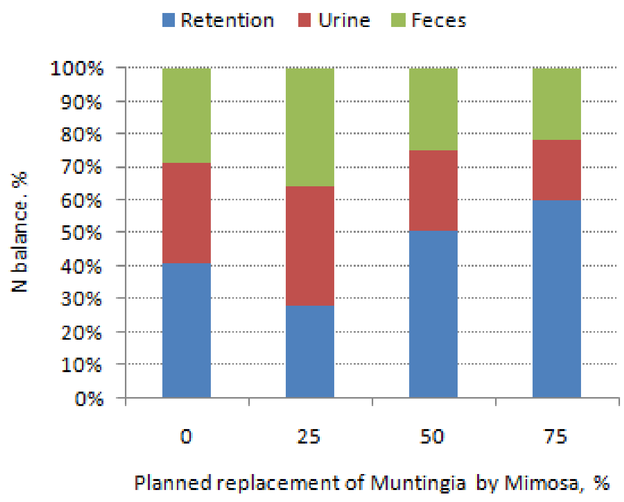


Figure 7. Per cent of the diet N excreted and retained according to the planned replacement of Muntingia by Mimosa

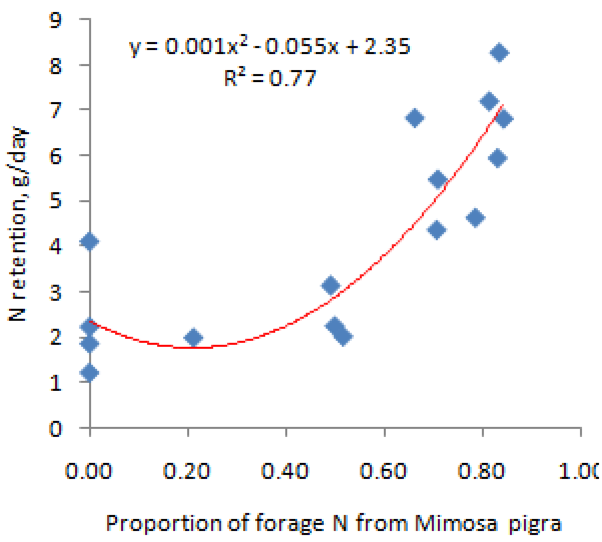


Figure 8. Relationship between proportion of forage protein from Mimosa on N retention as per cent of N digested

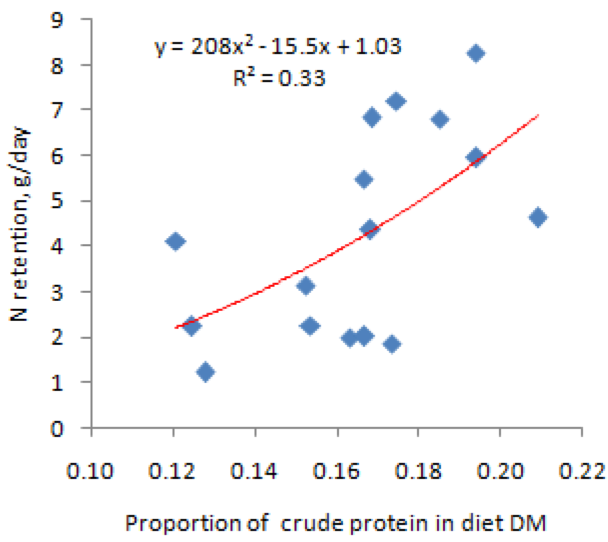
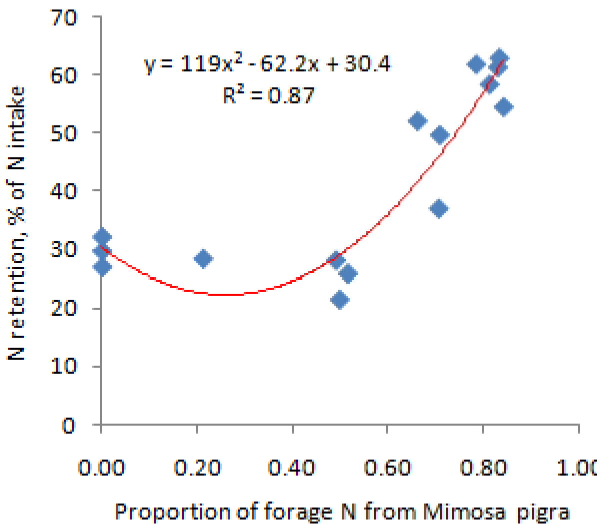


Figure 9. Relationship between proportion of crude protein in diet DM and N retention

A higher biological value for the protein in Mimosa is also inferred from the better relationship between N retention and proportion of diet N from Mimosa (R^2 =0.77; Figure 8) compared with N retention and proportion of crude protein in the diet DM (R^2 =0.33; Figure 9). Increases in N retention as per cent of N intake (Figure 10) and per cent of N digested (Figure 11), as the proportion of diet N from Mimosa was increased, provide more evidence for the high biological value of the Mimosa protein.



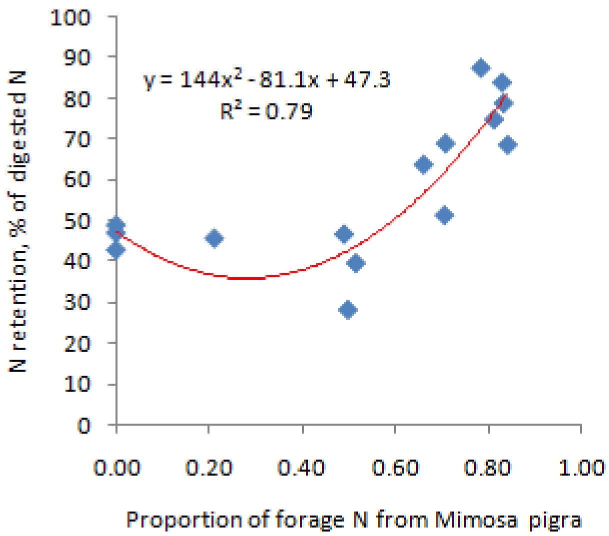


Figure 10. Relationship between proportion of forage protein from Mimosa and N retention as per cent of N intake

Figure 11. Relationship between proportion of forage protein from Mimosa and N retention as per cent of N digested

Rumen ammonia and ratios of methane to carbon dioxide in the eructed breath of the goats

The mean values of ruminal pH appeared (P=0.078) to be slightly lower on the diets containing Mimosa as were the values for rumen ammonia (P=0.142) (Table 5). The ratio of methane to carbon dioxide in the eructed breath of the goats was lowered by Mimosa at all levels (Figure 12) with the greatest reduction (50%) on the 50:50 ratio of the foliages (Figure 13). The per cent reduction in methane production was calculated on the basis that CO_2 production reflects energy utilization by the animal thus the ratio of methane to carbon dioxide in eructed gas is a measure of relative production of methane as a function of the intake of metabolizable energy (Madsen et al 2010; Leng and Preston 2010).

Table 5. Mean values of rumen traits and methane/carbon dioxide ratios in eructed breath of goats fed different ratios of Muntingia and Mimosa foliages

	MP0	MP25	MP50	MP75	SEM	Prob.
pH	6.9	6.3	6.2	6.5	0.168	0.078
NH3-N, mg/100ml	14.9	21	15.8	13.5	2.1	0.142
CH4/CO2	0.0314[a]	0.0239[b]	0.0178[c]	0.0252[b]	0.002	0.001
Per cent reduction in CH_4 due to Mimosa	0	24	43	20		

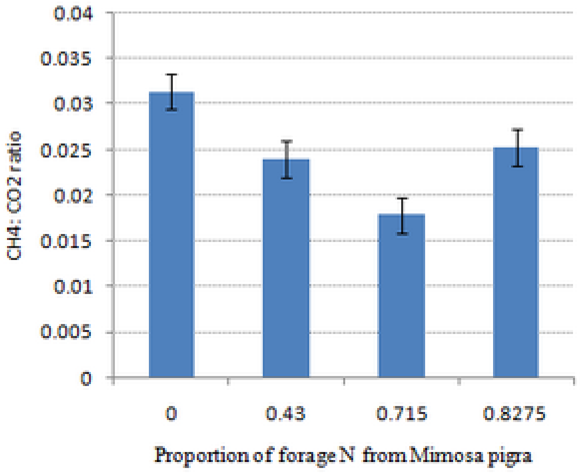


Figure 12. Relationship between ratio methane/carbon dioxide and proportion of forage N from mimosa

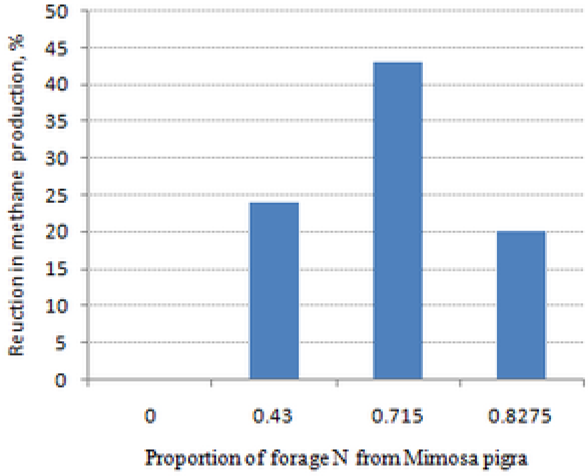


Figure 13. Reduction in methane production by increasing the proportion of foliage from mimosa

The improvements in most indices of nutritive value as *Mimosa pigra* replaced *Muntingia calabura* confirm the findings of Nguyen Thi Thu Hong et al (2008) that Mimosa has a high nutritive value for goats, almost certainly related to the Mimosa protein having good rumen escape properties due to its content of condensed tannins. The indications that methane: carbon dioxide ratios were reduced with Mimosa may also be a result of the tannin content as a positive role of tannin-rich plants in reducing enteric methane production has been reported for other leguminous plants rich in tannins (Puchala et al 2005; Hess et al 2006).

Conclusions

- Increasing the rate of replacement of N in *Muntingia calabura* by that in *Mimosa pigra*, in the diet of growing goats, led to increases in DM intake, apparent digestibility of crude protein and N retention.
- Methane production was reduced by replacing Muntingia N with Mimosa N with the greatest effect (42% reduction) with 72% of the diet N from mimosa.

Acknowledgments

The authors would like to express their gratitude to the MEKARN program, supported by Sida, and to the Norwegian Programme for Development, Research and Higher Education (NUFU), for their financial support. The research forms part of the requirement by the senior author for the MSc degree from Cantho University. The administration and teaching staff of An Giang University are gratefully acknowledged for providing resources and advice for conducting the research.

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Received 7 January 2011; Accepted 1 February 2011; Published 6 March 2011

Effect of supplements of *Gliricidia sepium* and *Sugar cane* on growth performance of goats fed foliage of *Tithonia diversifolia*

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Abstract

Twenty male, native goats approximately 8 months of age with average live weight of 12 kg were fed fresh foliage of Tithonia diversifolia as the basal diet. They were allocated to a completely randomized 2x2 factorial design with five animals per treatment. The factors were with or without a source of fermentable carbohydrate (sugar cane); and with or without a source of bypass protein (*Gliricidia sepium* foliage). Measurements were made of growth rate over a 120 day feeding trial and of apparent digestibility over a 5-day period following the feeding trial.

In the digestibility trial, total DM intake was increased by supplementation with either sugar cane or Gliricidia. Intake was lowest for goats fed fresh Tithonia foliage as the sole feed and highest when both sugar cane and Gliricidia were given together. Apparent digestibility of DM was increased by supplementation with sugar cane but not with Gliricidia. Apparent digestibility of crude protein was decreased by sugar cane supplementation and with a tendency to be increased by supplementation with Gliricidia.

In the feeding trial, DM intake and live weight gain were increased by supplementation with either sugar cane or Gliricidia but there was no further advantage for live weight gain in supplying both supplements at the same time. DM feed conversion tended to be improved by Gliricidia supplementation. As with live weight gain it appeared that either supplement improved DM feed conversion but the effects were not additive.

Key words: Bypass protein, digestibility, fermentable carbohydrate,

Introduction

Ruminants fed low quality forages require supplementation with critically deficient nutrients to optimize productivity (Preston and Leng 2009). A large increase in animal production can be achieved by alterations to the feed base and improving the ratio of protein absorbed relative to energy nutrients which is the primary factor that affects feed utilization efficiency. Ensuring an optimum balance of nutrients to improve microbial digestion in the rumen by supplementation automatically improves protein to energy ratio. Small amounts of escape (bypass) protein stimulate both productivity and efficiency of feed utilization (Preston and Leng 2009). It is important, therefore, to identify protein sources and economic mechanisms for producing diets that contain a high proportion of escape protein.

Trees and shrubs have been introduced into cropping and grazing systems to provide green fodder high in protein to supplement the available low protein forage. Recent studies, particularly of tree forages, show that anti-nutritional factors (ANF) like tannins, can affect animal nutrition in rather diverse ways (Singh et al 2003; Singh and Sahoo 2004). Formation of complexes of tannins with nutrients, especially proteins, has both negative and positive effects on their utilization (Reed 1995).

Mimosa pigra has been reported to manipulate the rumen through the presence of tannins (Thu Hong et al 2008) which can form reversible complexes with the protein thus enhancing the by-pass protein content of the diet (Preston and Leng 2009).

Sugar cane consists of three principal fraction: stalk, growing point and green leaves. The chemical composition and digestibility, which differ among these fractions, have been reported by several authors. Perez and Garcia (1975) found that whole sugar cane contained 16% total sugars (fresh basis) and 3.2% crude protein (CP); the DM content was 28-29% in the leaves, 16-18% in the growing point and 24-29% in the stalks. DM digestibility has been found to be between 69 and 72 % for the stalk or stalks mixed with leaves (Ffoulkes and Preston 1977). In contrast to the grasses the overall digestibility does not decrease with maturity in sugar cane, since increasing maturity is linked with accumulation of the soluble cell contents which more than offsets the decline in cell wall digestibility caused by lignifications of the cell structural cell wall carbohydrates (Preston 1977).

Tithonia diversifolia is a highly productive shrub that in widely distributed in all tropical latitudes. It is found in several of the upland regions in Lao PDR. Research in Vietnam showed that biomass yields exceed those from Guinea grass (Nguyen Van Sao et al 2010). These researchers reported annual yields of 25 tonnes/ha of DM and 6 tonnes/ha of crude protein. Of equal importance was the finding that soil fertility was improved after growing Tithonia. The leaves of Tithonia are readily consumed by goats in the fresh state (Pathoummalangsy and Preston 2008); however, growth rates when fed as the sole feed were poor (Preston T R , Unpublished data). Nguyen Van Sao et al (2010) showed that N retention was much lower in goats fed Tithonia as the sole feed compared with feeding of Tithonia supplemented with stylosanthes or Jackfruit foliages. Earlier work by Macheha and Rosales (2005) showed that the protein in Tithonia leaves was rapidly degraded in the rumen, which would explain the losses in the urine and the poor N retention and growth rates. The research by Pathoummalangsy and Preston (2008) confirmed the low N retention when Tithonia was the sole feed of goats. However, N retention was increased more than two-fold by supplementing the Tithonia with foliage from mulberry (*Morus alba*) or with a rapidly rumen fermentable carbohydrate such as cassava root chips.

The objective of the experiment described in this study was to investigate the potential of sugar cane and of Gliricidia sepium as sources of rumen fermentable carbohydrate and bypass protein, respectively, in a basal diet of Tithonia diversifolia fed to growing goats.

Materials and methods

Location and climate of the study area

The experiment was conducted at the Livestock Research Center of the National Agriculture and Forestry Research Institute, situated 40 km from Vientiane City. The climate in this area is divided into two seasons: dry and wet. The wet season is from May to October. Annual rainfall is in the range of 1400 – 1800 mm. The dry season is from November to April. The average minimum and maximum temperatures are about 15^0C and 32^0C, respectively. The experiment was carried out during the months July to October 2011.

Experimental design

Twenty male, native goats approximately 8 months of age with average live weight of 12 kg were fed fresh foliage of *Tithonia diversifolia* as the basal diet. They were allocated to a completely randomized 2x2 factorial design with five animals per treatment. The factors were

- With or without a source of fermentable carbohydrate (sugar cane)
- With or without a source of bypass protein (*Gliricidia sepium* foliage)

Individual treatments were:

TD; Fresh foliage of *Tithonia diversifolia* ad libitum

TD.SC: Tithonia and chopped sugar cane ad libitum

TD.GL: Tithonia ad libitum plus *Gliricidia sepium* foliage at 20 g (DM) per 1 kg live weight

TD.SC.GL: Tithonia ad libitum, sugar cane ad libitum and Gliricida sepium foliage at 10g (DM) per 1 kg live weight

The goats were confined in separate pens.

Experimental feeds

Tithonia and Gliricidia were planted in the Livestock Research Center. The foliage of Tithonia and Gliricidia were harvested daily in the afternoon then wilted overnight before feeding the next day. Whole sugar cane was purchased from farmers and chopped into pieces of 1-2 cm with a mechanical chopper.

Animals and management

A vaccination and de-worming program was applied before the commencement of the experiment. The animals were adapted to the pens and the experimental feed for 21 days before the start of the experiment. The feeding troughs were divided into two sections in order to accommodate the Tithonia and sugar cane. Branches of Gliricidia were hung above the feed troughs. Fresh feeds were offered twice per day at 08:00 and 14:00h. Water and mineral blocks were offered freely.

Data collection

The feeds offered and refused were recorded daily for individual animals. Samples of feeds offered and refused were taken weekly for chemical analysis. The goats were weighed every 14 days in the morning before feeding.

Chemical analyses

The DM content of the feeds offered and refused and of the feces was determined using a microwave oven (Undersander et al 1993). Determinationof N in feeds, feces and urine as donde according to AOAC (2000) procedures.

Statistical analyses

The data were analyzed using the General Linear Model (GLM) option of the ANOVA program in the Minitab (2000) software. Treatment means which showed significant differences at the probability level of P<0.05 were ompared with the Turkey test in the Minitab (2000) software.

Results

The chemical composition of feeds

The fresh leaves of Gliricidia had higher crude protein content than those of Tithonia (Table 1) but represented a lower proportion of the total foliage.

Table 1. The chemical composition of the feeds

	Dry matter (%)	**Crude protein** (% in DM)	**Organic matter** (% in DM)	**Proportion** (% fresh basis)
Tithonia				
Leaves	31.7	18.4	98.8	74.0
Stem	19.1	13.6	92.6	26.0
Gliricidia				
Leaves	26.8	26.4	98.3	60.0
Stem	16.4	12.4	99.1	27.0
Petiole	29.1	19.6	98.6	13.0
Sugar cane	26.3	1.8	99.7	

Intake and apparent digestibility

In the digestibility trial, total DM intake was increased by supplementation with either sugar cane or Gliricidia (Table 2). Intake was lowest for goats fed fresh Tithonia foliage as the sole feed and highest when both sugar cane and Gliricidia were given together (Table 3). Apparent digestibility of DM was increased by supplementation with sugar cane but not with Gliricidia (Table 2). By contrast, apparent digestibility of crude protein was decreased by sugar cane supplementation and with a tendency (P=0.11) to be increased by supplementation with Gliricidia.

Table 2. Mean values (main effects) for coefficients of apparent digestibility and N balance in goats fed Tithonia diversifolia supplemented with sugar cane and/or Gliricidia sepium

	Sugar cane			**Gliricidia sepium**			
	Without	**With**	**P**	**Without**	**with**	**P**	**SEM**
DMI	223	440	0.001	274	389	0.001	8.95
Urine	367	270	0.001	318	319	0.91	9.51
Apparent digestibility, %							
DM	67.4	75.8	0.001	71.0	72.2	0.20	0.66
OM	71.8	67.3	0.001	67.0	72.1	0.001	0.92
Crude protein	91.2	80.4	0.001	84.3	87.4	0.116	1.37

Table 2. Mean values of feed intake by goats feed differences levels of Tithonia diversifolia foliage in fresh and supplemented with Gliricidia sepuim and sugar cane .

	TD	**TDSC**	**TDGL**	**TDSCGL**	**SEM**	**Pro.**
Intake in fresh basis, g/d						
Tithonia leaves	469	219	354	256	5.68	0.0001
Tithonia stems	181	76.4	134	83.5	2.16	0.0001
Gliricidia leaves			396	228		
Gliricidia stems			154	70.9		
Glicidia petiole			84.1	51.0		
Sugar cane		683		613		
DM intake ,g/day						
Tithonia	158a	72.5d	119b	83.9c	1.85	0.0001
Gliricidia			156	88.0		
Sugar cane		180		161		
Total	158a	252b	276c	333d.	2.80	0.0001
g/kg LW	15.1a	24.7b	23.7b	31.0c	0.299	0.0001
Apparent digestibility,%						
DM	67.0	75.0	67.9	76.5	0.93	0.77
CP	91.0	77.6	91.4	83.3	1.94	0.17
OM	71.9	62.1	71.7	72.5	1.3011	0.001

abc mean values without common letter differ at P<0.05

In the feeding trial, DM intake (Figures 1 and 2) and live weight gain were increased by supplementation with either sugar cane or Gliricidia (Table 5) but there was no advantage for live weight gain in supplying both supplements at the same time (Table 6; Figures 3 and 4). DM feed conversion tended (P=0.11) to be improved by Gliricidia supplementation. As with live weight gain it appeared that either supplement improved DM feed conversion but the effects were not additive (Figures 5 and 6).

Table 5. Mean values (main effects) for changes in live weight and DM feed in goats fed *Tithonia diversifolia* supplemented with sugar cane or Gliricidia sepium

	Sugar cane			**Gliricidia sepium**			
	Without	**With**	**P**	**Without**	**With**	**P**	**SEM**
Live weight, kg							
Initial	10.1	9.6		9.6	10.0		0.683
Final	12.4	12.6		11.6	13.5		0.680
Final#	12.2	12.9	0.002	11.8	13.2	0.001	0.124
Daily gain, g	18.4	24.2	0.001	15.6	27.0	0.001	1.360
DMI, g/d	217	293	0	205	304	0.001	12.8
DM FCR	16.4	12.3	0.3	17.3	11.4	0.12	2.52

 # Corrected for differences in initial live weight

Table 6. Mean values for changes in live weight and DM feed conversion in goats fed *Tithonia diversifolia* (TD) supplemented with sugar cane (SC) and/or Gliricidia sepium (GS)

	TD	**TDSC**	**TDGL**	**TDSCGL**	**SEM**	**P**
Live weight, kg						
Initial	10.0	9.2	10.1	10.0	0.97	
Final	11.2[a]	12.0[ab]	13.7[b]	13.2[b]	0.85	0.001
Daily gain, g	8.49a	22.6b	28.3c	25.6bc	1.45	0.001
DMI, g/d	158a	252b	276b	333c	18.2	0.001
DM FCR	23.1	11.5	9.7	13.1	3.6	0.06

abc mean values without common letter differ at P<0.05

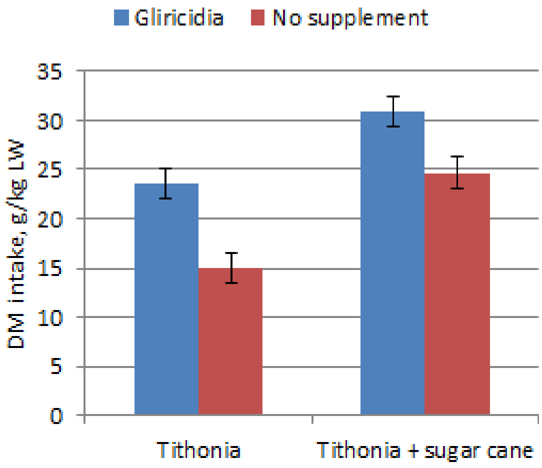

Figure 1. Effect of Gliricidia foliage on DM intake of goats fed Tithonia foliage supplemented or not with chopped sugar cane

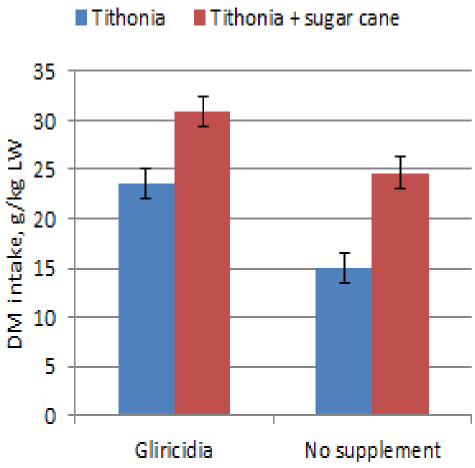

Figure 2. Effect of chopped sugar cane on DM intake of goats fed Tithonia foliage supplemented or not with with Gliricidia foliage

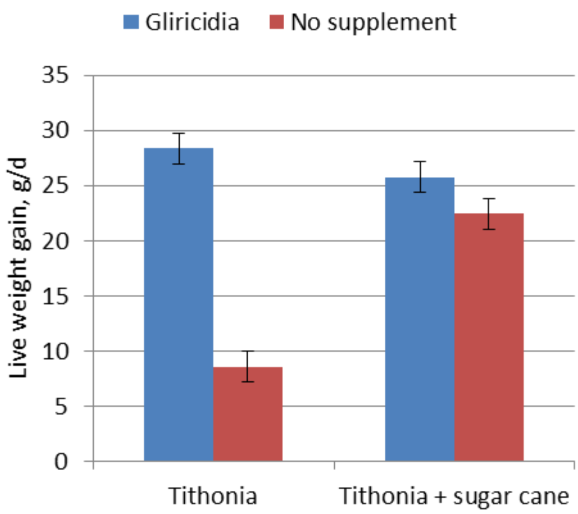

Figure 3. Effect of Gliricidia foliage on live weight gain of goats fed Tithonia foliage supplemented or not with chopped sugar cane

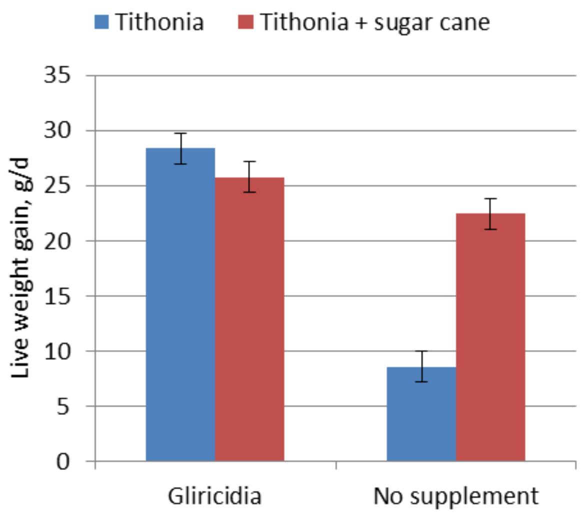

Figure 4. Effect of chopped sugar cane on live weight gain of goats fed Tithonia foliage supplemented or not with Gliricidia foliage

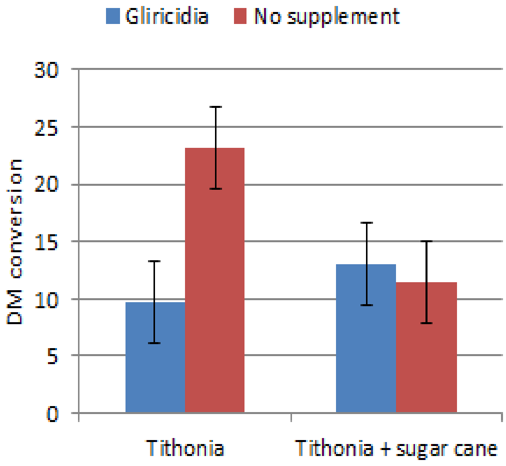

Figure 5. Effect of Gliricidia foliage on DM conversion of goats fed Tithonia foliage supplemented or not with chopped sugar cane

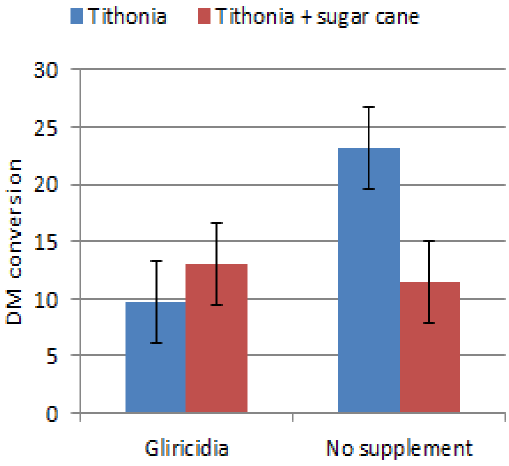

Figure 6. Effect of chopped sugar cane on DM conversion of goats fed Tithonia foliage supplemented or not with with Gliricidia foliage

Discussion

The crude protein in the leaves of fresh Tithonia was similar to the value of 20 % in DM reported by Pathoummalangsy and Preston (2008) and the values of 21% recorded by Mahecha and Rosales (2005) and 22 % recorded by Jama et al (2000). For the sugar cane the crude protein content of 1.7 % in DM is normal for this crop (Preston and Leng 2009).

The increase in voluntary intake and live weight gain when Tithonia was supplemented would appear to be a direct result of more protein becoming available at tissue level, presumably because part of the Gliricidia protein escaped the rumen fermentation and passed directly to the abomasum and intestine. There are several reports showing increases in live weight gain by supplementing forages with Gliricidia foliage: in cattle (Seijas et al 1994; Combellas et al 1996; Preston and Leng 2009); in goats (Abdulrazak et al 2006); and in sheep (Izaguirre et al 2011).

The specific case of Tithonia as the basal forage appears to be unique in that it has an apparently high potential nutritive value (eg: apparent digestibilities for DM of 67% and of crude protein of 91%), yet growth rates when fed as the sole forage were low (8.5 g/day). Both sugar cane and Gliricidia increased the growth rate threefold. Similar results were reported by Pathoummalangsy and Preston (2008), in this case reflected in relative increases in N retention to supplementation with cassava root chips and/or foliage of mulberry (*Mora albus).* These authors hypothesized that the effect of the cassava chips was to provide easily fermentable carbohydrate in the rumen to enhance the uptake of the ammonia resulting from the rapid degradation of the soluble protein in the Tithonia; and that the mulberry foliage acted as a source of bypass protein. In the case of the present experiment, it would appear that the sugar cane had a similar effect to the cassava chips used by Pathoummalangsy and Preston (2008); while the Gliricidia has a similar effect to the mulberry foliage used by Pathoummalangsy and Preston (2008). In both studies there were no further benefits in combining the two supplements, compared with either one fed alone.

Conclusions

- DM intake and live weight gain were increased and DM feed conversion tended to be improved when goats fed fresh foliage of *Tithonia diversifolia* were supplemented with either sugar cane or foliage of *Gliricidia sepium.*
- There were no further benefits by combining the two supplements.

Acknowledgements

The research was conducted at the Livestock Research Center (LRC) of NAFRI, Lao PDR.

I would like to express my gratitude to the MEKARN) project and The Norwegian Program for Development, Research and Education (NUFU) , for the financial support of this study and research; and to Associate Professor Dr. Vanthong Pheangvichit, Head of Research Unit National Agriculture and Forestry Research institute and Professor T R Preston for their supervision.

I would also like to extend sincere thanks to Professor Dr. Jan Berg, International Coordinator, NUFU project; Associate professor Dr. Ngo Van Man, MEKARN regional coordinator and Mr. Nguyen Dzung, NUFU project assistant, for their facilitation, help and support to the whole course. The farm staff in LRC provided valuable technical support during the research.

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